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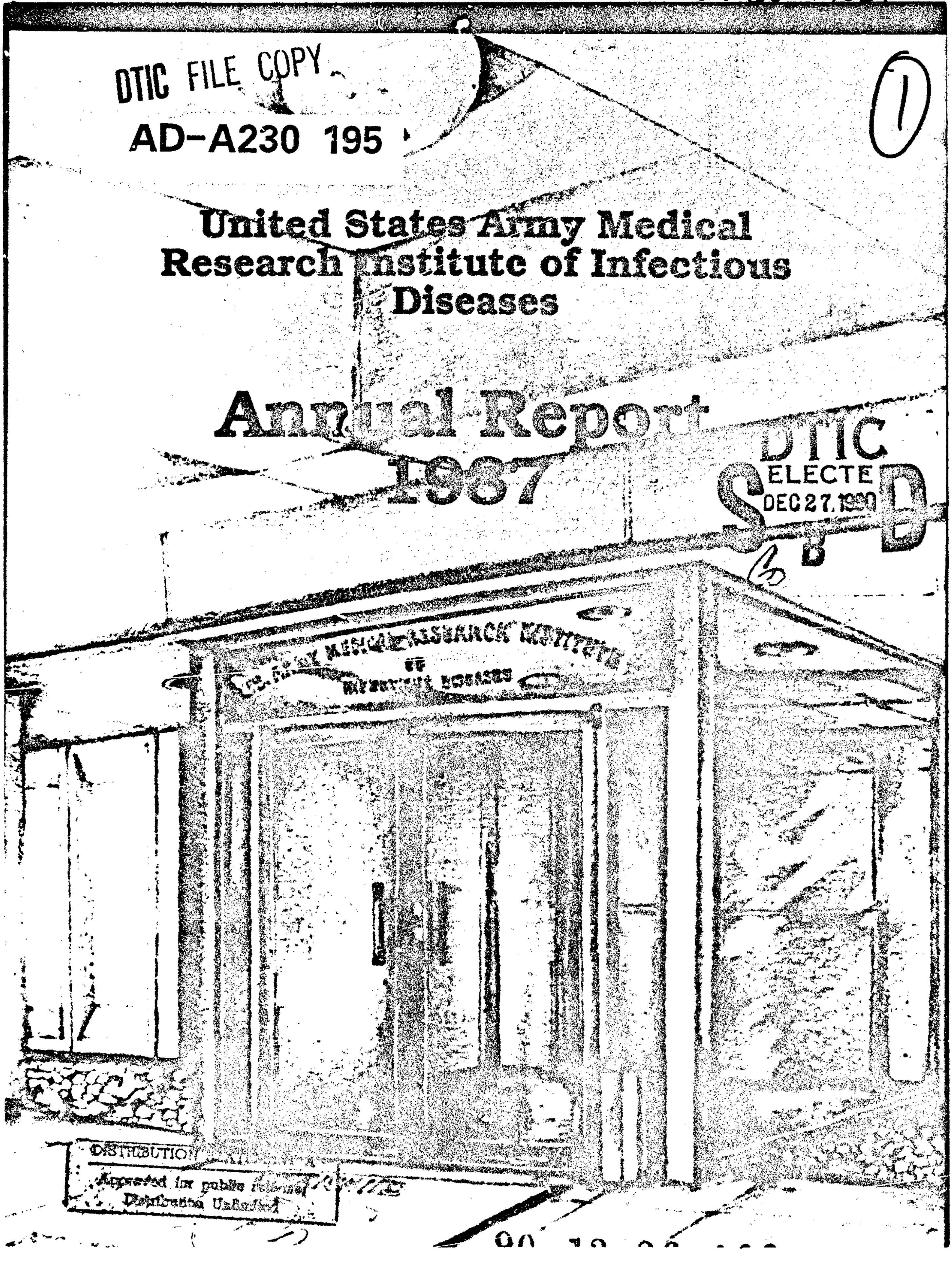
Annual Report 1987



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Annual Report
Fiscal Year 1987

United States Army
Medical Research Institute
of Infectious Diseases
Fort Detrick, Maryland

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A report of progress on the research program of the U.S. Army Medical Research Institute of Infectious diseases on Medical Defense Against Biological Agent (U) for Fiscal Year 1987 is presented.

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U.S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

FREDERICK, MARYLAND 21701-5011

U.S. ARMY MEDICAL RESEARCH

INSTITUTE OF INFECTIOUS DISEASES ANNUAL PROGRESS REPORT

FISCAL YEAR 1987

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Project 3M161102BS12
3M263763D807
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3M162770A871
3A161101A91C

1 October 1987

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EDITOR'S NOTE

This FY 1987 Annual Progress Report is a general review of research activities of the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, conducted on Medical Defense Against Biological Warfare Agents (U) under Projects 3M161102BS12, 3M263763D807, 3M463750D809, 3S464758D847, 3M162770A870, 3M162770A871, and In-House Laboratory Independent Research, Project 3A161101A91C.

In conducting the research described in this report, the investigators adhered to the "*Guide for the Care and Use of Laboratory Animals*," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

FOREWORD

The United States Army Medical Research Institute of Infectious Diseases (USAMRIID) has as its mission the development of strategies, products, information, procedures, and training for medical defense of U.S. Armed Forces against biological warfare threats as well as naturally occurring toxins and infectious agents of military importance that require special containment. The countermeasures include vaccines, drugs, diagnostic reagents, and medical management methods to reverse or minimize the effects of a biological attack or an endemic disease and to preserve fighting strength. USAMRIID is also a Department of Defense reference laboratory for disease agents of biological origin.

USAMRIID's leadership role and its reputation in medical defense against BW threats are extensive. Each year USAMRIID receives additional requests from talented postdoctoral candidates to spend time in the laboratories of internationally recognized USAMRIID investigators. In FY 87, National Research Council postdoctoral fellows came from the United Kingdom, the Republic of Korea, India, France, Sweden, the People's Republic of China, Japan, Senegal, and Finland. The Institute also welcomed visiting scientists and dignitaries from counterpart organizations in the United Kingdom, the Federal Republic of Germany, the People's Republic of China, France, the Republic of Korea, Australia, Japan, and Brazil. In March USAMRIID was the venue of the one-day international meeting *Hemorrhagic Fever with Renal Syndrome: Symposium on Progress in Pathobiology, Diagnosis and Treatment*. A USAMRIID representative was once again invited, by the Department of State and the Office of the Secretary of Defense, to participate as an official U.S. delegate and expert in the follow-on Experts' Meeting in Geneva in April 1987, as mandated by the 1986 Second Review Conference of the Biological Weapons Convention.

USAMRIID's proficiencies in dealing with disease outbreaks have been tested and proven many times over in civilian and military emergency situations, most recently, for example, with Korean hemorrhagic fever in U.S. marines stationed on Okinawa, and with the outbreaks of Ebola fever, Lassa fever, and Rift Valley fever in Africa. In these examples, USAMRIID scientists led or were members of the specialized teams who pooled expertise in infectious diseases and coordinated the successful efforts that resulted in rapid and reliable diagnoses and, in some cases, countermeasures.

There are many derivative benefits originating from the USAMRIID medical defense research programs, which impact, not only on the Army's state of readiness, but also on other federal agencies and foreign governments. USAMRIID is a major resource of scientific information and professional expertise for many other national agencies. The Institute serves as consultant to the Department of State on terrorism; to the Federal Bureau of Investigation on hostage negotiations and BW threat assessment; and to the Department of

Agriculture on treating and containing exotic disease outbreaks within the United States. Other government agencies, industrial laboratories, pharmaceutical houses, and foreign governments depend on USAMRIID for information on disease agent pathogenesis, diagnosis, prevention, and treatment. The Institute provides design and function recommendations relating to safety precautions and procedures in biological containment laboratories.

USAMRIID has the capability of deploying a unique medical team to evacuate patients by stringent isolation procedures. Such procedures are essential for health care providers, and especially for patients who have, or are suspected of having a high-hazard disease, such as a hemorrhagic fever.

USAMRIID provides diagnostic services, diagnostic assays and reagents, monoclonal antibodies, DNA segments, and a variety of vaccines, toxoids, antitoxins, and antisera to federal, state and local agencies, commercial organizations, and foreign governments. In FY87, the Institute provided vaccines and biologics to nearly 100 requestors, among them: the *Institut Pasteur* (in Paris, Dakar and Bangui); the Chemical Defence Establishment of the U.K.; the Centers for Disease Control (in Atlanta and Fort Collins, CO); the University of Lund, Sweden; our forces in the Republic of Korea; the Medical School of the University of Thessaloniki, Greece; Ciba-Geigy, Ltd. of Basel, Switzerland; the Yale University Arboviral Research Unit; and Harvard Medical School. The shipment of these products and their associated technologies are indicative of USAMRIID's increasingly important role - both nationally and internationally - in infectious disease research.

USAMRIID's approach to medical defense is both traditional and innovative. Active immunization with vaccines is still considered the ideal method of disease prevention, and has been proven the most effective method for controlling infectious diseases caused by conventional agents. Therefore, efforts in vaccine development have received high priority. It may well be impossible to develop vaccines against all potential agents. It is possible, though, with the powerful new tool of genetic engineering, to develop vaccines that can protect against an entire class of disease agents, such as the alphaviruses or the venoms of snakes of the family Elapidae.

To provide broad-spectrum protection against viruses, an antiviral drug development program was established in 1981. Through its auspices, thousands of compounds per year are screened for antiviral activity against the deadliest viruses known. Research is also being pursued on immunopotentiators to use in tandem therapy with antiviral drugs.

The development of a countermeasure to a threat has historically been a long process, often requiring 14 years or more. Today, those research techniques, known under the umbrella term "biotechnology" offer the potential for reducing the total time required by four to five years. Biotechnology has been identified as one of the five major technology thrusts of the Army. These techniques are being applied in all Institute research programs and include recombinant DNA technology, DNA probes, manipulation of plasmids and genomes, hybridoma and monoclonal antibody production, sequencing studies, liposome microencapsulation technology, immunopotentiators, and immunomodulators, including interferons and interleukins, and enzyme stabilization and immobilization techniques.

The development and application of computer artificial intelligence has opened new frontiers for the modification and manipulation of molecular configurations of therapeutic drugs. Analogs of effective drugs are being derived quite rapidly and more accurately than ever before. Once drug sites are identified for intervention, therapeutic drugs can be designed to react at these sites.

New vaccines, new treatment modalities, and new diagnostic assays for unusual diseases and toxins are all concrete evidence of the USAMRIID's increased productivity in 1987. These products and information, while generated for exotic and dangerous agents with biological warfare potential, have broad application to both military and civilian medicine. The Institute is proud to continue the tradition of Army science contributing to worldwide public health.

Questions and comments about this report are welcomed and may be addressed to:

Commander
USAMRIID
Fort Detrick
Frederick, Maryland 21701-5011

AUTHOR INDEX

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a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Middlebrook J M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7211
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Schmidt, J
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22. KEYWORDS (Precede EACH with Security Classification Code) (U) BW Defense; (U) Low Molecular Weight Toxins; (U)Microbial Toxins;(U) Biochemistry;(U) Therapy;(U) Lab Animals;(U) Guinea Pigs;(U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) The technical objective of this work unit is to provide the research base for the development of protective modalities from toxins. Recent advances in molecular genetics have demonstrated that many toxins can be cloned and produced in mass quantities. We are studying several toxins of high potential for BW use with these new techniques.

24. (U) Our approach is to study any and all aspects of the toxin including detection, genetics, synthesis, elaboration, structure, composition, pharmacology, mechanism of action, pathogenesis, and sensitivity to drugs. We seek to develop novel means of protection from botulinum and marine toxins as well as mycotoxin. Some of these are synthetic vaccines, CRM-based vaccines, toxin-blocking drugs or toxin-reversing drugs.

25. (U) 8610 - 8709 Monoclonal antibodies have been raised to 5-6 snake phospholipase A2 neurotoxins (PLA2's). One monoclonal was an extremely potent neutralizing antibody against crotoxin. This monoclonal was used as the antigen to develop antiidiotype antibodies to test as vaccine candidates. Rabbit antisera were used to investigate the immunological cross-reactivities of 14 PLA2 neurotoxins or enzymes. Extensive and complex relationships were defined and we determined that all PLA2 neurotoxins could be placed into 3 serogroupings. Five drugs were identified which have varying degrees of protective activity against beta-bungarotoxin. Twenty-seven mg type A and 75 mg type B were purified and stored for research purposes. Sufficient purified type C was obtained so that 20 residues of the light chain and 14 of the heavy chain were identified. Antisera to peptides based on botulinum toxin sequences were obtained and evaluated.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M161102BS12: Science Base/Medical Defense Against BW

WORK UNIT NO. S12-AA-001: Basic Studies of Conventional Toxins of Biological Origin and Development of Medical Defensive Countermeasures

PRINCIPAL INVESTIGATOR: J. L. Middlebrook, Ph.D.

ASSOCIATE INVESTIGATORS: R. Crosland, Ph.D.
J. Schmidt, Ph.D.
D. Leatherman

BACKGROUND

The BW potential of toxins has long been recognized. The Army has had a research program directed towards the development of protective modalities, primarily vaccines. The program was driven by a combination of intelligence information and a knowledge of which toxins were potent enough and could be prepared in quantities sufficient for delivery. Basically, these considerations kept our list of candidate BW toxins rather short. With the emergence of molecular genetics as a real and practical technology, the number of potential threat toxins has become enormous. It is clearly impractical to attempt to study each and every toxin. Rather, a program has been designed which is flexible enough to accommodate new threats rapidly, while developing information with representative toxins to build general principles for protection. At present, we are studying botulinum toxin, tetanus toxin, several snake phospholipase neurotoxins, several snake postsynaptic neurotoxins, ricin, snake mycotoxins, and diphtheria toxin.

An important element in many cases is to obtain the gene for the toxin either by synthesizing it (based on known protein sequence data) or cloning. With the gene in a suitable expression system (no trivial task we know), we can produce the toxin in large enough quantities for use in basic research/drug development or to be toxoided and tested as a vaccine. The vaccine, in turn, can be used to produce antibody for clinical use or detection systems. Alternatively, the gene can be chemically altered to produce nontoxic, but immunologically cross-reacting proteins (CRM) and then these can be tested for possible efficacy as vaccines.

SUMMARY

Monoclonal antibodies have been raised to five to six snake phospholipase A2 neurotoxins (PLA2) and development of monoclonals to four others is underway. One monoclonal was an extremely potent neutralizing antibody against crotoxin, but not other PLA2 neurotoxins. This monoclonal was used as the antigen to develop anti-idiotype antibodies to test as vaccine candidates. Using rabbit antibody against crotoxin as the capture reagent, we

obtained nine isolates. These anti-idiotype monoclonal antibodies are now being purified and will be tested as antigens for raising neutralizing antisera in guinea pigs. Rabbit antisera were used to investigate the immunological cross-reactivities of 14 PLA2 neurotoxins or enzymes. Extensive and complex relationships were defined and it was determined that all PLA2 neurotoxins could be placed into three serogroupings. Five drugs were identified that have varying degrees of protective activity against the venom from *Bungarus multicinctus* and the most toxic component of the venom, ß-bungarotoxin. Type A (27 mg) and type B (75 mg) were purified and stored for research purposes. Sufficient purified type C was obtained so that 20 residues of the light chain and 14 of the heavy chain were identified. Antisera to peptides based on botulinum toxin sequences were obtained and evaluated. As yet, the antisera produced react with, but do not neutralize botulinum toxin.

MEGA Mechanisms of action of bacterial exotoxins. Studies on botulinum toxin.

PRINCIPAL INVESTIGATOR: John L. Middlebrook, Ph.D.

During the past year we have expanded our scope of work such that our project title is outdated. In addition to bacterial toxins, we now study mechanistic and immunological aspects of several snake presynaptic neurotoxins.

In collaboration with Ivan Kaiser, University of Wyoming, we have found that monoclonal 1 to be an extremely potent neutralizing antibody against crotoxin, but not other PLA2 neurotoxins. This is the first report of a neutralizing monoclonal antibody to any PLA2 neurotoxin. We further found that monoclonal 1 completely inhibited the enzymatic activity of crotoxin. In contrast, another monoclonal (#5) also inhibited the enzymatic activity of crotoxin, but had no effect on the toxin's lethal effects. This is an important separation of enzymatic and neurotoxic activities of the toxin and may be a critical clue to understanding the molecular basis of crotoxin's action.

Because of the potent neutralizing activity of monoclonal 1, we chose to use it as the antigen to develop anti-idiotype antibodies for possible vaccine candidates. Mice were immunized with monoclonal 1 and routine hybridoma fusions were carried out. Using rabbit antibody against crotoxin as the capture reagent, we obtained nine isolates, which were double-cloned and ascites fluids obtained. These anti-idiotype monoclonal antibodies are now being purified and will be tested as antigens for raising neutralizing antisera in guinea pigs. While it is too early to assess their value in this respect, it is clear that the anti-idiotypes are recognized immunologically as crotoxin.

Using eight, highly purified PLA2 neurotoxins, rabbit antisera were raised and were used to investigate the immunological cross-reactivities of a total of 14 PLA2 neurotoxins or enzymes. Extensive and complex relationships were defined and we determined that all PLA2 neurotoxins could be placed into three serogroupings: the first was the crotalid and vipirid toxins, the second was the elapid toxins, and the third was ß-bungarotoxin alone. Monoclonal antibodies are in hand for each of the three groups and systems are now being developed which should detect any PLA2 neurotoxin.

Finally, work has continued with botulinum toxin. An MUA was filled with the National Institutes of Health to clone the gene for type C toxin; approval was granted. Considerable effort was required to obtain enough phage DNA to prepare a library. However, with the help of Mel Eklund (Seattle), we now have the requisite material, we anticipate and rapid progress. In addition, further work on the mechanism of action of botulinum toxin has demonstrated that changes in phosphorylation can be observed when synaptosomes are incubated with the toxin. Further work is underway to define the relationship(s) of these observations with our earlier finding on toxin-induced drops in cGMP levels.

MEGB Protein Chemistry and Structure-Function studies of *Clostridium botulinum* Neurotoxins

PRINCIPAL INVESTIGATOR: J. Schmidt, Ph.D.

Work was continued on the preparation of highly purified botulinum neurotoxins. To this end, semi-crude preparations of types A and B were obtained from Dr. Siegel. These had been grown in the fermenter, acid precipitated, extracted, and passed through a DE-52 column. I purified these toxins by my previously described procedure, with the exception that I further simplified the method and improved the yield. Supplies of highly purified botulinum neurotoxins, serotypes A, B, C, and E, are being maintained at levels adequate for research.

Due to low production by the bacterial strains employed here, efforts to improve the efficiency of purification of type C neurotoxin were continued. I found that, in contrast to published results, 50% of type C toxin remained inside the bacterium at the end of the growth period and had to be be extracted. Currently, I employ ammonium sulfate precipitation, followed by solubilization of the toxin with tris-phosphate buffer, then hydroxyl apatite chromatography as the first column in a four-column procedure. I obtained and evaluated a new strain of type C for toxin production. Although it did produce more toxin (relative to our strains), it did so chiefly in fortified, cooked- meat medium, which is unsuitable for large-scale work.

Sufficient purified type C was obtained so that I could then individually purify small amounts of the constituent heavy and light chains. These were placed on our gas-phase sequencer. Twenty residues of the light chain and 14 of the heavy chain were identified. In addition, whole type C toxin was digested with proteases, and the fragments were partially purified by HPLC. These were sent to Dr. Don Hunt at the University of Virginia for sequencing by mass spectrometry. Data so obtained were provided to Dr. Alcaide for cloning work.

Sequence data on type E tryptic peptides were also received from Dr. Hunt. Further, a preparation of type E was cleaved with cyanogen bromide, and sequences of the fragments so produced will be analyzed by mass spectrometry. Many of the methionine residues in type E proved to be resistant to cyanogen bromide and relatively large fragments predominated. The site of nicking was determined to be in the largest CNBr peptide.

In collaboration with Dr. Siegel, antisera to peptides based on botulinum toxin sequences were obtained and evaluated. Using a peptide sequence from the amino-terminal region of type E, antibodies were produced that bound well to the native toxin, but did not neutralize it. Similarly, antibodies to the amino-terminal region of type B could bind to the toxin; neutralization studies are incomplete at this time. Antisera to heavy chain regions of certain serotypes are also being produced in this ongoing study.

MEGF Cellular Mechanisms of Actions of Militarily Relevant Toxins

PRINCIPAL INVESTIGATOR: R. Crosland, Ph.D.

The objective is to develop drug treatments for poisoning caused by neurotoxins. Initial studies have focused on presynaptic neurotoxins which have phospholipase activity as their salient feature (e.g., ß-bungarotoxin, crotoxin, taipoxin, notexin) and on presynaptic neurotoxins which affect calcium channels (e.g., ß-leptinotarsin-h, omega-conotoxin). Efforts to date have been very productive in that I have found five drugs, currently in use for other purposes, which have varying degrees of protective activity against the venom from *Bungarus multicinctus* and the most toxic component of the venom, ß-bungarotoxin. The two most effective drugs are chloroquine and quinacrine, which decrease the lethality of ß-bungarotoxin 17-fold and 8.6-fold, respectively. Both drugs are currently used as anti-malarials in humans, thus greatly reducing the studies necessary to use the drugs against neurotoxins.

PRESENTATIONS

1. **Middlebrook, J. L.** 1987. Transport of protein toxins across membranes. Presented at the Annual Meeting of the Biochemistry Society, Leicester, England, April.

2. **Middlebrook, J. L.** 1987. Immunological relationships between snake phospholipase A2 neurotoxins. Presented at the International Conference on Biophysics, Jerusalem, Israel, August.

3. **Middlebrook, J. L., and L. I. Kaiser.** 1987. A neutralizing monoclonal antibody to crotoxin. Presented at the Annual Meeting of the Society for Toxinology, Singapore, China, June.

PUBLICATIONS

1. **Crosland, R. D.** 1987. Effect of chloroquine on the toxicity in mice of the venom and neurotoxins from *Bungarus multicinctus*. Submitted to *J. Pharmacol. Exp. Ther.*

2. **Kaiser, I. I., and J. L. Middlebrook.** 1987. Preparation of a crotoxin-neutralizing monoclonal antibody. Submitted to *Toxicon.*

3. **Middlebrook, J. L.** 1987. Cell surface receptors for protein toxins, pp. ---- . *In* L. L. Simpson (ed.), Academic Press, New York. In Press.

4. **Ronnberg, B., and J. Middlebrook.** 1987. Effects of macromolecular synthesis inhibition on diphtheria toxin cell-surface receptors, pp. ---- *In* F. Ferhrenbash (ed.), Proceedings of the Third Annual European Workshop on Bacterial Toxins. In Press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DAOG1522	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

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b. CONTRACT/GRANT NUMBER		87	7.0	2071
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a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Dalrymple, J M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7241
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Leppla, S H
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Schmaljohn C S

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Togavirus; (U) Flavivirus; (U) Bunyavirus; (U)Arenavirus (U)Anthrax; (U)Genes; (U)Antigen;(U)Diagnosis;(U)Lab Animals;(U)Mice;(U)RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To elucidate antigenic composition, replicative strategies, and specific gene functions for selected togaviruses, flaviviruses, bunyaviruses, arenaviruses and anthrax. To evaluate specific protein and nucleic acid constituents and gene products as potential diagnostic antigens, probes and immunogens with prophylactic potential. To develop methodology and provide a technical base for an improved BW defense program.

24. (U) Characterize structural proteins and nucleic acid of selected pathogens by biophysical and biochemical techniques; identify the genome regions responsible for important antigenic determinants or diagnostic probes. Ultimately define the replication strategy for a better understanding of mechanisms for either deducing targets for chemotherapeutic intervention or inducing a protective immune response.

25. (U) 8610 - 8709 Monoclonal antibodies to Crimean-Congo hemorrhagic fever virus include several capable of neutralizing viral infectivity and at least one capable of protecting from virus challenge. Synthetic peptides to Rift Valley fever virus are capable of immunizing animals, inducing neutralizing antibody, and conferring some protection against challenge. Recombinant vaccinia viruses expressing the G2 envelope glycoprotein offer promising human vaccine candidates. Molecular clones of Hantaan virus have shown great promise as diagnostic probes for various viral strains, and the expression of the S gene nucleoprotein gene product in a *Baculovirus* system has demonstrated the feasibility of this method for the production of valuable diagnostic antigens. The DNA sequences of the genes for both the protective antigen and the edema factor of anthrax toxin have been determined and the mechanism of interaction of the three toxin components elucidated.

DD FORM 1498 83 MAR EDITION OF MAR 63 IS OBSOLETE

PROJECT NO. 3M161102BS12: Science Base/Medical Defense Against EW

WORK UNIT NO. S12-AB-002: Basic Studies on Conventional Agents of Biological Origin and Development of Medical Defensive Countermeasures

PRINCIPAL INVESTIGATOR: J. M. Dalrymple, Ph.D.

ASSOCIATE INVESTIGATORS: C. S. Schmaljohn, Ph.D.
J. F. Smith, Ph.D.
S. H. Leppla, Ph.D.
J. R. Lowe, COL, Ph.D.
G. B. Knudson, Ph.D
S. F. Little, M.S.

BACKGROUND

The research concept being explored by the basic studies presented within this work unit includes an examination of the feasibility of employing biotechnology or molecular technology for the development of new and improved vaccines and/or diagnostic reagents. Although all of the agents under investigation represent real disease threats, the basic information developed and the strategies determined to be successful in the approach to vaccination or diagnosis should serve as valuable models for related disease producing agents.

Previous progress reports have emphasized the details of obtaining molecular clones and their DNA sequences, the generation of batteries of monoclonal antibodies, and the development of methods and techniques for the synthesis or expression of gene products. The state-of-the-art has progressed to the point that many of these clones, sequences, and monoclonal antibodies are now available, and experience with the associated technologies has increased the feasibility of obtaining similar reagents and information for other agents. In this report we see an expansion of the research into an investigation of the various methods for exploiting this new information in the pursuit of improved disease prevention and control.

SUMMARY

The successful molecular cloning, sequencing, and antigenic analysis of a variety of disease agents serves as a model for the molecular investigation of human pathogens and offers various approaches to agent detection, diagnosis, and protection. Basic research on Rift Valley fever virus (RVF) has revealed genome organization, viral replication strategy, antigenic determinants important for any successful human vaccine, and a determination of the genetic diversity among viral strains in nature. In this report, the use of

synthetic peptides generated from the DNA sequence analysis as well as recombinant vaccinia viruses containing RVF viral genetic inserts exhibit great potential for future human vaccine candidates. In addition, these studies further define the antigenic structure and variability of these agents, increase our ability to decipher mutations and other genetic alterations in these and related viruses, and greatly enhance the research planning for the study of other agents.

The recent description of the *Hantavirus* genus serves as a classical example of a group of closely related viruses, causing significant human disease of military importance, that were characterized and analyzed, primarily by molecular biotechnology. The successful cloning, sequencing. and analysis of the genes coding for the important viral proteins similarly revealed genome organization and replication strategy and provided the basis for their classification. In this report, the description of the utility of nucleic acid probes for the detection of viruses, the measurement of the genetic variability between various viral strains and isolates, and the expression of the nucleoprotein in a baculovirus system for producing non-infectious diagnostic antigens all serve to illustrate the application of these discoveries to practical military problems.

The investigation of anthrax toxin has been approached by many of the same technologies employed for the virus research. The successful DNA sequencing of protective antigen (PA) and edema factor (EF) and the identification of 24 separate antigenic determinants on PA by a battery of monoclonal antibodies again serve to illustrate the similarity in approach. The elucidation of the mechanism of interaction of the three components of the anthrax toxin provides important information for future anthrax vaccine development.

MEIA Molecular and Biological Characterization of Nairoviruses

PRINCIPAL INVESTIGATOR: J. F. Smith, Ph.D.

We are currently studying Crimean-Congo hemorrhagic fever virus (CCHF). The central objective of these studies is the development of a sufficiently large and diverse panel of monoclonal antibodies to enable i) antigenic structure studies to be carried out among various CCHF strains and other Nairoviruses, ii) rigorous identification of virus-coded structural and nonstructural proteins as well as the post-translational modifications carried out on these proteins, and iii) a definition of the viral antigens expressing neutralizing and protective epitopes.

Using ELISA, immunofluorescence, neutralization, and antigen production procedures established last year, we have performed two hybridoma fusions, which have resulted in approximately 130 hybridomas. The initial analysis of these monoclones indicates that about 65% of the antibodies are directed at the viral nucleocapsid protein with the remainder directed at other viral structural proteins. Several of these antibodies are capable of neutralizing virus infectivity in vitro, and at least one is capable of protecting infant mice in challenge studies. Preliminary studies of epitope conservation among members of the Nairovirus family with polyclonal antisera indicated that the surface glycoproteins, rather than the internal proteins, are the most conserved. This observation, together with the finding that the immunofluorescence assay is broadly cross-reactive within this group, suggest that previous epidemiology studies may not have discriminated CCHF from other Nairoviruses. We hope that the monoclonal antibodies will enable the construction of sensitive and specific assays which will not only allow this differentiation, but will discriminate various CCHF strains which differ in their virulence for man.

MEIB Analysis of Experimental Rift Valley Fever Vaccines

PRINCIPAL INVESTIGATOR: J. M. Dalrymple, Ph.D.

ASSOCIATE INVESTIGATOR: J. F. Smith, Ph.D.

Studies have been conducted in support of the objective of improved RVF virus and related Phlebovirus vaccines by a variety of approaches. Analysis of a large number of monoclonal antibodies as well as monospecific antisera have indicated that protective epitopes are expressed only on the G2 surface glycoprotein and that some of these epitopes are conformationally independent. Based on this information as well as physical mapping data provided by others, a series of 16 synthetic peptides, ranging in size from 9 to 14 residues, have been examined for their ability to induce antibodies reactive with native viral proteins as well as neutralizing protective antibodies. In addition, the expression of varied envelope glycoprotein genes and gene segments in recombinant vaccinia viruses have been examined as candidate vaccines. Genetic variation among various isolates of RVF viral strains has been examined by sequencing regions of the envelope glycoprotein genes known to code for immunogenic determinants. These studies are aimed at examining the natural diversity of strains against which any engineered vaccine would be required to protect.

Peptide immunization of rabbits or mice have shown that 14 peptides induced high-titer, anti-peptide antibodies; and 12 induced antibodies reactive with viral proteins in ELISA, immunoprecipitation, or western blot assays. These site-specific antibodies have proved very useful in elucidating the intracellular processing mechanisms occurring during virus glycoprotein synthesis. In addition, a single peptide consisting of only 12 amino acids (381-12), induced significant levels of neutralizing antibodies. In preliminary studies, 381-12 protected 10 to 30% of immunized mice. Current peptide studies are designed to i) improve the immunogenicity of 381-12, ii) to analyze longer peptides containing 381-12 sequences, and iii) to study sequences corresponding to other regions of the G2 molecule believed to contain protective epitopes.

Animal immunization with a large number of recombinant vaccinia viruses containing various gene constructs from RVF viral envelope glycoprotein genes have clearly demonstrated that any construct expressing mature G2 envelope glycoprotein is protective in the mouse protection assay. Recombinants expressing only the G1 envelope glycoprotein have exhibited only limited or no protective capacity. Recombinants that vary with respect to the initiation codons inserted exhibited differences in the non-structural precursor proteins translated; the same rules of protection apply, i.e., G2 glycoprotein expressed equals protection of mice.

Considerable nucleotide sequence homology exists between various natural geographic isolates of RVF virus from Africa. Natural isolates exist that are not recognized by the neutralizing and protective monoclonal antibody against an important determinant on the G2 envelope glycoprotein. Similarly, monoclonal antibody-resistant mutants were generated, and the precise nucleotide/amino acid changes that are responsible for the resistance of the mutant virus to the monoclonal antibody were identified. Mutant viruses so generated were still readily neutralized by polyclonal antisera to parent RVF virus and candidate vaccines still conferred protection against these mutants.

MEID Molecular and Biological Characterization of Hantaviruses

PRINCIPAL INVESTIGATOR: C. S. Schmaljohn, Ph.D.

Molecular techniques have been employed to further our understanding of the relationships of viruses in the newly established *Hantavirus* genus of Bunyaviridae as well as to develop diagnostic antigens and probes for viruses of this group.

Prototype Hantaan virus was compared to two viruses isolated from Korean hemorrhagic fever (KHF) patients in order to investigate the genetic stability of these viruses and to determine the similarity of viruses isolated from rodent versus human hosts. Hantaan virus was originally isolated from the Korean striped field mouse, *Apodemus agrarius corea*, in 1976 and was passaged in sero-negative *Apodemus* prior to adaptation for growth in cell culture in 1978. Lee virus was isolated by injecting viremic blood from a KHF patient into sero-negative *Apodemus* in 1979, and subsequently was adapted to cell culture. HoJo virus was isolated without animal passage by direct inoculation of a 1985 KHF patient's blood into Vero E6 cell culture. The M genome segments of these three viruses (which encode both of the viral envelope glycoproteins), were compared by nucleotide sequence analysis. Comparison of amino acids predicted from the nucleotide sequences demonstrated that Lee and HoJo vriuses shared 98.2% and 97.5% homology with Hantaan, respectively, and 98.7% homology with one another. In each of the three viruses, more differences were found in G1 than in G2 coding regions. These similarities were also reflected in plaque-reduction neutralization and hemagglutination inhibition studies performed with a variety of monoclonal and polyclonal sera. No significant differences between the three viruses were revealed with either test. These data demonstrate that the etiologic agent of KHF is nearly identical to Hantaan virus and suggest that the viruses do not undergo major or rapid antigenic changes in their rodent and human hosts.

Investigation of the relationships of viruses was extended to each of the four hantavirus serogroups by nucleic acid probe hybridization techniques, and the potential utility of these probes for detecting representative hantaviruses was evaluated. Recombinant DNA clones representing the M and S genome segments of prototype Hantaan virus were subcloned into plasmid vectors containing promotors for SP6 polymerase. Radiolabeled RNA transcripts were generated by copying the cDNA with SP6 polymerase and were hybridized to RNA from Hantaan, Seoul urban rat, Sapporo rat, Puumala, Prospect Hill, Lee, and HoJo viruses. Our results indicated that the RNA probes were very sensitive detectors of Hantaan and the two human viruses, were somewhat less sensitive for the rat viruses, and, as expected, were much less effective in detecting the more distantly related Puumala and Prospect Hill viruses. These data demonstrate the feasibility of using nucleic acid probes for the detection of a variety of hantaviruses. Such probes may have great utility in diagnostic areas where antibody probes are impractical, such as the probing of immune complexes for the presence of virus.

The cDNA clones of Hantaan virus have also been used to generate diagnostic antigen. Clones representing the small (S) genome segment of Hantaan virus (which encodes the viral nucleocapsid protein) were subcloned into a baculovirus expression vector by homologous recombination with *Autorgrapha californica* nuclear polyhedrosis virus (AcNPV). The foreign gene was inserted such that it replaced the polyhedrin gene of the baculovirus and thus was under the control of the strong polyhedrin promotor. Because AcNPV infects only lepidopteran (moth) cells and not mammalian cells or dipteran (mosquito) cells, high levels of expressed Hantaan nucleocapsid protein can be generated under reduced laboratory containment. We examined this expressed nucleocapsid protein for antigenicity and for reactivity with a variety of antisera to representative hantaviruses and also to sera from patients with hemorrhagic fever with renal syndrome. These studies revealed that the expressed protein reacts to high titer with antisera to Hantaan and Seoul urban rat viruses as well as with all Korean hemorrhagic fever patient sera examined. We observed lower reactivity with antisera to Puumala and Prospect Hill viruses and with sera from Nephropathia epidemica patients. The expressed protein reacted in all cases very similarly to authentic Hantaan virus protein and provides a means with which to generate large amounts of diagnostic antigen under safe conditions. The ability for scaling up antigen production by propagation of *S. frugiperda* cells in suspension culture also suggests there may be an adequate supply for the extensive serological testing anticipated.

MEDA Basic Research Studies for Protection Against Anthrax

PRINCIPAL INVESTIGATOR: S. H. Leppla, Ph.D.

ASSOCIATE INVESTIGATORS: J. R. Lowe, COL, Ph.D.
G. B. Knudson, Ph.D.
S. F. Little, M.S.

The DNA sequences of the genes for PA and EF proteins of anthrax toxin have been determined. Nested deletions of the lethal factor (LF) gene were obtained for use in DNA sequencing. Deletions of the PA and EF genes were generated for use in mapping antigenic and functional domains of the individual proteins.

Characterization of monoclonal antibodies against PA showed that the 36 available antibodies identify approximately 24 antigenic regions. An additional set of monoclonal antibodies was prepared against the purified amino terminal 20- kd trypsin fragment, which is poorly immunogenic when it exists as a constituent of the intact protein.

The mechanism by which the three protein components of anthrax toxin interact was not previously known. We have now discovered that PA binds to the surface of animal cells and is proteolytically cleaved at a single peptide bond, thereby exposing a site to which either LF or EF binds. Thus, PA has two binding sites--one for binding to the surface of animal cells, and a second that binds either of the other two toxin components. The latter site becomes exposed only after PA is proteolytically cleaved by a target cell enzyme. Alteration of the cleavage site by directed mutagenesis is expected to produce an inactive PA that may find use in future anthrax vaccines.

We demonstrated that biting flies and mosquitoes are capable of mechanically transmitting *B. anthracis* from infected to healthy animals.

Mutagenesis with the transposon TN*916* was used to obtain aromatic amino acid requiring strains of *B. anthracis*. These mutant strains are expected to be avirulent, and may serve as the basis for improved live vaccines.

PRESENTATIONS

1. **Battles, J. K., M. S. Collett, and J. M. Dalrymple.** 1986. Comparison of Rift Valley fever virus isolates and variants by nucleic acid sequencing of G2 envelope glycoprotein epitope genes. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

2. **Battles, J. K., and J. M. Dalrymple.** 1987. Nucleotide sequence comparison of geographic variants of Rift Valley fever virus and antibody escape mutants. Presented at the Annual Meeting of the American Society for Virology, Chapel Hill, NC, May-June.

3. **Knudson, G. B., and M. J. Turell.** 1987. Mechanical transmission of *Bacillus anthracis* by the stable fly, *Stomoxys calcitrans*. Presented at the 87th Annual Meeting of the American Society for Microbiology, March.

4. **Leppla, S. H., A. M. Friedlander, and E. M. Cora.** 1987. Proteolytic activation of anthrax toxin bound to cellular receptors. Presented at the Third European Workshop on Bacterial Protein Toxins, Uberlingen, West Germany, June-July.

5. **Morrill, J. C., M. S. Collett, and J. M. Dalrymple.** 1986. Evaluation of experimental Rift Valley fever virus vaccines in pregnant sheep. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

6. **Schmaljohn, C. S., A. L. Schmaljohn, and J. M. Dalrymple.** 1987. Coding strategy and expression of the M and the S genome segments of Hantaan virus. Presented at the XVIth Pacific Science Congress, Seoul, Korea, August.

7. **Schmaljohn, C. S.** 1987. Coding strategy and expression of the M and S genome segments of Hantaan virus. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

8. **Smith, J., L. Hodgson, M. Dombalagian, and A. Komoriya.** 1987. Induction of neutralizing antibodies to Rift Valley fever virus with synthetic peptides. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

PUBLICATIONS

1. **Ivins, B. E., G. Knudson, S. Welkos, and D. LeBlanc.** 1987. *Bacillus anthracis* as donor and recipient in filter mating transfers of Tn*916*. *Plasmid* **17**:78.

2. **Ivins, B. E., S. L. Welkos, G. B. Knudson, and D. J. LeBlanc.** 1987. Transposon Tn*916* mutagenesis in *Bacillus anthracis*. Submitted to *Infect. Immun.*

3. **Leppla, S. H., D. L. Robertson, S. L. Welkos, L. A. Smith, and M. H. Vodkin.** 1986. Cloning and analysis of genes for anthrax toxin components, pp. 275-278. *In* Flamagne, et al. (ed.), Bacterial protein toxins, Suppl. 15. Zentralblatt für bakteriologie mikrobiologie und hygiene. 1. Abteilung. Gustav Fischer, Stuttgart.

4. **Schmaljohn, C. S., G. B. Jennings, and J. M. Dalrymple.** 1987. Identification of Hantaan virus messenger RNA species, pp. 116-121. *In* Mahy and Kolakovsky (ed.), The biology of negative strand viruses. Elsevier Science Publishers, Amsterdam.

5. **Schmaljohn, C. S.** 1987. Hantaan virus, pp. ---. *In* -----(ed), Virus diseases of laboratory and captive animals. Elsevier Science Publishers, Amsterdam.

6. **Schmaljohn, C. S., H. W. Lee, and J. M. Dalrymple.** 1987. Detection of Hantaviruses with RNA probes generated from recombinant DNA. *Arch. Virol.* **95**:291-301.

7. **Schmaljohn, C. S., A. L. Schmaljohn, and J. M. Dalrymple.** 1987. Hantaan virus M RNA: coding strategy, nucleotide sequence, and gene order. *Virology* **157**:31-39.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DAOG1526	30 Oct 87	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS12	AC	003
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) Basic Studies Seeking Generic Medical Countermeasures Against Agents of Biological Origin

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
81 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	1.0	200
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathophysiology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Saviolakis, G A
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7181
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Bunner, D L
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) Mice; (U) Therapy; (U) Mammalian Peptides; (U) Neurotransmitters; (U) Leukotrienes; (U) RA I; (U)Lab Animals

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To study the basic mechanisms of action and the physiological effects on host vital systems of mammalian low molecular weight peptides, such as neurohormones, known as putative peptide neurotransmitters, and leukokines. To develop therapeutic interventions for military personnel and to mitigate the adverse effects of these peptides, and to develop methods for their detection.

24. (U) I. - In vitro studies, using neural cell cultures, for characterization of peptide and non-peptide receptors, receptor regulation and interactions, post-receptor biochemical events, and evaluation of pharmacologic agonists and drugs. II. In vivo studies, using small animals, for evaluation of peptide systemic effects and pharmacokinetics after respiratory delivery.

25. (U) 8610 - 8709 Several peptides were found to be inactive in regulating muscarinic cholinergic receptors previously detected in human retinoblastoma (RB) cells. Moreover, expression of this neurotransmitter receptor in RB cells was found to be unpredictable. Therefore, this system was considered to be unsuitable for continuous studies. A new in-vitro neural cell system, the PC-12 rat pheochromocytoma cell, was adopted, its culture requirements were established, and assays for catecholamine uptake, release and detection were developed. The effects of cholinergic ligands, substance P, insulin, and dermorphin on catecholamine release from PC-12 cells were evaluated. A rat model was developed for the study of pulmonary absorption and pharmacokinetics of peptides with saline or lipophilic vehicle aerosols. Insulin and interleukin-1 were studied and found to be bioeffective. Assays for these peptides were developed.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M161102BS12: Science Base/Medical Defense Against BW

WORK UNIT NO. S12-AC-003: Basic Studies Seeking Generic Medical Countermeasures Against Agents of Biological Origin

PRINCIPAL INVESTIGATOR: G. A. Saviolakis, LTC, M.D.

ASSOCIATE INVESTIGATOR: D. L. Bunner, COL, M.D.

BACKGROUND

Small molecular weight endogenous peptides, such as neuropeptides and monokines, have major effects on the physiological control of vital organ systems, such as cardio-respiratory, vascular, endocrine, and immunity, and on behavior. Due to their demonstrated potency, many of these peptides may be important as potential BW agents. Therefore, the purposes of this work unit are: (a) to assess the pathophysiological effects of selected groups of peptides, (b) to develop methods for their detection, and (c) to develop therapeutic interventions to mitigate their effects on military personnel.

SUMMARY

In vitro studies. Human Y79 retinoblastoma cells, previously shown to have muscarinic receptors, were continued to be used for studies of peptide effects on this cholinergic receptor subtype. The actions of several peptides on these receptors were evaluated but no effects were found. Continued evaluation of these cells was hampered by problems such as mycoplasma contamination, and changing phenotype of both available cells and also of a new cell clone obtained from the ATCC (American Tissue Culture Collection). For these reasons, studies with these cells were suspended and activities were focused on developing a new neural cell line for the purpose of our studies. The rat PC-12 pheochromocytoma cell was selected because of its properties as a sympathetic and chromaffin cell type and its ability to take up and release catecholamines in response to cholinergic and peptidergic stimuli. The growth and culture requirements of this cell were studied and its ability to release catecholamines in response to nicotine, K^+-induced depolarization, and peptides (insulin, dermorphin, substance P) were studied in preliminary experiments. Moreover, a small number of muscarinic receptors were found on undifferentiated PC-12 cells; receptors for insulin and interleukin 1 were not found. This cell line is now being used to study the effects of a number of peptides on catecholamine metabolism and muscarinic receptors. We hope that this system can be used generically for detecting neuroactive substances and for screening counteracting substances.

In vivo studies. Neurophysiological studies, designed to evaluate the effects of interleukin 1 and other monokines on discrete brain nuclei, were discontinued because of the premature departure from the laboratory of the associate investigator assigned to the project. Studies to evaluate the pulmonary absorption of peptides were resumed in collaboration with Dr. Creasia, Inhalation Toxicologist, assigned to Pathophysiology Division. Insulin was used as the peptide to develop a respiratory absorption model because of its well-defined biological effect, i.e., hypoglycemia, and because of the readily available radioimmunoassay (RIA) for its detection. Insulin, either in saline solution or incorporated in lipophilic vehicles, such as fusidic acid and azone, was delivered to rats by intratracheal instillation and also as an aerosol, and its effects by these routes were compared to those from intravenous administration. Insulin saline was not consistently effective. Intratracheal administration of insulin (mol wt 5640) (1 IU/rat) in fusidic acid or azone was rapidly effective, with significant change in blood glucose levels observed within 5 min of delivery and maximal hypoglycemia (blood glucose < 30 mg/dl) observed within 30 to 40 min. We observed similar effects after delivery of insulin in aerosol form; these were comparable in both potency and time-course to those we observed after intravenous administration. In some animals, the depression of blood glucose was sufficiently severe to cause death. Peak levels of insulin after 5 min of delivery and bioactive levels, higher than basal values, were detected by RIA as long as two h later. Interleukin 1 (mol wt 17,000) (50 ng/rat) in fusidic acid caused fever and Zn^{+2} depression within 4 to 6 h after intratracheal instillation. These studies demonstrated that the pulmonary alveolocapillary epithelium can transport peptides and that relatively small doses of transported peptides can have significant biological effects. Further studies to complete work on these peptides, and studies designed to evaluate the absorption of peptides of smaller size (mol wt 400 to 3,000) have been planned.

In support of in vitro and in vivo studies, and also to expand the technical base of the bioregulator programs, one technician was trained to perform various biochemical methodologies for peptide detection and bioeffects. These included: peptide separation techniques, such as classical and high performance liquid chromatography (HPLC), polyacrylamide gel electrophoresis, and isoelectric focusing. Peptides were detected by RIA; catecholamine were detected and quantitated by HPLC-electrochemistry (EC).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA302660	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 10 Dec 86	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AB	012
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) (U) Advanced Vaccine Development Studies on Rickettsia of Potential BW Threat

12. SUBJECT AREAS 0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	1.0	274
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Williams, J C
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Waag, D M
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) McCaul, T F

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Q Fever; U) Coxiella burnetii; (U) Vaccines; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) *Coxiella burnetii* is perceived to have significant potential as a BW agent. The currently available vaccine is reasonably protective, but highly reactogenic. An efficacious yet more safe vaccine needs to be developed and stockpiled to protect at-risk US troops. The objective is to proceed with evaluation of a chloroform-methanol extracted residue (CMR) vaccine to assess feasibility for use in humans. Simultaneously a new generation subunit vaccine is being sought, which can be produced readily without the requirement for high containment laboratories.

24. (U) Determine toxicity, safety, efficacy, and dose response to CMR vaccine. Extend animal model testing of CMR to identify and quantify humoral and cell-mediated immune responses. Proceed with human use evaluations if safety and efficacy are demonstrated. Define immunogenic subunits of *C. burnetii* to provide bases for development of a subunit vaccine.

25. (U) 8610 - 8709 The immune suppressive complex (ISC) virulence factor was expressed differently among 9 strains of *C. burnetii*. Strains expressing only smooth lipopolysaccharide(LPS) Phase I carried the most active ISC. None of the strains with only rough LPSII expressed the ISC. However, one strain isolated from heart valves did not express the ISC although it contained both smooth and rough LPS chemotypes. A case-controlled study to determine the incidence and risk for acquisition of Q fever has revealed that parturient cats are significant in outbreaks of a typical pneumonia. The role of cats in the spread of Q fever to humans may impact significantly of this zoonosis.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AB-012: Advanced Vaccine Development Studies on Rickettsia of Potential BW Threat

PRINCIPAL INVESTIGATOR: J. C. Williams, Ph.D.

ASSOCIATE INVESTIGATORS: D. M. Waag, Ph.D.
T. F. McCaul, Ph.D.

BACKGROUND

Coxiella burnetii is able to modulate the host immune response in positive and negative directions. These bivalent responses of the host may be both specific and non-specific. While animals injected with phase I cells show increased tumoricidal and bactericidal abilities, pathogenic reactions such as hepatomegaly, splenomegaly, liver necrosis, death, lymphocyte hyporesponsiveness, and antigen-specific negative modulation also occur. These adverse responses generated by the components of the immune suppressive complex (ISC) were the subject of the current study.

Inactivated phase I *C. burnetii* cells are effective in inducing the immunosuppressive activity in mice. Therefore, this activity is not the result of an infectious process, but of some microbial component. Phase I cells at low concentrations do not exert a toxic effect on lymphocytes cultured in vitro, but at high concentrations (>100 µg/ml), lymphocytes are killed. In fact, 5 µg of phase I whole cells (WCI) per ml as in-vitro recall antigen stimulates lymphocytes from saline-injected mice. However, when used as recall antigens with cultured lymphocytes from phase I (WCI) or reconstituted, phase I chloroform-methanol residue (CMRI)-injected animals, we observed substantial immunosuppression. The CMRI is efficacious as a vaccine and non-toxic at high concentrations (>100 µg/ml) in vitro. Therefore, structural features of the ISC are responsible for the induction of suppression in vivo and the initiation of lymphocytosis in vitro.

The recognition of *C. burnetii* infection as a focal or regional health problem for individuals in the animal industry is well accepted. An important question regarding this zoonosis is the role of pets in the spread of Q fever to humans. Retrospective clinical and seroepidemiologic data have lead to the hypothesis that this disease may be frequently involved in outbreaks of atypical pneumonia cases. Previously, in Nova Scotia, we initiated studies on the cause of pneumonia in 1979 and found that *C. burnetii* was the culprit in cases of atypical pneumonia. However, the exposure to cattle, sheep, and goats was not routinely associated as the cause of their infection. We have observed a significant number of cases of Q fever after the skinning of wild rabbits. Importantly, a recent report led to suspicion that exposure to parturient cats might explain discrete foci of outbreaks of Q fever in Nova Scotia. As a result of these observations, we designed a case-control study to determine the incidence and risk factors for acquisition of Q fever in Maritime Canada.

SUMMARY

In previous experiments, the phase I ISC was dissociated, but not inactivated, after extraction of phase I cells with chloroform-methanol. Also reconstitution of the phase I CMR with non-rickettsial chloroform-methanol extract (CME) and reagent grade lipids restored the ISC activity. The phase I lipopolysaccharide (LPS) alone or reconstituted with CME did not possess the properties of the ISC.

The expression of the ISC activity by different isolates of *C. burnetii* was tested. A survey of eight *C. burnetii* strains with structural variation in LPS indicated that phase I Ohio strain, phase I Henzerling, phase I Nine Mile, and phase I Nine Mile 514 expressed the ISC activity. Although the phase I, Nine Mile 514 strain carries the ISC, it is a semi-rough chemotype and does not express any rough LPS. Interestingly, the phase I cardiac valve-isolated, "KAV" and "PAV" strains, which express both the smooth (phase I), semi-rough, and rough (phase II), expressed significantly different properties of the ISC activity. Although organisms expressing the smooth and semi-rough LPS induce the ISC activity, not all organisms classified as phase I induce suppression in the murine system. Noteworthy was the diminution of ISC activity in strains possessing marked increase in the concentration of the rough LPS chemotype. Although some of these strains expressed both smooth and semi-rough LPS, they all expressed significant amounts of the rough LPS chemotype. Thus, the synthesis of LPS and expression of the ISC appear to be controlled coordinately and may reflect linkage on the chromosome, possibly under genetic control. The correlation between LPS structural variation and ISC may be related incidentally to the cell matrix receptor involved in anchoring the dithiothreitol-soluble and CME-active components.

Our studies indicate that exposure to the products of feline conception is a risk factor for acquisition of Q fever. The greatest risk follows exposure to stillborn kittens. Exposure to cat litters postpartem was the only other significant risk factor in the multivariate analysis. Several traditional risk factors for Q fever were present based on the univariate analysis, unadjusted for multiple comparisons. Individuals were at risk for the contraction of Q fever if they worked on a farm, slaughtered or dressed animals, and if they had contact with cat-, cattle-, sheep-, and tick-infested animals. Some of these risk factors obviously overlap, such as working on farms and being in contact with large domestic animals. Despite the weak association of this group of risk factors in the context of the overall study, in individual cases and from our knowledge of the biology, contact with cattle, sheep, and slaughter animals was the probable means whereby some of our cases acquired Q fever.

The question of how cats are infected is paramount to breaking the cycle of spread to man. Our study suggests that cat-associated Q fever may be prevalent in cases of respiratory illness of unknown origin. More case-control studies should be performed to determine the role of cats in the spread of Q fever and the source of feline infection.

PRESENTATIONS

1. **Waag, D., and J. C. Williams.** 1987. Identification of suppressor cells induced following injection of C57BL/10 ScN mice with phase I *Coxiella burnetii* whole cells. Presented at the American Physiology Society Annual Meeting, Federation of American Societies for Experimental Biology, Washington, D. C., April.

2. **Waag, D., J. C. Williams, K.-I. Amano, M. England, and J. Beveridge.** 1987. Lack of correlation between the ability to induce in vivo and in vitro pathogenic reactions and LPS phenotypes of *Coxiella burnetii* strains. Presented at the *Am. Soc. for Rickettsiology, Williamsburg, VA.*

PUBLICATIONS

1. **Amano, K-I, J. C. Williams, S. R. Missler, and V. N. Reinhold.** 1987. Structure and biological relationships of *Coxiella burnetii* lipopolysaccharides. *J. Biological Chem.* *262:*4740-4747.

2. **Her, G. R., S. Santikarn, V. N. Reinhold, and J. C. Williams.** 1987. Simplified approach to HPLC precolumn fluorescent labeling of carbohydrates: N-(2-pyridinyl)-glycosamines. *Carbohdr. Chem.* 76:129-139.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA302662	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	H. TERM	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AC	013
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY-88 -01			

11. TITLE (Precede with Security Classification Code) (U) Advanced Vaccine Development Studies on Viruses of Potential BW Threat

12. SUBJECT AREAS 0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	87 10	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	1.0	610
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Lupton, H W
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2405
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Barrera Oro, J G
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Cole, F E Jr.

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Junin Virus (U) Vaccines; (U) Lab Animals; (U) Monkeys; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Develop and test a live, attenuated Junin virus vaccine prepared against Argentine hemorrhagic fever for prophylactic treatment of at-risk personnel.

24. (U) Conduct preclinical tests to evaluate vaccine safety, immunogenicity, and stability. Conduct animal and in vitro studies to determine vaccine cross protection against heterologous Arenaviruses. Evaluate the vaccine constituents and production processes with goals of improved titer, stability, and immunogenicity. Design and conduct clinical and field trials to evaluate vaccine safety and efficacy.

25. (U) 8610 - 8709 Phase I clinical trials to determine the safety and immunogenicity of the live, attenuated, Candid #1 Junin vaccine continued. Six groups comprised of 39 individuals were studied, while study of a seventh group (5 persons) was initiated. In addition, a randomized, double-blind study in 14 Argentine volunteers (9 vaccinees, 5 placebo) was completed in Pergamino, Argentina. Thus far, a total of 70 volunteers have received the vaccine, with only 7 having detectable, BMN cell-associated viremias. The immune response rate among the 70 vaccinees was 100% as measured by lymphocyte transformation (LT), ELISA, PRN_{80}, or FA. Maximum geometric mean serum titers in responders were 1/285 for ELISA IgM, 1/132 for ELISA IgG, 1/300 for FA, and 1/56 for PRN_{80}. After 1 year, serologic responses were characterized by low and waning titers in most vaccinees, with 0%, 46%, and 70% of responders tested (*n=10 to 13*) positive by FA, ELISA, and PRN_{80}, respectively. However, LT stimulation index values against Junin virus remained unchanged at 1 year postvaccination in 100% of responders. This fact, in concert with our observation that the Candid #1 vaccine elicits a typical secondary response in JV-immune volunteers, suggests strongly that it induces persistent immunological memory, even though its humoral immunogenicity appears marginal. Continued under DA313526.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AC-013: Advanced Vaccine Development Studies on Viruses of Potential BW Threat

PRINCIPAL INVESTIGATOR: H. W. Lupton, COL, Ph.D.

ASSOCIATE INVESTIGATORS: J. G. Barrera Oro, M.D.
F. E. Cole, Jr., Ph.D.

BACKGROUND

A United Nations project jointly conducted by United States and Argentine investigators resulted in the development of Candid #1 strain Argentine hemorrhagic fever (Junin) vaccine at USAMRIID. Master seed, production seed, and vaccine were produced and preclinical testing was completed in conformity with Good Laboratory Practices and Good Manufacturing Practices Regulations, and in compliance with vaccine requirements for both the United States and Argentina. Preclinical data were documented in an Investigational New Drug (IND) submission. Clinical protocols were approved and the vaccine has been administered to small groups of research volunteers at USAMRIID since October 1985. Preliminary data from phase I clinical studies indicate that the vaccine is safe and immunogenic. Field testing of the vaccine will be conducted at the Instituto Nacional de Estudios sobre Virosis Hemorragicas in Pergamino, Argentina, over the next 3.5 years. Adequate experimental design has necessitated a small safety trial, a larger-scale safety trial, and an efficacy trial in seropositive individuals. The efficacy trial will be a double-blind, placebo -controlled study in 3,500 individuals selected from a population with a disease incidence of 75 cases per 10,000 individuals over two endemic seasons. Thus, a threefold reduction in disease will demonstrate vaccine efficacy with 95% confidence and an 80% power. Concurrently, a case-control study will be conducted to assist in selection of the high-risk population, and a risk analysis study in the endemic region will determine Junin viral activity in rodents.

SUMMARY

Phase I clinical trials to determine the safety and immunogenicity of the live, attenuated, Candid #1 Junin vaccine continued. Six groups comprised of a total of 39 individuals were studied, while study of a seventh group (five persons) was initiated. In addition, a randomized, double-blind study in 14 Argentine volunteers (nine vaccinees, five placebo) was completed in Pergamino, Argentina. Thus far, a total of 70 volunteers have received the vaccine, with only seven having detectable BMN cell-associated viremias. No significant changes in clinical or laboratory parameters have been attributable to the use of the vaccine. The immune response rate among the 70 vaccinees was 95%, as measured by lymphocyte transformation or ELISA, 86% by plaque -reduction neutralization(PRN$_{80}$, and 31% by fluorescent antibody (FA).

Antibody could be detected in all 70 by one or more of these tests. Maximum geometric mean serum titers in responders were 1/285 for ELISA IgM, 1/132 for ELISA IgG, 1/300 for FA, and 1/56 for PRN_{80}. After 1 year, serological responses were characterized by low and waning titers in most vaccinees, with 0%, 46%, and 70% of responders tested (n=10 to 13) positive by FA, ELISA, and PRN_{80}, respectively. However, lymphocyte transformation stimulation index values against Junin virus remained unchanged at 1 year postvaccination in 100% of responders. This fact, in concert with our observation that the Candid #1 vaccine elicits a typical secondary response in Junin-immune volunteers, suggests strongly that it induces persistent immunologic memory, even though its humoral immunogenicity appears marginal.

PRESENTATIONS

1. **Barrera Oro, J. G., C. MacDonald, R. Kenyon, J. Meegan, F. Cole, Jr., H. W. Lupton, and C. J. Peters.** 1987. Virus isolation and immune response in humans inoculated with a live, attenuated Junin virus (JV) vaccine. Presented at the VIIth International Congress of Virology, Edmonton, Alberta, Canada, August.

2. **Maiztegui, J. I., F. Feinsod, A. M. Briggiler, C. J. Peters, D. A. Enria, H. W. Lupton, A. M. Ambrosio, E. Tiano, M. R. Feuillade, G. Gamboa, O. Conti, D. Vallejos, C. MacDonald, and J. G. Barrera Oro.** 1987. Inoculation of Argentine volunteers with live-attenuated Junin virus vaccine. Presented at the VIIth International Congress of Virology, Edmonton, Alberta, Canada, August.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA302664	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AD	014
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) (U) Advanced Drug Development Studies Against Agents of Biological Importance

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	3.0	957
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Canonico, P G
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2290
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Kende, M
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Ussery, M A

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) Antiviral Drugs; (U) Pharmacology; (U) Viral Diseases; (U) Lab Animals; (U) Mice; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Identify effective drugs against viruses that are potential threats to military personnel; obtain data on toxicology, pharmacology, and metabolism of antiviral drugs and conduct preclinical and clinical studies to assess safety and efficacy in compliance with FDA regulations. Develop novel applications of drug delivery systems for antiviral chemotherapy. Develop adjuvants.

24. (U) Assess efficacy of potential antivirals against viruses in tissue cultures and in rodent models for Rift Valley fever virus (RVFV) and VEE. Evaluate toxicity and pharmacology of promising compounds in preclinical protocols conducted in rodents and nonhuman primates. Provide technical support for clinical protocols. Evaluate state-of-the-art technologies for improved drug delivery. Perform animal studies to assess efficacy of immunopotentiating compounds and drugs combinations as potential antivirals or vaccine adjuvants.

25. (U) 8610 - 8709 Two antivirals with low in vivo toxicity and high antiviral activity against a number of viruses were selected for advanced testing in the RVF mouse model. Eleven different immunomodulators were evaluated in mouse, guinea pig, or monkey models for their prophylactic and therapeutic efficacy against arboviruses; AVS- 1968, 1300, and 1018 showed excellent in vivo activity against Rift Valley fever virus (RVFV). An extract of *C. burnetti* exhibited excellent anti-RVFV activity even when a single dose was administered seven days before infection. Human recombinant α-2 interferon completely eliminated circulating yellow fever virus in the African green monkey when administered 24 but not 72 hrs after infection. Additional bulk quantities of the pyridinium prodrug of ribavirin were synthesized. Further testing in the Japanese encephalitis peripheral challenge murine model reconfirmed the activity observed when the prodrug was administered to mice infected with low doses of virus.

DD FORM 1498, 83 MAR — EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AD-014: Advanced Drug Development Studies Against Agents of Biological Importance

PRINCIPAL INVESTIGATOR: P. G. Canonico, Ph.D.

ASSOCIATE INVESTIGATORS: M. Kende, Ph.D.
M. A. Ussery, MAJ

BACKGROUND

Drug screening efforts at USAMRIID have identified a number of compounds with broad-spectrum antiviral activity against "exotic" RNA viruses. This research program addresses the continued evaluation of these lead compounds and their application as broad-spectrum antiviral agents.

Research activity is also directed at combination chemotherapy. Combinations of certain antivirals have been shown to exhibit significant synergy. The synergy of drugs, which enhances the immune system, is also of special interest. Finally, the number of natural products which nonspecifically activate the immune system, resulting in enhanced host resistance to viral infections, has grown significantly. USAMRIID's program is evaluating such compounds as antiviral agents. Particular attention has been given to compounds that are active orally and prodrugs or delivery systems that are targeted to the brain. Approaches are exploited for the specific targeted delivery of these immunomodulators, given either alone or in combination with other antivirals.

SUMMARY

The immunomodulator, CL-246,738 [3,6-bis(2-piperidinoethoxy) acridine hydrochloride], shown to have direct antiviral activity, was evaluated in a Venezuelan equine encephalomyelitis murine model and was found to be more active than the positive control, selenazole.

Two antivirals with low in-vivo toxicity and high antiviral activity against a number of viruses were selected for advanced testing in the Rift Valley fever (RVF) mouse model. Experiments were performed to define the optimal dosage schedule for AVS 79 [9-(beta-D-ribofuranosyl)purine-6 -thiocarboxamide]. AVS 79 was evaluated in combination with ribavirin. The two compounds are not antagonistic, but further work is necessary to define whether their combined activities are synergistic or additive. AVS 206 [1-(beta -D–ribofuranosyl)-1,2,4-triazole-3-carboxamidine hydrochloride] is being similarly evaluated and continues to show very high efficacy in vivo.

Eleven different immunomodulators were evaluated in mouse, guinea pig, or monkey models for their prophylactic and therapeutic efficacy against viruses representative of four arbovirus families. Three compounds: AVS 1968, 1300, and 1018, showed excellent in-vivo activity against RVF virus. All three compounds were orally active. The oral LD_{50} dose was 3-5-fold higher than the parenteral dose. All compounds eliminated circulating viral titers, induced interferon, induced natural killer cell reactivity, and returned elevated liver and heart functional serum enzymes to near normal levels. None of the compounds was effective against Venezuelan equine encephalomyelitis or Pichinde viral infections.

An extract of *C. burnetti* exhibited excellent anti-RVF virus activity, even when a single dose was administered 7 days before infection. Human recombinant α-2 interferon was shown to completely eliminate circulating yellow fever virus in African green monkeys when administered 24, but not 72 h, after infection.

Two immunomodulators, AVS 1300 or 1018, combined with ribavirin showed additive therapeutic effect in RVF virus-infected mice, but not against Pichinde-infected guinea pigs. AVS 1968 and ribavirin were highly synergistic against RVF virus but were not effective against Pichinde. In contrast, the combination of poly(ICLC) and ribavirin was effective in treating Pichinde virus-infected guinea pigs.

Additional bulk quantities of the pyridinium prodrug of ribavirin were synthesized. Further testing in the Japanese encephalitis peripheral challenge murine model reconfirmed the activity observed when the prodrug was administered prophylactically to mice infected with low doses of virus. It was not effective when given to mice infected with high doses of challenge virus. The prodrug was not effective when given therapeutically to mice infected peripherally with Venezuelan equine encephalomyelitis virus or intracranially with Japanese encephalitis virus. Pharmacokinetic analysis with radioactively labeled prodrug revealed brain levels of 1-2 µg of ribavirin per g tissue. These results indicate that brain levels of prodrug need to be increased 5- to 10-fold to obtain an optimal antiviral effect. New prodrug derivatives with slower rates of hydrolysis and release of ribavirin are being synthesized in an attempt to reach desired brain levels.

PRESENTATIONS

1. **Cosgriff, T. M., P. G. Canonico, L. Hodgson, D. Parrish, and T. Chapman.** 1987. Ribavirin: studies of the effects of the antiviral drug on platelet function. Presented at the XIth International Congress on Thrombosis and Haemostasis, Brussels, Belgium, July.

2. **Downs, M. B., M. A. Ussery, and P. G. Canonico.** 1987. Immunocytochemical studies of the kinetics of peripheral Japanese encephalitis virus (JEV) infection in C57 black mice. Presented at the Annual Meeting of the American Association of Anatomists, Washington, D. C., May.

3. **Kende, M., and P. G. Canonico.** 1987. Treatment of experimental viral infection with immunomodulators. Presented at the International Symposium on Immunomodulators and Nonspecific Host Defense Mechanisms Against Microbial Infections, West Berlin, West Germany, May.

4. **Kende, M., M. Contos, W. Rill, and P. G. Canonico.** 1987. Optimization of liposomal carriers for antiviral therapy. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

5. **Kende, M., W. Rill, J. Smith, M. Derevjanik, and P. G. Canonico.** 1987. Oral efficacy of an acridine derivative (AD) immunomodulator against Rift Valley fever virus (RVFV) infection in mice. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

PUBLICATIONS

1. **Canonico, P. G., M. Kende, and B. G. Gabrielson.** 1987. Carrier -mediated delivery of antiviral agents. Submitted to *Adv. Virus Res.*

2. **Kende, M., H. W. Lupton, and P. G. Canonico.** 1987. Treatment of experimental viral infections with immunomodulators, pp. ----. *In* Masihi and Lange (ed.), Imunomodulators and non-specific host defence mechanisms against microbial infections. Pergamon Journals, Ltd., Oxford (In Press).

3. **Kende, M., H. W. Lupton, W. R. Rill, P. Gibbs, H. B. Levy, and P. G. Canonico.** 1987. Ranking of prophylactive efficacy of poly(ICLC) against Rift Valley fever virus infection in mice by incremental relative risk of death. *Antimicrob. Agents Chemother.* 31:1194-1198.

4. **Kende, M., H. W. Lupton, W. L. Rill, H. B. Levy, and P. G. Canonico.** 1987. Enhanced therapeutic efficacy of poly(ICLC) and ribavirin combinations against Rift Valley fever virus infection in mice. *Antimicrob. Agents Chemother.* 31:986-990.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DAOB6410	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 10 Dec 86	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AE	015
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) (U)Advanced Non-system Development Studies on Conventional Agents of Biological Origin for Development of Medical Defensive Countermeasures

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	1.0	189
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Williams, J C
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Scott, G H
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Waag, D

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Lab Animals; (U) Bacillus anthracis; (U) Guinea Pigs; (U) Mice; (U) Hamsters; RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Prophylactic vaccines, therapeutic agents, and immunomodulators developed to protect US troops against potential BW agents must be effective when the microorganism is presented as an aerosol. The objective is to evaluate animal models of aerosol-induced infections and toxemias.

24. (U) Define and quantify the molecular aspects of clinical, pathological, and immunological changes that occur in vaccinated or treated animals when exposed to potential BW agents in aerosols.

25. (U) 8610 - 8709 Phase I whole cell and chloroform-methanol residue vaccines prepared by Salk Institute protected A/J mice from lethal challenge by aerosol and intraperitoneal routes. Aerosols did not induce antibodies against phase I lipopolysaccharide(LPSI), yet marked increases of antibody against surface proteins were measured. This surprising result contradicts the traditional view that antibodies against LPSI are a measure of immunity. Therefore, protein subunit vaccines may be efficacious. The resistance of *C. burnetii* to formalin treatment has led to the evaluation of gamma (^{60}Co) dose response to inactivate residual viable organisms in vaccine preparations. One thousand kilorads are required to kill 3.8×10^{10} organisms per ml of medium for the effective sterilization of vaccines and reagents.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AE-015: Advanced Non-system Development Studies on Conventional Agents of Biological Origin for Development of Medical Defensive Countermeasures

PRINCIPAL INVESTIGATOR: J. C. Williams, Ph.D.

ASSOCIATE INVESTIGATORS: G. H. Scott, Ph.D.
D. Waag, Ph.D.

BACKGROUND

Infection of laboratory animals and humans with virulent phase I *Coxiella burnetii*, the etiologic agent of Q fever, leads to progressive, but usually self-limiting, disease which is confirmed by the serological measurement of anti-*C. burnetii* antibodies. The distribution of Q fever is worldwide. About 5% of cases are chronic, involving liver granuloma or cardiac tissue (endocarditis). In acute disease, the temporal development of immunosuppression has been detected in mice and guinea pigs. In chronic disease of humans, an antigen-specific suppression circuit may be involved in the establishment and continuance of chronic Q fever. In order to learn more about the suppressor mechanisms, we are studying the mouse and guinea pig models more thoroughly. Our studies have centered around the immune suppressive complex (ISC) of *C. burnetii* and the immune mechanisms induced by this complex. The current phase I whole- cell vaccine is being replaced by a new candidate: chloroform-methanol extracted residue (CMR) from phase I whole cells. The CMR vaccine has been shown to be efficacious and non-reactogenic in animals, but is still difficult to produce from infected yolk sacs. Thus, an objective is to prepare a subunit vaccine by cloning the DNA of virulent *C. burnetii* and screening the clones for production of immunogenic proteins. This approach complements the biochemical approach of purifying immunogens and has the advantage of not requiring infectious microorganisms.

Vaccinogenic products generated via traditional fractionation procedures are being compared to recombinant DNA based vaccines. The A/J mouse is currently the animal model of choice for evaluating the efficacy of vaccines against virulent challenge.

SUMMARY

In previous studies we have tested the susceptibility of inbred strains of mice to infection by phase I *C. burnetii*. We are using the A/J strain as a model for testing the efficacy of Q fever vaccines. Therefore, we tested the ability of vaccinated A/J mice to develop a protective immune response against virulent *C. burnetii* administered as airborne particles or injected parenterally.

Groups of A/J mice were injected i.p. with 10 μg of inactivated, phase I *C. burnetii* cells or with 10 μg of a chloroform-methanol extracted residue from phase I cells (CMRI). A control group was injected with sterile PBS. Three weeks later, mice from each group were challenged with virulent phase I *C. burnetii* by aerosol exposure and by i.p. injection. Mice injected with either vaccine survived a challenge dose that killed 100% of the non-vaccinated mice when administered by aerosol, and 60% when administered i.p. Vaccine -induced protection was also reflected by a post-challenge reduction in splenomegaly, and perhaps most importantly, in the reduction of viable *C. burnetii* in the spleens of vaccinated and challenged mice.

Antibody titers against the phase I lipopolysaccharide (LPS-I) were markedly increased in previously vaccinated animals that were challenged with viable phase I cells administered i.p.; by contrast, aerosol challenge did not stimulate anti-LPS-I titers. This failure of vaccinated mice to develop antibody titers against LPS-I when infected by airborne *C. burnetii* was unexpected and requires further study.

To facilitate the preparation of non-infectious diagnostic materials for interchange among different laboratories, we examined the feasibility of inactivating *C. burnetii* with gamma irradiation (^{60}Co) without altering its immunological and antigenic properties. The amount of irradiation necessary to reduce the number of viable *C. burnetii* by 90% ranged from 121K rads for purified phase II organisms to 60 K rads for purified phase I cells. *Coxiella burnetii* organisms in all three preparations survived 500 K rads, but none survived 1000 K rads.

The antigenic properties of the organisms were not significantly altered by 1000 K rads. Mouse protection studies indicated that irradiation and formalin-inactivated organisms provided vaccinated mice with similar levels of protection against lethal *C. burnetii* challenge. Electron microscopic examination indicated that 1000 K rads of gamma irradiation did not destroy the morphology of the organisms, and we have experienced no difficulty in using gamma-sterilized cells in enzyme-linked immunosorbent assays.

PRESENTATIONS

1. **Scott, G. H., and J. C. Williams.** 1986. Responses of inbred mice to *Coxiella burnetii* infection. Presented at the 6th National Conference of the American Society for Rickettsiology, Williamsburg, VA, September.

PUBLICATIONS

1. **Scott, G. H., J. C. Williams, and E. H. Stephenson.** 1987. Animal models in Q fever: Pathologic responses of inbred mice to phase I *Coxiella burnetii*. *J. Gen. Microbiol.* *133*:691-700.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA302669	30 Oct 87	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AH	017
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) (U) Advanced Development for Rapid Diagnostic Procedures To Detect Agents of Biological Origin in Clinical Specimens

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	2.0	549
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR LeDuc, J W
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7341
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Meegan, J M
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Viral Diseases; (U) Bacterial Diseases; (U) Immunology; (U) Diagnosis; RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To develop, test, and perfect assays to rapidly detect and identify biological agents obtained from environmental samples or infected military personnel.

24. (U) Antigen and early (IgM) antibody-capture enzyme immunoassay technology has been the approach adopted.

25. (U) 8610 - 8709 Antigen detection and early antibody assays were developed and optimized for identification/diagnosis of disease caused by leptospira, and Junin (Argentine hemorrhagic fever), Sandfly fever (Naples), Japanese encephalitis, West Nile (WN) and Crimean-Congo hemorrhagic fever (CCHF) viruses. To optimize assays, monoclonal antibodies were developed, characterized, then tested for their ability to increase the sensitivity of rapid detection assays. Monoclonal antibodies to Sandfly fever (Sicilian and Naples), WN, Chagres, Rift Valley fever and CCHF viruses were studied. With Sandfly fever (Sicilian) virus variables influencing enzyme immunoassays, neutralization tests, and western blot assays were optimized.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AH-017: Advanced Development for Rapid Diagnostic Procedures to Detect Agents of Biological Origin in Clinical Specimens

PRINCIPAL INVESTIGATOR: J. W. LeDuc, LTC, Ph.D.

ASSOCIATE INVESTIGATORS: J. M. Meegan, CRD, Ph.D.
T. M. Cosgriff, COL, M.D.
T. G. Ksiazek, LTC, D.V.M.
R. R. Graham, MAJ, D.V.M.
C. A. Rossi

BACKGROUND

The goal of this study is to develop, then optimize, rapid, simple tests for identification of biological agents. We anticipate that samples containing unknown agents will be received from both environmental and clinical sources. Consequently, for each agent, an attempt has been made, not only to develop rapid antigen-detection assays, but also rapid immunoglobulin (Ig) M assays to measure antibodies in a single serum sample collected early in clinical disease, but after the period of antigen circulation.

In previous years, rapid assays for the detection of antigen and antibody were developed for a number of militarily relevant viral diseases. After optimization, many of these assays were field-tested under Work Unit 809-EA-001. In all cases, the antibody assays worked well under field conditions. However, there is a need to shorten all assay times, especially for the IgM test. Although antigen detection assays also functioned well, there is a need to increase antigen-detection sensitivity. Incorporation of monoclonal antibodies (MAB) into these assays can increase sensitivity, but not all MAB show this effect. Thus, the focus of our studies is to shorten the IgM assay, and develop and characterize MAB which will increase assay sensitivity when incorporated into antigen-detection assays.

SUMMARY

Antigen-detection and early antibody-detection assays were developed and optimized for identification/diagnosis of diseases caused by leptospira, and Junin (Argentine hemorrhagic fever), Sandfly fever (Naples), Japanese encephalitis, West Nile (WN) and Crimean-Congo hemorrhagic fever (CCHF) viruses. Generally, hyperimmune, polyclonal antisera were initially used to develop enzyme immunoassays. To optimize assays, collections of MAB were developed, characterized, then tested for their ability to increase the sensitivity of the polyclonal assays. This year, MAB to Sandfly fever (Sicilian and

Naples), WN, Chagres, Rift Valley fever, and CCHF viruses were developed, either in-house or on contract, then analyzed for usefulness in rapid diagnosis. Our results to date show that for most systems, the optimum conditions are a mixture of MAB as capture antibody, and polyclonal antisera as detector antibody.

Since these assays take 2 to 3 h to perform, we are attempting, through contract and in-house efforts, to develop faster assays while retaining sensitivity. Promising results have been obtained with filter-based assay systems, and there appears to be great potential for biosensor systems. However, many of these more rapid tests require MAB that are directed to two, separate, non-competing antigenic sites. We are developing and testing additional MAB with these additional MAB looking for those with these properties.

We recently commenced collaborative studies adapting our antibody assay to increase specificity and reproducibility by incorporating synthetic peptides and recombinant DNA-expressed proteins as antigens.

PRESENTATIONS

1. **Barrera Oro, J., C. MacDonald, R. Kenyon, J. Meegan, F. Cole, Jr., H. W. Lupton, and C. J. Peters.** 1987. Virus isolation and immune response in humans inoculated with a live-attenuated Junin virus (JV) vaccine. Presented at the VII International Congress of Virology, Edmonton, Alberta, Canada, August.

2. **Barrero Oro, J. G., C. MacDonald, A. I. Kuehne, B. C. Mahlandt, J. Spisso, J. M. Meegan, C. J. Peters, and H. W. Lupton.** 1986. Initial assessment in humans of a liver-attentuated vaccine against Argentine hemorrhagic fever (Candid #1). B. Virus isolation and serologic response. Presented at the II Argentine Congress of Virology, Cordoba, Argentina, October.

3. **Huggins, J. W., C. M. Hsiang, T. M. Cosgriff, M. Y. Guang, J. J. Smith, Z. A. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, X. E. Gui, K. W. Yuan, and T. M. Zhang.** 1987. Clinical therapeutic efficacy of intravenous ribavirin treatment of hemorrhagic fever with renal syndrome (HFRS): Randomized, double-blind placebo controlled trial in the People's Republic of China. Presented at the VII International Congress Virology, Edmonton, Alberta, Canada, August.

4. **Huggins, J. W., C. M. Hsiang, T. M. Cosgriff, M. Y. Guang, J. I. Smith, Z. A. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, X. E. Gui, K. W. Yuan, T. M. Zhang, and H. W. Lee.** 1987. Intravenous ribavirin therapy of hemorrhagic fever with renal syndrome (HFRS). Presented at the XVI Pacific Science Conference, Seoul, Korea, August.

5. **Kenyon, R., J. Barrera Oro, C. MacDonald, J. Meegan, and C. Peters.** 1987. Human lymphocyte transformation assay for Junin virus (Argentine hemorrhagic fever). Presented at the Annual Meeting of the American Society of Virology, Chapel Hill, NC, May-June.

6. **Koga, P., and J. Meegan.** 1986. Efficacy of vaccines as simulants for biological agents. Presented at the Joint Services Workshop on BW Agent Simulants, Aberdeen, MD, December.

7. **Malinoski, F. J., G. F. Meadors, H. Ramsberg, P. Stopa, and T. Ksiazek.** 1987. Safety and efficacy of a new Chikungunya virus vaccine: double-blind, placebo-controlled human trial. Presented at the VII International Congress of Virology, Edmonton, Alberta, Canada, August.

8. **Meegan, J. M.** Monoclonal antibodies: 1986. Application to diagnosis and seroepidemiology of arboviruses. Presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene, Denver, CO, December.

9. **Meegan, J. M., J. LeDuc, S. Garcia Franco, A. M. Ambrosio, and J. I. Maiztegui.** 1986. An ELISA for IgG and IgM antibodies to Junin virus. Presented at the II Argentine Congress of Virology, Cordoba, Argentina, October.

10. **Meegan, J., J. LeDuc, S. Garcia Franco, and J. Maiztegui.** 1986. Rapid diagnostic methods to detect Junin virus infections. Presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene, Denver, CO, December.

PUBLICATIONS

1. **Glass, G. E., J. E. Childs, G. W. Korch, and J. W. LeDuc.** Ecology and social interactions of sylvatic and commensal Norway rats (*Rattus norvegicucs)* populations in Baltimore, MD, U.S.A. Submitted to *J. Zoology (London).*

2. **Wood, O. L., J. M. Meegan, J. Morrill, and E. Stephenson.** Rift Valley fever. pp.   , Z. Dinter and B. Morein (Eds.), *Viral Infections of Ruminants.* Elsevier, Amsterdam (to be published 1988).

3. **Meegan, J. M., and C. Bailey.** Rift Valley fever. pp.   , *In* T. Monath (Ed.), *Epidemiology of Arthropod-borne viruses.* CRC Press, Boco Ratan, FL (to be published in 1988).

4. **Meegan, J. M., R. J. Yedloutschnig, B. A. Peleg, J. Shy, C. J. Peters, J. S. Walker, and R. E. Shope.** 1987. Enzyme-linked immunosorbent assay for detection of antibodies to Rift Valley fever virus in ovine and bovine sera. *Am. J. Vet. Res.* *48:*1138-1141.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA302670	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AI	018
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) Advanced Vaccine Development Studies on Toxins of Potential BW Threat

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0620 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	95 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	1.0	20
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Siegel, L S
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7211
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Microbial Toxins; (U) Vaccines; (U) Therapy; (U) Toxoids; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Evaluation and testing of toxoids and antitoxins for protection against botulinal neurotoxins. These neurotoxins are considered to have significant biological warfare potential and our at-risk forces should be immunized against them.

24. (U) Obtain botulinum toxin serotypes A-G in partially or highly purified state. Evaluate toxoids prepared from such materials for protection of personnel against botulinum toxin poisoning. Simultaneously evaluate antitoxins produced against botulinal toxoids as suitable prophylactic or therapeutic agents for botulinal toxin poisoning.

25. (U) 8610 - 8709 - To continue to evaluate the current botulinum vaccine [botulinum pentavalent (ABCDE) toxoid], and to establish a rational basis for reimmunization, we assayed 219 serum samples from immunized personnel for neutralizing antibodies to type A and B toxins. Because the neutralization test (a mouse bioassay) is expensive, time-consuming, and cumbersome, we established 2 ELISAs, with purified type A or B botulinum neurotoxin as the capture antigen, to determine antibody titers. We assayed > 100 samples in each ELISA and developed a database to correlate ELISA values for human sera with neutralization titers so that the ELISA may replace the neutralization test. Using the current vaccine in a guinea pig model system, investigations are continuing to develop an immunization schedule to produce the highest titers of neutralizing antibodies in the shortest time. In an ongoing project, we are evaluating synthetic peptides, produced according to known sequences of the neurotoxin molecule, for their efficacy as vaccines.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/ Drugs

WORK UNIT NO. 807-AI-018: Advanced Vaccine Development Studies on Toxins of Potential BW Threat

PRINCIPAL INVESTIGATOR: L. S. Siegel, Ph.D.

BACKGROUND

There are seven immunologically distinct neurotoxins (A-G) produced by the heterogeneous group of bacteria given the genus species designation *Clostridium botulinum*. Human botulism is associated with types A, B, E, and F. Although there have been few well-documented human cases reported, types C, D, and G have the potential of producing toxic effects in man. Toxoids (chemically inactivated but immunogenic toxins) for each serotype are used to elicit immunity to these toxins. The botulinum toxoid used for human immunization was prepared by treating types A, B, C, D, and E toxins with formaldehyde and combining them to form a composite immunogen. This toxoid, manufactured by the Michigan Department of Health under contract to the Army in the late 1960s, produces sustained measurable antibody levels only after a series of four injections administered over a period of 1 year. Mild side effects, including tenderness, redness, heat, and swelling at the site of injection, are common. A new, improved toxoid prepared from more highly purified neurotoxins is required. In addition to the current pentavalent (A-E) toxoid, the new product should include types F and G.

It is well documented that antibodies capable of neutralizing protein toxins have been induced by synthetic peptides derived from those toxins. Such synthetic peptides are ideal candidates for vaccines as they can be produced at a lower cost and would be safer than toxoids (minimal risk of reversion to toxicity, lower risk of adverse reactions).

SUMMARY

Approximately 180 persons at Fort Detrick are being immunized with the current botulinum vaccine [Botulinum Pentavalent (ABCDE) Toxoid]. To continue to evaluate this toxoid, and to establish a rational basis for reimmunization, we assayed serum samples from immunized personnel for neutralizing antibodies to type A and type B toxins. (The antibody response to type A correlates well with the response to types C, D, and E. Typically, the response to type B is the poorest.) Neutralization tests on sera, with a mouse bioassay with World Health Organization Standard Antiserum as a standard, were performed according to the standard neutralization procedure described by Centers for Disease Control in Atlanta, Georgia. We have determined titers for 23 persons who have completed the primary series of three immunizations, and for 98 individuals who have been on the immunization schedule for

varying times. Data have been obtained for paired sera from those 98 persons, obtained just prior to and approximately 2 weeks after the annual booster. Thus, a total of 219 serum samples have been assayed, by the mouse bioassay, for neutralizing antibodies to type A and type B neurotoxins.

Because neutralization tests are expensive, time-consuming, and cumbersome, we have established an ELISA, using purified type A or type B neurotoxin as the capture antigen, to assay for antibodies. We assayed 111 serum samples with the type A ELISA we developed, and demonstrated a linear correlation between ELISA titers and neutralization test results for type A (R=0.88). Using the type B ELISA, we assayed 144 serum samples. Statistical analyses of these data are in progress, but preliminary results indicate good correlation between ELISA and neutralization titers for type B.

With the current vaccine in a guinea pig model system, investigations are in progress to develop an immunization schedule to produce the highest titers of neutralizing antibodies in the shortest possible time. Groups of animals were immunized according to various regimens and blood samples were drawn at intervals. Serum samples are being assayed by an ELISA to determine antibody response over time.

In an ongoing project, we are evaluating synthetic peptides, produced according to known sequences of the neurotoxin molecule, for their efficacy as vaccines. Peptides were coupled to a carrier molecule [keyhole limpet hemocyanin (KLH)] and used to immunize rabbits. Sera from these animals was obtained and assayed for antibody. Using the peptide coupled to a different carrier molecule (bovine serum albumin) in an ELISA system, we demonstrated that the rabbits produced high titers of antibody to the peptide moieties of the KLH conjugates. More importantly, these antibodies also reacted with the purified native toxin in an ELISA. However, when assayed in the mouse bioassay neutralization test, these antibodies neutralized only low amounts of toxin. Thus, these particular peptides have limited potential as candidate vaccines. Additional peptides are being evaluated.

MIGB Evaluation and testing of toxoiding processes for the toxins of *C. botulinum*

PRINCIPAL INVESTIGATOR: L. S. Siegel, Ph.D.

Research has not been conducted in this area during the reporting period.

MIGC Evaluation, testing and field testing of antitoxins for botulinum toxins.

PRINCIPAL INVESTIGATOR: L. S. Siegel, Ph.D.

Research has not been conducted in this area during the reporting period.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA302668	30 Oct 87	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AG	019
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) Advanced Immunotherapy Studies Against Potential BW Viral Agents

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	2.0	265
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Jahrling, P B
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Lassa Virus; (U) Viral Diseases; (U) Lab Animals; (U) Monkeys; (U) Guinea Pigs; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To select, acquire, and test immune plasma and globulin fractions for protective efficacy and safety in prophylaxis and therapy of hemorrhagic fever virus infections that pose special problems for U.S. Forces sent to those areas where these diseases are endemic.

24. (U) Specific immune plasma is obtained by plasmapheresis from convalescent patients after naturally occurring infections with Lassa virus, Argentine hemorrhagic fever virus (Junin), and Ebola virus. Plasma units are tested by current blood bank procedures and for presence of protective (neutralizing) antibodies. Criteria are established for optimal therapeutic administration of the final products. Alternate strategies for acquiring high titered antibody are developed and tested.

25. (U) 8610 - 8709 Collecting and processing Lassa-immune plasma from convalescent patients in Liberia (LIB) and Sierra Leone (SL) were accelerated. The inventory of LIB plasma units suitable for therapy increased to 559, and SL units to 293. No donors were HIV-antibody positive, but 8% were excluded for hepatitis B surface-antigen reactivity. 158 Prospective new donors were identified by viral isolation or seroconversion in LIB, and screening procedures for Lassa-immune donors at additional blood banks in LIB and Nigeria were initiated. Field testing of the Lassa antigen-capture ELISA in LIB was begun, to ensure appropriate administration of specific immune therapy. Sterilization of immune plasma or globulin by chemical treatment with tri (n-butyl) phosphate to remove HIV and NON-B hepatitis viruses preserved neutralizing antibody and in vivo protective efficacy. Parallel studies with Junin-immune globulin and monoclonal antibodies indicated a requirement for both N- and cell-lytic activity to confer passive protection.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AG-019: Advanced Immunotherapy Studies Against Potential BW Viral Agents

PRINCIPAL INVESTIGATOR: P. B. Jahrling, Ph.D.

BACKGROUND

Lassa fever is a viral disease of considerable public health importance in regions of West Africa, particularly Liberia, Sierra Leone, and Nigeria, where several thousand cases are believed to occur annually. While serological data suggest that subclinical cases may occur, the case-fatality ratios among hospitalized cases are still high, variously estimated at 14 to 22% in Sierra Leone and 13 to 14% in Liberia. To increase survival rates, passive immunization of acutely ill patients is employed frequently. One of many problems in evaluating plasma efficacy is the variable quality of the plasma infused. One objective of this study is the identification of Lassa-convalescent patients to be recruited as plasma donors. Through this process, a pool of optimal donors was identified, and guidelines for identifying new donors were established. The feasibility of preparing IgG by various chromatographic procedures for intravenous treatment of human Lassa fever is established. However, there is concern that adventitious agents present in the original plasma pool might not be removed by processing, and thus might contaminate the final product. The viruses causing AIDS (HIV); and hepatitis A, B, and non-A, non-B are of principal concern. This work unit is designed to determine the safety and efficacy of various plasma fractionation methods for preparation of monomeric IgG and for removal of these agents. The availability of high-titer plasma has now facilitated the formal testing of passive immunization with plasma and globulin products for treatment of Lassa fever.

SUMMARY

Collection and processing of Lassa-immune plasma from convalescent patients in Liberia (LIB) and Sierra Leone (SL) was accelerated. The inventory of LIB plasma units suitable for therapy increased to 559, and SL units to 293. No donors were HIV-antibody positive, but 8% were excluded for hepatitis B surface antigen reactivity. Prospective new donors (158) were identified by virus isolation or seroconversion in LIB, and screening procedures for Lassa -immune donors at two additional blood banks in LIB and one in Nigeria were initiated. Field testing of the Lassa antigen-capture ELISA in LIB was begun, to ensure appropriate administration of specific immune therapy. Viremia titers of 3 $\log_{10}$ PFU/ml were detected without difficulty in a series of 38 field samples obtained from hospitalized patients early after onset, suggesting that the critical target population in most need of immune globulin therapy can be readily identified in the hospital. Sterilization of immune plasma by chemical

treatment with tri (n-butyl) phosphate to remove HIV and non-A and non-B hepatitis viruses preserved neutralizing antibody which continued to have protective efficacy in guinea pigs. The effect of this process on globulin prepared by aerosil precipitation followed by QAE chromatography will now be tested. If successful, this will add an extra degree of assurance that the final product (in monomeric form) is free of adventitious agents. Parallel studies with Junin-immune globulin and monoclonal antibodies indicated a requirement for both N- and cell-lytic activity to confer passive protection to guinea pigs. $F(ab')_2$ preparations of Junin-immune globulin neutralized virus, but failed to lyse cells and failed to protect. Likewise, only those neutralizing monoclonal antibodies which also lysed infected cells protected guinea pigs. The therapeutic utility of mouse monoclonals in guinea pigs and presumably primates may be limited by immune elimination of antibody when species lines are crossed. Circumvention of this problem by the design of chimeric antibodies is under investigation.

PUBLICATION

1. **Monson, M. H., A. K. Cole, J. D. Frame, J. R. Serwint, S. Alexander, and P. B. Jahrling.** 1987. Pediatric Lassa fever: a review of 33 Liberian cases. *Am. J. Trop. Med. Hyg.* **36**:408-415.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY			1. AGENCY ACCESSION DA308926	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 10 Dec 86	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AL	020
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) (U) Advanced Development Studies on Immunomodulators/Enhancers

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defence

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	1.0	569
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Anderson, A O
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Wood, O
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Mice; (U)Vaccines;(U)Microorganisms;(U)Aerosols;(U)Lab. Animals;(U)Hamsters;(U)GuineaPigs(U)RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Prophylactic vaccines, therapeutic agents, and immunomodulators developed to protect US troops against potential BW agents must be effective when the microorganism or toxin is presented in an aerosol. The objective is to determine the safety, efficacy, and dose response of prophylactics, therapeutics, and immunomodulators against an airborne challenge. Emphasis is on how effective products induce protection and why ineffective ones fail.

24. (U) Define and quantify the molecular aspects of clinical, pathological, and immunological changes that occur in vaccinated or treated animals when exposed to potential BW agents in aerosols. Apply advanced methodology to determine how a prophylactic or therapeutic induced protection; or, conversely to analyze why a product failed to instill protection.

25. (U) 8610 - 8709 Intraperitoneal priming with unmodified NDBR 103 yields protective efficacy against aerosol or s.c. challenge with Rift Valley fever virus (RVFV) which is superior to that of s.c. vaccination with RVFV vaccine and avridine. The basis for this cross protection appears to be transdiaphragmatic transport of antigen in peritoneal macrophages to lung-associated lymphatic tissues and to peripheral lymphatic tissues such as the spleen. Avridine produced bimodal effects on this transdiaphragmatic macrophage traffic. There was an initial reduction followed by overshoot compared to non-avridine controls. In each case, the rate of migration of avridine treated cells into respiratory lymphatic tissues was increased. Aerosol immunization with small particle aerosol of avridine and NDBR-103 failed to yield protection against either s.c. or aerosol challenge, despite induction of specific s-IgA and IgG.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807:	Industrial Base BW Vaccines/Drugs
WORK UNIT NO. 807-AL-020:	Advanced Development Studies on Immunomodulators/Enhancers
PRINCIPAL INVESTIGATOR:	A. O. Anderson, M.D.
ASSOCIATE INVESTIGATORS:	M. L. Pitt, Ph.D. O. Wood, Ph.D. C. York, B.S.

BACKGROUND

In other studies, intraperitoneal immunization with formalin-inactivated Rift Valley fever vaccine (NDBR-103), followed by intranasal boost, produced superior protection against s.c. or aerosol challenge with 10 LD_{50} doses of virulent RVF virus (ZH-501).

SUMMARY

Investigation of the mechanism for this cross-protective response which could not be induced by s.c. or intraduodenal vaccination revealed a novel traffic of peritoneal antigen-laden macrophages across the diaphragmatic lymphatics into the pleural cavity and lung with ultimate destination in the lung interstitium and bronchial lymphatic tissues.

The effect of the immunomodulator avridine on the transdiaphragmatic traffic of peritoneal macrophages to the lungs was investigated. Either 1:1 carbon/avridine mixture or carbon alone was injected i.p.into mice. The number of macrophages containing carbon in the pleural cavity, bronchial lavage, and blood was investigated 2, 7, 14, and 28 days post inoculation. The presence of avridine caused a slower traffic of peritoneal macrophages through the diaphragm initially in the first 7 days compared to carbon alone. The traffic of macrophages in avridine-treated mice increased at 14 and 28 days when traffic of macrophages in animals receiving only carbon decreased dramatically. There were no differences in the numbers of labeled monocyte/macrophages from the bronchial lavage or blood of animals from either group.

Aerosol immunization in the nose-only exposure unit with 5 weekly doses of avridine/RVF viral vaccine mixtures did not result in morbidity or mortality. Necropsies of unchallenged, aerosol-vaccinated mice also failed to reveal significant vaccine- or immunodulator-induced lesions in the lungs. Serology from these mice indicated that aerosol immunization stimulated both IgA and IgG antibodies which were specific for RVF virus.

When these mice were challenged with 10 LD_{50} of virulent ZH-501 RVF virus by aerosol or s.c., there were no survivors in either group. Since ELISA-detected antibodies were present, the failure of aerosol immunization to yield protection is puzzling. Mucosal immunization is known to suppress cellular cytotoxicity in other systems. If aerosol immunization induced antibody but suppressed "mucosal cytotoxic cells," these results may suggest important prophylactic activities of mucosal cytotoxic cells in respiratory immunity. Investigation of respiratory mucosal cytotoxic cells is in its infancy and warrants further exploration.

Enhanced immunoreactivity of lymphatic tissue lymphoid cells was induced by endocytic stripping of ligands from migrant lymphocyte surfaces in high endothelial venules (HEV). The endothelial cells of HEV are known to exhibit modest phagocytic activity along with high cytoplasmic expression of lysosomal enzyme activities. Intravenous infusion of opsonized microorganisms in Lewis rats failed to demonstrate any significant reticuloendothelial function of HEV, yet infusions of "opsonized," syngeneic leukocytes resulted in endocytosis, lysosomal enzyme activation, and destruction of these cells by HEV endothelial cells. In vitro treatment of thoracic duct lymphocytes (TDL) with low doses of labeled anti-immunoglobulins, anti-lymphocyte serum, and selected lectins resulted in ultrastructural evidence of endocytic stripping of the labeled ligand from the surface of lymphocytes as they emigrated from the blood across HEV. In vitro treatment of TDL with higher doses of anti-lymphocyte antibodies resulted in endocytosis and destruction of the labeled cells by HEV endothelial cells. Therefore, HEV endothelial cells exhibited active endocytic interactions with recirculating lymphocytes, while they were enroute from blood to lymphatic tissue, resulting in removal of the ligand from the lymphocyte surface. In the extreme case, heavily opsonized cells were destroyed. These interactions may constitute important mechanisms for monitoring recirculating lymphocytes. Removal of surface moieties from migrant cells may be responsible for the enhanced immunoreactivity of post-migrant lymphocytes, compared to that of cells isolated from peripheral blood or afferent lymph. Additional collaborative studies with Jerrold Ward of NCI revealed that the endocytic process may participate in the development of acquired immunodeficiency in HIV-infected patients because HIV viral antigens and the CD-4 receptors are detected in HEV of lymph node from patients with HIV infections. Immunodeficiency may be a result of the removal of CD-4 molecules from the surfaces of helper T-cells during migration across HEV. CD-4 molecules are required in the interaction between helper T-cells and antigen-presenting cells.

PRESENTATIONS

1. **Anderson, A. O.** 1987. Direct transdiaphragmatic traffic of peritoneal macrophages to the lung. Presented at the Ninth International Conference on Lymphatic Tissues and Germinal Centres in Immune Reactions, Oslo, Norway, August.

2. **Anderson, A. O.** 1987. Mucosal priming alters pathogenesis of Rift Valley fever. Presented at the Ninth International Conference on Lymphatic Tissues and Germinal Centres in Immune Reactions, Oslo, Norway, August.

3. **Anderson, A. O.** 1987. Endocytic stripping of ligands from migrant lumphocyte surfaces in high endothelial venules (HEV). Presented at the Ninth International Conference on Lymphatic Tissues and Germinal Centres in Immune Reactions, Oslo, Norway, August.

4. **Anderson, A. O., O. Wood, L. F. Fischbach, and M. L. M. Pitt.** 1987. Mucosal priming alters pathogenesis of Rift Valley fever. Presented at the Annual Meeting of the Federation of American Societies for Experimental Biology and Medicine, Washington, D. C., March.

PUBLICATIONS

1. **Anderson, A, O., O. L. Wood, A. D. King, and E. H. Stephenson.** 1987. Studies on anti-viral mucosal immunity with the lipoidal amine adjuvant Avridine, pp. 1781-1790. *In* J. Mestecky, J. R. McGhee, J. Bienenstock, and P. L. Orga (Eds.), Part B, Recent advances in mucosal immunology, Proceedings of the International Congress on Mucosal Immunology.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA305650	30 Oct 87	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AK	022
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) (U) Advanced Immunotherapy Studies Against Toxins

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	3.0	585
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathophysiology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Hewetson, J F
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7181
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Mereish, K A
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Poli, M A

22. KEYWORDS (Precede EACH with Security Classification Code) (U) BW Defense; (U) Military Medicine; (U) Mycotoxins; (U) Lab Animals; (U) Mice; (U) Diagnosis; (U) RA I; (U) Monkeys; (U) Toxins

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To develop methods for the field detection of mycotoxins in biological and environmental samples. To develop prophylactic and/or therapeutic agents for soldiers exposed to these toxins.

24. (U) Use immunoassays to detect mycotoxins in biological/environmental samples. Develop monoclonal and polyclonal antibodies for use as prophylactic agents. Develop stable toxin-protein conjugates for possible vaccine use.

25. (U) 8610 - 8709 High-titer goat antisera and monoclonal antibodies to saxitoxin have been produced with the potential for immunoprophylactic studies. Methodology for the immuno-detection of the major urinary metabolites of T-2 (HT-2 and tetraol) intoxication have been worked out and these products were detected in rats and monkeys after i.v. and oral exposure. Metabolism studies of brevetoxin demonstrated most of the metabolites were excreted in feces. An antisera raised against PbTx-3 cross reacts strongly with in vitro metabolites from hepatic cells, indicating a strong potential for screening of exposure to this toxin. Several physicochemical properties of microcystin have been determined and a new cyclic hepatapeptide was isolated and purified from Microcystis aeroginosa.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AK-022: Advanced Immunotherapy Studies Against Toxins

PRINCIPAL INVESTIGATOR: J. F. Hewetson, Ph.D.

ASSOCIATE INVESTIGATORS: K. A. Mereish, CPT, Ph.D.
M. A. Poli, Ph.D.
R. Solow, CPT

BACKGROUND

Small molecular weight toxins that can lead to death or illness upon contact or ingestion are of interest to the Army because of their potential as biological warfare agents. The toxins that are being investigated include T-2 mycotoxin and its metabolites, saxitoxin and its derivatives, brevetoxin and its derivatives, and microcystin. T-2 is a mycotoxin that occurs in moldy grain, causes alimentary toxic aleukia in humans and domestic animals, and has been implicated in biological warfare incidents in Southeast Asia and Afghanistan. Saxitoxin is a potent neurotoxin which acts on the sodium channel and has been responsible for many cases of paralytic shellfish poisoning. Brevetoxin, a polyether toxin, acts on the sodium channel at a different site than saxitoxin. It has been responsible for mass fish kills and human health problems. Microcystin, found in blue-green algae, is a cyclic peptide and a potent hepato toxin. It has been responsible for many deaths among livestock. The need for rapid, reliable, and quantitative methods for detection of the small molecular weight toxins and the development of antidotes for treatment of the resulting toxicoses is well established. Methods available for detection include variations in the radio immune assay (RIA), and enzyme-linked immunoassay (ELISA). The ELISA is most appropriate for field use as it does not use radioisotopes. Both the RIA and ELISA are available for T-2 and have been used for several studies. A saxitoxin ELISA is available but studies are continuing to improve and implement this assay for field use. A RIA is available for brevetoxin that now will be further developed with newly available antisera. An ELISA for brevetoxin is not yet available. Possible therapies that have been investigated for T-2 toxin are the glutathione prodrugs, anti-inflammatory glucocorticoids, intestinal adsorbing agents, anti–oxidants, and microsomal-inducing agents. Anti-T-2 antibodies have successfully reversed the toxic effects of T-2 in animals in other laboratories. Anti-saxitoxin antibodies reverse the certain toxicity of saxitoxin. Adaptation of ELISA technology for toxin detection and defining effective therapies for treatment of toxicosis resulting from exposure are major goals of this work unit.

SUMMARY

High-titer, anti-saxitoxin antisera suitable for detection assays and prophylactic studies were raised in rabbits and goats. An ELISA assay for saxitoxin is in place. Radioimmunoassays for the mycotoxins T-2, hydroxy T-2 (HT-2), tetraol, and diacetoxyscirpenol are also operational. Several monoclones against saxitoxin were produced and are being further evaluated.

Methods to detect the urinary metabolites of T-2 intoxication (HT-2 and tetraol) were created and tested. These metabolites were demonstrated in rats and monkeys after exposure to T-2. It is therefore possible to detect T-2 reliably after exposure in experimental animals by examining a urine sample obtained within 2 to 3 days after exposure.

Metabolism and excretion of the brevetoxins were studied. Excretion occurred primarily in the feces. The characterization of the observed metabolites is being studied in vitro with rat hepatic cells. Cellular metabolites cross react strongly with goat antisera raised against the brevetoxin, PbTx-3, and therefore indicate a strong potential in screening for exposure to these toxins. In addition, there is strong evidence that goat antisera can be used prophylactically or therapeutically for the management of neurotoxin shellfish poisoning.

Studies on *Microcystis aeruginosa* toxin characterized its physicochemical properties with regard to ultraviolet spectra, solubility in organic and inorganic solvents, and stability. The ionophoric properties of this toxin demonstrated more selectivity toward K^+ ions than Ca^{++} or Na^+ in an ion-picrate extraction method. In an three-phase, ion-picrate transfer method, selectivity was greater toward Ca^{++} than K^+ ions. Studies on in vitro toxicity in cultured rat hepatocytes showed no significant effect on enzymes released over 24 h and on the influx of Ca^{++}. A new cyclic hepatopeptide was isolated and purified from *Microcystis aeruginosa*.

MIHA Exploratory Development of Detection and Treatment of Marine Toxin Poisoning

PRINCIPAL INVESTIGATOR: J. F. Hewetson, Ph.D.

ASSOCIATE INVESTIGATORS: K. A. Mereish, CPT, Ph.D.
M. A. Poli, Ph.D.

Physicochemical Properties of Microcystin-a. The ultraviolet spectrum of microcystin-a in ethanolic solution (0.5 µg/ml) was characterized by a maximum at 242 nm with a molar absorbtivity of 69.91 x 10. The fluorescence intensity in ethanol was maximized at an excitation wavelength of 330 nm and 450 nm emission. Microcystin-a is freely soluble (> 2 mg/ml) in ethanol, methanol, and acetonitrile; and slightly soluble in water, ethylene octane, hexane, and heptane. It is stable for several weeks in water, ethanolic and acidic solutions at 4°C. However, it has a poor stability in alkaline solutions (pH > 8).

Ionophoric Properties. The ionophoric property of microcystin-a was tested. In the ion-picrates extraction method, microcystin-a was more selective toward K^+ ions than were Ca^{++} or Na^+. In the three-phase ion-picrates transfer method, selectivity was greater toward Ca^{++} than K^+ ions.

In vitro toxicity. In cultured rat hepatocytes, microcystin-a, strain 7820 at concentrations of 1 and 10 µg/ml, induced no significant effects on the amount of enzymes (lactate dehydrogenase, 5'-nucleotidase, acid phosphatase, and alkaline phosphatase) released over a 4 h. Also, there was no effect on the influx of Ca^{++} ions.

Isolation and Identification. A new cyclic hepatopeptide of *Microcystis aeruginosa* was purified by HPLC and analyzed by FAB fourier transform mass spectrometry. The new peptide has a molecular weight of 980, a demethylated microcystin-a—probably at the aspartic acid moiety.

A large quantity of goat anti-saxitoxin antisera has been produced and the IgG fraction isolated. This material competes with saxitoxin in the membrane-binding assay, shows good activity in an ELISA, and will be used for protection studies against saxitoxin and its various derivatives. High-titer rabbit sera has also been produced. Both the rabbits and goats continue to be boosted and bled. Several monoclones that appear to have good reactivity to saxitoxin in an ELISA have been produced and will be further evaluated.

We demonstrated the appearance and detection of T-2 metabolites in the urine of T-2 exposed animals. The results show that in both intramuscularly and orally exposed rats, HT-2 and tetraol could be detected and quantitated by RIA. All the detectable metabolites in the rats exposed intramuscularly were excreted within 3 days, whereas HT-2 and tetraol could be detected up to 9 days after oral exposure. Both metabolites were also detected in urine from monkeys exposed intramuscularly.

Techniques were devised for the isolation and purification of Kupffer cells from rat livers. These macrophages were tested for their sensitivity to microcystin; the results demonstrated profound changes in the morphology of the cells. In addition, the ability of the Kupffer cells to engulf *Candida* yeast was impaired in the presence of microcystin-a.

Metabolism and excretion of the brevetoxins were investigated with a rat model. Tritiated PbTx-3 administered intravenously was cleared rapidly from the bloodstream with less than 5% remaining after 2 min. Excretion occurred primarily via the feces, with 65% of the administrated dose excreted within 3 days. Isolated hepatic cells were used to study in vitro metabolism. HPLC analysis of cell culture medium indicated three peaks of excreted radioactivity that were more polar than the parent compound. Structural and immunological analyses of the peaks are in progress.

Goat antisera to PbTx-3 was prepared against a toxin-bovine serum albumin conjugate (BSA) by succinylation of the alcohol function of PbTx-3 followed by carbodiimide coupling of the PbTx-3 hemisuccinate to the lysine residues of BSA. The resulting high-affinity antibody was unable to differentiate between PbTx-2 and PbTs-3, which suggests specificity for the cyclic polyether backbone region of the molecule. Cellular metabolites from the hepatic cell cultures described above cross reacted strongly with the antiserum, indicating a strong potential for its use in screening for exposure to these toxins. In addition this anti-serum is capable of completely inhibiting the binding of ^{3}H-PbTx-3 to its pharmacologically active receptor site in vitro, suggesting a prophylactic or therapeutic potential in the management of neurotoxin shellfish poisoning. Studies evaluating this potential are underway.

PRESENTATIONS

1. **Mereish, K. A., R. W. Wannemacher, Jr., D. L. Bunner, and T. Krishnamurthy.** 1987. Composition of *Microcystis aeruginosa* strain 7820 toxin. Presented at the Conference on Natural Toxins from Aquatic and Marine Environments, Woods Hole, MA, August.

2. **Wannemacher, R. W., Jr., D. L. Bunner, K. A. Mereish, H. B. Hines, and R. E. Dinterman.** 1987. Biological and chemical stability of several natural toxins from aquatic and marine environments. Presented at the Conference on Natural Toxins from Aquatic and Marine Environments, Woods Hole, MA, August.

PUBLICATIONS

1. **Cozad, G. C., and J. F. Hewetson.** 1987. Influence of T-2 mycotoxin on host resistance to *Candida albicans* in mice. Submitted to *Infect. Immun.*

2. **Hewetson, J. F., J. G. Pace, and J. E. Beheler.** 1987. Detection and quantitation of T-2 mycotoxin in rat organs by radioimmunoassay. *J. Assoc. Off. Anal. Chem.* 70:654-657.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA303917	30 Oct 87	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	AC	001
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY 88 -01			

11. TITLE (Precede with Security Classification Code) (U) Vaccine, Q Fever

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense; 0601 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 04	89 01	DA	C. In-House

17. CONTRACT/GRANT
a. DATE EFFECTIVE | EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
87	1.0	352
88		

19. RESPONSIBLE DOD ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L
d. TELEPHONE NUMBER (include area code) 301-663-2833

20. PERFORMING ORGANIZATION
a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF PRINCIPAL INVESTIGATOR Williams, J C
d. TELEPHONE NUMBER (include area code) 301-663-7453
f. NAME OF ASSOCIATE INVESTIGATOR (if available) Meadors, G F
g. NAME OF ASSOCIATE INVESTIGATOR (if available) Waag, D M

21. GENERAL USE FIC
MILITARY/CIVILIAN APPLICATION: H

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Q Fever (U) Coxiella burnetti; (U) Vaccines; (U) Medical Defense; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) *Coxiella burnetii*, the causative agent of Q fever, is considered by most experts to have those properties which make it a potential candidate for BW. The existing vaccine to protect US troops against this threat is reasonably effective, but causes sterile abscesses in previously sensitized individuals. This institute has a broad program to improve the current vaccine.

24. (U) This work unit is dedicated to the transfer of research-level vaccine production technology to conditions for pilot-scale processing of the Q fever vaccine. Definition of the pilot-scale conditions will permit the orderly production of large volume lots of vaccine that are safe and efficacious.

25. (U) 8610 - 8709 Volunteers were evaluated for humoral, cell-mediated, and delayed hypersensitivity immune responses before and after vaccination with IND 610 Q fever vaccine. Post-vaccination mean values were significantly greater than pre-vaccination values for both humoral antibody and cell-mediated immune responses. The presence of humoral antibody levels correlated with cell-mediated responses. Delayed hypersensitivity did not correlate with humoral antibody levels or cell-mediated responses. Roughly 80% and 65% of vaccinated individuals responded with significantly increased mean antibody titers and mean cellular responses, respectively. A new candidate Q fever vaccine has been produced. This product will enter phase I human trials during FY88.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M463750D809: Development of Drugs and Vaccines Against Diseases of BW Importance

WORK UNIT NO. 809-AC-001: Vaccine, Q Fever

PRINCIPAL INVESTIGATOR: J. C. Williams, CDR, Ph.D.

ASSOCIATE INVESTIGATORS: G. F. Meadors, LTC, M.D.
D. M. Waag, Ph.D.

BACKGROUND

Coxiella burnetii, the causative agent of Q fever, is considered by most experts to have those properties which make it a potential candidate for biological warfare. The existing formaldehyde-inactivated, phase I whole cell vaccine is reasonably effective, but exhibits undesirable effects in previously sensitized individuals. A broad program is ongoing to develop and produce improved subunit vaccines.

SUMMARY

Individuals at risk of acquiring Q fever in the laboratory were evaluated by measurement of humoral antibody titer (ELISA), cell-mediated response (in vitro lymphocyte proliferation), and delayed hypersensitivity (skin test) before and after administration of a formaldehyde-inactivated, phase I vaccine (IND 610). The criteria used were that individuals positive by at least two of the tests would not be vaccinated. Vaccine was not given to 46% of the tested individuals. Of those receiving vaccine, no adverse reactions were noted. The responses before and after vaccination were evaluated to develop experimental values that may be predictive of protective immunity. No correlation between skin test data and either humoral or cell-mediated responses could be established. However, about 80% and 65% of humoral antibody and cell -mediated responses were two standard deviations (95% confidence interval) above the pre-vaccination mean values. The low conversion rate (80% and 65%) is probably due to the low tolerable dose of the phase I vaccine.

The pilot lot of the chloroform-methanol residue (CMR) vaccine for *C. burnetii* has been produced. Sterility tests and composition evaluations are ongoing. Pre-clinical testing will commence in FY88.

PRESENTATIONS

1. **Waag, D., and J. C. Williams.** 1987. Identification of suppressor cells induced following injection of C57BL/10 ScN mice with phase I *Coxiella burnetii* whole cells. Presented at the American Physiology Society Annual Meeting, Federation of American Societies for Experimental Biology, Washington, D. C., April.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA305651	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 10 Dec 86	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT
10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER	
a. PRIMARY	63750A	3M463750D809		AN	002	
b. CONTRIBUTING						
c. CONTRIBUTING	DA LRRDAP, FY 88 -01					

11. TITLE (Precede with Security Classification Code) (U) Vaccine, Advanced Development

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	1.0	37
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Medical Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Cosgriff, T M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2997
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Meadors, G F
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Vaccines; (U) Volunteers; (U) High Containment Medical Care; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) (1) Monitor immunizations of persons where work places them at risk of exposure to vaccine agents; enter data on safety and immunogenicity of experimental and licensed vaccines into computer data base. (2) Maintain facilities to transport and treat patients under conditions of total (P-4) biohazard containment to prevent the possible spread of highly pathogenic microorganisms/toxins to medical personnel or the environment.

24. (U) (1) Reactions to immunizations are carefully monitored to assess safety in humans. Immune responses are measured utilizing conventional serologic assays, skin testing, and lymphocyte studies. (2) Maximum containment facilities, are maintained in a state of readiness at all times to enable transport and treatment of persons suspected or known to be ill from highly pathogenic microorganisms/toxins.

25. (U) (1) Computer data entry for the Special Immunizations Program has progressed rapidly during the last year, with entry of pre-1983 data. Four thousand, five hundred out-patient visits were recorded in the last 12 months as part of this program. (2) The high-containment clinical facilities have continued to undergo updating during the last year. Medical personnel have maintained their skills in providing care under high-containment conditions through regular exercises.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE

PROJECT NO. 3M463750D809:	Development of Drugs and Vaccines Against Diseases of BW Importance
WORK UNIT NO. 809-AN-002:	Vaccine, Advanced Development
PRINCIPAL INVESTIGATOR:	T. M. Cosgriff, COL, M.D.
ASSOCIATE INVESTIGATOR:	N. A. Popovic, M.D., Ph.D. G. F. Meadors, III, LTC, M.D. D. M. Driscoll, CPT, AN K. E. Wilson, M.S., R.N.

BACKGROUND

Special Immunizations Program

In 1981, the Department of Occupational Medicine was formed within the Medical Division to provide a formal and closely supervised approach to managing the USAMRIID Special Immunizations Program. A physician was placed in charge. The purpose of this program is to administer both investigational and licensed vaccines to personnel at risk of exposure to infectious agents during laboratory studies and to monitor safety and serological responses.

High Containment Facilities

USAMRIID is one of the few medical institutes in the world which has specially designed high containment facilities to allow for the care of patients with illnesses secondary to contagious, highly virulent pathogens, while limiting risk of spread of disease outside the containment area and secondary infections in medical personnel.

The facilities include a high-containment clinical care unit, and a high-containment clinical laboratory unit. The medical instruments in these units are the most modern available and provide the capabilities for critical care delivery, surgery, and complete laboratory studies. There are also mobile containment units for patient transport and field laboratory studies.

SUMMARY

Special Immunizations Program

Data entry in the Special Immunizations Program has been computerized, resulting in more efficient assessment of vaccine safety and efficacy. Older immunization data (pre 1983), not previously computerized, was also added to the present computer files. Approximately 2500 entries have been made during the summer months of 1987.

Participants in the Special Immunizations Program are closely monitored by the staff and, in the last 12 months, approximately 4500 outpatient clinic encounters were recorded. From the scientific viewpoint, the immunization program provides a unique opportunity to monitor individuals over long periods of time with regard to response to vaccinations. The data provide important evidence for vaccine efficacy and the correlation of immune responses to protection afforded by the vaccines.

High Containment Facilities

During the last year the high-containment patient isolation unit has continued to be updated. The medical and nursing staff assigned to operate the high-containment facilities have continued to gain valuable experience in providing patient care under the constraints imposed by the special environment. Regularly scheduled training exercises and provision of actual patient care provide the opportunity to gain necessary experience.

Through regularly scheduled exercises, the Aeromedical Isolation Team has maintained its skills in the isolation and transportation of patients suspected to have diseases associated with highly dangerous pathogens. The flight training program is coordinated with the West Virginia Air National Guard. During these periods of training, the isolation team transports patients with simulated illness to test the feasibility of new equipment and the latest techniques of high- containment isolation.

The High-Containment Clinical Laboratory continues to be involved in animal studies of virulent pathogens. This involvement keeps the laboratory in a state of readiness should its services be needed for evaluation of human disease. In the last year a mobile high-containment laboratory was also established.

PRESENTATIONS

1, **Popovic, N. A.** 1987. Smallpox and Monkeypox. Presented at the Medical Defense Against Biological Warfare and Highly Communicable Infectious Agents Cours, USAMRIID, Frederick, MD, August.

2. **Driscoll, D. M.** 1986. Containment and care of individuals suspected of exposure to a high hazard infectious disease. Presented to Critical Care Residents, Walter Reed Army Medical Center, Washington, DC, November.

3. **Driscoll, D. M., and J. D. Mims.** 1986. High hazard disease, patient containment and evacuation. Presented, U.S. Army Advanced Course on Infection Control, Bethesda, MD, December.

4. **Driscoll, D. M., and J. D. Mims.** 1987. Protection, safety and performance standards for individuals working in a high hazard patient care unit. Feasibility of performing critical care technical skills in a positive pressure suit to provide safe quality care. Presented to Critical Care Staff Physicians, Walter Reed Amry Medical Center, Washington, DC, January.

5. **Driscoll, D. M.** 1987. The capabilities of the Aeromedical Isolation Team. Presented, Aerospace Medicine Residence Course. San Antonio, TX, May.

6. **Driscoll, D. M.** 1987. The capabilities of USAMRIID to provide high containment patient care utilizing both the mobile and fixed high containment units. Presented, USAMRIID Hemostasis Symposium, Leesburg, VA, May.

PUBLICATIONS

1. **Wilson, K. E., and D. M. Driscoll.** 1987. Mobile high containment isolation: a unique patient care modality. *Am. J. Infect. Cont.* **15**:120-124.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY		1. AGENCY ACCESSION DA305993	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 10 Dec 86	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	BA	004
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) (U) Ribavirin

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	1.0	135
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Canonico, P G
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2290
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Huggins, J W
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Ribavirin; (U) Antiviral Drug; (U) Sandfly Fever; (U) Virus; (U) Prophylaxis; (U) Treatment; (U) RA I; (U) Volunteers

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Develop the drug ribavirin as an antiviral for treatment of viral diseases of military importance.

24. (U) Perform clinical trials to evaluate efficacy and toxicity in accordance with regulatory requirements.

25. (U) 8610 - 8709 A research protocol for a prospective, prophylactic clinical study in volunteers evaluating the efficacy of ribavirin against dengue 2 virus was completed. The protocol remains to be reviewed by various committees before being submitted for approval to the Food and Drug Administration.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE

PROJECT NO. 3M463750D809: Development of Drugs and Vaccines Against Diseases of BW Importance

WORK UNIT NO. 809-BA-004: Ribavirin

PRINCIPAL INVESTIGATOR: P. G. Canonico, Ph.D.

ASSOCIATE INVESTIGATOR: J. W. Huggins, Ph.D.

BACKGROUND

Although most viral diseases are not associated with high mortality and are self-limiting, certain disease outbreaks, such as the influenza pandemic of 1918-19, can be associated with great loss of life. Exotic viral diseases about which we know very little and with which we are presently unable to cope, occur in many areas of the world. Many viruses with the potential for inducing illness of high morbidity and mortality remain endemic in certain areas of the world; notable examples are Rift Valley fever, Lassa fever, and Ebola in Africa; Argentinian and Bolivian hemorrhagic fevers in South America; and hemorrhagic fever with renal syndrome in Asia. Numerous other examples can easily be cited. Broad-spectrum antiviral agents would be welcome insurance against the threat of viral outbreak during military operations.

A number of compounds have recently been approved by the Food and Drug Administration for the prevention or treatment of virus-induced diseases. These compounds have extremely narrow spectra of activity. In contrast, the new antiviral drug, ribavirin, appears to provide broad-spectrum activity. It was the purpose of this research to evaluate in humans the efficacy of putative antivirals against viruses of military importance.

SUMMARY

A research protocol for a prospective, prophylactic clinical study in volunteers evaluating the efficacy of ribavirin against dengue 2 virus was completed. The protocol remains to be reviewed by various committees before being submitted for approval to the Food and Drug Administration.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA303505	30 Oct 87	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	EA	005
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) Rapid ID and Diagnosis System

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense; 0601 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 03	88 01	DA	C. In-House

17. CONTRACT/GRANT
a. DATE EFFECTIVE | EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
87	1.2	426
88		

19. RESPONSIBLE DOD ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L
d. TELEPHONE NUMBER (include area code) 301-663-2833

20. PERFORMING ORGANIZATION
a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF PRINCIPAL INVESTIGATOR LeDuc, J W
d. TELEPHONE NUMBER (include area code) 301-663-7244
f. NAME OF ASSOCIATE INVESTIGATOR (if available) Meegan, J
g. NAME OF ASSOCIATE INVESTIGATOR (if available)

21. GENERAL USE FIC

MILITARY/CIVILIAN APPLICATION: H

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Vaccines; (U) Medical Defense; (U) Viral Diseases; (U) Immunological Reagents; (U) Antigens; (U) Rapid Diagnosis; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To develop, standardize, then conduct field tests of developed, rapid diagnosis assays. To support extramural contracts in rapid diagnosis. To assess availability and suitability of commercial reagents for use in rapid detection assays for viruses affecting U.S. military personnel.

24. (U) Employ immunoassay methods to develop and evaluate rapid assays for diagnosis of viral diseases of natural or BW threat to the military. When possible, test these assays at field laboratories using epidemiologically relevant samples. Develop and supply for extramural rapid diagnosis projects standardized reagents, safety-tested and efficacy-tested.

25. (U) 8610 - 8709 Field testing of rapid identification/diagnosis assays for Hantaan virus (Korean hemorrhagic fever) was extended to endemic foci in the People's Republic of China and the Republic of Korea, and to an epidemic among U.S. Marines from Okinawa training in Korea. Assays developed for chikungunya and Junin (Argentine hemorrhagic fever) viruses were adapted, then used to support efficacy studies of two new live-virus vaccines. Broadly cross-reactive assays developed for leptospirosis diagnosis were successfully tested on samples from previous outbreaks. Throughout the year, reagents and scientific consultation were supplied to extramural contractors funded by USAMRDC, USDAMMDA, and USACREDEC.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M463750D809: Development of Drugs and Vaccines Against Diseases of BW Importance

WORK UNIT NO. 809-EA-005: Rapid Identification and Diagnosis System

PRINCIPAL INVESTIGATOR: J. W. LeDuc, LTC, Ph.D.

ASSOCIATE INVESTIGATORS: J. M. Meegan, CDR, U.S.N., Ph.D.
T. G. Ksiazek, LTC, D.V.M.
R. R. Graham, MAJ, D.V.M
C. A. Rossi (DA Intern)

BACKGROUND

The objectives of this study are to conduct field tests of rapid diagnosis/identification assays; to evaluate clinical samples to establish if rapid diagnosis can best be achieved by detecting antigen or antibody; and to provide a technical support base for USAMRIID, U.S. Army Medical Material Development Activity (USAMMDA), and U.S. Army Chemical Research Development and Engineering Command (USACRDEC) extramural programs aimed at developing novel rapid detection/identification systems. The main technology used is the enzyme immunoassay. Field tests were conducted in areas of the world where the viruses are endemic. In earlier studies, assays were transported to OCONUS laboratories in developing countries, and testing revealed they were durable, rapid, simple, sensitive, and reproducible. However, the sensitivity of antigen-detection assays must still be improved.

Our main goal is to detect antigen in clinical or environmental samples within 2 to 3 h. Since the first indication that a disease is present might be an infected serviceman, and because some diseases do not produce a detectable antigenemia during clinical disease, we made an attempt to develop rapid antibody-detection assays for each agent. In previous years, we established that assays for the detection of antigen, and in some cases early IgM antibody, can rapidly diagnose patients presenting with a number of militarily relevant diseases.

SUMMARY

Field tests of rapid identification/diagnosis assays for Hantaan virus (Korean hemorrhagic fever) were extended through a second year, in part as support of an antiviral drug study, at endemic sites in People's Republic of China and in Republic of Korea. Results indicated that at admission, diagnosis is best established by demonstration of IgM antibody. This rapid diagnostic assay system was deployed when an unknown disease outbreak occurred in Marines from Okinawa on a training exercise (operation Bear Hunt) with Republic of Korea troops in Korea. Field teams rapidly identified

the disease which caused 14 cases (two fatalities) as Hantaan virus-induced, Korean hemorrhagic fever, and thus facilitated patient management and allowed treatment in Korea of selected cases with the experimental antiviral drug shown effective in our China studies.

Assays developed for chikungunya and Junin (Argentine hemorrhagic fever) viruses were optimized, then used to support efficacy studies of two new live-virus vaccines. A broadly cross-reactive assay was developed for leptospirosis diagnosis, and it was successfully tested on samples from previous outbreaks. Detection of anti-leptospira IgM antibody allows rapid diagnosis on the day of presentation.

Support of extramural contract programs was expanded to USAMMDA and USACRDEC. This support included developing, characterizing, then supplying specific antibodies; supplying safe, inactivated antigens; and providing scientific consultation services.

PRESENTATIONS

1. **Barrera Oro, J., C. MacDonald, R. Kenyon, J. Meegan, F. Cole, Jr., H. W. Lupton, and C. J. Peters.** 1987. Virus isolation and immune response in humans inoculated with a live-attenuated Junin virus (JV) vaccine. Presented at the VII International Congress of Virology, Edmonton, Alberta, Canada, August.

2. **Barrero Oro, J. G., C. MacDonald, A. I. Kuehne, B. C. Mahlandt, J. Spisso, J. M. Meegan, C. J. Peters, and H. W. Lupton.** 1986. Initial assessment in humans of a liver-attentuated vaccine against Argentine hemorrhagic fever (Candid #1). B. Virus isolation and serologic response. Presented at the II Argentine Congress of Virology, Cordoba, Argentina, October.

3. **Binn, L. N., W. H. Bancroft, K. H. Eckels, R. H. Marchwicki, D. R. Dubois, L. V. S. Asher, J. W. LeDuc, C. J. Trahan, and D. S. Burke.** 1987. Inactivated hepatitis A virus vaccine produced in human diploid MRC-5 cells. Presented at the International Symposium on Viral Hepatitis and Liver Disease, London, England, May. (*J. Med. Virol.* 21:25a).

4. **Huggins, J. W., C. M. Hsiang, T. M. Cosgriff, M. Y. Guang, J. I. Smith, Z. A. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, X. E. Gui, K. W. Yuan, T. M. Zhang, and H. W. Lee.** 1987. Intravenous ribavirin therapy of hemorrhagic fever with renal syndrome (HFRS). Presented at the XVI Pacific Science Conference, Seoul, Korea, August.

5. **Huggins, J. W., C. M. Hsiang, T. M. Cosgriff, M. Y. Guang, J. J. Smith, Z. A. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, X. E. Gui, K. W. Yuan, and T. M. Zhang.** 1987. Clinical therapeutic efficacy of intravenous ribavirin treatment of hemorrhagic fever with renal syndrome (HFRS): randomized, double-blind placebo controlled trial in the People's Republic of China. Presented at the VII International Congress of Virology, Edmonton, Alberta, Canada, August.

6. **Kenyon, R., J. Barrera Oro, C. MacDonald, J. Meegan, and C. Peters.** 1987. Human lymphocyte transformation assay for Junin virus (Argentine hemorrhagic fever). Presented at the Annual Meeting of the American Society of Virology, Chapel Hill, NC, May-June.

7. **Koga, P., and J. Meegan.** 1986. Efficacy of vaccines as simulants for biological agents. Presented at the Joint Services Workshop on BW Agent Simulants, Aberdeen, MD, December.

8. **LeDuc, J. W., and A. Antoniades.** 1987. Severe hemorrhagic fever with renal syndrome in Greece: clinical and epidemiological characteristics, and isolation of the etiological agent. Presented at the 16th Congress of the Pacific Science Association, Seoul, Korea, August.

9. **LeDuc, J. W., and A. Antoniadis.** 1987. Severe hemorrhagic fever with renal syndrome in Greece: clinical and epidemiological characteristics, and isolation of the etiological agent. Presented at the VII International Congress of Virology, Edmonton, Alberta, Canada, August.

10. **Lemon, S. M., J. T. Stapleton, J. W. LeDuc, D. Taylor, and L. N. Binn.** 1987. A cell culture-adapted variant of hepatitis A virus (HAV) selected for resistance to neutralizing monoclonal antibody retains virulence in owl monkeys. Presented at the International Symposium on Viral Hepatitis and Liver Disease, London, England, May (*J. Med. Virol.* 21:16a-17a).

11. **Meegan, J. M.** 1986. Monoclonal antibodies: application to diagnosis and seroepidemiology of arboviruses. Presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene, Denver, CO, December.

12. **Meegan, J. M., J. LeDuc, S. G. Franco, A. M. Ambrosio, and J. I. Maiztegui.** 1986. An ELISA for IgG and IgM antibodies to Junin virus. Presented at the II Argentine Congress of Virology, Cordoba, Argentina, October.

13. **Meegan, J., J. LeDuc. S. G. Franco, and J. Maiztegui.** 1986. Rapid diagnostic methods to detect Junin virus infections. Presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene, Denver, CO, December.

14. **Stapleton, J. T., J. W. LeDuc, L. N. Binn, and S. M. Lemon.** 1987. Lack of neutralizing activity in fecal abstracts following experimental hepatitis A virus (HAV) infection in man and owl monkeys. Presented at the International Symposium on Viral Hepatitis and Liver Disease, London, England, May (*J. Med. Virol.* 21:17a-18a).

PUBLICATIONS

1. **Antoniadis, A., D. Grekas, C. A. Rossi, and J. W. LeDuc.** 1987. Isolation of a *Hantavirus* from a severely ill patient with hemorrhagic fever with renal syndrome in Greece. *J. Infect. Dis.* 156:1010-1013.

2. **Antoniadis, A., J. W. LeDuc, and S. Daniel-Alexiou.** Clinical and epidemiological aspects of hemorrhagic fever with renal syndrome (HFRS) in Greece. *Eur. J. Epidemiol.* 3:295-301.

3. **Binn, L. N., W. H. Bancroft, K. H. Eckels, R. H. Marchwicki, D. R. Dubois, L. V. S. Asher, J. W. LeDuc, C. J. Trahan, and D. S. Burke.** 1987. Inactivated hepatitis A virus vaccine produced in human diploid MRC-5 cells. *J. Med. Virol.* (In Press).

4. **Childs, J. E., G. E. Glass, G. W. Korch, and J. W. LeDuc.** 1987. Prospective seroepidemiology of hantaviruses and population dynamics of small mammal communities of Baltimore, MD, U.S.A. *Am. J. Trop. Med. Hyg.* 37:648-662.

5. **Childs, J. E., G. W. Korch, G. E. Glass, J. W. LeDuc, and K. V. Shah.** 1987. Epizootiology of *Hantavirus* infections in Baltimore: isolation of a virus from Norway rats and characteristics of infected rat populations. *Am. J. Epidemiol.* 126:55-68.

6. **Glass, G. E., J. E. Childs, G. W. Korch, and J. W. LeDuc.** Ecology and social interactions of sylvaatic and commensal Norway rats (*Rattus norvegicus*) populations in Baltimore, MD, U.S.A. Occasional Papers in the Museum of Natural History, University of Kansas (In Press).

7. **Keenan, C. M., S. M. Lemon, L. N. Binn, and J. W. LeDuc.** 1987. Hepatitis A infection. Animal model of human disease. *Comp. Path. Bull.* 19:3-5.

8. **LeDuc, J. W.** 1987. Epidemiology and ecology of the California serogroup viruses. *Am. J. Trop. Med. Hyg.* 37:60S-68S.

9. **LeDuc, J. W.** 1987. Epidemiology of Hantaan and related viruses. *Lab. Anim. Sci.* 37:413-418.

10. **LeDuc, J. W.** 1987. Epidemiology of hemorrhagic fever viruses. Submitted to *Rev. Infect. Dis.*

11. **LeDuc, J. W., K. M. Johnson, and J. Kawamata.** 1986. Hantaan and related viruses, pp. 1B1 - 1B3. *In* A. M. Allen and T. Nomura (ed.), Manual of microbiologic monitoring of laboratory animals. NIH Publication No. 86-2498.

12. **LeDuc, J. W., and F. P. Pinheiro.** 1987. Oropouche fever. pp. , *In* T. P. Monath (Ed.), Epidemiology of arthropod-borne viral diseases, CRC Press, Boca Ratan, FL (to be published in 1986-1987) (In Press).

13. **Lemon, S. M., S. F. Chao, R. W. Jansen, L. N. Binn, and J. W. LeDuc.** 1987. Genomic heterogeneity among human and nonhuman strains of hepatitis A virus. *J. Virol.* 61:735-742.

14. **Meegan, J. M., and C. Bailey.** Rift Valley fever. pp. , *In* T. Monath (Ed.), Epidemiology of arthropod-borne viruses. CRC Press, Boca Ratan, FL (to be published in 1986-1987).

15. **Meegan, J. M., R. J. Yedloutschnig, B. A. Peleg, J. Shy, C. J. Peters, J. S. Walker, and R. E. Shope.** 1987. Enzyme-linked immunosorbent assay for detection of antibodies to Rift Valley fever virus in ovine and bovine sera. *Am. J. Vet. Res.* 48:1138-1141.

16. **Niklasson, B., and J. W. LeDuc.** 1987. Epidemiology of nephropathia epidemica in Sweden. *J. Infect. Dis.* 155:269-276.

17. **Niklasson, B., J. LeDuc, K. Nystrom, and L. Nyman.** 1987. Nephropathia epidemica; incidence of clinical cases and antibody prevalence in an endemic area of Sweden. *Epidemiol. Infect.* 99:559-562.

18. **Pinheiro, F. P., and J. W. LeDuc.** 1987. Mayaro fever. pp. , *In* T. P. Monath (Ed.), Epidemiology of arthropod-borne viral diseases. CRC Press, Boca Ratan, FL (to be published in 1986-1987) (In Press).

19. **Shortridge, K. F., H. W. Lee, J. W. LeDuc, T. W. Wong, G. W. Chau, and L. Rosen.** 1987. Serological evidence of Hantaan-related viruses in Hong Kong. *Trans. R. Soc. Trop. Med. Hyg.* 81:400-402.

20. **Trahan, C. J., J. W. LeDuc, E. C. Staley, L. N. Binn, R. H. Marchwicki, S. M. Lemon, C. M. Keenan, and W. H. Bancroft.** 1987. Induced oral infection of the owl monkey (*Aotus trivirgatus*) with hepatitis A virus. *Lab. Anim. Sci.* 37:45-50.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA311563	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	AK	007
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP FY 88- 01			

11. TITLE *(Precede with Security Classification Code)* Vaccine, Chikungunya

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
b. CONTRACT/GRANT NUMBER		87	1.0	146
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS *(include zip code)* Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Lupton, H W
d. TELEPHONE NUMBER *(include area code)* 301-663-2833	d. TELEPHONE NUMBER *(include area code)* 301-663-7241
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR *(if available)* Cole, F E, JR
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR *(if available)* Ramsburg, H H

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Military Medicine; (U) BW Defense; (U) Vaccines; (U) Volunteers; (U) Chikungunya; (U) Clinical Trials; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23. (U) Develop and test a live, attenuated chikungunya vaccine for prophylactic treatment of at-risk military personnel.

24. (U) Conduct preclinical tests to evaluate vaccine safety and efficacy. Conduct animal and/or in vitro studies to demonstrate cross protection against heterologous viruses. Design and conduct clinical trials and laboratory tests to evaluate vaccine safety and efficacy.

25. (U) 8610 - 8709 Phase I clinical evaluation of the CHIK vaccine (IND-2426) began in September 1986 under a protocol approved by The Surgeon General's HSRRB. Five groups were studied: 15 vaccine recipients and 14 placebo controls. Seronegative volunteers were given 10^5 PFU of vaccine s.c. Only minor clinical abnormalities were observed among principals and controls. All vaccinees seroconverted, with peak PRN80 titers occurring on day 28 postvaccination. ELISA IgM titers rose after 10 days and peaked on day 21. Less than 1 log10 of viremia was detectable in 4 of 13 vaccine recipients tested thus far, generally for only 1 day. Attenuated, strain ZH-548, Rift Valley fever master and production seeds and a candidate vaccine are in final test stage at The Salk Institute. In a monkey neurovirulence test, animals were injected with either the production seed, candidate vaccine, vaccine control fluid, parent virus, or live attenuated Smithburn vaccine. The candidate and the Smithburn vaccine were similar in the level of neuropathology induced; the vaccine elicited neurological signs in 1 or 5 monkeys, whereas 4 of 5 monkeys that received Smithburn vaccine showed neurological signs. In comparison, monkeys given parent virus had neuropathology and neurological signs significantly more severe. Immunofluorescence studies revealed no evidence of virus in brain, cord or other tissues taken from monkeys that received candidate vaccine, in contrast to parent virus recipients, in which virus was demonstrated in 90% of CNS tissues and 25% of all others.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M463750D809: Development of Drugs and Vaccines Against Diseases of BW Importance

WORK UNIT NO. 809-AK-007: Vaccine, Chikungunya

PRINCIPAL INVESTIGATOR: H. W. Lupton, COL, Ph.D.

ASSOCIATE INVESTIGATORS: F. E. Cole, Jr., Ph.D.
H. H. Ramsburg, M.S.

BACKGROUND

Chikungunya (CHIK), an arthropod-borne alphavirus that produces a dengue-like illness in man, is found throughout Africa, Southeast Asia, the western Pacific, and India; it often causes epidemics. Despite widespread geographic distribution, individual strains of CHIK are closely related antigenically, thereby allowing a vaccine to provide broad-spectrum protection against heterotypic strains of this virus, in addition to the antigenically related viruses of O'Nyong Nyong, Mayaro, and Ross River. With CHIK strain 15561, a southeast Asian, human isolate from a mild case of CHIK fever, we developed a vaccine by a series of plaque-to-plaque passages in certified MRC-5 cells. Master and production seeds and vaccine have been produced and preclinically tested in compliance with both the Good Laboratory Practices and the Good Manufacturing Practices Regulations. An Investigational New Drug submission was approved by the Office of Biological Research and Review of the FDA in July, 1986 (IND 2426).

Until 1975, when deaths occurred in South Africa, Rift Valley fever virus was confined to sub-Saharan Africa, rarely causing serious illness in man. In 1977, the virus spread to Egypt where an increased incidence of lethal human disease made self-evident the need for a more effective vaccine. In 1982, investigators at USAMRIID and the University of Alabama, Birmingham, received a grant from the U.S./Israel BARD Fund to initiate a program to develop a live, attenuated vaccine by passage of the virus in the presence of mutagenic chemicals. A candidate vaccine was developed, and master and production seeds and candidate vaccine were prepared in vaccine -quality MRC-5 cells by The Salk Institute. Final tests are in progress.

SUMMARY

Phase I clinical evalutions of the new live, attenuated CHIK vaccine (CHIK 181/clone 25) were begun in September 1986 in accordance with a protocol approved by The Surgeon General's HSRRB. Five groups of volunteers were studied by the double-blind technique, with a total of 15 principals and 14 placebo-inoculated controls. Seronegative volunteers were given 10^5 PFU of CHIK vaccine or placebo s.c. Minor clinical abnormalities were seen occasionally in both principal and control groups. All vaccinated volunteers

seroconverted as measured by the 80% plaque-reduction serum neutralization test (PRN_{80}). Peak PRN_{80} titers occurred on day 28 postvaccination, and ranged from 1:80 to 1:2560. IgM levels, measured by ELISA, rose after 10 days and peaked on day 21. Less than 1 $\log_{10}$ of viremia was detectable in 4 of 15 vaccine recipients tested thus far. Of these, three were viremic on day 6 or 7 and one on days 2 and 5 postvaccination. Clinicals trials on the new CHIK vaccine are still in progress.

Attenuated, strain ZH-548, Rift Valley fever master and production seeds and a candidate vaccine are undergoing final testing at The Salk Institute. In a monkey neurovirulence test, animals were injected with either the production seed; candidate vaccine; vaccine control fluid; parent virus; a virulent strain (ZH-501); or live, attenuated Smithburn vaccine. Neurological signs were seen in five of five monkeys that received parent virus, five of five that received ZH-501, and four of five that received Smithburn vaccine. In contrast, none of five production seed and one of five vaccine monkeys showed neurological signs. Further, monkeys in the candidate vaccine and production seed groups had microscopic evidence of minimal to mild generalized inflammation in the central nervous system (CNS) without neuronal necrosis. Similar neuropathological results were seen in monkeys that received the Smithburn vaccine. In contrast, monkeys that received the parent virus developed a substantial degree of inflammatory disease, including neuronal necrosis, throughout the neuraxis. Immunofluorescence studies revealed no evidence of virus in brain, cord, or other tissues taken from monkeys that received candidate vaccine, in contrast to parent virus recipients, in which virus was demonstrated in 90% of CNS tissues and 25% of all others. Only one sample (spinal cord) from monkeys receiving production seed or the Smithburn vaccine had evidence (minimal) of viral replication in neuronal tissues, as demonstrated by immunofluorescence. Assays for live virus in these tissues are in progress.

PRESENTATION

1. **Malinoski, F. J., G. F. Meadors, H. H. Ramsburg, P. J. Stopa, and T. G. Ksiazek.** 1987. Safety and efficacy of a new chikungunya virus vaccine: double-blind, placebo-controlled human trial. Presented at the VII International Congress of Virology, Edmonton, Alberta, Canada, August.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA305652	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 10 Dec 86	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	64758A	3S464758D847	AN	002
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY-88 -01			

11. TITLE (Precede with Security Classification Code) (U) Vaccine, Advanced Development

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	1.0	1
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Medical Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Cosgriff, T M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2997
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Meadors, G F
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) RA I (U) Vaccines; (U) Volunteers; (U) Phase I, Phase II, and Phase III Clinical Trials

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Conduct Phase I (safety and tolerance), Phase II (efficacy), and Phase III (field trials) testing of experimental vaccines for prophylaxis of diseases of unique military importance, particularly those with potential as biological warfare threats.

24. (U) Experimental vaccines which have undergone immunogenicity and safety testing in preclinical studies will be studied in rigorous clinical trials after extensive scientific and ethical reviews.

25. (U) 8610 - 8709 Studies have been conducted on Rift Valley fever, Q-fever, and Chikungunya vaccines. Results to date indicate that these vaccines are safe and immunogenic.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3S464758D847: Medical Defense Against Diseases of BW Importance

WORK UNIT NO. 847-AN-002: Vaccine, Advanced Development

PRINCIPAL INVESTIGATOR: T. M. Cosgriff, COL, M.D.

ASSOCIATE INVESTIGATORS: G. F. Meadors, LTC, M.D.
F. J. Malinowski, CPT, M.D.

BACKGROUND

As part of the task of developing vaccines to meet the biological warfare threat, Phase I and Phase II clinical trials of candidate vaccines are conducted by the Medical Division. If these trials demonstrate that a vaccine is safe and immunogenic, Phase III studies are conducted in larger numbers of volunteers, and when possible, in endemic areas.

SUMMARY

Rift Valley Fever Vaccine. During fiscal year 1987, the randomized intralot potency study of 19 of the 20 existing lots of Rift Valley fever vaccine, inactivated, freeze-dried, TSI-GSD-200, continued. Over 75 persons have participated in this study. We anticipate that a further 3 years will be required before the data can be analyzed. The vaccine appears to be safe and effective in protecting at-risk laboratory workers from the disease.

Q-fever Vaccine. During fiscal year 1987, Q-fever vaccine, inactivated, freeze-dried, NDBR 105, continued to undergo clinical testing. Over 150 volunteers have participated in the study to date. We have observed no significant adverse reactions, and no cases of laboratory-acquired disease have occurred among vaccinated persons. No correlation is apparent between skin test results and measurements of cellular and humoral immunity, before or after immunization. There are also no consistent changes in these parameters following immunization.

Chikungunya Vaccine. During fiscal year 1987, 29 volunteers were immunized during initial safety and efficacy trials of Chikungunya vaccine, live, attenuated, dried, TSI-GSD-218. No significant adverse reactions have occurred. Minor complaints have been equally common in vaccinated and control groups. The vaccine has been uniformly effective in eliciting neutralizing antibody in all recipients.

PRESENTATIONS

1. **Malinoski, F. J., G. F. Meadors, H. Ramsberg, P. Stopa, and T. Ksiazek.** 1987. Safety and efficacy of a new chikungunya virus vaccine: double-blind, placebo-controlled human trial. Presented at the VIIth International Congress of Virology, Edmonton, Alberta, Canada, August.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA308927	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 10 Dec 86	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8 DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	64758A	3S464758D847	BA	003
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY 88 -01			

11. TITLE (Precede with Security Classification Code) (U) Ribavirin

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense; 0601 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
85 04	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	1.0	827
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Canonico, P G
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2290
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Huggins, J W
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Antiviral; (U) Ribavirin; (U) Chemotherapy; (U) Junin Virus; (U) Hantaan Virus; (U) Hemorrhagic Fever with Renal Syndrome; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Develop the drug ribavirin as an antiviral for the treatment of viral diseases of military importance.

24. (U) Conduct Phase III clinical trials appropriate for the development and FDA approval of ribavirin for the prophylaxis or treatment of serious viral infections. Establish liaison with medical authorities in appropriate areas to study the diseases. Design clinical trials and obtain appropriate U S and host country study clearances for conducting human trials. Conduct clinical trials to evaluate efficacy and toxicity in accordance with regulatory requirements.

25. (U) 8610 - 8709 A clinical trial of the efficacy of ribavirin in treating clinically ill patients with the Chinese variant of hemorrhagic fever with renal syndrome (HFRS) was continued at Hubei Medical College, Wuhan, People's Republic of China, and Korea. A total of 244 and 38 subjects were enrolled in the study in China and Korea, respectively. Treatment was shown to decrease the risk of dying by fivefold. Ribavirin shortened the duration of each clinical phase and had marked protective effect on renal functions.

PROJECT NO. 3S464758D847: Medical Defense Against Diseases of BW Importance

WORK UNIT NO. 847-BA-003: Ribavirin

PRINCIPAL INVESTIGATOR: P. G. Canonico, Ph.D.

ASSOCIATE INVESTIGATOR: J. W. Huggins, Ph.D.

BACKGROUND

Ribavirin (1-ß-D-ribofuranosyl-1,2,3-triazole-3-carboxamide), a nucleoside analogue with a close structural resemblance to guanosine, has been found to significantly inhibit a broad spectrum of both DNA and RNA viruses. Ribavirin is the only antiviral agent which has been shown to have efficacy in the therapy of respiratory syncytial viral infection. Other clinical studies have shown that ribavirin is effective in the treatment of Lassa fever in man. In our studies, patients with an admission viremia of $\geq 10^{5.6}$ tissue culture infectious doses per ml were found to have a $\geq$ 73% fatality. Treatment with intravenous ribavirin within the first 6 days of illness reduced the mortality rate to 8%, compared to 43% in those treated after day 6.

This work unit expands these clinical trials by examining the efficacy of ribavirin against two other militarily relevant viral diseases, hemorrhagic fever with renal syndrome (HFRS) and Argentine hemorrhagic fever (AHF).

SUMMARY

Geographically diverse but clinically similar human diseases caused by Hantaan viruses and characterized by fever, hemorrhage, and renal damage in five overlapping phases are known collectively as hemorrhagic fever with renal syndrome (HFRS). Existing therapy consists of intensive fluid and electrolyte management and symptomatic treatment. The antiviral drug ribavirin has demonstrated significant antiviral activity against 13 viral isolates of HFRS in cell culture and is protective in a suckling mouse model. A prospective, randomized, double-blind, placebo-controlled, clinical trial of ribavirin for the treatment of patients with a clinical diagnosis of HFRS within 7 days of fever onset was conducted in the People's Republic of China (PRC) and in Korea. Subjects were randomized to receive intravenous ribavirin (33 mg/kg loading dose; 16 mg/kg every 6 h for 4 days; 8 mg/kg every 8 h for 3 days), or placebo. In a nine-site study covering two seasons in Hubei Province, PRC, 244 patients (57 in 1985-86 and 187 in 1986-87) met the study criteria for analysis (clinical diagnosis, serologically confirmed by IgM ELISA). A similar single-season study in Korea at two study sites including the U.S. Army's 121 Evacuation Hospital and the Capital Army Hospital (ROK) in Seoul treated 38 subjects in 1986-87. Statistical analysis proved random assignment

of patients between treatment groups. Treatment reduced mortality from 10 of 118 in the placebo group to 3 of 126 in the ribavirin group (p=0.041 by Fisher's exact test); no mortality occurred in either group in Korea. Patients treated by the fourth day of fever showed the most marked improvement with a fivefold lower risk of dying. This may be partially explained by the protective effect of ribavirin on the kidney. Ribavirin treatment decreased serum creatinine, blood urea nitrogen, proteinuria, days of hypotension, duration and magnitude of hypertension, maximum white blood counts, and hemorrhagic manifestations by increasing platelets, decreasing petechiae, ecchymosis, and edema. Ribavirin shortened the duration of each clinical phase, with significant effects on the hypotensive and oliguric phase duration, while retaining an earlier onset of the recovery (polyuric) phase.

PRESENTATIONS

1. **Cosgriff, T. M., P. G. Canonico, L. Hodgson, D. Parrish, and T. Chapman.** 1987. Ribavirin: studies of the effects of the antiviral drug on platelet function. Presented at the XIth International Congress on Thrombosis and Haemostasis, Brussels, Belgium, July.

2. **Huggins, J. W., C. M. Hsiang, T. M. Cosgriff, M. Y. Guang, J. I. Smith, Z. A. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, X. E. Gui, K. W. Yuan, T. M. Zhang, and H. W. Lee.** 1987. Intravenous ribavirin therapy of hemorrhagic fever with renal syndrome (HFRS). Presented at the XVI Pacific Science Congress, Seoul, Korea, August.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA302626	30 Oct 87	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A870	AP	131
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) (U) Risk Assessment and Evaluation of Viral Agents and Their Vectors

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	1.0	100
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Linthicum, K J
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2775
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Logan, T M
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Turell, M J

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Viral Diseases (U) Arthropod Transmission; (U) Entomology; (U) RA I; (U) Lab Animals (U)Gerbils

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Identify arthropods and vertebrates associated with the maintenance and transmission of medically important arboviruses to man and define ecologic and environmental factors influencing the ability of arthropods to transmit viruses. Information will allow for development of methods for predicting relative risk of military personnel to arbovirus infections and for designing specific vector control strategies.

24. (U) Ecologic and environmental factors relating to an arthropod's ability to transmit viruses are studied under natural and controlled environments.

25. (U) 8610 - 8709 Data from the advanced very high resolution radiometer on the National Oceanic and Atmospheric Administration's polar-orbiting meteorological satellites were used to monitor ecological parameters associated with Rift Valley fever (RVF) viral activity in Kenya and Zambia. Thematic mapper data from Landsat satellites have been used to identify specific breeding habitats of RVF vector species and determine when flooding occurs. Six species of possible enzootic vectors of this disease have been tested in the laboratory and found able to transmit the virus to animals. Survival of infected specimens was found to be equal to that of non-infected specimens.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M162770A870:	Risk Assessment, Prevention, and Treatment of Infectious Diseases
WORK UNIT NO. 870-AP-131:	Risk Assessment and Evaluation of Viral Agents and Their Vectors
PRINCIPAL INVESTIGATOR:	K. J. Linthicum, CPT, Ph.D.
ASSOCIATE INVESTIGATORS:	T. M. Logan, CPT, Ph.D. M. J. Turell, Ph.D.

BACKGROUND

Previous research incriminated floodwater *Aedes* mosquitoes, which breed in discrete, well-defined habitats known as dambos, as the endemic/enzootic vectors of Rift Valley fever (RVF) virus in sub-Saharan Africa. Remote sensing technology has been shown to be a tool for monitoring RVF viral activity in areas where it is known to be endemic. Previous attempts to obtain information on the vector competence of the mosquitoes that are believed to be responsible for the disease have been hampered because of the unavailability of large numbers of specimens.

SUMMARY

Data from the advanced very high resolution radiometer on board the National Oceanic and Atmospheric Administration's polar-orbiting, meteorological satellites have been used to infer ecological parameters associated with RVF viral activity in Kenya and to develop an indicator of potential viral activity. The indicator of potential viral activity is produced by weighting a green vegetation index derived from the satellite data. The satellite data are continuously analyzed and correlated with rainfall, mosquito vector population levels, the flooding of mosquito vector breeding habitats, and isolation of virus from mosquito vectors. The high correlation between the satellite-derived green vegetation index and the ecological parameters associated with RVF virus indicated that satellite data can serve as a forecasting tool for RVF. Currently ecological parameters associated with RVF viral activity is being monitored by these methods in Kenya and Zambia.

Thematic mapper data from Landsat satellites has been used to identify specific breeding habitats of RVF vector species by analyzing various electromagnetic channels which can detect vegetation patterns unique to these habitats. The data have also been used to detect the presence of water in these habitats and hence determine the likelihood of mosquito vector emergence in the area. These data are essential for timely and efficient control operations.

Thousands of field-collected specimens were sent to USAMRIID, after artificial flooding of breeding habitats, for us to conduct vector competence studies. Seven African *Aedes* species (*mcintoshi, circumluteolus dentatus,*

cumminsii, sudanensis, palpalis, and fowleri), suspected of being involved to some extent in the enzootic cycle of RVF in sub-saharan Africa, were tested for their ability to become infected and transmit the virus. Ability to transmit varied greatly among the species. *Aedes mcintoshi* had the lowest transmission rate (7%) after oral exposure to virus. *Aedes palpalis* and *A. fowleri* had 43% and 37% transmission rates, respectively. Infected *A. mcintoshi* survived equally as well as uninfected specimens in the laboratory. In contrast, infected *Culex pipiens* did not survive as well as uninfected specimens.

PRESENTATIONS

1. **Knudson, G. B., and M. J. Turell.** 1987. Mechanical transmission of *Bacillus anthracis* by the stable fly, *Stomoxys calcitrans*. Presented at the 87th Annual Meeting of the American Society for Microbiology, March.

2. **Linthicum, K. J., and C. L. Bailey.** 1987. Application of remote sensing imagery to the ecology of Rift Valley fever virus. Presented at the 1987 Annual Meeting of The American Mosquito Control Association, Seattle, Washington, April.

3. **Linthicum, K. J., C. L. Bailey, F. G. Davies, and C. J. Tucker.** 1986. Use of satellite remote sensing imagery to predict Rift Valley fever virus activity in Kenya. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

4. **Turell, M. J., and C. L. Bailey.** 1986. Effect of environmental temperature on the replication, dissemination, and transmission of Rift Valley fever virus by *Aedes fowleri*. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

5. **Turell, M. J., R. F. Tammariello, and C. L. Bailey.** 1986. Reduced recovery of Rift Valley fever virus associated with assay of larval pools. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

6. **Turell, M. J., R. F. Tammariello, J. F. Smith, and C. L. Bailey.** 1987. Reduced recovery of Rift Valley fever virus associated with assay of mosquito larval pools. Presented at the VII International Congress on Virology, Edmonton, Alberta, Canada.

PUBLICATIONS

1. **Davies, F. G., and K. J. Linthicum.** 1986. The Sudan dioch (*Quelea quelea aethiopica*) and Rift Valley fever. *Trans. Roy. Soc. Med. Hyg.* **80**:171-172.

2. **Faran, M. E., M. J. Turell, W. S. Romoser, R. G. Routier, P. H. Gibbs, T. L. Cannon, and C. L. Bailey.** 1987. Reduced survival of adult *Culex pipiens* infected with Rift Valley fever virus. *Am. J. Trop. Med. Hyg.* **37**:403-409.

3. **Linthicum, K. J., F. G. Davies, A. Kairo, and C. L. Bailey.** 1987. Rift Valley fever virus disease in Kenya. *In* Viral diseases in Africa. Proceedings of the Scientific, Technical and Research Commission of the Organization of African Unity Symposium on Viral Disease in Africa Affecting Plants, Animals and Man. Nairobi, Kenya (In Press).

4. **Linthicum, K. J., F. G. Davies, A. Kairo, C. L. Bailey, H. F. Kaburia, and K. J. Lindquist.** 1987. Field ecological studies on Rift Valley fever virus, pp. 97-108. *In* Advances in the diagnosis, treatment and prevention of immunizable diseases in Africa. Proceedings of the Seventh Annual Medical Scientific Conference, Nairobi, Kenya.

5. **Linthicum, K. J., F. G. Davies, and J. Kamau.** 1987. Predation on emerging adult mosquitoes by *Brachydeutera munroi* (Diptera:Ephydridae), pp. 178-181. Proceedings of the 72nd Annual Meeting of the New Jersey Mosquito Control Association.

6. **Linthicum, K. J., C. L. Bailey, F. G. Davies, C. J. Tucker.** 1987. Use of satellite remote sensing imagery to predict Rift Valley fever virus activity in Kenya. *In* Viral diseases in Africa. Proceedings of the Scientific, Technical and Research Commission of the Organization of African Unity Symposium on Viral Diseases in Africa Affecting Plants, Animals and Man. Nairobi, Kenya (In Press).

7. **Linthicum, K. J., C. L. Bailey, F. G. Davies, and C. J. Tucker.** 1987. Detection of Rift Valley fever viral activity in Kenya by satellite remote sensing imagery. *Science* **235**:1656-1659.

8. **Romoser, W. S., M. E. Faran, and C. L. Bailey.** 1987. Newly recognized route of arbovirus dissemination from the mosquito (*Diptera:Culicidae*) midgut. *J. Med. Entomol.* **24**:431-432.

9. **Turell, M. J., and C. L. Bailey.** 1987. Transmission studies in mosquitoes (Diptera:Culicidae) with disseminated Rift Valley fever virus infections. *J. Med. Entomol.* **24**:11-18.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA302630	30 Oct 87	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AA	130
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory Development Studies on Toxins of Biological origin for Development of Medical Defensive Countermeasures

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	2.0	559
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Friedlander A M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) York, C G
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Hines, H B

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Low Molecular Weight Toxins; (U) Rapid Detection; (U) Vaccines; (U) Lab Animals; (U)Mice; RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To define the respiratory toxicity of agents of potential BW importance; elucidate pathogenesis of intoxications induced by aerosols, to include determination of the sequence of events leading to protective immunity. Data obtained will provide the basis for evaluation of prophylactic and therapeutic regimens developed to protect deployed US forces.

24. (U) Develop animal models and define the clinical, pathological, and immunological changes during intoxications. Characterize immune defenses within the respiratory tract. Information is used to provide basis for efficacy of vaccination and therapy procedures.

25. (U) 8610 - 8709 HPLC and gas chromatography-mass spectrometry have been used to trace the metabolism of T-2 toxin in monkeys and to allow detection of T-2 at 100 picograms. Assays for saxitoxin have been developed which allow detection at 1 nanogram. These analytic procedures have also been helpful in developing decontamination protocols for brevetoxin. An inexpensive disposable nebulizer has been characterized aerobiologically and will be useful in studies requiring disposable equipment. A new particle sizing-system has been made operational, allowing us to better characterize our aerosol equipment.

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AA-130: Exploratory Development Studies on Toxins of Biological Origin for Development of Medical Defensive Countermeasures

PRINCIPAL INVESTIGATOR: A. M. Friedlander, COL, M.D.

ASSOCIATE INVESTIGATORS: J. L. Middlebrook, Ph.D.
H. B. Hines, Ph.D.
S. M. Naseem, Ph.D.
C. G. York, M.S.

BACKGROUND

Medical defense, in the form of therapeutic drugs or vaccines to protect against a potential BW agent, must be effective when the agent is delivered by aerosol. Our investigations are designed to define the respiratory toxicity of potential agents, to identify and characterize commercially available equipment that will simplify the conduct of such studies, to develop new equipment when necessary, and to develop analytical chemistry methodologies for potential toxin threats.

SUMMARY

High-performance liquid chromatography (HPLC) and gas chromatography-mass spectroscopy (GC-MS) for detection of several toxins have been evaluated. The in vivo metabolism of T-2 toxin by cynomolgous monkeys was investigated by HPLC. Urine and blood analyses showed that T-2 was rapidly transformed and T-2 tetraol was the ultimate product. T-2 was cleared from the blood in 72 h but persisted in the urine for several days. GC-MS analysis applied to trichothecenes allowed detection of 100 pg of toxin.

An HPLC procedure for saxitoxin was made operational with a detection limit of 1 ng.

Studies on brevetoxins by HPLC and mouse bioassay have shown that strong acids and bases can be used for decontamination.

Additional preliminary studies have demonstrated that T-2, saxitoxin, and microcystin can alter arachidonic acid metabolism in cultured cells.

The final design of the nose-only aerosol exposure system, designed at in-house, has been installed and has become the standard unit for our nose-only aerosol studies. A new nebulizer (Retec) has been evaluated and

compares favorably with the standard Collison nebulizer. The new unit is inexpensive and will be especially useful when decontamination is required. A Differential Mobility Particle Sizer was procured and is being used to extend the range of our ability to characterize the performance of various aerosol dissemination and sampling devices.

PUBLICATIONS

1. **Stephenson, E. H., R. B. Moeller, C. G. York, and H. W. Young.** 1987. Nose only vs whole body aerosol exposure for induction of upper respiratory infections of laboratory mice. Submitted to *Am. Indust. Hyg. Assoc.*

2. **Naseem, S. M., and K. A. Mereish.** 1987. Phorbol-mediated changes in arachidonic acid metabolism and phospholipid turnover in cultured human endothelial cells. Submitted to *Biochem. Biophys. Acta.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA308918	30 Oct 87	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AD	131
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code)
(U) Exploratory Vaccine Development Studies on Conventional Agents of Potential BW Threat

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT
a. DATE EFFECTIVE — EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)
87	8.0	2548
88		

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Jahrling, P B
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Kenyon, R H
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Crumrine, M H

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Viral Diseases; (U) Vaccines; (U) Lab Animals; (U) Guinea Pigs; (U) Monkeys; (U) RA I;(U)Rodents

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To isolate, study, and characterize agents of potential BW threat. To obtain immunogens that elicit protective immunity and to devise effective regimens to protect US military personnel in the field.

24. (U) Naturally occurring and laboratory-derived strains are molecularly and biologically characterized and assessed for virulence or attenuation in susceptible animal models. Attenuated strains are tested for ability to induce cross-reactions with virulent strains. Protective immunity elicited by inactivated antigens is determined.

25. (U) 8610 - 8709 The search to develop adult rodent and primate models for Crimean-Congo hemorrhagic fever (CCHF) was expanded to include 19 inbred mouse strains, guinea pigs, and hamsters. One CCHF viral strain produced viremia in rhesus monkeys, and is being passaged to increase virulence. Junin vaccine protected all rhesus monkeys challenged with Machupo virus, in the absence of cross-reactive N-Ab. To explain cross protection, cell-mediated immunity to arenaviruses is being assessed in Junin vaccine recipients and in animal models. The importance of mucosal immunity and its biologic basis was defined in mouse models for Rift Valley fever viral infection and immunization. Morphometric variants of *C. burnetii* were characterized by immune electron microscopy to relate antigenic and virulence determinants. In anthrax studies, the previously described antigens EA-1 and EA-2 were evaluated for their abilities to protect guinea pigs. Additional animals with high titers to surface polysaccharides were not protected. Further studies with a *B. subtilis* recombinant strain containing the PA gene confirmed safety and efficacy of this vaccine. Bionetics Laboratories continues to provide molecular biology and immunology support on a task-order, protocol-directed basis.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AD-131: Exploratory Vaccine Development Studies on Conventional Agents of Potential BW Threat

PRINCIPAL INVESTIGATOR: P. B. Jahrling, Ph.D.

ASSOCIATE INVESTIGATORS: A. O. Anderson, COL, M.D.
M. H. Crumrine, LTC, Ph.D.
A. M. Friedlander, COL, M.D.
R. H. Kenyon, Ph.D.
H. W. Lupton, COL, Ph.D., D.V.M.

BACKGROUND

Basic studies on the pathophysiology and immunology of conventional agents of potential BW threat are essential to the systematic development of protective vaccines. Among the arenaviruses, four are significant human pathogens. Lassa virus causes severe, often fatal Lassa fever in tens of thousands of patients in West Africa annually. Junin and Machupo viruses are associated with Argentine and Bolivian hemorrhagic fevers, respectively, and have the documented potential to cause devastating outbreaks in South America. Lymphocytic choriomeningitis virus (LCMV) has a world-wide distribution and has caused significant morbidity in human populations naturally exposed to aerosols from infected animals. All these viruses are highly infectious via the aerosol route and have the demonstrated potential to cause explosive outbreaks under artificial conditions. The aerosol potentials for anthrax and Q-fever (caused by *Coxiella burnetii*) are also well documented. An understanding of the protective immune mechanisms, especially mucosal and cellular immune responses responsible for recovery and protection against acute disease, are prerequisite for the development of effective vaccines and therapeutic measures, and are the focal points for this research unit.

SUMMARY

Cross-protective relationships and mechanisms among Old-world and New-world arenaviruses were examined in detail, by using guinea pig and monkey models. Naturally attenuated Lassa viral strains freshly isolated from Liberian patients effectively immunized animals against prototype Lassa viral challenge, but not LCMV. Most attenuated LCMV strains protected against Lassa viral challenge, but one, LCMV strain 13-065, potentiated subsequent challenge with most Lassa strains in guinea pigs. Junin vaccine, Candid #1, protected guinea pigs and monkeys against heterologous challenge with Machupo virus in the absence of cross-neutralizing antibody. The pathophysiology, hematological, and coagulation abnormalities of Machupo

infection were defined, providing insight into arenaviral hemorrhagic fevers in general. Cell-mediated immunity to arenaviruses was assessed primarily with Junin vaccine recipients and animal models. An adaptation of the lymphocyte transformation (LT) test with limiting dilutions to estimate the numbers of committed cells was demonstrably more sensitive and precise than other measures of cell-mediated immunity in vaccine recipients for at least 1 year. This assay is being adapted to Machupo, Lassa, and Crimean-Congo hemorrhagic fever (CCHF) viruses.

The search to develop adult rodent and primate models for CCHF was expanded to include 19 inbred mouse strains, guinea pigs, and hamsters. One CCHF strain produced viremia in rhesus monkeys, and is being passed to increase virulence. The importance of mucosal immunity and its biological basis was defined in mouse models for Rift Valley fever (RVF) viral infection and immunization. The biological basis for the ability of intraperitoneal priming, but not i.v., s.c., or intraduodenal priming, to elicit respiratory mucosal immunity and systemic immunity was discovered.

Mucosal pathogenesis studies are defining the receptors responsible for neurotropism spread of Venezuelan equine encephalomyelitis (VEE) virus. Morphometric variants of *C. burnetii* were characterized by immune electron microscopy to relate antigenic and virulence determinations. This information will be useful in our investigation of the role of these surface antigens in attachment and penetration into host cells. In anthrax studies, the previously described antigens EA-1 and EA-2 were evaluated for their abilities to protect guinea pigs. Additional animals with high titers to surface polysaccharides were not protected. Further studies with a *B. subtilis* recombinant strain containing the protective antigen (PA) gene confirmed safety and efficacy of this vaccine. Bionetics Laboratories continued to provide molecular biology and immunology support on a task-order, protocol-directed basis.

MGLA Exploratory Development of Killed and Attenuated Lassa Virus Vaccines

PRINCIPAL INVESTIGATOR: P. B. Jahrling, Ph.D.

Cross-protective relationships and mechanisms among Old-world and New-world arenaviruses were examined in detail. Of 58 fresh strains of Lassa virus (identified by neutralization), isolated from Liberian patients, 17 were avirulent for strain 13 guinea pigs, and elicited uniform protection against Lassa strain Josiah. Nine of the 17 "naturally attenuated" strains came from adult patients with a mild disease course; five more came from adult patients with a mild disease course; five more came from severely ill pregnant women, and three from infants. Unlike related Old-world arenaviruses (mopeia and Mobala), these naturally attenuated variants were indistinguishable from virulent Liberian Lassa by cross-neutralization testing. None protected against LCMV (WE), but LCMV (ARM) protected against the 41 virulent Lassa strains in the absence of cross-neutralizing antibody.

Immunological potentiation or enhancement of arenaviral infection was demonstrated for the first time. Guinea pigs immunized with LCMV strain 13-065, from Argentina, and challenged with Lassa strain Josiah developed higher viremias and died more quickly (11 vs 17 days) than non-immunized controls. This observation may preclude the use of LCMV-vaccinia constructs for immunization against Lassa. The mechanism is under investigation. No cross-protection or enhancement was detected with guinea pigs immunized with attenuated Old-world strains (e.g., LCMV, Mopeia, Ippy, Mobala, or Liberian strains) and virulent New world viruses (Junin and Machupo). Conversely, Junin vaccine (Candid #1) did not protect or enhance Lassa viral challenge, although it did protect against Machupo challenge in guinea pigs and monkeys.

Successful immunization against Machupo challenge by Junin vaccine afforded an opportunity to define more precisely the pathogenesis of virulent Machupo infection with respect to hematological and coagulation abnormalities, clinical chemistries, cellular immunology, and pathophysiologic events. Immunization with Candid #1 protected 12 of 12 rhesus monkeys against Machupo virus, while infection of control monkeys caused all 12 to die, 11 acutely (days 17 to 24), and 1 on day 42. Survival times did not correlate with age or weight. All control, challenged monkeys developed erythematous facial and abdominal rashes, hemorrhagic nasal discharges, and bloody diarrhea. All became anorectic and dehydrated, Viremia titers peaked at 5-6 log PFU viruses per ml 17 days after inoculation, coinciding temporally with modest peak elevations in serum enzymes including ALT, AST, LDH, GGT, HBDH, CK, and CKMB. Mean serum creatinine and BUN levels did not vary significantly. There was little histologic evidence for disseminated intravascular coagulation (DIC); yet, coagulation testing revealed a pattern suggestive of DIC, including prolonged prothrombin and partial prothromboplastin times, decreased coagulation factor levels, increased FDP levels, and decreased platelet counts. Platelet functions were also impaired both in terms of aggregation and granule release. Infectious viral burdens were highest in adrenal, liver, kidney, and spleen, corresponding with the distribution of Machupo antigens detected by immunofluorescence. In contrast, clinical signs, serum chemistries, coagulation parameters, and platelet function tests all remained normal for Candid #1-immunized, Machupo-challenged monkeys. These studies provide insight into critical virus-induced events characterizing arenaviral hemorrhagic fever in primates. Analogous studies of Lassa viral infection in control and immunized monkeys are in progress.

MGLD Characteristics of Virulent and Attenuated Junin Virus Infections

PRINCIPAL INVESTIGATOR: R. H. Kenyon, Ph.D.

We use lymphocyte transformation (LT) as a test for cell-mediated immunity to Junin virus. We added a limiting dilution procedure to this assay to estimate the number of cells committed to recognition of the Junin viral antigens. By this procedure, we detected approximately 80,000 committed cells at 4 months post vaccination. This procedure is probably a more accurate measure of cell-mediated immunity than the more commonly used stimulation index. With several recent vaccinees, we are following these responsive cells over 1 year. We will compare this measure of immunity with measures of humoral immunity, and will attempt to relate these measures to protection. We also have attempted to measure cell-mediated immunity by in vitro determinations of interleukin-2 and interleukin-2R by ELISA techniques. Although we can detect both in response to specific antigen, neither appears sufficiently sensitive enough to be of value.

The human LT assay for detection of immunity to Junin virus also works (with modifications) for guinea pigs. For LT assays with monkeys, we found that adherent cells must be removed and homologous serum added to the system for the assay to function well. Present studies are devoted to determining ways to increase the sensitivity and versatility of this assay to determine cell-mediated immunity to CCHF, Machupo, and Lassa viruses, as well as to Junin virus.

There presently is no satisfactory adult animal model for CCHF. We attempted, without success, to cause disease in any of 19 different, inbred strains of adult mice and 13 different strains of weanling mice inoculated with the virus. We also were unable to cause disease in adult inbred or outbred guinea pigs or adult inbred hamsters. However, cyclophosphamide immunosuppression of guinea pigs followed by infection with the virus was associated with weight loss in all animals and death in 20%. Virus was found in lymph nodes and adrenal glands, but there was not significant pathology. We also attempted to produce disease in rhesus and African green monkeys. One CCHF strain caused viremia and anorexia in rhesus. Present studies are focused on the effect of passage history on virulence of the infecting inoculum. Suckling mice inoculated i.p. or intracerebrally are our only consistently lethal model for CCHF. We studied the pathogenesis of the virus in this model, and, contrary to Russian reports, found high titers of virus in liver early in infection, and, later, in the brain. Examination of tissues for pathology showed the greatest damage in hematopoietic centers of the spleen and liver, and in the brain and adrenal glands.

MGDA Exploratory Research for the Protection Against Anthrax

PRINCIPAL INVESTIGATOR: M. H. Crumrine, LTC, Ph.D.

ASSOCIATE INVESTIGATORS: J. W. Ezzell, Ph.D.
B. E. Ivins, Ph.D.

Dr. John Ezzell has continued studies to define the role of various antigens in protection against anthrax infections and their usefulness in rapid diagnosis. The previously described antigens (EA-1 and EA-2) were evaluated for their ability to protect guinea pigs against challenge with virulent *Bacillus anthracis*. Additionally, conjugated surface polysaccharide from vegetative anthrax cells was evaluated for it's ability to protect against anthrax challenge. Guinea pigs with high post-immunization antibody titers to these antigens were not protected from challenge by virulent anthrax. Further studies using monoclonal antibodies to EA-1 and the polysaccharide suggest that these antigens are on the surface of non-encapsulated organisms and are probably masked during infection with virulent encapsulated organisms. For this reason, antibodies to these antigens probably are not involved in protection. The polysaccharide antigens appear to be useful in the rapid identification of *B. anthracis* vegetative cells but not spores.

In other studies, red blood cells of fatally infected guinea pigs lysed near the time of death with high levels of hemoglobin released into the serum and urine. When serum cholesterol levels were raised to greater than 100-120 mg/dl by using a special diet, no hemolysis occurred. The significance of this observation in the pathogenesis of anthrax is being investigated.

Dr. Bruce Ivins, in collaboration with Mr. Steve Little, has continued the evaluation of recombinant organisms and their ability to produce protective immunity to anthrax infections.

Further studies with a *B. subtilis* recombinant strain containing the PA gene have confirmed the previous protection studies and the safety of this live-vaccine candidate in guinea pigs.

Dose-range experiments to determine the optimum dose and schedule for this candidate vaccine show that the number of organisms required to produce immunity exceeds that for the veterinary vaccine. An immunization schedule similar to the veterinary vaccine produces similar results. Other studies of proteolytic cleavage fragments of the PA protein have shown that the protective epitope is contained in the intact molecule and on a 65-kd trypsin fragment of PA. The 20-kd trypsin fragment of the PA molecule does not contain the protective epitope. The PA gene has been recloned into a nonproteolytic strain of *B. subtilis*. The recombinant clones will be tested for the amount of PA produced and the amount of proteolytic nicking that occurs.

Dimethylglycine was evaluated as a potentiator of the immune response to anthrax vaccination. This compound may augment protection against anthrax infection in animals given the licensed human vaccine.

MGBI *Coxiella burnetii* Genetic and Cellular Aspects

PRINCIPAL INVESTIGATOR: A. M. Friedlander, COL, M.D.

ASSOCIATE INVESTIGATOR: T. McCaul, Ph.D.

A number of morphologically-variant forms of *C. burnetii* have been described, which include a large cell variant (LCV), a small cell variant (SCV), and an endospore-like differentiation with the LCV. Results of immunoelectron microscopy have been used to understand the antigenic relationship between these variants. This information will be useful in our investigation of the role of these surface antigens in attachment and penetration into host cells. Antibodies to lipopolysaccharide (LPS) I revealed that epitopes for LPS exist on the SCV, with little or no LPS I detectable on either the LCV or the endospore. The antigens detected by polyclonal antibody to the chloroform-methanol residue (CMR) exist on both the LCV and SCV but not on the endospore. The LCV was unique in being the only variant to react with antibody to the 29.5-kd major surface protein. It appears that the LPS and CMR antigens present on SCV and LCV and the 29.5-kd protein present on the LCV are all undetectable on the endospore. These ongoing studies will be useful in characterizing the relationships among the variants of *C. burnetii*.

MGBA Mucosal Immunity: Response to Aerosol Challenge with Toxins, Microbes, and other Biologic Agents; Immunomodulators and Mucosal Immunity

PRINCIPAL INVESTIGATOR: A. O. Anderson, COL, M.D.

We discovered the biological basis for the ability of intraperitoneal priming to elicit respiratory mucosal immunity and systemic immunity. In addition to the known route of lymphatic drainage of the peritoneal cavity via diaphragmatic lymphatics to anterior mammary and peritoneal cavity and perithymic lymph nodes to the blood, there was also a significant traffic of peritoneal macrophages ("antigen-laden" cells) across diagphragmatic lymphatics, through the pleural space, and into the lung interstitium and/or bronchus-associated lymphatic tissue. This route of mononuclear cell migration to the lung has not been previously described. Monoclonal antibodies (MAB) specific for the G-1 and G-2 polypeptides of RVF virus were completely protective against aerosol challenge when combined together but resulted in heavy mortalities if used separately. Monoclone-G2 treated mice died of encephalitis· MAB-G1-treated mice died of hepatitis. We discovered a mouse model of RVF viral vaccine-induced anaphylaxis in the C_3H/HeJ mouse; C_3/OuJ; BDF_1, and Swiss mice were resistant. The mechanism of this susceptibility is being investigated by molecular biology techniques with cDNA probes for IgE, IgA, and eFcR. A program in molecular biology of mucosal immunology was initiated by Maryann Vaney. Mucosal pathogenesis studies are defining the receptors responsible for the neurotropism spread of VEE.

MGIL Bionetics Research, Inc. Contract Research Support (formerly Meloy Laboratories)

PRINCIPAL INVESTIGATOR: H. W. Lupton, COL, Ph.D., D.V.M.

During the past year, Bionetics Laboratory, Inc., has provided collaborative molecular biology and immunology research support for in-house investigators on a task-order, protocol-directed basis. Activities included molecular cloning, DNA sequencing, plasmid DNA preparation, peptide synthesis, antigen conjugation, hybridoma development, and monoclonal antibody production.

Significant molecular biology support has been provided through amino acid sequences of selected antigenic regions of RVF virus and multiple toxins; DNA sequence analyses relating to cloned segments of Q fever, botulinum toxin, a proteinaceous toxin, alphaviral glycoproteins E1 and E2, myotoxin, EF gene of *B. anthracis*, and a unique primer; expression of genetic material in Cos cells; conduct of genetic library screening; library construction in lamba ORF-8 cloning vector; mRNA isolation from snake glands; and construction of specialized vectors for genetic engineering of vaccinia virus. Polyclonal antibodies were produced in rabbits against the antiviral drug ribavirin. Work continued in an effort to develop hybridomas secreting monoclonal antibodies specific for 10 separate antigens.

PRESENTATIONS

1. **Anderson, A. O.** 1987. Direct transdiaphragmatic traffic of peritoneal macrophages to the lung. Presented at the Ninth International Conference on Lymphatic Tissues and Germinal Centres in Immune Reactions, Oslo, Norway, August.

2. **Anderson, A. O.** 1987. Mucosal priming alters pathogenesis of Rift Valley fever. Presented at the Ninth International Conference on Lymphatic Tissues and Germinal Centres in Immune Reactions, Oslo, Norway, August.

3. **Kenyon, R. H., and C. J. Peters.** 1987. Actions of complement on Junin virus. Presented at the Symposium on Hemostatic Impairment in Viral Hemorrhagic Fevers, Leesburg, VA, May.

4. **Kenyon, R. H., and C. J. Peters.** 1987. Expression of Junin antigens on the surface of infected Vero cells. Presented at the Symposium on Hemostatic Impairment in Viral Hemorrhagic Fevers, Leesburg, VA, May.

5. **Kenyon, R. H., and C. J. Peters.** 1986. Immune response of guinea pigs to Junin virus (JV). Presented at the Second Annual Congress of Virology, Cordoba, Argentina, October.

6. **Kenyon, R. H., and C. J. Peters.** 1986. Actions of complement on Junin virus (JV) and on JV-infected cells. Presented at the Second Annual Congress of Virology, Cordoba, Argentina, October.

7. **Kenyon, R. H., D. Pifat, P. Jahrling, R. Condie, J. Maiztegui, D. Brantley, and C. J. Peters.** 1987. Use of heterologous antibody and F(ab')2 portions for protecting guinea pigs infected with Junin virus (JV). Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

PUBLICATIONS

1. **Ezzell, J. W., Jr.** 1986. *Bacillus anthracis*, pp. 21 - 25. *In* C. L. Gyles and C. O. Thoen (ed.), Pathogenesis of bacterial infections in animals. Iowa State University Press, Ames.

2. **Ezzell, J. W., Jr.** 1987. Sporicidal activity of Alcide Exspor and sodium hypochlorite on *Bacillus anthracis* spores. Submitted to *Appl. Environ. Microbiol.*

3. **Ivins, B. E.** 1987. The search for a new-generation human anthrax vaccine. Submitted to *Clin. Immunol. Newslett.*

4. **Kenyon, R. H., D. E. Green, J. I. Maiztegui, and C. J. Peters.** 1987. Viral strain-dependent differences in experimental Argentine hemorrhagic fever (Junin virus) infection of guinea pigs. Intervirology (In Press).

5. **Kenyon, R. H., and C. J. Peters.** 1987. Actions of complement on Junin virus. Submitted to *Rev. Infect. Dis.*

6. **Peters, C. J., P. B. Jahrling, C. T. Liu, R. H. Kenyon, K. T. McKee, Jr, and J. G. Barrera Oro.** 1987. Experimental studies of arenaviral hemorrhagic fevers. *Curr. Topics Microbiol Immunol.* 134:5-63.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA302646	30 Oct 87	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AE	134
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory Vaccine Development Studies on Toxins of Potential BW Threat

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT
a. DATE EFFECTIVE | EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
87	1.0	96
88		

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Anderson, A O
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Meade, B
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Vaccines (U) Cardiotoxin; (U) Lab Animals; (U) Rabbits; (U) Cobrotoxin; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To define the toxicity potential of natural toxins of BW importance and to develop vaccines, leading to protective immunity, that protect deployed military personnel from intoxication by natural toxins.

24. (U) Develop immunogical systems to detect natural toxins in biological systems and protective vaccines either by chemical inactivation (toxoiding) or by synthesis of non-toxic peptides that mimic antigenic determinants on the toxin molecule.

25. (U) 8610 - 8709 We demonstrated in a mouse model that active immunization with formalin toxoid of a cobra post-synaptic neurotoxin provided significant levels of protection to lethal toxin challenge. We determined that immunization of guinea pigs with a specific carrier-conjugated synthetic peptide induced production of cobrotoxin-neutralizing antibodies. We developed immunological detection systems for cobrotoxin and ricin employing polyclonal antibodies. Polyclonal antibodies to conotoxin GI were prepared.

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PROJECT NO. 3M162770A871:	Medical Defense Against Biological Warfare
WORK UNIT NO. 871-AE-134:	Exploratory Vaccine Development Studies on Toxins of Potential BW Threat
PRINCIPAL INVESTIGATOR:	A. O. Anderson, LTC, M.D.
ASSOCIATE INVESTIGATOR:	B. D. Meade, Ph.D.

BACKGROUND

The goal of our research is to develop immunological systems that detect natural toxins in biological samples, and vaccines that protect from intoxication by these toxins. The toxins currently under investigation are ricin, a protein synthesis-inhibiting toxin from the castor bean, *Conus geographus*; and the toxins from elapid snakes, specifically the cardiotoxins, the phospholipases A_2, and the post-synaptic neurotoxins. We are taking two complementary approaches to vaccine development. The first is the traditional approach of chemical inactivation, or toxoiding. The second is the chemical synthesis of non-toxic peptides that mimic protective, antigenic determinants on the toxin molecule.

SUMMARY

We studied the feasibility of using active immunization to protect mice from post-synaptic neurotoxemias with a toxoid immunogen, prepared by formalin-inactivation of cobrotoxin, the post-synaptic neurotoxin from *Naja naja atra*. The mean lethal dose of cobrotoxin in immunized mice was about 10 times higher than in control mice. In addition, we demonstrated that mice with high levels of IgG antibody to cobrotoxin, as measured by solid-phase immunoassay, were more likely to survive toxin challenge. Thus, active immunization is potentially an effective countermeasure to these toxins, although it appears that high antibody levels are required. Although the formalin toxoid is immunogenic, the stability of the toxoid must be improved because the toxoid reverts to active toxin during storage. The studies will also be extended to determine the duration of immunity; the optimal dose, route, and schedule of immunization; and the degree of protection provided against other, related toxins.

To determine if synthetic peptides have potential as protective immunogens, we immunized guinea pigs with either free or carrier-conjugated peptides. Peptides for test were selected by applying epitope-predicting algorithms to known structures of the post-synaptic neurotoxins. Two of the conjugated peptides induced antibodies that recognized native cobrotoxin in a solid-phase immunoassay, and one of the peptides led to the

production of toxin-neutralizing antibodies. This effective peptide will be tested further in mouse-active protection studies; additional synthetic peptides will be tested in guinea pigs.

We have developed immunological detection systems, employing rabbit and goat polyclonal sera, for cobrotoxin and ricin. Rabbit and goat polyclonal antisera have been prepared to conotoxin GI, a 13-amino acid peptide neurotoxin. These antisera will be used to develop a detection assay for ricin and cobrotoxin.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA302650	30 Oct 87	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AF	135
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code)
(U) Exploratory Immunotherapy Studies on Toxins of Potential BW Threat

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	7.0	2071
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathophysiology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Wannemacher, R W, JR
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7181
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Siegel, L S
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Pace, J G

22. KEYWORDS (Precede EACH with Security Classification Code) (U)Military Medicine;(U)Low Molecular Weight; RA I Toxins;(U)T-2 Toxin;(U) Saxitoxin;(U) Rapid Detection;(U) Lab Animals;(U) Mice;(U) Therapy

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To develop the ability to detect toxins in biological samples; to study the mechanisms of action; and to develop and evaluate biologics and selected compounds for prevention and treatment of diseases induced by toxins of military importance.

24 (U) Develop new technology for fermenter-type production of sufficient toxin for isolation, purification, alteration, and detection studies; Use HPLC and mass spectrometry (MS) plus immunology to detect agents and their metabolites in biological fluids. Immunogenicity of various antigens will be utilized to make experimental toxoids/vaccines.

25. (U) 8610 - 8709 By aerosol or parenteral routes T-2 toxin is rapidly metabolized with the major urinary excretory products being 3'OH HT-2 and T-2 tetraol. In contrast to this toxin, the macrocyclic trichothecenes appeared to be much more slowly metabolized and excreted, which may explain their higher toxicities. Models have been developed to study mechanisms of action and therapeutic approaches to treatment of microcystin and brevetoxin. A 30-min exposure to 0.1% sodium hypochlorite will inactivate saxitoxin, tetrodotoxin, microcystin, and brevetoxin. These latter toxins are all slowly absorbed through the skin of guinea pigs.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AF-135: Exploratory Immunotherapy Studies on Toxins of Potential BW Threat

PRINCIPAL INVESTIGATOR: R. W. Wannemacher, Jr., Ph.D.

ASSOCIATE INVESTIGATORS: D. L. Bunner, COL, M.D.
C. B. Templeton, CPT, D.V.M., Ph.D.
D. A. Creasia, Ph.D.
J. G. Pace, Ph.D.
L. S. Siegel, Ph.D.
E. C. Hauer, M.S.
W. L. Thompson, M.S.

BACKGROUND

A number of low molecular weight toxins are potential biological warfare agents. In order to develop a medical defense against these toxins, it is necessary to study their toxicity, pathophysiology, and pharmacokinetics. This information will be used to develop rational therapies and means of detection. Our earlier studies were done on a component of "yellow rain," i.e., the trichothecene mycotoxins. Work on these toxins is being phased down and the technology developed is being used to study other small-size toxins that are also potential biological defense warfare agents. These include the marine toxins (saxitoxin, tetrodotoxin, and brevetoxin), blue-green algal toxins (microcystin and anatoxin-a), and coral toxins (palytoxin).

SUMMARY

Monkeys were injected i.v. with [^{3}H]-T-2 mycotoxin, most of the radioactivity appeared in the urine and feces at the end of 5 days. In urine, the major metabolites were 3'OH HT-2 and T-2 tetraol. In guinea pigs exposed to [^{3}H]-T-2 toxin by the aerosol route, 93% of the radiolabel was metabolized and excreted in the urine and feces. The major urinary metabolites were T-2 tetraol, a more-polar unknown, and glucuronide conjugates. In the urine of monkeys exposed i.v. to sublethal doses of T-2 mycotoxin, metabolites of this toxin were detected by immunoassays and mass spectral analysis. Again, 3'OH HT-2 and T-2 tetraol were the major metabolites. In contrast to the T-2 toxin, in vivo distribution and metabolism studies of [^{3}H)-verrucarin A and verrucarol in guinea pigs showed 51 and 46%, respectively, of the total radiolabel was excreted in the urine and feces by day 9. This suggests a slow rate of metabolism and excretion of the macrocyclic trichothecenes, which may explain the higher toxicity observed with some of these compounds.

At low doses in the monkey, T-2 mycotoxin caused emesis and marked reduction in food intake. In vitro T-2 had multiple effects on cell membrane mechanisms. In vivo rat studies indicated that T-2 and T-2 tetraol were equally effective in inhibiting protein synthesis and acute toxicity. This is in contrast to the *in* vitro data which suggest that T-2 mycotoxin is 1000 times more potent than tetraol in inhibiting protein synthesis. These data all emphasize the multi-organs effects of T-2 toxin and its metabolites. They also raise the question as to whether inhibition of protein synthesis is the major mechanism of action of this group of toxins.

The brevetoxins produced some severe cardiorespiratory alterations as well as some centrally mediated effects. About 55% of this toxin is extracted in the in vitro liver perfusion studies. This suggests a lower rate of hepatic metabolism which would leave more toxin available for effects on other peripheral and central tissues. Brevetoxin is poorly absorbed through skin.

Intravenous injection of microcystin results in marked liver lesions. These ultrastructural changes correlated with biochemical changes, such as increased serum-liver specific enzymes, decreased P:O ratios and destabilization of desmosomal proteins. These effects were not observed when microcystin was added to isolated mitochondrial preparations or hepatocytes. This toxin, when added to hepatocytes, did cause a disruption of the attachment matrix, followed by loss of cytoplasmic enzymes, but not membrane-associated enzymes. This effect was blocked by cholic acid, suggesting bile acids may protect. Microcystin inhibited the uptake of cholic and taurocholic acids which suggests that they share the same transport sites. When applied to the skin in dimethylsulfoxide, microcystin was not absorbed as rapidly as T-2 mycotoxin. The results of the in vivo skin toxicity of microcystin agree with the in vitro absorption rates.

Saxitoxin and tetrodotoxin were absorbed through the skin at much slower rates than T-2 mycotoxin. Brevetoxin, saxitoxin, tetrodotoxin, and microcystin were all inactivated with a 30-min exposure to 0.1% sodium hypochlorite. This is a 50-fold lower concentration than that required to inactivate T-2 mycotoxin. Tetrodotoxin was inactivated by high and low pH's while microcystin was relatively stable.

MGHB

PRINCIPAL INVESTIGATOR: R. W. Wannemacher, Jr., Ph.D.

ASSOCIATE INVESTIGATORS: D. L. Bunner, COL, M.D.
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D. A. Creasia, Ph.D.
E. C. Hauer, M.S.

When [^{3}H]-T-2 mycotoxin was injected i.v. into monkeys, 95% of the radioactivity appeared in the urine and feces by day 5. At a dose of 14 µg of T-2 per kg, two out of six monkeys developed emesis, and all reduced their food intake by 50%. At doses of 0.32 and 0.5 mg/kg, T-2 mycotoxin produced emesis and decreases in food intake but had little effect on platelet function. T-2 metabolites were detected in the urine of the latter two groups of monkeys by immunoassay and mass spectrum analysis. Sensitivity but not specificity appeared to be equivalent for the two techniques.

Previous work with T-2 mycotoxin aerosols showed that inhalation of T-2 was at least 10 times more toxic than T-2 administered i.v. to the rat and mouse. This work has now been extended to the guinea pig in which inhalation of T-2 aerosol was found to be twice as toxic than by the intravenous route. Rats exposed to T-2 toxin via aerosol or i.v. challenge did develop metabolic acidosis but had normal blood pCO_2 and elevated pO_2. T-2 metabolites can produce significant hemoglobin abnormalities, which, in part, may be the reason for the marked elevation in pO_2 seen during toxemia.

A 5-min pre-exposure of L-6 cells to T-2 mycotoxin (0.04 ng/ml) resulted in a significant reduction in protein synthesis and decreased uptake of glucose and calcium. A concentration of 4 ngof T-2 toxin per ml was required to cause a loss of cellular LDH. Thus, we concluded that T-2 mycotoxin had multiple effects on cell membrane transport and, at high concentrations, impaired cellular membrane integrity. These effects occurred rapidly, within 10 min of exposure to T-2.

A new class of macrocyclic trichothecene mycotoxins (myrotoxin B) was found to be 100 times more toxic than T-2 mycotoxin in the mouse bioassay, and 20 times more potent as a skin irritant. Thus, myrotoxin B appears to have the highest toxicity of the trichothecene mycotoxins evaluated to date.

Infusion of brevetoxin (PbTx-2) into the conscious rat, resulted in a dose-related decrease in respiratory rate, core and peripheral temperatures, and heart rate but had no effect on arterial blood pressure or blood gases. An antibody against PbTx-2 offered some protection against the respiratory effects of this toxin.

Saxitoxin, tetrodotoxin, and microcystin are absorbed at a much slower rate through the skin in the presence of dimethylsulfoxide (DMSO) than is T-2 mycotoxin. Brevetoxin is poorly (less than 1%) absorbed through the skin in the presence of DMSO.

All of the newer toxins are inactivated by 1% sodium hypochlorite. Tetrodotoxin was rapidly inactivated at high or low pH, while microcystin was relatively stable.

A number of computer programs have been adapted for use with various analytical instruments and personal computers. They will be valuable tools in literature surveys, data acquisition and storage, visual aid production, and data analysis.

MGIIG

PRINCIPAL INVESTIGATOR: J. G. Pace, Ph.D.

ASSOCIATE INVESTIGATORS: N. A. Robinson, CPT. Ph.D
W. L. Thompson, M.S.

T-2 toxin had no effect on liver, heart, or blood adenine nucleotide concentrations. In vivo rat studies showed T-2, tetraol, and several protein synthesis inhibitors cause rapid and prolonged inhibition of protein synthesis, leading to death.

In vivo distribution and metabolism studies of radiolabeled verrucarin A and verrucarol in guinea pigs showed 51 and 46%, respectively, of the total radiolabel was excreted in urine and feces by day 9. Three urinary unknown metabolites have been isolated and are being identified by mass spectrometry.

Fate and distribution of T-2 toxin, given by aerosol, was studied in guinea pigs. The total deposition of T-2 was 27 $\pm$ 4%. By 14 days, 92.9% of the radiolabel was metabolized and excreted in the urine and feces. The major urinary metabolites were T-2 tetraol, a more-polar unknown, and glucuronide conjugates.

Fate and distribution of T-2 in the primate model showed the toxin was metabolized and eliminated mainly as 3'-OH T-2 and tetraol. We have determined the pharmacokinetic parameters.

In vitro - de-epoxy T-2 and tetraol were one to two logs less toxic than the parent compounds in the protein synthesis inhibition assay.

In vitro liver perfusion studies showed that brevetoxin (PbTx-3) was extracted (E=0.55) by liver, metabolized to five polar unknowns, and eliminated via bile. In vitro and in vivo clearance rates were in good agreement, suggesting the perfusion model can be used to predict pharmacokinetic parameters for PbTx-3.

Microcystin, added to attached hepatocytes, caused a rapid disruption of the attachment matrix, followed by loss of cytoplasmic enzymes, but not membrane-associated enzymes. This effect was blocked by cholic acid, suggesting bile acids may protect. Microcystin inhibited the uptake of cholic and taurocholic acids, which is consistent with the hypothesis that the toxin and bile acids share the same transport system.

Microcystin had no apparent effect on a number of other established tissue culture cell lines.

In vitro microcystin had no effect on mitochondrial swelling or respiration which suggests that the toxin is not an ionophore. In vivo rat studies showed microcystin caused liver enlargement, cloudy swelling of mitochondria, and rough endoplasmic reticulum, endoplasmic reticular whorls, and loss of desmosomes. These ultrastructural changes correlated with biochemical changes, such as increased serum liver-specific enzymes, decreased P:O rations, and destabilization of desmosomal proteins.

MGGC

PRINCIPAL INVESTIGATOR: L. S. Siegel, Ph.D.

No additional studies have been done on the effects of treatment with a candidate drug, 3,4-diaminopyridine, for the treatment of botulinum intoxication. The earlier work has been submitted for publication.

PRESENTATIONS

1. **Mereish, K. A., R. W. Wannemacher, Jr., D. L. Bunner, and T. Krishnamurthy.** 1987. Composition of *Microcystis aeruginosa* strain 7820 toxin. Presented at the Conference on Natural Toxins from Aquatic and Marine Environments, Woods Hole, MA, August.

2. **Miura, G. A., K. A. Bostian, T. W. Geisbert, J. D. White, and J. G. Pace.** 1987. In vivo and in vitro effects of microcystin -LR on rat liver. Presented at the Conference on Natural Toxins from Aquatic and Marine Environments, Woods Hole, MA, August.

3. **Robinson, N. A., W. L. Thompson, and J. G. Pace.** 1987. Effect of microcystin-LR on bile acid uptake in isolated rat hepatocytes. Presented at the Conference on Natural Toxins from Aquatic and Marine Environments, Woods Hole, MA, August.

4. **Templeton, C. B., and D. A. Creasia.** 1987. Changes in arterial blood gases, temperature and plasma lactate concentrations in rats exposed to intravenous or aerosol T-2 mycotoxin. Presented at the Tenth Annual Conference on Shock, Montreal, Canada, June.

5. **Templeton, C. B., and M. A. Poli.** 1987. Cardiorespiratory effects of brevetoxin PbTx-2 in conscious rats. Presented at the Annual Meeting of the Society for Toxinology, Singapore, China, June.

6. **Thompson, W. L., M. B. Allen, and K. Bostian.** 1987. The effects of microcystin on monolayers of primary rat hepatocytes. Presented at the Annual Meeting of the Society for Toxinology, Singapore, China, June.

7. **Wannemacher, R. W., Jr., D. L. Bunner, and R. E. Dinterman.** 1987. Comparison of the toxicity and absorption of algal toxins and mycotoxins after dermal exposure in guinea pigs. Presented at the Annual Meeting of the Society for Toxinology, Singapore, China, June.

8. **Wannemacher, R. W., Jr., D. L. Bunner, K. A. Mereish, H. B. Hines, and R. E. Dinterman.** 1987. Biological and chemical stability of several natural toxins from aquatic and marine environments. Presented at the Conference on Natural Toxins from Aquatic and Marine Environments, Woods Hole, MA, August.

9. **Wannemacher, R. W., Jr., R. E. Dinterman, W. L. Thompson, and B. B. Jarvis.** 1987. Toxicological studies on a new class of macrocyclic trichothecenes. Presented at the Annual Meeting of the American Society for Toxicology, Washington, DC, March.

PUBLICATIONS

1. **Bunner, D. L., and E. R. Morris.** 1987. Alteration of multiple cell membrane functions in L-6 myoblasts by T-2 toxin: an important mechanism of action. Submitted to *Toxicol. Appl. Pharmacol.*

2. **Bunner, D. L., and E. R. Morris.** 1987. Cell membrane effects of T-2 mycotoxin in L-6 myoblasts, pp. ----. *In* --------- (ed.), Proceedings of the International Society for Toxinology. (In Press).

3. **Creasia, D. A., and R. J. Lambert.** 1987. Acute respiratory tract toxicity of the trichothecene mycotoxin, T-2 toxin, pp. ----. *In* V. R. Beasly (ed.), Trichothecene mycotoxicosis: pathophysiologic effects. CRC Press, Boca Raton (In Press).

4. **Creasia, D. A., J. D. Thurman, L. J. Jones, III, M. L. Nealley, C. G. York, R. W. Wannemacher, Jr., and D. L. Bunner.** 1987. Acute inhalation toxicity of T-2 mycotoxin in mice. *Fundam. Appl. Toxicol.* 8:230-235.

5. **Davio, S. R., and D. A. Creasia.** 1987. Passive immunization against saxitoxin administered intravenously or via the respiratory tract. Submitted to *Toxicon.*

6. **Kemppainen, B. W., R. T. Riley, J. G. Pace, F. J. Hoerr, and J. Joyave.** 1986. Evaluation of monkey skin as a model for *in vitro* percutaneous penetration and metabolism of [^{3}H]T-2 toxin in human skin. *Fundam. Appl. Toxicol.* 7:367-375.

7. **Pace, J. G.** 1986. Metabolism and clearance of T-2 mycotoxin in perfused rat livers. *Fundam. Appl. Toxicol.* 7:424-433.

8. **Pace, J. G., and C. F. Matson.** 1987. Stability of T-2, HT-2, and T-2 tetraol in biological fluids. Submitted to *Appl. Environ. Microbiol.*

9. **Pace, J. G., M. R. Watts, and W. J. Canterbury.** 1987. T-2 mycotoxin inhibits mitochondrial protein synthesis. *Toxicon* (In Press).

10. **Thompson, W. L., M. B. Allen, and K. A. Bostian.** 1987. The effects of microcystin on monolayers of primary rat hepatocytes, pp. ----. *In* --------- (ed.), Proceedings of the International Society for Toxinology. (In Press).

11. **Thompson, W. J., J. G. Pace, and J. C. O'Brien.** 1987. In vitro metabolism of T-2 mycotoxin. Submitted to *Fundam. Appl. Toxicol.*

12. **Wannemacher, R. W., Jr., D. L. Bunner, and R. E. Dinterman.** 1987. Comparison of toxicity and absorption of algal toxins and mycotoxins after dermal exposure in guinea pigs, pp. ----. *In* --------- (ed.), Proceedings of the International Society for Toxinology. (In Press).

13. **Wannemacher, R. W., Jr., R. E. Dinterman, W. L. Thompson, and B. B. Jarvis.** 1987. Toxicological studies of a new class of macrocyclic trichothecene. *The Toxicologist* 7:208.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DAOG3811	30 Oct 87	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AK	139
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory Development Studies Seeking Rapid Diagnostic Procedures to Detect Agents of Biological Origin in Clinical Specimens

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
81 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	2.0	538
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR LeDuc, J W
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Knauert, F K
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Ezzell, J W

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Viral Diseases; (U) Immunology; (U) Lab Animals; (U) Rats; (U) Biotechnology; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To develop technology for rapid diagnosis and identification of BW agents in the military clinical and environmental sphere. Field diagnosis will enhance the medical protection of US military personnel.

24. (U) To develop and refine state-of-the-art nucleic acid probes and other methods for virus detection and identification.

25. (U) 8610 - 8709 Nucleic acid filter hybridization techniques were perfected to probe target nucleic acid in clinical specimens. Pretreatment of tissues with a combination of methods improved the precision of these assays, but the sensitivity did not approach that of model systems. Further studies to increase sensitivity are underway. Preliminary attempts to combine immunological detection as a pretreatment followed by a probe assay have been encouraging. Combining immune complex isolation methods with probes appears useful for detecting nucleic acid in immune complexes. A system to use RNA instead of DNA probes was developed, and comparative sensitivity studies are in progress. *In situ* hybridization techniques have been successful in detecting target nucleic acid in fresh frozen sections, but as yet not in other slide specimens.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AK-139: Exploratory Development Studies Seeking Rapid Diagnostic Procedures to Detect Agents of Biological Origin in Clinical Specimens

PRINCIPAL INVESTIGATOR: J. W. LeDuc, LTC, Ph.D.

ASSOCIATE INVESTIGATOR: F. K. Knauert, Ph.D.

BACKGROUND

Recent technological advancements have made nucleic acid hybridization an attractive alternate method for detecting viruses and other pathogens in clinical and environmental samples. We used a cloned sequence complementary to the M segment RNA of Rift Valley fever (RVF) virus to develop such an assay, and have compared its usefulness to current procedures for detecting and identifying pathogens. In the model system we developed with virus- infected Vero cells and infectious cell media, we routinely detected 0.5-1 x 10^4 PFU of virus. This standard assay can detect RVF viral RNA sequences in inactivated vaccine samples, and RVF virus in aerosolized samples. We attempted to transfer this technology to the detection of RVF virus in clinically and epidemiologically relevant samples, including human and sheep sera, tissue homogenates from aborted fetuses of RVF virus-infected sheep, and tissue homogenates of infected mosquitoes. We found that while we were able to successfully detect RVF viral RNA, we could not achieve the overall sensitivity realized in our model system. The primary problem in these samples is the presence of excessive amounts of cellular proteins and nucleic acids. This material nonspecifically adsorbs to the nitrocellulose filters, competes with viral RNA for limited binding sites, clogs the filter, and increases the nonspecific binding of the DNA probe. We are modifying the standard procedure to eliminate these problems and to increase the sensitivity and specificity of this assay.

SUMMARY

Clinical samples from various tissues interfere with nucleic acid filter hybridization techniques and reduce the sensitivity of the assay. Pretreatment of tissues by a combination of chemical, biochemical, and physical methods improved the precision of these assays, but the sensitivity still does not approach that of model systems. Because no combination of pretreatments has universal application, we studied a number of alternate methods for removing interfering contaminants. One strategy is an adaptation of the "capture" principal employed in ELISA procedures. Immunologically captured viral material was eluted from the solid phase so that it could be directly immobilized onto nitrocellulose, then subjected to the standard hybridization

procedure. We were able to detect RNA from samples prepared in this manner with sensitivity comparable to the direct filtration method. This same approach works for capturing immune complexes (with C1Q), then probing for target nucleic acid. We also developed methods to exploit the reported increased sensitivity of RNA probes. We constructed a library of clones in RNA transcription vectors, and comparative sensitivity testing with DNA probes is in progress. A second use of these vectors is to determine if we can use different RNA segments to develop probes that can distinguish different geographical isolates of RVF virus. Preliminary results indicate we can distinguish between two geographically distinct RVF viral strains. Since we can foresee a need to detect antigen in histological specimens, we developed *in situ* hybridization techniques and successfully used them to detect target nucleic acid in fresh frozen, infected mouse liver sections. Ongoing studies hope to extend these methods to probe for target RNA in other histological sections.

PRESENTATIONS

1. **Knauert, F. K., J. C. Morrill, and M. J. Turell.** 1985. A nucleic acid hybridization assay for detecting Rift Valley fever virus. Presented at the 34th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Miami, FL, November.

2. **Knauert, F. K.** 1986. Use of a DNA probe to detect Rift Valley fever virus RNA in epidemiologically relevant specimens. Presented at the The American Society for Virology Annual Meeting, Santa Barbara, CA, June.

3. **Knauert, F. K., A. D. King, and B. Kelly.** 1986. An *in situ* hybridization assay for detecting Rift Valley fever virus (RVFV) in mouse liver sections. Presented at the 35th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Denver, CO, December.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA0G3815	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AH	146
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory Development Studies Seeking Generic Medical Defensive Countermeasures Against Agents of Biological Origin

12. SUBJECT AREAS 1503 Defense; 0605 Clinical Medicine; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
81 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	2.0	648
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Canonico, P G
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2290
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Ussery, M A
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Pifat, D Y

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U)Antiviral Drugs; (U)Pharmacology; (U)Viral Diseases; (U)Lab Animals;(U) Mice;(U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Conduct laboratory studies to develop novel antiviral drugs by identifying potential targets for pharmacologic intervention. These drugs are needed to treat soldiers who become exposed to virulent viral agents.

24. (U) Describe mechanisms of action and metabolism of new drugs and provide analytical support for drug analysis. Perform structure-activity analyses to identify new analogs for synthesis. Apply approaches of molecular virology and cell biology to define the molecular basis of virus binding, uptake, uncoating, replication, and maturation.

25. (U) 8610 - 8709 328 Compounds were tested for in vitro antiviral activity against Rift Valley fever virus. 39 Compounds showed antiviral activity; 7 showed very strong activity. Active compounds are undergoing further development, including additional in vitro testing and in vivo evaluation in mouse models. The antiviral program against dengue viral infection has continued with the evaluation of 77 new compounds. Two of these compounds were identified to have very high activity against dengue 1 and 3 and have been resynthesized in bulk for in vivo evaluation. The development of a murine leukemia model that mimics the course of the human acquired immune deficiency syndrome (AIDS) has been undertaken in support of the Institute's AIDS program. The model will be used to screen candidate antiviral agents and immunomodulators, separately and in combination, in order to identify promising therapeutic drugs for the treatment of AIDS.

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AH-146: Exploratory Development Studies Seeking Generic Medical Defensive Countermeasures Against Agents of Biological Origin

PRINCIPAL INVESTIGATOR: P. G. Canonico, Ph.D.

ASSOCIATE INVESTIGATOR: M. A. Ussery, MAJ, Ph.D.
D. Y. Pifat, Ph.D.
M. Kende, Ph.D.
B. Gabrielsen, Ph.D.
J. T. Rankin, Jr., LTC, Ph.D.

BACKGROUND

This research program has focused on the discovery and development of agents or procedures which will be effective against a broad range of "exotic" RNA viruses. The program maintains an extensive antiviral drug screening effort. The larger portion of compounds evaluated are obtained through no -cost, technical exchange agreements with the private sector; while others are synthesized, based on known leads. Initial testing consists of in vitro assays against a battery of RNA viruses as well as rodent models. Viruses in the Institute-based screen included dengue, Rift Valley fever (RVF), Crimean -Congo hemorrhagic fever (CCHF), and Venezuelan equine encephalomyelitis. Animal models include two retroviruses in support of the Institute's human -acquired immune deficiency syndrome (AIDS) program.

Another approach being evaluated is the targeting of antiviral agents to specific tissue sites. This approach involves the synthesis of compounds which have an inherent capacity to concentrate in specific organs, or coupling of antivirals to carriers that will promote the site-specific delivery of the drug.

We are pursuing studies on the molecular basis of drug action to identify potential targets for drug interactions as well as for assessing the potential for untoward toxicity.

SUMMARY

Three hundred and twenty-eight compounds were tested for in vitro antiviral activity against RVF virus. Thirty-nine compounds showed antiviral activity of which seven showed very strong activity. Active compounds are undergoing further development, including additional in vitro testing and in vivo evaluation in mouse models.

The antiviral program against dengue viral infection has continued with the evaluation of 77 new compounds with either known activity against the related flaviviruses, yellow fever virus or Japanese encephalitis virus, or compounds that are related to active anti-dengue compounds identified the preceding year. We found two of these compounds to have very high activity against dengue 1 and 3; these have been resynthesized in bulk for in vivo evaluation

A study was performed to test the biological activity of erythropoietin (EPO) in adult rhesus monkeys. We established that human cloned EPO at a dose of 450 U/kg significantly elevated hematological parameters such as red blood cell counts, hematocrit, and hemoglobin. Erythropoietin was used to reverse the anemia induced by high doses of ribavirin. These experiments showed that EPO partially reversed a ribavirin-induced anemia without deleterious side effects.

An in vitro screening assay for CCHF virus was established. We found that the virus replicated and plaqued efficiently in SW13 cells. A number of other cell lines are currently being evaluated to determine their relative sensitivity to a variety of drugs.

Work was continued on the production and characterization of monoclonal antibodies specific for Junin virus. Eight neutralizing monoclonal antibodies have been obtained, two of which react differentially with the surface of infected cells. These monoclones will be linked to potential antiviral compounds for experiments involving the specific targeting of drugs to infected cells.

The development of a murine leukemia model that mimics the course of the AIDS has been undertaken in support of the Institute's AIDS program. The model will be used to screen candidate antiviral agents and immunomodulators, separately and in combination, in order to identify promising therapeutic drug for the treatment of AIDS.

The antiviral active compound, 1-(ß-D-ribofuranosyl)-1,2,4-triazole-3-carboxamidine HCl (AVS 206) was synthetically converted to a series of (un) substituted 1,4,5,6-tetrahydropyrimidine hydrochlorides in which the amidine functionally had been masked within a six-membered ring. The corresponding non-cyclic N,N'-dimethylamidine of the parent amidine (206) was also prepared. These compounds were submitted for in vitro/vivo screening.

The amidines, 2-(ß-D-ribofuranosyl)-thiazole (and selenazole)-4-carboxamidine hydrochloride have been prepared. Both were prepared in small amounts in five steps from tiazofurin and selenazofurin. All intermediates were characterized analytically and spectroscopically and submitted for testing.

An analogue series of 7- substituted polycyclic natural products based upon the structure of 7-deoxynarciclasine (AVS 360) have been prepared in collaboration with Drs. George Pettit of Arizona State University and Ernst Schubert, Pharm-Eco Labs.

4-Acetyl-4-phenylpiperidine (AVS 999) has been structurally characterized by NMR (^{1}H, ^{13}C) and its pruity determined. Its water-soluble hydrochloride salt has been synthesized for ease of administration during in vivo studies. Oxidation products and other derivatives of the parent compound are being prepared in an attempt to ascertain the source of biological antiviral activity.

PRESENTATIONS

1. **Downs, M. B., M. A. Ussery, and P. G. Canonico.** 1987. Immunocytochemical studies of the kinetics of peripheral Japanese encephalitis virus (JEV) infection in C57 black mice. Presented at the Annual Meeting of the American Association of Anatomists, Washington, D. C., May.

2. **Kende, M., and P. G. Canonico.** 1987. Treatment of experimental viral infection with immunomodulators. Presented at the International Symposium on Immunomodulators and Nonspecific Host Defense Mechanisms Against Microbial Infections, West Berlin, West Germany, May.

3. **Kende, M., M. Contos, W. Rill, and P. G. Canonico.** 1987. Optimization of liposomal carriers for antiviral therapy. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

4. **Kende, M., W. Rill, J. Smith, M. Derevjanik, and P. G. Canonico.** 1987. Oral efficacy of an acridine derivative (AD) immunomodulator against Rift Valley fever virus (RVFV) infection in mice. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

5. **Watts, D. M., M. A. Ussery, and C. J. Peters.** 1987. Effects of ribavirin on the replication of Crimean-Congo hemorrhagic fever virus. Presented at the Annual Meeting of the American Society for Virology, Chapel Hill, NC, May-June.

PUBLICATIONS

1. **Canonico, P. G., M. Kende, and B. G. Gabrielson.** 1987. Carrier -mediated delivery of antiviral agents. Submitted to *Adv. Virus Res.*

2. **Kende, M., H. W. Lupton, and P. G. Canonico.** 1987. Treatment of experimental viral infections with immunomodulators, pp. ----. *In* Masihi and Lange (ed.), Imunomodulators and non-specific host defence mechanisms against microbial infections. Pergamon Journals, Ltd., Oxford (In Press).

3. **Kende, M., H. W. Lupton, W. R. Rill, P. Gibbs, H. B. Levy, and P. G. Canonico.** 1987. Ranking of prophylactive efficacy of poly(ICLC) against Rift Valley fever virus infection in mice by incremental relative risk of death. *Antimicrob. Agents Chemother.* **31:**1194-1198.

4. **Kende, M. H. W. Lupton, W. L. Rill, H. B. Levy, and P. G. Canonico.** 1987. Enhanced therapeutic efficacy of poly(ICLC) and ribavirin combinations against Rift Valley fever virus infection in mice. *Antimicrob. Agents Chemother.* **31:**986-990.

5, **Neenan, J. P., S. M. Opitz, K. M. Borges, P. G. Canonico, and M. A. Ussery.** 1987. Nucleoside dialdehydes as inhibitors of RNA viruses and adenosylhomocysteine hydrolase. Submitted to *J. Med. Chem.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA0G3810	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AB	150
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY88- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory Development Studies of Conventional Agents of Biological origin for Development of Medical Defensive Countermeasures

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16 PERFORMANCE METHOD
80 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	11.0	3039
c. TYPE	d. AMOUNT	88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division,USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Linthicum, K J
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Liu, C T
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Turell, M J

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) Arboviruses; (U)Anthrax (U) Vaccines; (U) Lab Animals; (U) Mice (U) Rats; (U) Guinea Pigs; (U) RA I

23. TECHNICAL OBJECTIVE 24 APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To define the disease spectrum of arboviruses to include vector and reservoir competence, pathogenesis of viral strains, and therapy and immunoprophylaxis.

24. (U) Longitudinal epidemiological studies correlate clinical manifestations with seroconversions and identify sites for in-depth ecological studies to recover viral strains from reservoirs. Ecological and genetic factors relating to vector and reservoir competence are studied under controlled conditions.

25. (U) 8610 - 8709 Laboratory studies have shown that Crimean-Congo hemorrhagic fever (CCHF) virus can be transmitted vertically and horizontally be *Hyalomma truncatum* ticks. Following experimental infection of Dugbevirus in an African tick, *Rhipicephalus appendiculatus*, and a North American tick, *Rhipicephalus sanguineus*, both species were able to transmit the virus to a host animal. Significant observations on the physiological affects of Pichinde virus on infected animals is now possible by closely monitoring heart functions by using the innovative techniques of cannula implantation in the common carotid artery and external jugular vein. Stable flies and mosquitoes were able to transmit lethal infections of Bacillus anthracis to mice and guinea pigs mechanically. Vectorial capacity of *Aedes aegypti* for dengue 2 virus was significantly enhanced by the concurrent ingestion of microfilariae of *Brugia malayi*.

PROJECT NO. 3M162770A871:	Medical Defense Against Biological Warfare
WORK UNIT NO. 871-AB-150:	Exploratory Development Studies of Conventional Agents of Biological Origin for Development of Medical Defensive Countermeasures
PRINCIPAL INVESTIGATOR:	K. J. Linthicum, CPT, Ph.D.
ASSOCIATE INVESTIGATOR:	M. J. Turell, Ph.D. C. T. Liu, Ph.D. T. M. Logan, CPT, Ph.D.

BACKGROUND

Highly pathogenic viruses, with invertebrate and vertebrate vectors, are known to be potential threats to United States military personnel in endemic areas. Many of these viruses cause severe human disease, mainly hemorrhagic fevers, which includes Rift Valley fever (RVF), Crimean-Congo Hemorrhagic fever (CCHF), and Dugbe. In most cases, little is known about the epidemiology and life cycles of these viruses or on pathogenesis, therapy, or immunoprophylaxis of the viral diseases. The main emphasis of this work unit is to evaluate the natural threat and impact of the group of highly pathogenic viruses by accurately establishing the incidence and distribution of apparent and inapparent infections and determining epidemiological, ecological, and agent-related factors influencing endemic viral activity; and to alter the highly pathogenic nature of these agents by defining the pathogenic mechanisms involved in the disease processes and developing therapeutic measures to intervene in hemorrhagic fever.

SUMMARY

For the first time in the laboratory, *Hyalomma truncatum* ticks have been orally infected with CCHF virus. Vertical and horizontal transmission has been demonstrated. Studies on African and North American species of ticks have shown that transmission of Dugbe virus to the host animal is possible after experimental infection.

Parameters reflecting a change in capillary permeability to albumin were measured in Pichinde virus-infected guinea pigs on day 10 post inoculation, and only a reduced lymphatic pressure was seen when compared to that in non-infected animals. Results of other studies indicate that a decrease in body weight after Pichinde viral infection is not parallel to the change in body surface area. Very significant developments in the technology of remotely sensed, rectal temperature data have been made. Sensors are also available for measuring blood pressure and heart rate. These measurements may become a part of our experimental protocols in the future.

Vector capacity of *Aedes aegypti* for dengue 2 virus was significantly enhanced by the concurrent ingestion of microfilariae of *Brugia malayi*. Stable flies and mosquitoes were also able to transmit lethal infections of *Bacillus anthracis* mechanically to mice and guinea pigs. We showed mosquito inoculation to be an effective bioassay for certain toxins and antitoxins. *Culex pipiens* with disseminated RVF viral infections had significantly reduced survivals compared to their siblings without disseminated infections.

MGLJ Studies of Arbovirus Infection, Dissemination, and Transmission in Vectors

PRINCIPAL INVESTIGATOR: K. J. Linthicum, CPT, Ph.D.

ASSOCIATE INVESTIGATOR: T. M. Logan, CPT, Ph. D.

AND

MGLM Experimental Transmission of CCHF Virus by Ticks

PRINCIPAL INVESTIGATOR: T. M. Logan, CPT, Ph.D.

ASSOCIATE INVESTIGATOR: K. J. Linthicum, CPT, Ph.D.

Crimean-Congo hemorrhagic fever virus has been isolated from 29 different species of ticks; however, up to recently, there has virtually been no quantitative data available regarding the vector competence of these arthropods. *Hyalomma truncatum* ticks were orally infected with CCHF virus for the first time in the laboratory. Vertical transmission from larvae to nymphs to adults and horizontal transmission to host has been demonstrated. Horizontal transmission of CCHF has also been demonstrated in inoculated *Rhipicephalus appendiculatus* ticks. The virus can persist in both species for many months in the laboratory. We observed no transovarial transmission.

The ability of ticks to become infected with and horizontally transmit Dugbe virus to a host has been studied. One strictly African species, four North American species, and one species found both in Africa and North America were inoculated with virus and studied over a period of 12 months. All species became infected. *Rhipicephalus appendiculatus* and R. *sanguineus* were able to transmit the virus to a host animal.

MGLC Pathophysiology of Arenavirus Disease

PRINCIPAL INVESTIGATOR: C. T. Liu, Ph.D

A leukotriene antagonist (LY-171883) has been shown to be ineffective with the selected doses and routes of administration in the treatment of Pichinde virus-infected strain 13 guinea pigs.

Parameters reflecting a change in capillary permeability to albumin were measured in Pichinde virus-infected guinea pigs 10 days post inoculation. We did not observe a change in capillary permeability of albumin at this time. Lymphatic pressure was lower in infected animals, but we saw no difference in lymphatic flow and frequency of lymphatic contraction.

We examined the relation between body weight and body surface area changes in Pichinde virus-infected guinea pigs. We found that a sharp decrease in body weight after Pichinde viral infection was not parallel to the change of body surface area; the longer the infection lasted, the smaller the degree of body surface area changes.

The percent changes in body surface area between controls with different body weights and Pichinde virus-infected guinea pigs of corresponding ages were compared over 14 days at various time intervals. We concluded that body surface area should be used to unify the organ functions, metabolic rate, or dosages for drug administration in control and virus-infected animals. The relationship between body weight and age of strain 13 guinea pigs from birth to 14 weeks has been determined daily.

Virus-infected groups, with either NIH or high-fat - 15% corn oil diet always consumed less food after day 6. The high-fat diet failed to modify the mean survival (14 days) after Pichinde viral infection. The mean survival time was 15.8 days with fat supplements.

Significant progress was made in the development of the technology of chronically implanting cannulas into both the common carotid artery and external jugular vein of strain 13 guinea pigs. Diurnal changes of blood pressure and heart rate and cardiac output can now be studied from the same animal with or without Pichinde viral infection over a longer time.

MGLK Factors Affecting Vector Competence of Mosquitoes for Rift Valley Fever Virus

PRINCIPAL INVESTIGATOR: M. J. Turell, Ph.D.

Studies conducted with inoculated mosquitoes showed that once the extrinsic incubation period for a mosquito species had been completed, the ability to transmit RVF virus was not affected by the time after inoculation, number of cycles completed, or whether the mosquito probed, partially engorged, or fed to repletion. Similarly, when mosquitoes had multiple probes during a single blood meal (i.e., when they fed on several different animals), transmission rates were not affected by the order of feeding. This, combined with our earlier study showing that *Cx. pipiens* with disseminated RVF viral infections were less likely to feed to repletion than siblings without a disseminated infection, indicates that the vectorial capacity of mosquitoes with a disseminated infection may be enhanced.

Intrathoracic inoculation of mosquitoes was shown to be an efficient, quantitative, bioassay for several presynaptic snake neurotoxins (crotoxin, notexin, taipoxin, and *Naja naja atra* PLA2 toxins), as well as two algal toxins (microcystin and saxitoxin) and a scorpion toxin (A.a. H IT). The LD_{50} for each of these toxins was about 1 ng per mosquito. Mosquito inoculation was also an effective bioassay for quantifying antitoxin activity for both a polyclonal guinea pig anti-taipoxin and a monoclonal anti-crotoxin. Surprisingly, we observed no excess mortality in mosquitoes inoculated with botulinum A, tetanus, ricin, conotoxin, or anthrax toxins.

Mosquitoes collected in RVF virus enzootic areas of Africa: *Aedes circumluteolus*, *Aedes mcintoshi (=Aedes lineatopennis)* and *Culex antennatus* collected in Kenya; *Aedes palpalis* collected in the Central African Republic; *Culex pipiens* collected in Egypt; and *Aedes fowleri* collected in Senegal were evaluated for their potential to vector RVF virus. While each of these species transmitted lethal RVF viral infections to hamsters, *Cx. pipiens, Ae. fowleri,* and *Ae. palpalis* were the most efficient vectors. Studies are currently underway to further delineate factors (i.e., environmental temperature) that might affect the ability of these species to serve as vectors of RVF virus.

After probing on guinea pigs with anthrax bacteremias of ca. $10^{8.6}$ CFU per ml of blood, both stable flies and mosquitoes (*Aedes aegypti* and *Aedes taeniorhynchus*) were able to transmit lethal anthrax infections to both guinea pigs and mice, even when these arthropods were held at room temperature for 4 h after exposure to the bacteremic guinea pig before being allowed to continue feeding on the susceptible animal.

PRESENTATIONS

1. **Knudson, G. B., and M. J. Turell.** 1987. Mechancial transmission of *Bacillus anthracis* by the stable fly, *Stomoxys calcitrans*. Presented at the Annual Meeting of The American Society of Microbiology Atlanta, GA, March.

2. **Turell, M. J., and C. L. Bailey.** 1986. Effect of environmental temperature on the replication, dissemination, and transmission of Rift Valley fever virus by *Aedes fowleri*. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

3. **Turell, M. J., R. F. Tammariello, and C. L. Bailey.** 1986. Reduced recovery of Rift Valley fever virus associated with assay of larval pools. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

4. **Turell, M. J., R. F. Tammariello, J. F. Smith, and C. L. Bailey.** 1987. Reduced recovery of Rift Valley fever virus associated with assay of mosquito larval pools. Presented at the VII International Congress on Virology in Edmonton, Alberta, Canada.

PUBLICATIONS

1. **Davies, F. G., and K. J. Linthicum.** 1986. The Sudan dioch (*Quelea quelea aethiopica*) and Rift Valley fever. *Trans. Roy. Soc. Med. Hyg.* 80:171-172.

2. **Faran, M. E., M. J. Turell, W. S. Romoser, R. G. Routier, P. H. Gibbs, T. L. Cannon, and C. L. Bailey.** 1987. Reduced survival of adult *Culex pipiens* infected with Rift Valley fever virus. *Am. J. Trop. Med. Hyg.* 37:403-409.

3. **Gargan, T. P., II, G. C. Clark, D. J. Dohm, M. J. Turell, and C. L. Bailey.** 1987. Vector potential of selected North American mosquito species for Rift Valley fever virus. *Am. J. Trop. Med. Hyg.* (In Press).

4. **Linthicum, K. J., F. G. Davies, A. Kairo, and C. L. Bailey.** 1987. Rift Valley fever virus disease in Kenya, pp. . *In* Viral diseases in Africa. Proceedings of the Scientific, Technical and Research Commission of the Organization of African Unity Symposium on Viral Disease in Africa Affecting Plants, Animals and Man. Nairobi, Kenya (In Press).

5. **Linthicum, K. J., F. G. Davies, A. Kairo, C. L. Bailey, H. F. Kaburia, and K. J. Lindquist.** 1987. Field ecological studies on Rift Valley fever virus. *In* Advances in the diagnosis, treatment and prevention of immunizable diseases in Africa, pp. 97-108. Proceedings of the Seventh Annual Medical Scientific Conference, Nairobi, Kenya. Paper 21/86,

6. **Linthicum, K. J., F. G. Davies, and J. Kamau.** 1987. Predation on emerging adult mosquitoes by *Brachydeutera munroi* (Diptera:*Ephydridae*), pp.178-181. Proceedings of the 72nd Annual Meeting of the New Jersey Mosquito Control Association.

7. **Liu, C. T.** 1987. Observations of the in situ contracting heart of guinea pigs infected with Pichinde virus. *Life Sci.* 41:2313-2317.

8. **Liu, C. T.** Energy balance and growth rate of outbred and inbred guinea pigs. *Am. J. Vet. Res.* (In Press).

9. **Liu, C. T., and C. J. Peters.** 1987. Improvement of cardiovascular functions with a sulfidopeptide leukotriene antagonist in a guinea pig model of viral hemorrhagic fever. *Pharmacologist* 29:196.

10. **Liu, C. T., C. J. Peters, and G. G. Pinter.** 1987. Bilateral cervical lymph collection and measurement of capillary permeability to albumin in strain 13 guinea pigs. *Fed. Proc.* 46:349.

11. **Meegan, J. M., and C. L. Bailey.** 1987. Rift valley fever. *In* T. P. Monath (ed.), Epidemiology of arthropod-borne viral diseases. CRC Press, Inc. (In Press).

12. **Morrill, J. C., G. B. Jennings, H. Caplen, M. J. Turell, A. J. Johnson, and C. J. Peters.** 1987. Pathogenicity and immunogenicity of a mutagen-attenuated Rift Valley fever virus immunogen in pregnant ewes. *Am. J. Vet. Res.* 48:1042-1047.

13. **Romoser, W. S., M. E. Faran, and C. L. Bailey.** 1987. Newly recognized route of arbovirus dissemination from the mosquito (*Diptera:Culicidae*) midgut. *J. Med. Entomol.* 24:431-432.

14. **Peters, C. J., P. B. Jahrling, C. T. Liu, R. H. Kenyon, K. T. McKee, Jr., and J. G. Barrera Oro.** 1987. Experimental studies of arenaviral hemorrhagic fevers. *Curr. Topics Microbiol. Immunol.* 134:5-68.

15. **Turell, M. J.** Horizontal and vertical transmission of viruses by insect and tick vectors, pp. . Epidemiology of arthropod-borne viral diseases, T. Monath, (ed.), CRC Press Inc. (In Press).

16. **Turell, M. J., and C. L. Bailey.** 1987. Transmission studies in mosquitoes (*Diptera:Culicidae*) with disseminated Rift Valley fever virus infections. *J. Med. Entomol.* 24:11-18.

17. **Turell, M. J., and G. B. Knudson.** 1987. Mechanical transmission of *Bacillus anthracis* by stable flies (*Stomoxys calcitrans*) and mosquitoes *Aedes aegypti* and *Aedes taeniorhynchus*. *Infect. Immun.* 55:1859-1861.

18. **Turell, M. J., T. N. Mather, A. Spielman, and C. L. Bailey.** 1987. Increased dissemination of dengue 2 virus in *Aedes aegypti* associated with concurrent ingestion of microfilariae of *Brugia malayi*. *Am. J. Trop. Med. Hyg.* 37:197-201.

19. **Watts, D. M., T. G. Ksiazek, K. J. Linthicum, and J. Hoogstraal.** 1987. Crimean-Congo hemorrhagic fever, pp. . *In* T. P. Monath (ed.), Epidemiology of arthropod-borne viral diseases. CRC Press, Inc. (In Press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA307135	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	H. TERM	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	131
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) Isolation and Characterization of Immunogenic Components of Anthrax Toxin

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense; 0601 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
85 04	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	3.0	120
c. TYPE	d. AMOUNT	*88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Bacteriology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Welkos, S L
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7341
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Ivins, B
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Greene, R.

22. KEYWORDS (Precede EACH with Security Classification Code) (U) BW Defense; (U) Lab Animals; (U) Mice; (U) RA I (U) Guinea Pigs; (U) Rabbits; (U) Anthrax Toxin; (U) Lac Fusion Proteins; (U) Cloning

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To isolate polypeptide fragments of anthrax toxin components by using plasmid cloning vectors that allow construction of hybrid genes. To characterize the structure, immunogenicity, and biological activity of the hybrid toxin proteins. This work may ultimately lead to an improved anthrax vaccine to protect military personnel.

24. (U) Isolate protective antigen (PA)-lac Z gene fusions and characterize these fusions by using restriction endonuclease digestion and agarose gel electrophoresis. Once purified, the different PA-lac hybrids will be assayed by in vitro and in vivo techniques. Purified, fully characterized hybrid proteins might be conjugated with other anthrax antigenic material to form a new vaccine.

25. (U) 8610 - 8709 We studied the efficacy of live anthrax vaccines and the mechanisms of host resistance to lethal infection by the spore vaccine in mice. Inbred strains of mice differed in their susceptibility to the Sterne spore vaccine. Most strains were resistant (R), whereas susceptible (S) A/J mice were killed by low doses of Sterne. The Hc gene encoding complement component C5 was associated with resistance. All R strains (inbred, backcross, F2, and recombinant inbred) were C5-positive whereas S strains were C5-negative. A/J mice were passively protected from lethal challenge by C5-positive serum. Immunization of R, but not S, mice with Sterne protected them against virulent challenge, but only with doses of Sterne approaching lethality. We cloned the anthrax protective antigen (PA) gene in *Bacillus subtilis*. The recombinant strain was safe, elicited anti-PA antibody, and protected R mice against challenge.

*This work will be continued under Accession Number DA308918.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3A161101A91C: Independent Laboratory In-House Research

WORK UNIT NO: 91C-00-131: Isolation and Characterization of Immunogenic Components of Anthrax Toxin

PRINCIPAL INVESTIGATOR: S. L. Welkos, Ph.D.

ASSOCIATE INVESTIGATORS: B. E. Ivins, Ph.D.
R. S. Greene, Ph.D.

BACKGROUND

Bacillus anthracis is an important pathogen of animal and of people exposed to infected animals or their products. Anthrax is a significant biological warfare threat due to the stability of its spores, the nonspecific symptoms of the infection, and the severity of the systemic disease. Our research goals are to understand the pathogenesis of anthrax and the basis of host susceptibility, and to identify essential vaccine epitopes of anthrax toxin.

Bacillus anthracis produces an exotoxin that is essential for the manifestation of disease and is immunogenic. This toxin is composed of three protein components: protective antigen (PA), edema factor (EF), and lethal factor (LF), consisting primarily of PA; domestic animals are immunized with a toxin-producing spore vaccine. The human vaccine is safe, but requires frequent boosters and may not provide sufficiently broad protection. The animal vaccine is effective but potentially reactogenic.

A major focus of anthrax research is to clone and express the genes encoding the individual components of anthrax toxin. Initial investigations focused on cloning the gene for PA. This protein is required to mediate the toxic effects of EF and LF and it induces protective immunity. We cloned a fragment of DNA from *B. anthracis* into an *Escherichia coli* plasmid vector, but expression of the PA gene was low.[1] To further analyze the PA gene, we sequenced the DNA (in collaboration with Dr. F. Eden-McCutchan, Meloy Laboratories, and COL. J. Lowe, Bacteriology Division, USAMRIID). The information we obtained has been valuable in research performed by several in-house and academic investigators. It facilitated our research for an effective, prototype, live vaccine. We transferred the PA gene from a recombinant *E. coli* strain into *B. subtilis*, a gram-positive, cloning strain. Two recombinants of *B. subtilis*, PA1 and PA2, were isolated; each stably produced high levels of PA.[2]

We are making additional, collaborative approaches to the cloning and optimal expression of virulence factors and vaccine antigens. Initially we used *E. coli* fusion vectors to isolate vaccine able to produce truncated PA fusion proteins.[3] However, the recombinants did not produce detectable levels of the proteins, and we are not presently pursuing this work. Current studies include development of methods to enhance expression by the PA-producing, *B. subtilis* recombinant; mutagenesis of the *B. anthracis* genome; and subcloning toxin component genes into *E. coli* expression plasmids.

An animal model is required to identify host responses in innate and acquired immunity to anthrax, and to test the efficacy of new vaccines. We have characterized the responses of inbred mice to virulent *B. anthracis* strains and to live vaccine strains. In previous work, we found that inbred mouse strains differed significantly in their susceptibilities to lethal toxicity and infection by both the virulent and attenuated vaccine strains of *B. anthracis* infection.

SUMMARY

Inbred mouse strains differed greatly in their susceptibility to infection by Sterne, the toxin-positive, capsule-negative, spore vaccine strain of *B. anthracis*. Most mouse strains were relatively resistant (R), whereas Sterne produced a lethal, systemic infection and intoxication at low doses in susceptible (S) A/J mice.[4] A single, major gene (*Hc*), encoding complement component 5 (C5), was associated with host resistance to lethal, Sterne infection. All R strains had a functional gene, whereas S strains were deficient in C5. A/J mice could be passively protected from lethal Sterne challenge by C5-positive sera, but not by sera from C5-negative, congenic mice. Also, resistance was linked to production of C5 in individual backcross and F2 hybrid mice. Finally, the responses of recombinant, inbred mice support the hypothesis that *Hc* has a major role in host resistance to Sterne. Currently, we are investigating the cellular responses of Sterne-resistant mice with a functional *Hc* gene.

The inbred mice were tested for their suitability as vaccine test animals. Sublethal doses of Sterne did not protect A/J mice from challenge with fully virulent, *B. anthracis* strains. In contrast, R mice, such as CBA/J, were partially or completely protected from virulent challenge, but only with doses of Sterne approaching the lethal dose. The CBA/J mice were also protected against anthrax with a live, recombinant, *B. subtilis* vaccine. We previously cloned the PA gene into *B. subtilis*.[2] The recombinant strain produced PA, was safe when administered in high doses to inbred mice, elicited anti-PA antibodies, and protected CBA/J mice against virulent challenge.

Collaborative efforts to clone, analyze, and screen putative vaccine antigens are ongoing. Dr. Robert Green (a visiting scientist) transferred the PA gene into a new, chimeric, phage-plasmid vector; he is studying PA expression in this system. We mutagenized the *B. anthracis* genome with transposon Tn*916*. We showed that *B. anthracis* can donate or receive the conjugative transposon, and that Tn*916* integrates randomly into the *B. anthracis* genome. Mutants deficient in aromatic amino acid biosynthesis were isolated and will be tested as live, vaccine candidates.

PRESENTATIONS

1. **Ivins, B. E., G. B. Knudson, S. L. Welkos, and D. J. LeBlanc.** 1986. *Bacillus anthracis* as donor and recipient in filter mating transfer of Tn*916*. Presented at the 10th Annual Meeting of the Mid-Atlantic Extrachromosomal Elements, Virginia Beach, VA, October.

PUBLICATIONS

1. **Ivins, B. E.** 1987. The search for a new-generation human anthrax vaccine. Submitted to *Clin. Immunol. Newslett.*

2. **Ivins, B. E., S. L. Welkos, G. B. Knudson, and D. J. LeBlanc.** 1987. Transposon Tn*916* mutagenesis in *Bacillus anthracis*. Submitted to *Infect. Immun.*

4. **Leppla, S. H., D. L. Robertson, S. L. Welkos, L. A. Smith, and M. H. Vodkin.** 1986. Cloning and analysis of genes for anthrax toxin components, pp. 275-278. *In* Flamagne, et al. (ed), Bacterial protein toxins, Suppl. 15. Zentralblatt für bakteriologie mikrobiologie und hygiene. 1. Abteilung. Gustav Fischer, Stuttgart.

5. **Welkos, S. L.** 1987. Protective efficacy and safety of live anthrax vaccines for mice. Submitted to *Microbial Pathogen.*

6. **Welkos, S. L., A. M. Friedlander, D. M. Becker, and T. J. Keener.** 1987. Pathogenesis and control of resistance to the Sterne strain of *Bacillus anthracis*. Submitted to *Microbial Pathogen.*

LITERATURE CITED

1. **Vodkin, M.H., and S. H. Leppla.** 1983. Cloning of the protective antigen gene of *Bacillus anthracis*. *Cell* 34:693-697.

2. **Ivins, B. E., and S. L. Welkos.** 1986. Cloning and expression of the *Bacillus anthracis* protective antigen gene in *Bacillus subtilis*. *Infect. Immun.* 54:537-542.

3. Welkos, S. L. 1986. FY86 Annual Report of the United States Army Medical Research Institute of Infectious Diseases, pp.151-153.

4. Ivins, B. E., S. L. Welkos, G. B. Knudson, and D. J. LeBlanc. 1987. Transposon Tn*916* mutagenesis in *Bacillus anthracis*. Submitted to *Infect. Immun.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA309249	30 Oct 87	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	H. TERM	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	LA	132
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code)
Medical Defensive Studies on Crimean-Congo Hemorrhagic Fever Virus

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
85 12	87 12	DA	C. In-House

17. CONTRACT/GRANT
a. DATE EFFECTIVE
EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
87	1.0	175
*88		

19. RESPONSIBLE DOD ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases
b. ADDRESS (include zip code)
Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL
Huxsoll, D L
d. TELEPHONE NUMBER (include area code)
301-663-2833

20. PERFORMING ORGANIZATION
a. NAME
Disease Assessment Division, USAMRIID
b. ADDRESS
Fort Detrick, MD. 21701-5011
c. NAME OF PRINCIPAL INVESTIGATOR
Watts, D M
d. TELEPHONE NUMBER (include area code)
301-663-7244
f. NAME OF ASSOCIATE INVESTIGATOR (if available)
g. NAME OF ASSOCIATE INVESTIGATOR (if available)

21. GENERAL USE FIC
MILITARY/CIVILIAN APPLICATION: L

22. KEYWORDS (Precede EACH with Security Classification Code) (U) RA I; (U) Crimean-Congo Hemorrhagic Fever; (U) Arbovirus; (U) Lab Animals; (U) Mice; (U) Guinea Pigs; (U)Nairovirus; (U) Bunyaviridae

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To evaluate and develop virological and immunological techniques, and an animal model system representing human Crimean Congo hemorrhagic fever (CCHF) virus. The knowledge gained will strengthen the U.S. military's capabilities to combat natural or BW CCHF human viral infections.

24. (U) Evaluate techniques for propagating and detecting CCHF virus; the most sensitive, specific, and practical methods will be made standard. By a standard approach, an animal species will be selected for CCHF modeling. Candidate techniques and the animal model will be perfected for use in implementing comprehensive, multidisciplinary, biomedical investigations.

25. (U) 8610 - 8709 Among several techniques evaluated for diagnosing CCHF, an enzyme immunoassay was most effective for detecting CCHF viral antibody, and the newborn-mouse inoculation and vertebrate cell-culture assays were best for detecting CCHF virus. Several hundred CCHF virus specific, monoclonal antibodies were produced for defining the molecular characteristics of the CCHF virion, and for determining whether antigenic differences exist among CCHF viral strains from Asia, Europe, Africa, and the Middle East. Ribavirin markedly inhibited the replication of representative CCHF strains, thus demonstrating its potential as a therapeutic drug, and studies to develop/improve vector control strategies for CCHF were initiated.

*This work is continued under Accession Number DA308918.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE

PROJECT NO. 3A161101A91C: Independent Laboratory In-House Research

WORK UNIT NO: 91C-LA-132: Medical Defensive Studies on Crimean Congo Hemorrhagic Fever Virus

PRINCIPAL INVESTIGATOR: D. M. Watts, Ph.D.

BACKGROUND

Crimean-Congo hemorrhagic fever (CCHF) is a severe and often fatal, tick-borne, viral disease of man. The geographical distribution, which includes several countries of Africa, Asia, Middle East, and Europe, is among the largest of all areas of recognized, arthropod-borne, viral diseases affecting human populations. Morbidity rates appear to be higher than for other arboviral diseases, and mortality rates during outbreaks have ranged from 15 to more than 50 percent. Despite the recognized human health significance, effective prevention and treatment strategies are lacking. This can be attributed largely to the lack of and/or unreliable biotechnology needed to generate knowledge required to understand the ecology, epidemiology, pathogenesis, and molecular biology of CCHF virus. Thus, the objective for this research is to establish the technological capability for developing effective strategies to combat the natural or biological warfare-induced threat posed by this virus to U.S. military forces.

SUMMARY

Our studies, aimed at perfecting diagnostic techniques for CCHF, were designed to evaluate in vivo and in vitro assays for detecting infectious virus, viral antigens, and virus-induced antibody. Crimean-Congo strains, originally isolated in Europe, Asia, and Africa, generally replicated to higher titers in newborn mice than in SW-13 and Vero cells. However, because the difference was not significant for most viral strains, further studies employing specimens from experimentally infected animals and from naturally infected humans and animals are needed to obtain a more definitive assessment. Other cell lines are under study, including *Dermacentor variabilis* tick cells and primary duck embryo cells. Initial results indicated that tick cells were more promising as an assay system than vertebrate cells, but attempts to reproduce this observation have not been successful. Preliminary data suggested that primary duck embryo cells were more susceptible to CCHF viral infection than any other cell line evaluated, but further studies are needed to generate a more conclusive assessment of these cells.

Either the detection of virus-specific antigens and/or IgM antibody during the acute phase of infection has been employed successfully as a rapid diagnostic approach for numerous viral diseases. Experiments with virus-infected SW-13 cells as a model system indicated that virus-specific antigen could be detected by indirect fluorescent antibody (IFA) as early as 12-h post

infection. A newly developed enzyme-linked immunosorbent assay (ELISA) was shown to be an effective and rapid approach for detecting viral antigen and virus-specific IgM antibody, thus demonstrating its overall utility for rapid diagnosis.

Because existing serology techniques are incapable of conclusively demonstrating CCHF virus-specific, we initiated studies to develop a more reliable technique. Preliminary results indicated that both the IFA and the ELISA are suitable for detecting antibody, but whether or not antibody induced by related viral antigens can be excluded remains to be determined. Compared to the IFA and ELISA, antibody titers measured by plaque-reduction neutralization were extremely low, thus precluding use of this technique for demonstrating antibody specificity.

Studies to induce disease resembling CCHF in laboratory animals are underway. Outbred mice, from a few days to 1 month old, developed a fatal infection after intracerebral inoculation. Exposure by other routes, including i.p. and s.c., produced a fatal infection only in newborn mice. However, whether this pathogenesis resembles that caused by CCHF viral infection of humans remains to be determined. Inbred mice, guinea pigs, and rhesus and African green monkeys became infected after exposure to CCHF virus, but did not develop overt illness.

Studies to evaluate ribavirin as a potential therapeutic candidate for CCHF were extended to include representative viruses from Europe, Asia, and Africa. Replication of all the viruses in Vero cells was markedly inhibited by the drug. Although there is not a CCHF animal model that parallels human disease, the inhibition effect was significantly greater than that observed in simultaneous tests with Rift Valley fever, where the drug was effective in several animal models.

PRESENTATIONS

1. **Watts, D.M., M.A. Usssery, and C.J. Peters.** 1987. Effects of ribavirin on the replication of Crimean-Congo hemorrhagic fever virus. Presented at the Annual Meeting of the American Society for Virology, University of North Carolina, Chapel Hill, NC, May-June.

PUBLICATIONS

1. **DeFoliart, G.R., P.R. Grimstad, and D.M. Watts.** 1987. Advances in mosquito-borne arboviruses/vector research. *Ann. Rev. Entomol.* 32:479-505.

2. **DeFoliart, G.R., D.M. Watts, and P.R. Grimstad.** 1986. Changing patterns in mosquito-borne arboviruses. *Am. J. Mosq. Control Assoc.* 2:437-455.

3. **Watts, D.M., D.S. Burke, B.A., Harrison, A. Nisalak, and R.W. Whitmire.** 1987. Effects of temperature on the transmission of dengue virus type 2 by *Aedes Aegypti. Am. J. Trop. Med. Hyg.* 36:143-152.

4. **Watts, D.M., T.G. Ksiazek, K.J. Linthicum, and H. Hoogstraal.** 1987. Crimean-Congo hemorrhagic fever, pp. . *In* T. G. Monath (ed), Epidemiology of arthropod-borne viral diseases (In Press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA303476	30 Oct 87	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	H. TERM	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	138
b. CONTRIBUTING				
c. CONTRIBUTING	None			

11. TITLE (Precede with Security Classification Code) (U) Application of Recombinant DNA Technology to Develop New Generation of Q fever Vaccines

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense; 0601 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 02	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	1.0	78
c. TYPE	d. AMOUNT	*88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Williams, J C
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Bolt C R
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) DNA Recombinant Technology; (U) Vaccine; (U) Q Fever; (U) Coxiella burnetii (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) *Coxiella burnetii* is perceived to be a prime candidate for biological warfare (BW). Current vaccines are reasonably effective against this rickettsial disease, but cause many side-effects. This is a pioneering study to determine the feasibility of using DNA recombinant technology to develop an entire new generation of safe and highly effective vaccines against Q fever. If successful, U.S. troops can be immunized without lost time due to sterile abscesses and sore arms.

24. (U) Standard techniques of gene cloning will be used to transform a host bacterial cell with *Coxiella burnetii* DNA. Screening for proteins in recombinant clones will be pursued by a number of techniques. Any clone producing an antigen that is recognized by specific antisera becomes a candidate for a vaccine seed. This study was approved by Institutional Biosafety Committee of WRAIR representing DoD and the Office of Recombinant DNA Activities, National Institutes of Health (NIH).

25. (U) A gene library from the DNA of *C. burnetii* has been constructed for cosmid vector pHC79. An immunoreactive gene product from clone pJB196 was identified as a 62 kd polypeptide by Western blot and in vitro translation system. Expression of the 62 Kd and a 14 Kd polypeptide was under the control of a heat shock promoter (HSP) in recombinant *E. coli*. Subcloning of pJB196 and sequencing 3 Kb of that DNA revealed two open reading frames, encoding a polypeptide of 58.3 Kd, and 10.5 KD along with two ribosomal on binding sites. A transcriptional control element on the 5' side of the initiation codom resembled a HSP (at -452). Four sequences were highly homologous to the 62 Kd protein from *C. burnetii* ($\leq$ 50%). Three are from *Mycobacteria*. The other is from *E. coli*, detected as a gene that complements or suppresses a temperature-sensitive RNAase activity. Therefore this protein is conserved in phylogenetically distant bacterial genera.

*This work is continued under Accession Number DA302660.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3A161101A91C: Independent Laboratory In-House Research

WORK UNIT NO. 91C-00-138: Application of Recombinant DNA Technology to Develop New Generation of Q Fever Vaccines

PRINCIPAL INVESTIGATOR: J. C. Williams, Ph.D.

ASSOCIATE INVESTIGATOR: C. R. Bolt, MA

BACKGROUND

Coxiella burnetii, the cause of Q fever, is unique among the pathogenic rickettsiae because it replicates in the microbicidal milieu of the phagolysosome, and it carries out a developmental cycle which consists of sporogenic differentiation with both binary and unequal cell division. Humans can be infected after exposure to only one virulent phase I microorganism by aerosols generated by infected, parturient animals. Microorganisms spread systemically from the lung induce a primary, slowly resolved, disease course which closely mimics influenza. In approximately 5% of infected individuals, the time course is extended, thereby producing chronic hepatitis and occasionally endocarditis. The acute and chronic forms of the disease are treated with tetracycline. However, chronic endocarditis disease requires long-term (1 to 2 years) treatment with tetracycline or rifampicin. Chemotherapy is successful only after surgery to replace the affected mitral or aortic valve.

Recent studies indicate that the surface proteins of *C. burnetii* may be sufficiently immunogenic to be used as subunit vaccines. There are approximately 30 to 35 surface-exposed proteins of *C. burnetii*, with certain of these participating as primary antigens. High levels of antibodies and a cooperative cellular immune response against specific protein antigens are required for protection against Q fever. The classical approach to making Q fever vaccine led to the production of phase I whole cell and phase I subfractions which were efficacious but also caused undesirable side effects. However, the arduous task of growing and producing *C. burnetii* is quite expensive and hazardous, requiring special handling in containment facilities.

In theory, recombinant DNA technology offers as an advantage the subunit vaccine. Once DNA encoding protective immunogens are cloned, the immunizing antigen purified, and humoral and cellular immune responses elicited, cultivation of the parasite will not be necessary. A subunit vaccine may circumvent the pathogenic potential of the current investigational phase I whole cell vaccine. Because classical genetic analysis of *C. burnetii* is difficult, recombinant DNA studies may also contribute to a basic understanding of the molecular biology of parasitism.

SUMMARY

During this reporting period, we were concerned with the cloning of two tightly linked genes and sequencing of DNA. Recombinant *E. coli* expressing *C. burnetii* antigens was selected from a cosmid bank of genomic DNA. Lysates of recombinant *E. coli* carrying *C. burnetii* genes reacted with immune sera from different sources. A *C. burnetii*-specific antigenic activity was associated with a 58.3-kd polypeptide, which had an apparent M_r of 62 kd on denaturing polyacrylamide gels. This polypeptide was highly expressed in *E. coli* under regulation of an endogenous promoter. A promoter with the properties of heat shock control (HSP) and two open reading frames, designated CB htp A and B, was inferred from the sequence data.

The identification of a single HSP that controls both CB htp A and B suggests that we have discovered an operon involved in stress response. This is not surprising since *C. burnetii* must cope with a wide range of environmental stimuli that includes transmission to the eukaryotic host via aerosols and the poikilothermic tick vector. Also, the mammalian host may trigger heat shock and other stress (i.e. microbicidal activities) responses, as *C. burnetii* is engulfed into the phagolysosome.

The CB htp B gene was also homologous to a gene of *M. tuberculosis* and *M. leprae*. In fact, this polypeptide is found in most of the *Mycobacteria* and represents the dominant antigen of that genus. Thus, this gene or a portion thereof is apparently conserved in phylogenetically distant bacteria. Other bacteria also contain an homologous protein conserved between both gram-negative and positive bacteria. This apparently "common antigen" with M_r of 65 kd, described originally in *Pseudomonas aeruginosa*, would be a candidate for a larger set of conserved proteins, which includes the homologous polypeptides from *Coxiella* and *Mycobacteria*.

Literature on heat shock stress responses also suggest possible identification of CB htp A and B. These proteins would be candidates for the gro ES and gro EL, respectively, of *E. coli*. As in *C. burnetii*, gro E has been shown to exist as bicistronic operon.

We investigated the intracellular localization of the homologous 60-65-kd polypeptide in three *Mycobacterial* species. In both *M. leprae* and *M. bovis*, the protein has been reported to be in the soluble fraction. However, membrane association has been implicated in the case of *M. tuberculosis*. CB htp B shows this same behavior both as a cloned product in *E. coli* and in its natural state in *C. burnetii.*

The purpose for cloning this polypeptide was to assess its efficacy as a subunit vaccine against Q fever. The protein was immunogenic in mice, and polyclonal antibodies raised against it crossreacted with *C. burnetii* and the recombinant *E. coli* clone from which it was extracted. Therefore, the antigen may be able to mediate protection against *C. burnetii* and other pathogens that

express homologous sequences. We are currently studying the epitopes which specify the *Coxiella*-specific determinants of the polypeptide with monoclonal antibodies.

PRESENTATIONS

1. **Vodkin, M. H., and J. C. Williams.** 1987. Cloning of an antigen from *Coxiella burnetii* and its homology to polypeptides in other bacteria. Presented at the USAMRIID-Cornell Symposium, Cornell University, Ithaca, NY, May.

2. **Vodkin, M. H., and J. C. Williams.** 1987. Cloning and expression of a major antigen gene of *Coxiella burnetii* homologous to a protein in *Mycobacteria* and *Escherichia coli.* Presented at Modern Approaches to New Vaccines, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, September.

PUBLICATIONS

1. **Williams, J. C., and M. H. Vodkin.** 1987. Metabolism and genetics of Chlamydias and Rickettsias. *J. Clin. Microbiol.* (In Press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA305755	30 Oct 87	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	H. TERM	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	139
b. CONTRIBUTING			LA	
c. CONTRIBUTING	None			

11. TITLE (Precede with Security Classification Code) (U) In Vitro Effect of Hemorrhagic Fever Viruses on Endothelial Cells

12. SUBJECT AREAS 0613 Microbiology; 0605 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 12	87 06	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE | EXPIRATION

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
87	1.0	158
*88		

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Medical Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Lewis R M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7655
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: L	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Endothelial Cells; (U) Coagulation Factors; (U) Hemostasis; (U) Hemorrhagic Fever Viruses (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Devise an in vitro model to determine the pathogenesis of the hemostatic derangement produced by hemorrhagic fever viruses. This work will ultimately benefit military personnel exposed to these viruses either in endemic areas or because of their use in biological warfare.

24. (U) Endothelial cell cultures utilizing human, bovine, and hybrid cells will be infected with hemorrhagic fever viruses and the effects on these cells, particularly with regard to hemostatic functions, will be determined.

25. (U) 8610 - 8709 Endothelial cell cultures infected with Pichinde virus are deficient in their response to prostaglandin activators. Hepatoma (HEPG2) cells and monocyte-like (U937) cells, which have been established in the laboratory because of their importance for coagulation. have been shown to allow viral infection. U937 cells, after differentiation by phorbol ester, produce 10-fold more Rift Valley fever virus than controls. Studies to define the mechanism of this phenomenon are underway. In addition, a system is being developed to measure the integrity of cell-cell adhesion for endothelial cells. This system will be used to evaluate the effects of immune mediators and viruses on cell junctions.

*This work will be continued under Accession Number DAOG1519.

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PROJECT NO. 3A161101A91C: Independent Laboratory In-House Research

WORK UNIT NO. 91C-LA-139: In Vitro Effect of Hemorrhagic Fever Viruses on Endothelial Cells

PRINCIPAL INVESTIGATOR: R. M. Lewis, Ph.D.

BACKGROUND

The United States Army Medical Research Institute of Infectious Diseases conducts an active medical defense program on several viral hemorrhagic fever viruses of biological warfare potential and natural disease threats in endemic areas. Mortality rates of 70% to 90% are not uncommon in some outbreaks in which the patients undergo massive hemorrhages in the tissues and intestines.

Human endothelial cells line blood vessels. Beside providing a smooth surface for blood flow, the endothelium exerts numerous biochemical controls over both blood clotting enzymes and platelets. If the mechanisms of these cells were altered in disease, serious bleeding abnormalities could result. The initial thrust of this research was to establish cultures of endothelial cells in the laboratory and to measure the biochemicals important for maintenance of proper blood flow. This model would allow us to determine the direct effect of hemorrhagic fever viruses on endothelial cells and to measure the indirect effects by immune mediators, which may be altered during infection by these viruses.

Endothelial cell cultures infected with Pichinde virus were shown to be deficient in their response to prostaglandin activators. Hepatoma (HEPG2) cells and monocyte-like (U937) cells, established in the laboratory because of their importance for coagulation, have been infected with a number of viruses of interest. U937 cells, after differentiation by phorbol ester, produce 10-fold more Rift Valley fever virus than untreated cells. Studies to define the mechanism of this phenomenon are underway.

SUMMARY

This study has achieved the technical goals previously set. A system is being developed to measure the integrity of cell-cell adhesion for endothelial cells and will be used to evaluate the effects of immune mediators and viruses on cell junctions. We anticipate that important information about the effects of hemorrhagic fever viruses on these cells will be obtained.

PRESENTATIONS

1. **Lewis, R. M., P. B. Jahrling, B. Y. Griffin, and T. M. Cosgriff.** 1987. The effects of hemorrhagic fever virus infection on endothelial cells. Presented at the XIth International Congress on Thrombosis and Haemostasis, Brussels, Belgium, July.

2. **Lewis, R. M., J. Morrill, C. J. Peters, and T. M. Cosgriff.** 1987. U937 cell differentiation and replication of Rift Valley hemorrhagic fever virus. Presented at the XIth International Congress on Thrombosis and Haemostasis, Brussels, Belgium, July.

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1. **Lewis, R. M., T. M. Cosgriff, B. Y. Griffin, J. Rhoderick, and P. B. Jahrling.** 1987. Immune serum increases arenavirus replication in monocytes. Submitted to *J. Gen Virol.*

2. **Lewis, R. M., T. M. Cosgriff, C. J. Peters, and J. C. Morrill.** 1987. Differentiation of a human monocytic cell line associated with increased production of Rift Valley fever virus by infected cells. *J. Med. Virol.* 23:207-215.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA306147	30 Oct 87	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
10 Dec 86	H. TERM	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	LA	140
b. CONTRIBUTING				
c. CONTRIBUTING	None			

11. TITLE (Precede with Security Classification Code) (U) Cloning of Military Relevant Toxin Genes for Novel Vaccine Development

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 12	88 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		87	1.0	158
c. TYPE	d. AMOUNT	*88		
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Smith, L A
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7211
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Baksi, K
MILITARY/CIVILIAN APPLICATION: L	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Alcaide, C

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense;(U) Lab Animals; (U) Cloning; (U) Vaccines; (U) Snakes; (U) Toxin; (U) Genes;(U) Arachnids;(U)RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) The objectives of this research are to clone, sequence, and express the genes coding for snail conotoxins; scorpion toxins; and the snake toxins, myotoxin, cobrotoxin, cardiotoxin, crotoxin, taipoxin, and bungarotoxins. The knowledge gained is expected to lead directly to the development of novel vaccines for these toxins to protect U.S. Army personnel in foreign areas.

24. (U) Three approaches have been or will be used in the cloning of the above toxin genes: (1) synthetic gene cloning, (2) cDNA cloning, and (3) genomic cloning. The genes will be inserted into a suitable vector (plasmid) and transformed into *Escherichio coli*. Cloned DNA will be analyzed with oligonucleotide probes homologous to toxin gene sequences and the DNA, subsequently, sequenced. The products of the clones will be examined immunologically and the genes will be manipulated into producing maximum quantities of products for vaccine development. Finally, site-specific mutagenesis will be employed at the molecular level to produce non-toxic, antigenic proteins.

25. (U) 8610 - 8709 In 1987, we report (1) the cloning and sequencing of a gene encoding myotoxin A from *Crotalus virdis virdis*, (2) the cloning and sequencing of a gene encoding an alpha scorpion neurotoxin from *Androctonus australis* Hector, and (3) the expression of the alpha scorpion toxin cDNA gene in monkey cos cells.

*This work will be continued under Accession Number DA0G1519.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3A161101A91C: Independent Laboratory In-House Research

WORK UNIT NO: 91C-LA-140: Cloning of Military Relevant Toxin Genes for Novel Vaccine Development

PRINCIPAL INVESTIGATOR: L. A. Smith, Ph.D.

ASSOCIATE INVESTIGATORS: P. Bougis, Ph.D.
C. Alcaide, Ph.D.
K. Baksi, Ph.D.

BACKGROUND

Wounds from poisonous animals are an occupational hazard in both the agricultural and fishing industries in many parts of the world. For example, in Asia, more than 10,000 deaths from snake bites are reported annually. A more serious consideration beyond the epidemiological aspects of poisonous stings and bites is the possibility that an adversary could use recombinant DNA technology to clone the gene(s) coding for the toxin protein(s) from animal venoms and produce large quantities of the toxin as a biological warfare weapon. The chemical nature of low molecular weight, protein toxins is such that they make ideal candidates for cloning.

Recombinant DNA technology is the most efficient way to produce sufficient amounts of these toxins for vaccine development. This technology provides a powerful set of tools with which to manipulate DNA molecules, and allows investigators to design and produce organisms of different strains which will produce the desired proteins in large quantities.

The objectives of this study are to clone, sequence, and express the genes coding for the toxic factors from snail, snake, and scorpion venoms, with the hope that this may lead to the development of novel vaccines.

SUMMARY

Purified toxins and antibodies against the purified toxins have been prepared for all the toxins described below.

Snail conotoxin. A synthetic gene for conotoxin was prepared on the Biosearch DNA synthesizer. The gene was inserted into pUC8 vector and cloned into *Escherichia coli*. The gene will now be cloned into an *E. coli* secretory expression vector and its expression studied.

Rattlesnake myotoxin A. A synthetic gene for myotoxin A was prepared on the Biosearch DNA synthesizer. The gene was inserted into pUC8 and cloned into *E. coli*. This gene was then inserted into a ß-lactamase, secretory expression vector and subcloned into *E. coli*. The product(s) from this system

are presently being analyzed by various techniques, such as sodium dodecyl -polyacrylamide gel electrophoresis and Western blot analyses. In addition to the cloning of a synthetic gene, mRNA was isolated from the glands of *Crotalus virdis virdis*. A cDNA library was generated from this mRNA. One cDNA that hybridized to oligonucleotide probes prepared to myotoxin A was sequenced. Expression studies are in progress at Bionetics Research, Inc.

Cobra snake cardiotoxin, cobrotoxin, and PLA2. High molecular weight DNA was prepared from the liver of *Naja naja atra*, and a genomic library was constructed in lambda fix. Cloned DNA hybridizing to oligonucleotide probes prepared to cardiotoxin and cobrotoxin are presently being analyzed by restriction mapping and nucleotide sequencing.

Scorpion toxins. Messenger RNA was prepared from the glands of *Androctonus australis* Hector. A cDNA library was generated with the mRNA. Ten clones were isolated that hybridized to oligonucleotide probes prepared to AaHII. Two clones were chosen and sequenced. Both clones contained AaHII sequences. One of the clones was truncated and the other was a full-length cDNA gene. The gene has been inserted into a vector compatible for expression in monkey *Cos* cells. Expression studies of the monkey cells were performed at Bionetics Research, Inc.; preliminary evidence suggests that the AaHII toxin is expressed and transported into the culture medium. Results were based on immunoassays. Northern and Southern blots also yielded supportive data indicating that transfection of the recombinant DNA into the monkey cells was successful. Western blot analysis, radioimmunoassays, binding studies, bioassays, and amino-acid and end-group analysis experiments are in progress.

PRESENTATION

1. **Smith, L.A.** 1987. Cloning of low molecular weight protein toxins for vaccine development. Presented at the Satellite Symposium on Neurotoxins at the International Society on Neurotoxins, La Guaria, Venezuela, May-June.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA307174	2. DATE OF SUMMARY 30 Oct 87	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 10 Dec 86	4. KIND OF SUMMARY H. TERM	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	141
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) Molecular Approaches to Alphavirus Vaccines

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
85 04	89 01	DA	C. In-House

17. CONTRACT/GRANT
a. DATE EFFECTIVE | EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
87	1.0	122
*88		

19. RESPONSIBLE DOD ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases
b. ADDRESS (include zip code)
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c. NAME OF RESPONSIBLE INDIVIDUAL
Huxsoll, D L
d. TELEPHONE NUMBER (include area code)
301-663-2833

20. PERFORMING ORGANIZATION
a. NAME
Virology Division, USAMRIID
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Fort Detrick, MD 21701-5011
c. NAME OF PRINCIPAL INVESTIGATOR
Dalrymple, J M
d. TELEPHONE NUMBER (include area code)
301-663-2665
f. NAME OF ASSOCIATE INVESTIGATOR (if available)
g. NAME OF ASSOCIATE INVESTIGATOR (if available)

21. GENERAL USE FIC
MILITARY/CIVILIAN APPLICATION L

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) Vaccines; (U) Alphavirus; (U) RA I; (U) Recombinant DNA; (U) Monoclonal Antibodies; (U) Cross-protection

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) The objectives of this research are to: a) develop the ability to rapidly identify DNA sequences in structural protein coding genes of alphaviruses and related virus variants, b) define the major antigenic determinants of alphavirus pathogens of military medical importance and prepare diagnostic reagents, c) evaluate the feasibility of incorporating different viral genes or gene segments into a vaccine virus vector, and d) evaluate the potential prophylactic value of broadly reactive alphavirus immunogens derived from recombinant DNA technology.

24. (U) Investigate alphaviruses that are antigenically closely related. Lymphocyte hybridomas will be prepared, and specific antibodies to determinants associated with neutralization and/or animal protection will be emphasized. Genes coding for the structural proteins of selected alphaviruses will be cloned and sequenced. Sequences conserved among alphaviruses will be used for rapid sequence analysis of different isolates and virus mutations. Sequences will be inserted into vaccinia virus as a vector for the expression of potentially protective immunogens.

25. (U) 8610 - 8709 Lymphocyte hybridomas producing monoclonal antibodies to 3 alphaviruses, Chikungunya (CHIK), Mayaro (MAY), and O'Nyong-nyong (ONN) have been prepared and protective antibodies located by using an infant-mouse, passive-protection system. Molecular clones (cDNA) have been obtained for 2 strains of CHIK as well as ONN and MAY viruses and sequencing is in progress. Clones containing the virion structural protein genes were identified by a synthetic oligonucleotide probe representing conserved alphavirus 3' sequence. Identification and expression of gene sequences reflecting protective antigenic determinants is the next logical extension of this research.

*This work will be continued under Accession Number DAOG1522.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE

PROJECT NO. 3A161101A91C: Independent Laboratory In-House Research

WORK UNIT NO. 91C-00-141: Molecular Approaches to Alphavirus Vaccines

PRINCIPAL INVESTIGATOR: J. M. Dalrymple, Ph.D.

BACKGROUND

The problem under investigation involves the identification of antigenic determinants on various alphaviruses of military medical importance and their incorporation into a common expression vehicle by recombinant DNA technology. A successful result would be the development of a replicating, polyvalent vaccine capable of conferring protection against many related pathogens belonging to the same genus or family. Proof of the concept and feasibility of the methods employed should allow application to other viral families containing numerous pathogens.

SUMMARY

Lymphocyte hybridomas producing monoclonal antibodies to three alphaviruses: Chikungunya (CHIK), O'Nyong Nyong (ONN), and Mayaro (MAY), were prepared in large numbers, but few, or none in the case of MAY, were capable of neutralizing the virus. A second fusion of isolated and structural polypeptides of CHIK has increased the number of neutralization-positive monoclonal antibodies. We will perform similar fusions with ONN and MAY viral antigens. We developed a mouse-protection assay using peripheral inoculation of infant mice (6- to 12-days old). Using this system, we showed numerous, passively adminstered, monoclonal antibody-containing, ascitic fluids to be capable of protecting against an otherwise lethal infection with CHIK virus. These data clearly identified protective determinants separate from those involved in in-vitro neutralization and provided us with the immunological tools necessary to identify the specific peptide sequence(s) responsible.

Molecular cDNA cloning of the structural protein genes of three alphaviruses has resulted in extensive libraries that encompass (hopefully!) the entire coding region of interest. Restriction mapping of ONN clones (obtained via USAMRDC contract support) suggests that virtually the entire genome has been cloned. Sequencing the cDNA clones representing the structural protein genes is in progress. Precise identification of gene location for CHIK and MAY cDNA clones is lacking, but sequencing and mapping studies are in progress. Once sequencing for precise identification and location are complete, subcloning gene segments and entire genes into expression vectors will be employed to identify DNA loci coding specifically for protective determinants.

Even though sequencing is not yet completed, ONN clones will be subcloned into vaccinia vectors to examine any potential problems with alphavirus protein expression in the recombinant vaccinia virus system. If successful, subgenomic regions will be similarly expressed and the products examined for their ability to induce protection from lethal viral challenge. Comparable gene regions from other pathogenic viruses (identified by hybridization of homologous sequences) will also be expressed to examine the feasibility of polyvalent alphaviral vaccines.

PRESENTATION

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APPENDIX A

PUBLICATIONS BY

UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

FY 87

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APPENDIX B

CONTRACTS, GRANTS, MIPRS, AND PURCHASE ORDERS IN EFFECT

UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

FY 87

CONTRACT NUMBER	TITLE, INVESTIGATOR, INSTITUTION
DAMD-17-84-C-4245	Study of Antigenic Structures of Botulinum Neurotoxin. B. Das Gupta, University of Wisconsin, Madison, WI.
PO-84-PP-4845	Expression of Immunogenic Virus Proteins in Eukaryotic Vector Systems. J. Hay, Uniformed Services University of the Health Sciences, Bethesda, MD.
DAMD-17-84-C-4130	Molecular Basis of Paralytic Neurotoxin Action on Voltage-Sensitive Sodium Channels. W. A. Catterall, University of Washington, Seattle, WA.
DAMD-17-85-C-5009	Development of General Antisera for Trichothecenes. C. E. Cook, Research Triangle Institute, Chemistry and Life Sciences Group, Research Triangle Park, NC.
DAMD-17-85-C-5071	Chemical Preparation Laboratory for IND Candidate Compounds, E. M. Schubert, Pharm-Eco Laboratories, Inc., Simi Valley, CA.
DAMD-17-86-C-6019	Enzyme Immunoassay for T-2 Tetraol (SBIR 85.II). P. H. Duquette, Bio-Metric Systems, Inc., Eden Prairie, MN.
DAMD-17-85-C-5199	Identification and Selective Acquisition of Chemicals and Drugs for Antiviral Chemotherapy. E. L. Stephen, Technassociates, Inc., Rockville, MD.
DAMD-17-85-C-5224	Diagnosis and Management of Trichothecene Toxicosis in the Swine Model. W. B. Buck, University of Illinois, Urbana, IL.

DAMD-17-86-C-6075	Mechanism of Action of Low Molecular Weight Toxins in the Cardiovascular System. W. T. Woods, University of Alabama, Birmingham, AL.
DAMD-17-85-C-5202	Preparation and Characterization of Antiparalytic Shellfish Poison Poly and Monoclonal Antibodies for Development of Identification and Prophylaxis Techniques. P. H. Duquette, Bio-Metric Systems, Inc., Eden Prairie, MN.
DAMD-17-85-C-5241	Pathophysiology and Toxicokinetic Studies of Blue-green Algae Intoxication in the Swine Model. V. Beasley, University of Illinois, Urbana, IL.
DAMD-17-85-C-5167	Use of Recombinant DNA Techniques for the Production of a More Effective Anthrax Vaccine. D. L. Robertson, Brigham Young University, Provo, UT.
DAMD-17-85-C-5212	Genetic and Physiological Studies of *Bacillus anthracis* Related to Development of an Improved Vaccine. C. B. Thorne, University of Massachusetts, Amhearst, MA
DAMD-17-85-C-5226	Rift Valley Fever Virus: Molecular Biologic Studies of the M Segment RNA for Application in Disease Prevention. M. Collett, Molecular Genetics, Minnetonka, MN.
DAMD-17-85-C-5283	Functional Consequences of Chemical Modification of the Saxitoxin Binding Site on Neuronal Sodium Channels. B. K. Krueger, University of Maryland, Baltimore, MD.
DAMD-17-85-C-5171	Characterization of the *P. brevis* Polyether Neurotoxin Binding Component in Excitable Membranes. D. G. Baden, University of Miami, Coral Gables, FL.
DAMD-17-85-C-5204	Metabolism, Mass Spectral Analysis and Mode of Action of Trichothecene Mycotoxins. C. J. Mirocha, University of Minnesota, St. Paul, MN.

DAMD-17-85-C-5232	Genetically-engineered Poxviruses and the Construction of Live Recombinant Vaccines. E. Paoletti, New York Department of Health and Health Research, Albany, NY.
DAMD-17-85-C-5276	Development of Systems for Delivery of Antiviral Drugs, W. Shannon, Southern Research Institute, Birmingham, AL.
DAMD-17-85-C-5285	Therapeutic Approaches to the Treatment of Botulism. L. L. Simpson, Jefferson Medical College, Philadelphia, PA.
DAMD-17-86-C-6044	Enhancement of Antiviral Agents Through the Use of Controlled Release Technology. T. R. Tice, Southern Research Institute, Birmingham, AL.
DAMD-17-85-C-5282	Alphavirus Epitopes of Vaccine Relevance. G. A. Cole, University of Maryland, College Park, MD.
DAMD-17-85-C-5266	Human Hybridomas for Exotic Antigens. M. Cohn, The Salk Institute for Biological Studies, La Jolla, CA.
DAMD-17-85-C-5274	Chemical Synthesis *Coxiella burnetii* Lipopolysaccharides: Structural Characterization, Chemical Synthesis and Immunogen. V. N. Reinhold, Harvard School of Public Health, Boston, MA.
DAMD 17-85-C5280	Mechanism of Action of Tetanus Toxin. M. Klempner, New England Medical Center Hospitals, Boston, MA.
MIPR 85-MM-5511	Preparation and Structural Analysis of Toxins, and Modeling of Toxins, Antibody Binding Sites and Antiviral Drugs. K. B. Ward, Washington, DC.
DAMD-17-86-C-6041	Synthesis of Nucleoside Analogues with Potential Antiviral Activity against Negative Strand RNA Virus Targets. R. D. Walker, Birmingham University, Birmingham, England.

DAMD-17-86-C-6002	Synthesis of Nucleoside Mono- and Dialdehydes as Antiviral Agents. J. P. Neenan. Rochester Institute of Technology, Rochester, NY.
DAMD-17-86-C-6001	Rare 2-Substituted Purine Nucleosides. V. Nair, University of Iowa, Iowa City, IA.
DAMD-17-86-C-6012	Chiral Acyclic Nucleosides: Potential Broad Spectrum Antivirals. E. Abushanab, University of Rhode Island, Kingston, RI.
DAMD-17-86-C-6011	Synthesis Laboratory for USAMRIID Selection Panel. J. A. Secrist, III, Southern Research Institute, Birmingham, AL.
DAMD-17-85-C-5274	Drug Development against Viral Diseases of Military Importance. J. A. Secrist, II. Southern Research Institute, Birmingham, AL.
DAMD-17-86-C-6047	Detoxication of Mycotoxins and Other Toxins and Compounds of Military Interest. A. Meister, Cornell University Medical College, New York, NY.
DAMD-17-86-C-6161	A Core Facility for the Study of Neurotoxins of Biological origin. L. L. Simpson, Jefferson Medical College, Philadelphia, PA.
DAMD-17-86-C-6017	Studies of Infection and Dissemination of Rift Valley Fever Virus in Mosquitoes. W. S. Romoser, Ohio University, Athens, OH.
DAMD-17-86-C-6017	Enterotoxins: Synthetic Peptide Approach to Study of Structure/Function and Immune Properties. H. M. Johnson, University of Central Florida, Gainesville, FL.
DAMD-17-86-C-6055	Cloning Sequencing and Structural Manipulation of the Enterotoxin D and E Genes from *Staphylococcus aureus*. J. J. Iandola, Kansas State University, Manhattan, KS.
MIPR 86-MM-6502	Rapid Screening and Structural Characterization of Biological Toxins. M. Ross, Naval Research Laboratory, Washington, DC.

DAMD-17-86-G-6011	Hemorrhagic Fever with Renal Syndrome (HFRS) (Korean Hemorrhagic Fever). H. W. Lee, Korea University College of Medicine, Seoul, Korea.
DAMD-17-86-C-6162	Development of New Immunogens and a Controlled Release Delivery System for Oral Immunization against Staphylococcal Enterotoxin B. T. R. Tice, Southern Research Institute, Birmingham, AL.
DAMD-17-86-C-6110	Development of Synthetic Immunizing Agents against Staphylococcal Enterotoxins. A. K. Judd, SRI International, Menlo Park, CA.
DAMD-17-86-G-6002	Double Blind, Placed Controlled Clinical Trial of Ribavirin Therapeutic Efficacy in the Treatment of Epidemic hemorrhagic Fever. C. -M. Hsiang, Hubei Medical College, Wuhan, Peoples' Republic of China.
DAMD-17-86-C-6118	Biology of Immunomodulators. J. D. Gangemi, University of South Carolina, Columbia, SC.
DAMD-17-86-C-6154	Evaluation of Immune Response Modifying Compounds Utilizing Virus-Specific Human T Lymphocyte Clones. M. Cohn, Georgetown University, Washington, DC.
DAMD-17-86-C-6117	Efficacy and Mode of Action of Immune Response Modifying Compounds against Alphaviruses and Flaviviruses. P. S. Morahan, Pennsylvania College of Medicine, Philadelphia, PA.
DAMD-17-86-C-6121	Screening of Immunoenhancing Drugs with Antiviral Activity against Members of the Arena-, Alpha-, and Adenoviridae. P. A. LeBlanc, University of Alabama, Birmingham, AL.
DAMD-17-86-C-6166	Effects of Immunomodulatory Drugs on T Lymphocyte Activation and Function. C. Tsoukas, Scripps Clinic and Research Foundation, La Jolla, CA.

DAMD-17-86-C-6043	Neurotoxin Binding Site on the Acetylcholine Receptor. T. L. Lentz, Yale University, New Haven, CT.
DAMD-17-86-C-6056	Mechanisms of Action of Clostridial Neurotoxins on Dissociated Mouse Spinal Cord Neurons in Cell Culture. G. Bergey, University of Maryland at Baltimore, MD.
DAMD-17-86-C-6134	Biosystematics of *Aedes (Neomelaniconion)*. T. J. Zavortink. University of San Francisco, San Francisco, CA.
PO-86-PP-6307	Acute T-2 Intoxication Physiologic Consequences and New Therapeutic Approaches. G. Feuerstein, Uniformed Services University of the Health Sciences, Bethesda, MD.
DAMD-17-86-C-6064	Structure/Function Studies of Presynaptic Neurotoxins. P. B. Sigler, University of Chicago, Chicago, IL.
DAMD-17-86-C-6061	Crotoxin: Structural Studies, Mechanism of Action and Cloning of its Gene. I. I. Kaiser, University of Wyoming, Laramie, WY.
DAMD-17-86-C-6059	An In Vitro System for Studying Presynaptically Acting Neurotoxins. B. D. Howard, Regents of the University of California, Los Angeles, CA.
DAMD-17-86-C-6060	Synthesis and Testing of Tetrodotoxin and Batrachotoxin Antagonists. L. Toll, SRI International, Menlo Park, CA.
DAMD-17-86-C-6062	Receptor Binding and Membrane Transport of Botulinum Toxins. J. R. Dankert, University of Central Florida, Gainesville, FL.
DAMD-17-86-C-6057	Development and Testing of an In Vitro Assay for Screening of Potential Therapeutic Agents Active Against Sodium Channel Neurotoxins. G. B. Brown, University of Alabama, Birmingham, AL.
DAMD-17-86-C-6058	Mass-Screening of Curarimimetic Neurotoxin Antagonists. J. Schmidt, State University of New York, Albany, NY.

DAMD-17-86-C-6160	Mechanism of the Presynaptic Neurotoxin Tetanus Toxin. T. B. Rogers, The University of Maryland, Baltimore, MD.
DAMD-17-86-C-6063	Structure-Function Relationship of Hydrophyiidae Postsynaptic Neurotoxins. A. Tu, Colorado State University, Fort Collins, CO.
DAMD-17-86-C-6120	Development of Methods for Carrier-Mediated Targeted Delivery of Antiviral Compounds Using Monoclonal Antibodies. M. I. Dawson, SRI International, Menlo Park, CA.
DAMD-17-86-C-6013	Research in Drug Development Against Viral Diseases of Military Importance (Biological Testing). W. Shannon, Southern Research Institute, Birmingham, AL.
DAMD-17-86-C-6119	Combination Chemotherapy Using Immune Modulators and Antiviral Drugs Against Togaviruses and Bunyaviruses. S. Baron, Medical Branch, University of Texas, Galveston, TX.
DAMD-17-86-C-6028	Determination of the In Vitro and In Vivo Activity of Compounds Tested Against Punta Toro Virus. Ro Sidwell, Utah State University, Logan, UT.
DAMD-17-86-C-6107	Research in Drug Development for Therapeutic Treatment of Neurotoxin Poisoning: Studies of Conotoxins. R. Almquist, SRI International, Menlo Park, CA.
DAMD-17-87-C-7007	The Mechanism of Action of Ribavirin on Bunyavirus Infected Cells. J. L. Patterson. Children's Hospital Corporation, Cincinatti, OH.
DAMD-17-86-C-6169	Colonization and Containment of *Hyalomma marginatus* Rufipes for Studies on Transmission of Congo-Crimean Hemorrhagic Fever. D. E. Sonenshine, Old Dominion University Research Foundation, Norfolk, VA.
DAMD-17-86-C-6042	Drug Development Against Viral Disease (Biological Testing). G. Tignor. Yake University, New Haven, CT.

PO-86-PP-6811	Dermorphin as a Behavioral and Autonomic Modulator. G. Feuerstein, Uniformed Services University of the Health Sciences, Bethesda, MD.
PO-86-PP-6813	The Regulation of a Post-Translational Peptide Acetyltransferase: Strategies for Selectively Modifying the Biological Activity of Neural and Endocrine Peptides. W. R. Millington, Uniformed Services University of the Health Sciences, Bethesda, MD.
PO-86-PP-6814	Regulatory Peptides: Behavioral and Neurochemical Effects. A. H. Barrett, Uniformed Services University of the Health Sciences, Bethesda, MD.
DAMD-17-87-C-7069	Diagnosis and Prevention of Infection by Phlebotomus Fever group Viruses. D. H. L. Bishop, Natural Environmental Research Council, Swindon, U.K.
DAMD-17-86-C-6239	Research, Development, and Delivery of Second Generation Fiber Fluorescent Immunoassay Instruments. T. R. Glass, ORD, Cambridge, MA.
DAMD-17-86-G-6016	Epidemiology and Epizootiological Investigations of Hemorrhagic Fever Viruses in India. P. M. Tukei, Virus Research Center (KMRI), Nairobi, Kenya.
DAMD-17-86-G-6032	Epidemiological and Epizootiological Investigation of Filoviruses in the Central African Republic. A. J. Georges, Pasteur Institute, Paris, France.
DAMD-17-86-C-6173	Immunological Techniques for Detection of Fungal and Dinoflagellate Toxins. F. S. Chu, University of Wisconsin, Madison, WI.
DAMD-17-86-C-6234	Synthetic Vaccines for the Control of Arenavirus Infections. M. Buchmeier, Scripps Clinic and Research Foiundation, La Jolla, CA.
DAMD-17-87-C-7090	Active Antitoxic Immunization Against Ricin Using Synthetic Peptides. A. K. Judd, SRI International, Menlo Park, CA.

DAMD-17-87-C-7007	In Vitro and In Vivo Measurement of Percutaneous Penetration of Low Molecular Weight Toxins of Military Interest. B. W. Kemppainen, Auburn University, Al.
DAMD-17-87-C-7111	Interferon Inducers Against Infectious Diseases. J. Bello. Roswell Park Memorial Institute,Buffalo, NY.
DAMD-17-87-C-7101	Epidemiology of Hantavirus Infectious in Baltimore. J. E. Childs, Johns Hopkins University, Baltimore, MD.
DAMD-17-87-C-7140	Potential Vaccine for Anthrax. R. Doyle, University of Louisville, Louisville, KY.
DAMD-17-87-C-7005	Immunologic Approach to the Identification of Vaccines to Various Toxins. T. Chanh. Southwest Foundation for Biomedical Research, San Antonio, TX.
DAMD-17-87-C-7114	Development of a Toxic Knowledge System. H. L. Trammel, University of Illinois, Urbana-Champaign, IL.
DAMD-17-87-C-7019	Freshwater Cyanobacteria (Blue-Green) Toxins: Isolation and Characterization. W. Carmichael, Wright State University School of Medicine, Dayton, OH
DAMD-17-87-C-7002	Dinoflagellate Toxins Responsible for Ciguatera Food Poisoning. D. M. Miller, Southern Illinois University, Carbondale, IL.
DAMD-17-87-C-7014	Production of Antigens and Antibodies for Rapid Diagnosis of Arbovirus Diseases. R. E. Shope, Yale University, New Haven, CT.
DAMD-17-87-C-7001	Binding Assays for the Quantitative Detection of *P. brevis* Polyether Neurotoxins in Biological Samples and Antibodies as Therapeutic Aids. D. G. Baden, University of Miami, Miami, FL.
DAMD-17-87-C-7155	Mode of Action of Membrane Perturbing Agents: Snake Venom Cardiotoxins and Phospholipases A. J. Fletcher, Hahnemann University, Philadelphia, PA.

PO-87-PP-7809	Marine Toxins: Automatic Toxicology and Therapeutic Strategies. G Feuerstein, Uniformed Services University of the Health Sciences, Bethesda, MD.
DAMD-17-87-C-7093	Toxin Production and Immunoassay Development. I. Palytoxin. D. C. Vann, Hawaii Biotechnology Group, Inc., Aica, Hawaii.
DAMD-17-87-C-7154	Expression of Yellow Fever Antigens and Infectious Virus from Cloned cDNA. C. Rice, Washington University School of Medicine, St. Louis, MO.
DAMD-17-87-G-7009	3Rd European Workshop on bacterial Protein Toxins. F. J. Fehrenbach, Robert Koch Institute, Berlin, West Germany.
DAMD-17-87-C-7137	Peptide Transport Through the Blood-Brain Barrier. W. M Pardridge, Regents of the University of California, Los Angeles, CA.
DAMD-17-87-G-7003	Ecology and Epidemiology of Crimean-Congo Hemorrhagic Fever Virus Transmission in the Republic of Senegal. J. P. Digoutte, Institute Pasteur de Dakar, Senegal.
DAMD-17-87-C-7123	Research Pathology and Special Techniques Support Services. W. C. Hall, Pathology Associates, Inc., Ijamsville, MD.
PO-87-PP-7852	The 3-D Structures of some Diarrheal Causing Bacterial Toxins. M. Sax, Veterans Administration, Iowa City, IA.
DAMD-17-87-C-7110	Determinants of Infectivity of Pathogens in Vector Ticks. A. Spielman, Harvard University, Boston. MA.
PO-87-PP-7803	Investigation on Toxins and Venoms by Novel MS Techniques. T. Krishnamurthy, Chemical Reseach and Development Center, Aberdeen Proving Ground, MD.
DAMD-17-87-G-7027	Conference on Mycotoxins and Phycotoxins, June 1987. B. B. Jarvis, University of Rhode Island, Kingston, RI.

PO-87-PP-7853	Microvascular Physiologic and Anatomic Responses of the Guinea Pig to Experimental Arenavirus Infection. M. Katz, Veterans Administration. Iowa City, IA.
DAMD-17-87-G-7019	Seroepidemiological Survey for Congo-Crimean Hemorrhagic Fever and Hantaan Virus. A. Antoniades, Aristotelian University of Thessaloniki, Thessaloniki, Greece.
PO-87-PP-7825	Immunoassay Procedures for Fiber Optic Sensors. F. S. Ligler, Naval Research laboratory, Washington, DC.
DAMD-17-87-C-7257	Mechanisms of Action of Low Molecular Weight Toxins in the Cardiovascular System. W. T. Woods, University of Alabama, Birmingham, AL.
DAMD-17-87-G-7023	3-Day Symposium on Neurotoxins as Tools in Neurochemistry. J. O. Dolly, Imperial College of Science and Technology, La Guaira, Venezuela.
DAMD-17-87-C-7233	T Cell Responses to Arenavirus Infections. G. A. Cole, University of Maryland, College Park, MD.
DAMD-17-87-C-7188	Neurotoxin and Epitope Structural Studies. D. S. Hunt, University of Virginia, Charlottesville, VA.
DAMD-17-87-C-7259	Molecular Strategy for the Construction of a Genetically Engineered Vaccine for Venezuelan Equine Encephalitis Virus. R. E. Johnston, Raleigh, NC.
PO-87-PP-7829	Development and Evaluation of Supercritical Fluid Chromatography (SFC) and SFS-Mass Spectrometry for Analysis of Trichothecenes, Marine Toxins and Neurotoxins. R. D. Smith, U. S. Department of Energy, Richland, WA.
PO-87-PP-7835	1987 U. S. Summer Faculty Research and Engineering Program. G. G. Outterson, U. S. Army Research Office, Research Triangle Park, NC.

PO-87-PP-7858	Development and Evaluation of Immunomodulators of Hemopoietic and Immunologic Mechanisms. G. G. Tsoukos, Uniformed Services University of the Health Sciences, Bethesda, MD.
PO-87-PP-7857	Collaborative Research Program on Seafood Toxins: II. S. W. Page, U. S. Food and Drug Administration, Washington, DC.

APPENDIX C

PRESENTATIONS
(Abstracts)

UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

FY 87

- **Anderson, A. O.** 1987. Direct transdiaphragmatic traffic of peritoneal macrophages to the lung. Presented at the Ninth International Conference on Lymphatic Tissues and Germinal Centres in Immune Reactions, Oslo, Norway, August.

- **Anderson, A. O.** 1987. Mucosal priming alters pathogenesis of Rift Valley fever. Presented at the Ninth International Conference on Lymphatic Tissues and Germinal Centres in Immune Reactions, Oslo, Norway, August.

- **Anderson, A. O.** 1987. Endocytic stripping of ligands from migrant lumphocyte surfaces in high endothelial venules (HEV). Presented at the Ninth International Conference on Lymphatic Tissues and Germinal Centres in Immune Reactions, Oslo, Norway, August.

- **Anderson, A. O., O. Wood, L. F. Fischbach, and M. L. M. Pitt.** 1987. Mucosal priming alters pathogenesis of Rift Valley fever. Presented at the Annual Meeting of the Federation of American Societies for Experimental Biology and Medicine, Washington, D. C., March.

- **Anderson, G. W., Jr., and J. F. Smith.** 1986. Rift Valley fever virus (RVFV) maturation at the plasma membrane of rat hepatocytes as revealed by immunoelectron microscopy. Presented at the Society of Alumni, Johns Hopkins University, for the student research paper competition, Baltimore, MD, November.

- **Anderson, G. W., Jr., and J. F. Smith.** 1986. Rift Valley fever virus (RVFV) maturation at the plasma membrane of rat hepatocytes as revealed by immunoelectron microscopy. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

- **Balady, M. A.** 1987. Primary CTL response in mice immunized with vaccinia viral vectors expressing Rift Valley fever viral glycoproteins. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

Barerra Oro, J. G., C. MacDonald, A. L Kuehne, B. C. Mahlandt, J. Spisso, J. M. Meegan, C. J. Peters, and H. W. Lupton. 1986. Ensayos iniciales en humanos de una vacuna atenuada contra la fiebre hemorrhagica Argentina (Candid #1). B. Aislamiento de virus y respuesta serologica.

Barrera Oro, J. G., K. T. McKee, A. L Kuehne, B. C. Mahlandt, F. Cole, and H. W. Lupton. 1986. Vacuna de virus Junin atenuado Candid #1. Immunogenicidad y capacidad protectora de animales de laboratorio. Presented at the Second Annual Congress of Virology, Cordoba, Argentina, October.

Battles, J. K., M. S. Collett, and J. M. Dalrymple. 1986. Comparison of Rift Valley fever virus isolates and variants by nucleic acid sequencing of G2 envelope glycoprotein epitope genes. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

Battles, J. K., and J. M. Dalrymple. 1987. Nucleotide sequence comparison of geographic variants of Rift Valley fever virus and antibody escape mutants. Presented at the Annual Meeting of the American Society for Virology, Chapel Hill, NC, May-June.

Bunner, D. L. 1987. Recent experiences in Gulf war casualty management. Presented at the Vesicant Workshop, Columbia, MD, February.

Bunner, D. L., and E. R. Morris. 1987. Cell membrane effects of T-2 mycotoxin in L-6 myoblasts. Presented at the Annual Meeting of the Society for Toxinology, Singapore, China, June.

Cooke, S. D., and L. L. Rhodes, Jr. 1987. Visualization of the rabbit glottis and endotracheal intubation. Presented at the National Capital Area Branch, American Association for Laboratory Animal Science, Washington, D. C., August.

Cosgriff, T. M., P. G. Canonico, L. Hodgson, D. Parrish, and T. Chapman. 1987. Ribavirin: studies of the effects of the antiviral drug on platelet function. Presented at the XIth International Congress on Thrombosis and Haemostasis, Brussels, Belgium, July.

Creasia, D. A. 1987. Respiratory tract immunization of rats with ovalbumin. Presented at a workshop at the International Symposium for Inhalation Toxicology, Germany, March.

Creasia, D. A., and S. R. Davio. 1987. Passive immunization against saxitoxin administered via respiratory tract. Presented at the Conference on Natural Toxins from Aquatic and Marine Environments, Woods Hole, MA, August.

Downs, M. B., M. A. Ussery, and P. G. Canonico. 1987. Immunocytochemical studies of the kinetics of peripheral Japanese encephalitis virus (JEV) infection in C57 black mice. Presented at the Annual Meeting of the American Association of Anatomists, Washington, D. C., May.

Faris, R. M., F. M. Feinsod, T. A. Morsy, A. El Misiry, M. S. Gabal, A. J. Shah, and S. El Said. 1986. Population-based study of human cutaneous leishmaniasis in Northern Sinai. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

Hasty, S. E., A. L. Schmaljohn, D. S. Stec, and J. M. Dalrymple. 1986. Comparison of geographic isolates of Sindbis virus. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

Huggins, J. W., C. M. Hsiang, T. M. Cosgriff, M. Y. Guang, J. I. Smith, Z. A. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, X. E. Gui, K. W. Yuan, T. M. Zhang, and H. W. Lee. 1987. Intravenous ribavirin therapy of hemorrhagic fever with renal syndrome (HFRS). Presented at the XVI Pacific Science Congress, Seoul, Korea, August.

Ivins, B. E., G. B. Knudosn, S. L. Welkos, and D. J. LeBlanc. 1986. *Bacillus anthracis* as donor and recipient in filter mating transfer of Tn*916*. Presented at the Tenth Annual Meeting of the Mid-Atlantic Extrachromosomal Elements, Virginia Beach, VA, October.

Kende, M., and P. G. Canonico. 1987. Treatment of experimental viral infection with immunomodulators. Presented at the International Symposium on Immunomodulators and Nonspecific Host Defense Mechanisms Against Microbial Infections, West Berlin, West Germany, May.

Kende, M., M. Contos, W. Rill, and P. G. Canonico. 1987. Optimization of liposomal carriers for antiviral therapy. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

Kende, M., W. Rill, J. Smith, M. Derevjanik, and P. G. Canonico. 1987. Oral efficacy of an acridine derivative (AD) immunomodulator against Rift Valley fever virus (RVFV) infection in mice. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

Kenyon, R. H., J. Barerra Oro, C. MacDonald, J. Meegan, and C. J. Peters. 1987. Human lymphocyte transformation assay for Junin virus (Argetnine hemorrhagic fever). Presented at the Annual Meeting of the American Society for Virology, Chapel Hill, NC, May-June.

Kenyon, R. H., and C. J. Peters. 1987. Actions of complement on Junin virus. Presented at the Symposium on Hemostatic Impairment in Viral Hemorrhagic Fevers, Leesburg, VA, May.

Kenyon, R. H., and C. J. Peters. 1987. Expression of Junin antigens on the surface of infected Vero cells. Presented at the Symposium on Hemostatic Impairment in Viral Hemorrhagic Fevers, Leesburg, VA, May.

Kenyon, R. H., and C. J. Peters. 1986. Immune response of guinea pigs to Junin virus (JV). Presented at the Second Annual Congress of Virology, Cordoba, Argentina, October.

Kenyon, R. H., and C. J. Peters. 1986. Actions of complement on Junin virus (JV) and on JV-infected cells. Presented at the Second Annual Congress of Virology, Cordoba, Argentina, October.

Kenyon, R. H., D. Pifat, P. Jahrling, R. Condie, J. Maiztegui, D. Brantley, and C. J. Peters. 1987. Use of heterologous antibody and F(ab')2 portions for protecting guinea pigs infected with Junin virus (JV). Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

Knauert, F. K., A. D. King, and B. D. Kelly. 1986. An *in situ* hybridization assay detecting Rift Valley fever virus (RVFV) RNA in mouse liver sections. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

Knudson, G. B., and M. J. Turell. 1987. Mechanical transmission of *Bacillus anthracis* by the stable fly, *Stomoxys calcitrans.* Presented at the 87th Annual Meeting of the American Society for Microbiology, March.

LeDuc, J. W. 1987. Epidemiology of viral hemorrhagic fevers. Presented at the Symposium on Hemostatic Impairment in Viral Hemorrhagic Fevers, Leesburg, VA, May.

LeDuc, J. W., and A. Antoniades. 1987. Severe hemorrhagic fever with renal syndrome in Greece: clinical and epidemiological characteristics and isolation of the etiological agent. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

Leppla, S. H., A. M. Friedlander, and E. M. Cora. 1987. Proteolytic activation of anthrax toxin bound to cellular receptors. Presented at the Third European Workshop on Bacterial Protein Toxins, Uberlingen, West Germany, June-July.

Lewis, R. M., P. B. Jahrling, B. Y. Griffin, and T. M. Cosgriff. 1987. The effects of hemorrhagic fever virus infection on endothelial cells. Presented at the XIth International Congress on Thrombosis and Haemostasis, Brussels, Belgium, July.

Lewis, R. M., J. Morrill, C. J. Peters, and T. M. Cosgriff. 1987. U937 cell differentiation and replication of Rift Valley hemorrhagic fever virus. Presented at the XIth International Congress on Thrombosis and Haemostasis, Brussels, Belgium, July.

Liu, C. T., and C. J. Peters. 1987. Improvement of cardiovascular functions with a sulfidopeptide leukotriene antagonist in a guina pig model of hemorrhagic fever. Presented at the Annual Meeting of the American Society for Pharmacology and Experimental Therapy, Honolulu, HI, August.

Liu, C. T., C. J. Peters, and G. G. Pinter. 1987. Bilateral cervical lymph collection and measurement of capillary permeability to albumin in strain 13 guinea pigs. Presented at the American Physiology Society meeting, Federation of the American Society for Experimental Biology and Medicine, Washington, D.C., April.

MacDonald, C., A. M. Briggiler, K. T. McKee, F. Feinsod, J. Morrill, P. Gibbs, C. J. Peters, J. I. Maiztegui, and J. G. Barerra Oro. Ensayos iniciales en humanos de una vacuna viva atenuada contra la fiebre hemorrhagica Argentina (Candid #1). A. Semilogia y laboratorio clinico. Presented at the Second Annual Congress of Virology, Cordoba, Argentina, October.

MacDonald, C., K. McKee, C. Peters, T. Cosgriff, F. Feinsod, and J. Barerra Oro. 1987. Initial clinical assessment of humans inoculated with a live-attenuated Junin virus (JV) vacine. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

Malinowski, F. J., G. F. Meadors, H. Ramsberg, P. Stopa, and T. Kziazek. 1987. Safety and efficacy of a new chikungunya virus vaccine: human phase II double-blind, placebo-controlled trial. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

Meegan, J., J. LeDuc, S. Garcia Franco, and J. Maiztegui. 1986. Rapid diagnostic methods to detect Junin virus infection. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

Meegan, J. M., J. LeDuc, S. Garcia Franco, and J. I. Maiztegui. 1986. An ELISA test for IgG and IgM antibodies to Junin virus. Presented at the Second Annual Congress of Virology, Cordoba, Argentina, October.

- **Mereish, K. A., R. W. Wannemacher, Jr., D. L. Bunner, and T. Krishnamurthy.** 1987. Composition of *Microcystis aeruginosa* strain 7820 toxin. Presented at the Conference on Natural Toxins from Aquatic and Marine Environments, Woods Hole, MA, August.

- **Middlebrook, J. L.** 1987. Transport of protein toxins across membranes. Presented at the Annual Meeting of the Biochemistry Society, Leicester, England, April.

- **Middlebrook, J. L.** 1987. Immunological relationships between snake phospholipase A2 neurotoxins. Presented at the International Conference on Biophysics, Jerusalem, Israel, August.

- **Middlebrook, J. L., and I. I. Kaiser.** 1987. A neutralizing monoclonal antibody to crotoxin. Presented at the Annual Meeting of the Society for Toxinology, Singapore, China, June.

- **Miura, G. A., K. A. Bostian, T. W. Geisbert, J. D. White, and J. G. Pace.** 1987. In vivo and in vitro effects of microcystin -LR on rat liver. Presented at the Conference on Natural Toxins from Aquatic and Marine Environments, Woods Hole, MA, August.

- **Morrill, J. C., M. S. Collett, and J. M. Dalrymple.** 1986. Evaluation of experimental Rift Valley fever virus vaccines in pregnant sheep. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

- **Nuzum, E. O., C. A. Rossi, E. H. Stephenson, and J. W. LeDuc.** 1986. Aerosol infectivity of Hantaan and related viruses in outbred Wistar rats. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

- **Peters, C. J.** 1987. Viral hemorrhagic fevers: significance, diagnosis, and therapy. Presented at the Third Annual Clinical Virology Symposium, Clearwater Beach, FL, April.

- **Pitt, M. L. M., and A. O. Anderson.** 1987. Direct transdiaphragmatic traffic of peritoneal macrophages to the lung. Presented at the Annual Meeting of the Federation of American Societies for Experimental Biology and Medicine, Washington, D. C., March.

- **Pifat, D. Y., R. H. Kenyon, A. Sanchez, and C. J. Peters.** 1986. Identification of protective epitopes on Junin virus using monoclonal antibodies. Presented at the Second Annual Congress of Virology, Cordoba, Argentina, October.

- **Poli, M. A.** 1987. Metabolism and excretion of the brevetoxin PbTx-3 in rats and isolated rat hepatic cells. Presented at the Annual Meeting of the Society for Toxinology, Singapore, China, June.

- **Robinson, N. A., W. L. Thompson, and J. G. Pace.** 1987. Effect of microcystin-LR on bile acid uptake in isolated rat hepatocytes. Presented at the Conference on Natural Toxins from Aquatic and Marine Environments, Woods Hole, MA, August.

- **Schmaljohn, C. S.** 1987. Coding strategy and expression of the M and S genome segments of Hantaan virus. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

- **Schmaljohn, C. S., A. L. Schmaljohn, and J. M. Dalrymple.** 1987. Coding strategy and expression of the M and the S genome segments of Hantaan virus. Presented at the XVIth Pacific Science Congress, Seoul, Korea, August.

- **Smith, J., L. Hodgson, M. Dombalagian, and A. Komoriya.** 1987. Induction of neutralizing antibodies to Rift Valley fever virus with synthetic peptides. Presented at the International Congress of Virology, Edmonton, Alberta, Canada, August.

- **Smith, L. A.** 1587. Cloning of low molecular weight protein toxins for vaccine development. Presented at the Satellite Symposium on Neurotoxins, International Society on Neurotoxins, La Guaria, Venezuela, May-June.

- **Templeton, C. B., and D. A. Creasia.** 1987. Changes in arterial blood gases, temperature and plasma lactate concentrations in rats exposed to intravenous or aerosol T-2 mycotoxin. Presented at the Tenth Annual Conference on Shock, Montreal, Canada, June.

- **Templeton, C. B., and M. A. Poli.** 1987. Cardiorespiratory effects of brevetoxin PbTx-2 in conscious rats. Presented at the Annual Meeting of the Society for Toxinology, Singapore, China, June.

- **Thomas, W. J., J. G. Barerra Oro, G. R. French, F. Cole, A. Shelokov, C. J. Peters, and H. W. Lupton.** 1986. Vacuna de virus Junin atenuado Candid #1. Estabilizadores y estabilidad del lote 85-1. Presented at the Second Annual Congress of Virology, Cordoba, Argentina, October.

- **Thompson, W. L., M. B. Allen, and K. Bostian.** 1987. The effects of microcystin on monolayers of primary rat hepatocytes. Presented at the Annual Meeting of the Society for T.xinology, Singapore, China, June.

- **Trusal, L. R., and D. C. Hybner.** 1987. Release of enzymes from neuronal cells exposed to taipoxin from *Oxyuranus scutellatus*. Presented at the Annual Pacific-Asian Congress on Plant, Animal, and Microbial Toxins, Singapore, China, June.

- **Turell, M. J., and C. L. Bailey.** 1986. Effect of environmental temperature on the replication, dissemination, and transmission of Rift Valley fever virus by *Aedes fowleri*. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

- **Turell, M. J., R. M. Tamariello, and C. L. Bailey.** 1986. Reduced recovery of Rift Valley fever virus associated with assay of larval pools. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

- **Vodkin, M. H., and J. C. Williams.** 1987. Cloning of an antigen from *Coxiella burnetii* and its homology to polypeptides in other bacteria. Presented at the USAMRIID-Cornell Symposium, Cornell University, Ithaca, NY, May.

- **Vodkin, M. H., and J. C. Williams.** 1987. Cloning and expression of a major antigen gene of *Coxiella burnetii* homologous to a protein in *Mycobacteria* and *Escherichia coli*. Presented at Modern Approaches to New Vaccines, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, September.

- **Waag, D., and J. C. Williams.** 1987. Identification of suppressor cells induced following injection of C57BL/10 ScN mice with phase I *Coxiella burnetii* whole cells. Presented at the American Physiology Society Annual Meeting, Federation of American Societies for Experimental Biology, Washington, D. C., April.

- **Watts, D. M., M. A. Ussery, and C. J. Peters.** 1987. Effects of ribavirin on the replication of Crimean-Congo hemorrhagic fever virus. Presented at the Annual Meeting of the American Society for Virology, Chapel Hill, NC, May-June.

- **Wannemacher, R. W., Jr., D. L. Bunner, and R. E. Dinterman.** 1987. Comparison of the toxicity and absorption of algal toxins and mycotoxins after dermal exposure in guinea pigs. Presented at the Annual Meeting of the Society for Toxicology, Singapore, China, June.

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GLOSSARY

AcNPV	*Autorgrapha californica* nuclear polyhedrosis virus
AIDS	Acquired immune deficiency syndrome
A/J	Mouse strain
ATCC	American tissue culture collection
BARD	United States-Israel Bi-National Agricultural Research and Development Fund
BMN	Blood monocytes
BSA	Bovine serum albumin
BW	Biological warfare
CCHF	Crimean-Congo hemorrhagic fever
cDNA	Complementary DNA
cGMP	Cyclic guanidine monophosphate
CHIK	Chikungunya virus
CME	Chloroform-methanol extract
CMR	Chloroform-methanol-extracted residue
CNS	Central nervous system
CRM	Cross-reacting material
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
EC	Electrochemistry
EF	Edema factor
ELISA	Enzyme-linked immunosorbent assay
EPO	Erythropoietin

FA	Fluorescent antibody
FDA	Food and Drug Administration
G1, G2	Glycoproteins
GC-MS	Gas chromatography-mass spectroscopy
HEV	High endothelial venules
HFRS	Hemorrhagic fever with renal syndrome
HIV	Human immunodeficency virus
HPLC	High-pressure liquid chromatography
HSRRB	Human Subject Research Review Board
HSP	Heat shock promoter
ICLC	Polyriboinosinic: polyribocytidylic acid
IFA	Indirect fluorescent antibody
Ig	Immunoglobulin
IgG	Immunoglobulin G
IND	Investigational New Drug
ISC	Immune suppressive complex
KHF	Korean hemorrhagic fever
KLH	Keyhole limpet hemocyanin
LCV	Large cell variant
LCMV	Lymphocytic choriomeningitis virus
LD_{50}	Median lethal dose
LF	Lethal factor
LIB	Liberia
LPS	Lipopolysaccharides

LT	Lymphocyte transformation
MAB	Monoclonal antibodies
MAY	Mayaro (Virus)
mRNA	Messenger RNA
MUA	Memorandum of Understanding and Agreement
NCI	National Cancer Institute
NIH	National Institutes of Health
NMR	Nuclear magnetic resonance
ONN	O'Nyong-nyong (Virus)
PA	Protective antigen
PFU	Plaque-forming units
PLA2	Phospholipase A2
PRC	People's Republic of China
PRN	Plaque-reduction neutralization
QAE	QAE chomatography
R	Resistant
RIA	Radioimmunoassay
RNA	Ribonucleic acid
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
S	Susceptibility
SCV	Small cell variant
SL	Sierra Leone
TDL	Thoracic duct lymphocytes

VEE	Venezuelan equine encephalomyelitis
WCI	Whole cells phase I

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