APPENDIX E

Status Report on Research on Serological Testing for Detection of L. *Tropica* Infection

STATUS REPORT

RESEARCH ON SEROLOGICAL TESTING FOR DETECTION OF *L. TROPICA* INFECTION

Introduction

In June 1997 researchers at the Portland VA Environmental Hazards Research Center (PEHRC) reported preliminary results from experimental *L. tropica* serological testing conducted by the University of Washington, Infectious Disease Research Institute and Corixa Corporation on 102 serum samples (this number later increased to 200). These samples were from Gulf War veterans participating in a case-control study at PEHRC. Approximately 2/3 of the samples provided were identified by the PEHRC as cases. The working case definition developed by PEHRC is defined as follows:

> *A "Case" is a respondent who must have at least one of the following signs or symptoms:*
>
> - *Muscle/joint pain*
> - *Cognitive changes including memory loss, confusion, inability to concentrate, mood swings, and/or somnolence*
> - *Diarrhea*
> - *Skin or mucous membrane lesions*
> - *Unexplained fatigue*
>
> *Onset must have been during or after deployment to the Gulf. Symptoms must have persisted for one month or longer and occurred during the three-month period preceding a clinical evaluation. To be selected as a case, a veteran's symptoms could not be explained by a diagnosable medical condition.*

Controls and cases were identified from responses to a mailed questionnaire. Cases and controls received a medical workup at the Portland VAMC.

Dr. Steve Reed of the Infectious Disease Research Institute and Corixa Corporation performed the serological tests using an experimental procedure. Of the 102 samples provided 10 had elevated antibody titers to a synthetic *L. tropica* antigen. Of these 10, 6 were cases, 2 were controls, and 2 were neither (the latter individuals were originally classified as cases by the questionnaire, but their symptoms resolved at the time of physical examination). The number of cases and controls that tested positive on this experimental serological test was approximately equal to the overall proportion of cases and controls in the total set of 102 serum samples tested.

The observation that 6 of the 10 samples testing positive were cases raised concerns that *L. tropica* might be more prevalent than originally thought among Gulf War veterans (only 37 clinically-proven cases of leishmaniasis (viscertropic and cutaneous)

had been identified in Gulf War veterans prior to these findings). This preliminary finding also raised concern over the safety of the U.S. blood supply.

To resolve these questions and concerns several meetings of a subcommittee of the Research Working Group (RWG) of the Persian Gulf Veterans Coordinating Board (PGVCB) were held in Washington, DC and in Portland, OR between July and September 1997 (Attachment 1 is a list of meeting dates and sites). Besides members of the RWG, experts on *L. tropica,* and blood supply safety and monitoring from inside and outside the government were consulted (Attachment 2 is list of all participants in these meetings). The overall purpose of the meetings was to:

- Evaluate the validity of Corixa Corporation's experimental serological assay;
- Evaluate the interpretability of the preliminary PEHRC data;
- Establish an understanding of the implications, if any, to veterans and the general public;
- Establish a set of action items.

Description of Experimental Serological Test

The test developed by Dr. Reed and his associates utilizes a cloned genomic fragment encoding a portion of a 210-kDa *L. tropica* protein. The recombinant protein encoded by this fragment, Lt1r4, contains a 33-amino acid repeat that has been shown to react with serum from Gulf War veterans with viscerotropic leishmaniasis. Prior to the application of this serological test on the PEHRC subjects, Dr. Reed and his group tested this antigen for detection of *L. tropica* antibodies in serum of Gulf War veterans with known *L.* tropica infection (these veterans were among the 37 with known *L. tropica* infection). ELISAs (enzyme-linked immunosorbent assay) were performed on the sera of eight Gulf War veterans with culture-proven viscerotropic leishmaniasis (seven of whom had *L. tropica* demonstrated as the causative agent; the *Leishmania* from the other patient was not available in sufficient quantity) and four patients with laboratory confirmed but culture-negative viscerotropic leishmaniasis. The researchers used as comparison groups 10 serum specimens from patients from Turkey with cutaneous leishmaniasis, presumably due to *L. tropica*, and 18 presumably uninfected American Red Cross blood donors. The mean reactivity to the Lt1r4 antigen was significantly greater for sera from patients with viscerotropic as well as cutaneous leishmaniasis than for the control group. The mean absorbance for the normal sera was, however, above 0.4, which is higher than would be optimal, and Dr. Reed's group is looking at different antigens that might be less reactive with normal sera. No hybridization was observed between the Lt1r4 insert and *Trypanosoma cruzi* or with *Leishmania* that causes new world cutaneous leishmaniasis. However, the insert did hybridize with *L. donovani*, the agent that causes visceral leishmaniasis.

Preliminary Results of PEHRC Study

During an August 15, 1997 meeting in Portland, OR, Dr. Linda McCauley (PEHRC) reviewed data on the veteran population used in this part of the Center study. These veterans served in the Gulf between 8/90 and 8/91. The researchers identified 3533 potentially eligible veterans. They excluded 75 who had been previously tested and

22 who were deceased. From this number they chose a random sample of 1751, and mailed questionnaires to 1651. Of these, 675 sent back the response, 454 were invited for examinations, and 227 examinations were completed (as of August 1997). The testing of volunteers included extensive lab panels, a complete physical exam (8 hours, including 4 hours of neurological/computer tests), and rheumatology evaluation.

Of the 200 Portland subjects for whom serologic testing was complete as of August 15, 1997, approximately two-thirds were defined as cases according to the working case definition described above. The other third were controls. Eighteen (of 200) subjects tested positive on the serologic test. Positive was defined as an absorbence on the ELISA of greater than three standard deviations above a control sample of Red Cross donors. Twelve of the 18 positives (or two-thirds) were cases. This proportion is the same as the proportion of cases in the overall group of 200. None of the persons testing positive on the serologic screen had current symptoms indicative of leishmaniasis.

Dr. Dennis Bourdette of the PEHRC reviewed the clinical findings in the sero-positive cases. A panel of four physicians reviewed these findings. The findings were unremarkable. Most veterans self-reported exposure to sand and half of them self-reported insect bites. 6/18 reported GI symptoms in the Gulf. 7/12 reported current cognitive complaints, 8/12 chronic fatigue, 6/12 unexplained muscle/joint problems, and 2/12 unexplained GI symptoms. There was no case with clinical spleno/hepatomagaly, and none with intermittent fever, typical signs of viscerotropic leishmaniasis.

Report on Serologic Testing in the CDC Pennsylvania Air National Guard Study

Also at the August 15, 1997 meeting in Portland OR, Dr. William Reeves (CDC) reviewed the experience with this serologic test in 154 vets who served in the Gulf War with the Pennsylvania 193rd National Guard (40% of the unit). During most of their deployment, the unit was stationed at the Riyahd airport in Saudi Arabia. One of these veterans was a confirmed case of viscerotropic leishmaniasis (one of the 37 previously identified), so there definitely was exposure, although sand fly activity is minimal during the winter, when the war took place. Of the 154, 16.9% were Lt1r ELISA positive, but only 4.5% were positive by an immunofluorescense assay used by CDC and there was no correlation between the results of the two tests. The veterans were 4.5% seropositive to the sand-fly fever virus, and again there was no cross-correlation (historically, there has been a co-segregation of *Leishmania* and sand fly fever virus). Severity of illness in these veterans was not correlated with Lt1r ELISA or to the season of deployment. There was no correlation to any of a list of specific symptoms. All the blood work was the same, although the eosinophil counts appeared to be higher in the Lt1r ELISA positive veterans. Serum chemistries were the same except for serum glucose. There were no liver or kidney findings.

Conclusions Regarding the Portland/Corixa Testing

As a result of the meeting in Portland, OR it was concluded that Dr. Reed=s serologic test for *L. tropica* should be viewed as extremely preliminary and as a research hypothesis-generating tool. At this point, there is no correlation between the result on this test and Gulf War veterans' current symptoms or medical status. The subcommittee

concluded that the new data did not provide compelling new evidence that Gulf War veterans are ill as a result of a *L. tropica* infection causing viscerotropic leishmaniasis.

At the July 9, 1997 meeting in Washington, DC concern was expressed by the subcommittee that an experimental serologic test was administered to subjects at PEHRC for which the investigators could not provide to the subjects reliable information on the clinical significance of a positive test. By the August 15, 1997 meeting in Portland the PEHRC researchers had revised their informed consent forms to reflect the use of this research tool. Subjects who test positive now are asked to return for a repeat serologic test and are referred for an examination by an infectious disease specialist who was not affiliated with the PEHRC.

Given the findings of the visit, the subcommittee agreed that it was appropriate for the Portland group to continue to administer the serologic test in order to provide additional data to assess the test's usefulness.

Blood Supply Safety

At the July 9, 1997 meeting the subcommittee discussed the safety of the US blood supply in light of the evidence known at that time. The subcommittee, which included HHS/CDC, HHS/FDA, VA and DoD participants, concluded that:

1. The evidence from the PEHRC study, though suggestive, could not be used to conclude that there was significant infection among Gulf War veterans. A determination of infection prevalence would have to wait for improved antibody testing.

2. There has been no additional evidence of infection among the Gulf War veteran population resulting from new diagnoses of leishmaniasis. The total number of documented cases of leishmaniasis (37) has not changed in several years. Walter Reed Army Medical Center and the Centers for Disease Control and Prevention are able to track diagnoses of leishmaniasis because they are the sole sources of the main treatment, pentavalent antimony. No new case of leishmaniasis (identified as a result of a call for treatment for a Gulf War veteran) among Gulf War veterans have emerged from either Walter Reed or CDC.

3. Based on historical records, there is no evidence, beyond five documented cases in England, of blood-blood transmission of leishmaniasis in the worldwide population.

4. Lastly, current prohibitions on blood donation by individuals with any symptoms of illness would serve to screen out symptomatic cases of leishmaniasis.

The general conclusion of the subcommittee of the RWG was that current evidence does not support a ban on donations of blood from Gulf War veterans. The results of the meeting in Portland, OR served to affirm these conclusions.

Next Steps

Despite reassurance that there is no threat to the US blood supply and that there is no new compelling evidence to suggest *L. tropica* is a cause of Gulf War veterans' illnesses, the subcommittee of the RWG met on September 10, 1997 to consider what additional steps are needed.

As a result of the interest in developing diagnostic tests for *L. tropica* infection, both for Gulf War veterans and for veterans of future deployments, the subcommittee recommended the development of a reference panel of antisera to *Leishmania* species. The purpose is to facilitate an interagency effort to address the following immediate and long-term **goals**.

1. To determine whether infection by *L. tropica* is a cause of unexplained chronic illnesses in Gulf War veterans.

    a. If such an association is found, to determine the prevalence of *L. tropica* in Gulf War veterans and to identify individuals with visceral disease who may need treatment.

    b. If such an association is found, to determine whether infection with *L. tropica* is transmissible by blood transfusion.

2. To develop assays that could be used in the future to monitor active duty service personnel and veterans for significant infections by *Leishmania* species.

The subcommittee envisioned a "staged" effort to accomplish the stated goals.

1. Investigation of Available Assays

An effort should be made to identify and characterize serologic assays that could be used to select for further study a group of veterans who potentially harbor visceral infections with *L. tropica*. Although serologic assays may not be highly sensitive for this purpose (due to possible weak antibody responses in persons who develop visceral leishmaniasis), assays specific for antibodies to *L. tropica* could still be used for case finding (as with the PEHRC research).

The subcommittee therefore proposed that a panel of reference positive and negative sera should be created to qualify candidate assays for the purpose of case finding. This provisional panel (herein called a "qualification panel") would be designed to determine, at a crude level (i.e., based on a small number of samples), whether the candidate assay:

a) detects antibodies to *L, tropica*;

b) cross-reacts with antibodies to other *Leishmania* species;

c) generates false-positive reactions with sera from healthy, unexposed individuals; and,

d) generates false-positive reactions in medical conditions that could cause confounding of a serologic test.

The subcommittee recommended a concurrent effort to develop a large panel (herein called a "validation panel") that could be used to accurately determine the sensitivity and specificity of candidate serologic tests for antibodies to *L. tropica* or other *Leishmania* species.

The subcommittee also recommended that support should be given to development of skin tests or other tests for cellular immunity to *Leishmania* as a basis for confirming serologically diagnosed infections or as an alternate strategy for screening persons with an exposure history, an undiagnosed skin lesion or a systemic disease suggestive of leishmaniasis.

2. Case Finding for Intensive Clinical Studies

If a candidate serologic assay is deemed to have useful sensitivity (including reactivity in known cases of *L. tropica*) and adequate specificity based on its performance with the "qualification panel," then the subcommittee proposed that an effort should be undertaken to identify and enroll seropositive veterans in clinical studies to investigate the possibility of *L. tropica*. This study would be on a small scale, focusing primarily on veterans with chronic constitutional symptoms. An example of such a study is the PEHRC effort.

3. Epidemiologic Survey

If case finding reveals detection of *L. tropica* in persons seropositive by the candidate assay, then the working group recommended a broader epidemiologic survey in Gulf War veterans. A decision whether or not to use the same assay in an epidemiologic study would depend on the performance characteristics of the assay both in the clinical study and on testing with the "validation panel." Ideally, supplementary tests (such as Western blot) could be used to improve upon the diagnostic specificity of screening in a large survey.

4. Studies of Blood Recipients

If case finding in veterans were to reveal detection of viscerotropic leishmaniasis in persons seropositive by the candidate assay, the working group recommended that "lookback studies" should be initiated to determine whether recipients of blood components donated by persons harboring *L. tropica* may have contracted the infection from transfusion.

ATTACHMENT 1

SUBCOMMITTEE TO REVIEW STATUS OF LEISHMANIASIS RESEARCH

Dates and Locations of Meetings

July 2, 1997 – Washington, DC (teleconference)

July 9, 1997 – Washington, DC

August 15, 1997 – Portland, OR (Portland VA Environmental Hazards Research Center)

September 10, 1997 – Washington, DC

ATTACHMENT 2

SUBCOMMITTEE TO REVIEW STATUS OF RESEARCH ON LEISHMANIASIS

Participants at Meetings

This is a list of all individuals who participated at one or more of the meetings to discuss leishmaniasis.

Department of Veterans Affairs - Office of Research and Development
John R. Feussner, M.D. Chair – Research Working Group
Timothy R. Gerrity, Ph.D. Chair, Subcommittee

Department of Veterans Affairs – Office of Environmental Hazards and Public Health
Frances Murphy, M.D. – Environmental Agents Service

Department of Veterans Arrairs – Cincinnati VA Medical Center
Gary Roselle, M.D. Director for Emerging Pathogens

Department of Veterans Affairs - Portland VA Environmental Hazards Center
Dennis Bourdette, M.D.
Wendy Johnston, M.D.
Linda McCauley, Ph.D.
Michael Riscoe, Ph.D.
Peter Spencer, Ph.D.

Department of Health and Human Services – Office of the Assistant Secretary for Health
CAPT. Bryan Jones, United States Public Health Service
James Mathews

Department of Health and Human Services – Centers for Disease Control and Prevention
Drue Barrett, Ph.D., National Center for Environmental Health
Mary Chamberland, National Center for Infectious Disease
Rima Khabbaz, Ph.D., National Center for Infectious Disease
Barbara Herwaldt, Ph.D., National Center for Infectious Disease
Thomas Navin, M.D. National Center for Infectious Disease
William Reeves, M.D., National Center for Infectious Disease
Phillip Talboy, National Center for Environmental Health

Department of Health and Human Services - Food and Drug Administration
Paul Aebersold, Ph.D., Center for Biologics and Evaluation Research
Robin Biswas, M.D., Center for Biologics and Evaluation Research
Jay Epstein, M.D., Center for Biologics and Evaluation Research
Richard Kenney, M.D., Center for Biologics and Evaluation Research
Brian Malkin, Office of the Commissioner
Hira Nakhasi, Ph.D., Center for Biologics and Evaluation Research

Curt Scribner, Center for Biologics and Evaluation Research
Ed Tabor, Center for Biologics and Evaluation Research
Robin Billings, Center for Biologics and Evaluation Research

Department of Health and Human Services – National Institutes of Health
Franklin Neva, M.D., National Institute of Infectious and Allergic Diseases
John Ferguson, M.D., Office of Medical Applications of Research

Department of Defense – Office of the Assistant Secretary of Defense/Health Affairs
Patricia Collins
CAPT David Trump

Department of Defense – Office of the Special Assistant for Gulf War Illnesses
Capt Steve Torrey
Kelley Brix, M.D., SRA International

Department of Defense - Walter Reed Army Institute of Research
COL. Sam Martin, Walter Reed Army Institute for Research
MAJ. Jeff Ryan, Walter Reed Army Institute for Research

Department of Defense - Naval Medical Research Institute
CAPT Craig Hyams
LTC. Allan McGill, Naval Medical Research Institute Detachment, Peru

Persian Gulf Veterans Coordinating Board
CDR David Edman
COL Edward Elson
William Kistner

Presidential Advisory Committee on Gulf War Veterans Illnesses
Joseph Cassels, M.D.

University of Washington, Infectious Disease Research Institute and Corixa Corporation, Seattle, Washington
Davin Dillon, Ph.D.
Raymond Houghton, Ph.D.
Steven Reed, Ph.D.

University of Maryland – Department of Medicine
T.E. Woodward, M.D.