INVESTIGATIONS OF CROSS IMMUNITY BETWEEN LEISHMANIA TROPICA (JE--ETC(U))

SEP 79  B E BEACHAM

DAMD17-79-C-9033
Investigations of Cross Immunity Between Leishmania tropica (Jericho) and Leishmania braziliensis in Experimentally Infected Mystromys albacaudatus

Bruce E. Beacham, M.D.

University of Virginia Medical Center
Department of Dermatology
Box 134
Charlottesville, Virginia 22908

DTIC ELECTED
JUN 1 4 1982
S
H

immunophylaxis to cutaneous leishmaniasis

Methods have been outlined for storage and reconstitution of various leishmania strains to be used as a vaccine. Investigations of cross immunity between L. Tropica (Jericho) and L. braziliensis panamensis were made utilizing the African white tailed rat, Mystromys albacaudatus, model. It was established that an ulcerogenic dose of L. Tropica (Jericho) and L. braziliensis (panamensis) was $2 \times 10^6$ promastigotes. Preliminary results indicated that L. tropica (Jericho) infected M. albacaudatus may develop immunity to infection with not only the homologous strain but also against L. braziliensis panamensis.
"Investigations of Cross Immunity Between Leishmania tropica (Jericho) and Leishmania braziliensis panamensis in Experimentally Infected Mystomys albicaudatus"

First Annual Report

Bruce E. Beacham, M.D.

September 1979

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD 17-79-C-9033

University of Virginia
Charlottesville, Virginia 22908

Approved for public release; distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.
ABSTRACT

It has been observed that after recovery from *Leishmania mexicana* infection Rhesus monkeys are resistant to challenge by *Leishmania b. braziliensis* but not *Leishmania b. panamensis* (Lainson and Bray 1966). Since this report a successful human leishmanial vaccine for *L. tropica* has been developed (Naggan, et al., 1970/1972) and an animal model has been described for cutaneous leishmaniasis (1977 American Society of Parasitology Meetings -- Dr. Larry Hendricks). We are investigating whether the immunization of *Mystromys albicaudatus* by *L. tropica* (Jericho strain) adequately protects these animals against *L. b. braziliensis*.

Methods have been outlined for storage and reconstitution of the various leishmania strains which will be used as a vaccine. The strains include *L. tropica* (Jericho), *L. braziliensis* and *L. panamensis*, all of which have been isolated from human cases and cryogenically stored.

Initially we investigated cross immunity utilizing three central experiments:
1. To establish the infective dose of *L. tropica* (Jericho) promastigotes and *L. b. braziliensis* promastigotes needed to infect 50 percent of *Mystromys albicaudatus*;
2. To establish the approximate length of time needed for immunity to develop after initial immunization with *L. tropica* (Jericho) (animals were re-challenged with an homologous strain of *L. tropica* (Jericho) at monthly intervals after self-healing of the initial ulcer);
3. To test the immunogenicity of a variety of dosages of *L. tropica* (Jericho) promastigotes when challenged with *L. b. panamensis* and *L. b. braziliensis* promastigotes.

Thus far, we have established an optimal ulcerogenic dose of *L. tropica* (Jericho) newly isolated strain and *L. b. braziliensis* (panamensis) to be $2 \times 10^6$ promastigotes. The incubation period depends upon varied dosages from an average of 14 days in the case of the highest dose of *L. tropica* (Jericho old strain) to an average of 30 days with .2cc *L. tropica* (Jericho new strain) with lesions ranging from 5mm to 1.5cm, respectively. Preliminary results indicated that *L. tropica* (Jericho new strain) infected *Mystromys albicaudatus* may impart immunity against infection with not only the homologous strain but also against *L. b. panamensis*. 
FOREWORD

The purpose of this report is to bring to attention the results of investigations dealing with possible cross immunity between L. tropica and L. braziliensis panamensis in an animal model. At this point in time, it would appear that there is some preliminary evidence that cross immunity does exist utilizing M. albacaudatus as an animal model. The preliminary nature of the following report must be underscored because animals which have been challenged must be observed clinically for adequate periods and biopsies and cultures must be obtained before refractoriness to challenge can be claimed.

Although the search for a vaccine has been unsuccessful to date, it is hoped that future results from this study may further contribute understanding to the development of such immunotherapy.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences-National Research Council (DHEW Publication No. 78-23, Revised 1978).
TABLE OF CONTENTS

ABSTRACT ................................................................. 4
FOREWORD ................................................................. 5

I. STATEMENT OF THE PROBLEM ..................................... 7
II. BACKGROUND ........................................................... 7
III. APPROACH TO THE PROBLEM .................................... 10
IV. RESULTS WITH DISCUSSION OF RESULTS ....................... 11
V. CONCLUSIONS ........................................................ 13
VI. RECOMMENDATIONS ................................................ 13

LITERATURE CITED ..................................................... 17

List of Tables

TABLE I
Initial inoculation of Mystromys albacaudatus
with L. tropica (Jericho) and L. b. braziliensis for
determination of optimal dose ........................................ 12

TABLE II
Initial inoculation of Mystromys albacaudatus
with L. tropica (Jericho old & new) and L. b.
braziliensis (panamensis) .............................................. 14

TABLE III
Mystromys albacaudatus inoculated with L. tropica
(Jericho) and subsequently challenged with 2x10⁶ L.
b. braziliensis (panamensis) and an homologous
strain of 2x10⁶ promastigotes .......................................... 15

6.
I. STATEMENT OF THE PROBLEM

If suitable experimental animals are successfully vaccinated with promastigotes of *L. tropica* (Jericho) solid immunity will develop to challenge *L. b. braziliensis* or *L. b. panamensis* promastigotes. Part of this hypothesis is supported by the work of Lainson and Bray (1966) as mentioned above and part by the rich history of the use of related species of parasites or species with reduced virulence to prevent disease.

II. BACKGROUND

The use of related species of parasites or species with reduced virulence is a well established form of prevention of disease in man. This method of immunization in parasitic disease to date has been limited to scattered reports of success of zooprophylaxis occurring with malaria, Babesiosis and Trypanosomiasis (Nelson, 1974). These reports demonstrate amelioration or prevention of disease by exposure to heterologous infections of animal origin.

Leishmania investigators, for a considerable length of time, have addressed the antigenic relationships of different species and strains of leishmania — in particular, relationshipsexisting between new- and old-world disease forms (Adler, 1964). Adler and Cunha (1964) demonstrated that patients recovered from typical oriental sores were immune to subsequent challenge with *Leishmania mexicana*. Thus, prior infection with recovery from a nonmetastasizing cutaneous leishmaniasis might provide immunity to other forms of new-world leishmaniasis in man. This hypothesis was confirmed in animals in 1966 by Lainson and Bray who demonstrated that rhesus monkeys recovered from *L. mexicana* infection were refractory to challenge with *L. b. braziliensis* but were easily infected by *L. b. panamensis*. In 1966 Lainson and Shaw reported a human volunteer immune to *L. mexicana* infection but completely susceptible to Panamanian cutaneous leishmaniasis. They concluded that *L. mexicana* and the causative agent of Panamanian cutaneous leishmaniasis were antigenically distinct, thus ruling out the use of *L. mexicana* as a vaccination source for Panamanian cutaneous leishmaniasis.

The above work was reported over ten years ago, but unfortunately no further progress has been made in the development of an effective human vaccine against new-world leishmanial disease. This hiatus can perhaps be explained by: (1) The difficulty encountered in evaluating immunity in humans, and (2) the lack of a suitable animal model which could be adequately immunized without significant metastatic leishmanial disease.

Recent developments suggest that the above two obstacles may be overcome. First, Bayram, et al (1970), reported on the successful vaccination of a small group of young adults in Israel with a new strain of leishmania isolated from humans residing in the Jericho region of Israel. Effective immunity could be produced in approximately four to six weeks after healing.
of the initial cutaneous ulcer with significant reduction in the attack rate of cutaneous old-world leishmaniasis in military personnel stationed in an endemic area (Naggan, et al, 1972). More recently, Kaufman, et al (1978), reported a gradual decline in the rates of takes of inoculations utilizing the same strain of L. tropica as used by Naggan in 1968. In 1968, Naggan reported an 85.7 percent take. In 1975, this rate was reduced to 21.3 percent take. The authors felt that L. tropica tends to lose its virulence after prolonged storage and multiple passages. They demonstrated that using a new strain, isolated just a few months before the vaccination trial was performed, resulted in a greater than 60 percent positive take rate. This loss of virulence secondary to long storage and in multiple passages has been reported in numerous other parasitic strains (Gunders, et al, 1972; Manson-Bahr, 1964; Heyneman, 1971). Adler and Zuckerman (1948) were able to infect volunteers with an L. tropica strain maintained for 22 years although the incubation period of eight months was unusually long. It is also not known whether this phenomenon is very critical in cryogenically stored leishmania strains.

In addition to the above statements, it should also be noted that no significant complications were reported in the vaccinations of approximately 1,200 soldiers with L. tropica (Jericho). It also should be noted that Naggan's results indicate that immunity which was thought to only be acquired after the healing process has commenced may be at least partially acquired as early as three to six weeks after inoculation.

Second, an ideal animal model for the study of cutaneous leishmaniasis has been found (Endricks, 1977). Mystromys albocaudatus is easily infected with conventional ulcer-producing doses of two million promastigotes of L. tropica. These ulcers self-cure in approximately three months and there has been no evidence of metastatic spread of the leishmanial disease. Furthermore, this animal has an average life span of four to five years making it ideal for relatively long-term evaluation of the immunologic status of the immunized and nonimmunized animals.

Because of these two relatively recent developments it would appear that ideal conditions exist to obtain more specific information concerning the cross immunity between old- and new-world leishmaniasis.

The approaches to immunological prophylaxis in protozoal infections can be divided into passive and active immunization:

Passive immunization in protozoal disease has centered around experience with Plasmodium Falciparum malaria in man (Cohen and Sadun, 1976); McGregor and Carrington, 1961). The antibody is directed against the merozoites and prevents the reinvasion of the red blood cell by blocking the attachment of the parasite to the erythrocyte membrane. However, these antibodies are variant-specific and substantial problems were encountered in the development of a vaccination program against malaria (Brown, 1976).

Active immunization has been investigated in protozoal diseases by four methods. (1) The first method, perhaps least acceptable in humans, is
the use of standardized doses of normal infective stages with the development of disease which is terminated by an appropriate antiparasitic drug. (2) The second method, most practical at present, is the use of related species with reduced virulence. (3) The third method is the use of artificially attenuated infective stages. (4) The fourth method is the use of in vitro organisms from which specific antigens may be isolated and used to immunize.

The most desirable approach to the development of a vaccine for humans would be the use of attenuated human strains of leishmania which are antigenically related to L. braziliensis and have reduced virulence. In the event that a solid cross immunity between L. braziliensis and leishmania strains with reduced virulence can be developed utilizing a rodent model, further work utilizing primates and eventually humans could proceed. It would also be appropriate to investigate the immunologic status of one animal model in a more extensive manner utilizing in vitro and in vivo measures of both humoral and cell-mediated immunity.

Cell-mediated immunity and macrophage function significantly influence the degree, course and final outcome of leishmanial infection. Participation of cell-mediated immunity is well documented in various leishmanial animal models, including the guinea pig and mouse (Blewett, et al, 1971; Turk and Bryceson, 1971; Semma and You, 1973; Preston, et al, 1971/1972; Skov and Thoeng, 1974). The degree of effectiveness of cell-mediated immunity may determine the clinical manifestations of the leishmanial disease (Turk and Bryceson, 1971). Disseminated cutaneous leishmaniasis most closely correlates with the lack of effective cell-mediated immunity and the recidivens type of leishmaniasis is characterized by healed disease with only a very few parasitized histiocytes. The role of the macrophage in acquired immunity in leishmanial infection has not been clearly defined. There is also good evidence that the macrophage is not the sole controller of parasite burden in chronically infected animals and most likely acts in conjunction with antibody response to the organism (Herman and Farrell, 1977).

The development of a positive delayed skin test can be correlated with the in vitro production of lymphokines and monokines in the development of blast transformation (Blewett, et al, 1971). It would be useful to establish a correlation between time of vaccination and time of adequate immunity as detected by in vitro cell-mediated measurements such as described above. It was previously thought that immunity would not develop until several weeks or months after the initial ulcer of cutaneous leishmaniasis had healed (Senekjian and Beattle, 1941; Porterman, 1944). However, observations made in Nagar's study (1975) and again in follow-up studies reported by Kourman in 1978 revealed the development of at least partial immunity in soldiers before the beginning of the healing phase of the ulcer. If there is a correlation between the measurement of cell-mediated immunity and refractiveness to infection with cutaneous leishmaniasis, a longer than necessary waiting period prior to entering an endemic area would be obviated.

Additionally, by recording and correlating cell-mediated immune responses in vaccinated diseased animals exposed to various cutaneous leishmanial species, a scale might be constructed which might serve as a guideline
to the prognosis of existing disease or detection of factors associated with
the breakdown in immunity. Since adequate immunization is essential to the
development of a successful vaccine, several other avenues of immunization
might be mentioned. It has been suggested that the use of amastigotes, the
disease producing entity in humans, might be more antigenic than the usual
promastigote form (personal communication). The last avenue open at this
time would be the utilization of irradiated killed promastigotes of L. braziliensis.
Precedence of this exists in malaria with the radiation of attenuated sporozoites
(Musenzerwitz, Vanderberg and Nost, 1969) as well as parasitized erythrocytes
(We1de and Sadun, 1967) in an attempt to develop vaccines and has met with
little success because of resistance secondary to strain variation (Brown, 1976).
Other discouraging results using this approach were reported by Lemma and Cole
(1974) who were unable to induce immunity against L. enriettii in guinea pigs
utilizing irradiated promastigotes of an homologous strain.

Finally, since most of this hypothesis relies on the use of closely
antigenically related species, how does one determine what parasite is causing
disease when challenge may produce a lesion? In the event this problem arises,
there now exists a reliable sensitive rapid means of identification of various
In their preliminary study they were able to consistently differentiate between
Leishmania donovani, Leishmania tropica and Leishmania braziliensis.

III. APPROACH TO THE PROBLEM

We have already determined that $2 \times 10^6$ promastigotes of L. tropica
(Jericho strain) injected intradermally, or even subcutaneously, in a properly
shaved region over the back of Hymenomys albicaudatus will produce an ulcer in
approximately 30 days. This ulcer has been observed to self-heal in approxi-
mately two to three months, at which time the animals are reported to be
refractory to challenge with homologous strains of L. tropica (Jericho)
(personal experience and personal communication). However, as mentioned in
the background section, we have observed that as many as 25 percent of the
initially inoculated animals developed ulcers when challenged with homologous
strains. It should also be noted that these 25 percent developed the smallest
primary lesions after the first inoculation.

In order to maintain the ulcers produced during vaccination, the
area surround the ulcer must be depilated by shaving with a #40 shaving
head, followed by a 30-second massage using a cream depilatory at weekly
intervals.

In order to produce the vaccine which was utilized, it was necessary
to reconstitute cryogenically stored leishmania obtained from Dr. Larry Hendricks
of the Walter Reed Army Institute of Research. The promastigotes were re-
constituted as per the method of Hendricks, et al (1973), and various con-
centrations established after five to six days of growth in 30 percent fetal
calf serum in Schneider's insect media revised.
Our hypothesis was tested in vivo since this is the most direct and practical method. We also utilized various sized groups of animals to (1) establish the infective dose (50) for the L. tropica (Jericho) vaccine and L. braziliensis panamensis inoculant, (2) determine the approximate length of time needed for homologous immunity to develop after initial immunization with L. tropica (Jericho), and (3) define the immunogenicity of a variety of dosages and schedules of vaccinations of L. tropica (Jericho) promastigotes when challenged with L. b. panamensis and L. b. braziliensis.

The precise experiments were:

1. 10 animals inoculated with $0.5 \times 10^6$ L. tropica (Jericho)
   10 animals inoculated with $1 \times 10^6$ L. tropica (Jericho)
   10 animals inoculated with $2 \times 10^6$ L. tropica (Jericho)

   10 animals inoculated with $1 \times 10^6$ L. braziliensis panamensis
   10 animals inoculated with $2 \times 10^6$ L. braziliensis panamensis

2. Sham controls inoculated with vehicle and challenged with L. braziliensis (10 animals).

3. Forty animals inoculated with $2 \times 10^6$ L. tropica (Jericho):
   healed 
   lesions 

   3 months
   without
   lesions 
   challenge
   with $2 \times 10^6$
   L. braziliensis panamensis
   challenge
   with $2 \times 10^6$
   L. braziliensis panamensis

Forty animals inoculated with $2 \times 10^6$ L. tropica (Jericho):

2nd inoculation
$2 \times 10^6$ L. tropica

3 months
healed lesions or none at all
challenge
with $2 \times 10^6$
L. braziliensis panamensis

Additional studies which would be of great interest would be to compare the immunogenicity of newly isolated L. tropica (Jericho) to our older, cryogenically stored material. The newly isolated promastigotes could be obtained from Israel from Dr. Grebliatt of the University of Hadassah.

IV. RESULTS WITH DISCUSSION OF RESULTS

Secondary to various delays in the onset of this project, such as late delivery dates of equipment secondary to gas shortages and the unexpected long healing period of many of the ulcers, only preliminary results are available at this time. Thus far, as depicted in Table I, we have established an
<table>
<thead>
<tr>
<th>Number of Animals</th>
<th>Species and Strain</th>
<th>Inoculum Size</th>
<th>Number Infected</th>
<th>Incubation Period</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td><em>L. tropica</em> (old Jericho)</td>
<td>$5 \times 10^6$</td>
<td>3</td>
<td>5 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>10</td>
<td><em>L. tropica</em> (old Jericho)</td>
<td>$2 \times 10^6$</td>
<td>5</td>
<td>4 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>10</td>
<td><em>L. tropica</em> (old Jericho)</td>
<td>$2 \times 10^7$</td>
<td>7</td>
<td>3 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>10</td>
<td><em>L. tropica</em> (new Jericho)</td>
<td>$2 \times 10^6$</td>
<td>9</td>
<td>3 weeks</td>
<td>12 weeks</td>
</tr>
<tr>
<td>10</td>
<td><em>L. braziliensis panamensis</em></td>
<td>$2 \times 10^6$</td>
<td>8</td>
<td>2 weeks</td>
<td>21 weeks</td>
</tr>
<tr>
<td>10</td>
<td><em>L. braziliensis panamensis</em></td>
<td>$2 \times 10^7$</td>
<td>4</td>
<td>4 weeks</td>
<td>21 weeks</td>
</tr>
</tbody>
</table>
optical ulcerogenic dose of L. tropica (Jerdicho) newly isolated strain and L. brancilleni parasitica to be 3/100 percent rate. Additionally, L. tropica (Jerdicho) old strain (two years old) needed a higher number of preparations to effectively produce a lesion. This most likely represents a statorxy phenomenon which has been described by many investigators. The results also indicate a clear difference between L. tropica (Jerdicho) and L. brancilleni parasitica in reaction of the infection — L. brancilleni parasitica demonstrating a duration of infection from 8 to 12 months longer compared with one to three months with L. tropica (Jerdicho).

As a clear control ten lizards, all inoculated with non-ulcerogenic susceptant lizards, these ten animals were then inoculated with 3 x 10^6 L. brancilleni parasitica after which nine developed ulcers.

Table II presents the initial results of the first inoculation of L. brancilleni parasitica with old and newly isolated strains of L. tropica (Jerdicho). Most of these animals were males because of previous poor results in successfully inoculating females in repeated preliminary studies. A total of 11 animals have been inoculated with L. tropica (Jerdicho) utilizing the old strain in 14 and the new strain in 6. The incubation period depended upon varicel factors from an average of 14 days in the case of the highest dose of L. tropica (Jerdicho old strain) to an average of 3 days with the L. tropica (Jerdicho new strain) with lesions ranging from 3 to 10 days respectively. In general, the new strain needed to need a slightly longer incubation period, have a longer healing time and result in a larger lesion.

Table III perhaps presents the most exciting results concerning gross immunity. Preliminary results indicated that L. tropica (Jerdicho new strain) infected lizards all responded to infection with not only the homologous strain but also against L. brancilleni parasitica. These results, if confirmed with greater numbers, should suggest that a future, more medically significant, experiment would be the use of L. brancilleni parasitica as the challenging agent.

II. CONCLUSION

As one can now see, we have some data primarily in vivo which may support the existence of gross immunity between L. tropica (Jerdicho) and L. brancilleni parasitica. It also is obvious that we need to await the full of the data which will not be available for as long as eight to ten months. The fact that this data will not be available until then is primarily because of the larger than predicted time for ulcer healing.

III. PROTOCOL

The University of Michigan School of Medicine has developed a comprehensive fellowship program with personal support through the Rockefeller Foundation. This program, administered through the Department of Medicine, is actively engaged in basic and clinical research in Southeastern Brazil,
<table>
<thead>
<tr>
<th>Date of Innoculation</th>
<th>Number of Animals</th>
<th>Species and Strain</th>
<th>Innoculum Size</th>
<th>Number Infected</th>
<th>Incubation Period</th>
<th>Duration</th>
<th>Size of Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/79</td>
<td>10</td>
<td><em>L. tropica</em> (old Jericho)</td>
<td>$2 \times 10^7$ pros (0.1ml)</td>
<td>9</td>
<td>20 days</td>
<td>4 wks</td>
<td>0.5 cm</td>
</tr>
<tr>
<td>2/79</td>
<td>4</td>
<td><em>L. tropica</em> (old Jericho)</td>
<td>$2 \times 10^2$ pros (0.1ml)</td>
<td>4</td>
<td>9 days</td>
<td>16 wks</td>
<td>0.5 cm</td>
</tr>
<tr>
<td>2/79</td>
<td>2</td>
<td><em>L. braziliensis panamensis</em></td>
<td>$2 \times 10^3$ pros (0.1ml)</td>
<td>1</td>
<td>14 days</td>
<td>6 mos</td>
<td>1.0 cm</td>
</tr>
<tr>
<td>3/79</td>
<td>20</td>
<td><em>L. tropica</em> (new Jericho)</td>
<td>$2 \times 10^6$ pros (0.1ml)</td>
<td>20</td>
<td>25 days</td>
<td>10-24 wks</td>
<td>1.5 cm</td>
</tr>
<tr>
<td>4/79</td>
<td>25</td>
<td><em>L. tropica</em> (new Jericho)</td>
<td>$2 \times 10^6$ pros (0.2ml)</td>
<td>25</td>
<td>42 days</td>
<td>2-4 mos</td>
<td>1.5 cm</td>
</tr>
<tr>
<td>8/79</td>
<td>11</td>
<td><em>L. tropica</em> (new Jericho)</td>
<td>$2 \times 10^6$ pros</td>
<td>Pending</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3/79</td>
<td>9</td>
<td><em>L. tropica</em> (new Jericho)</td>
<td>$2 \times 10^6$ pros</td>
<td>Pending</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
**TABLE III**

*Trypanosoma albugooides inoculated with *L. tropica* (Jericho) and subsequently challenged with 2x10⁶ *L. braziliensis panamensis* and an homologous strain of 2x10⁶ promastigotes.

<table>
<thead>
<tr>
<th>Originally Infected Type and Strain</th>
<th># of Infected Animals</th>
<th>Challenge Type &amp; Strain</th>
<th># Animals Infected With Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. tropica</em> (old Jericho)</td>
<td>5</td>
<td><em>L. tropica</em> (new Jericho)</td>
<td>1</td>
</tr>
<tr>
<td><em>L. tropica</em> (new Jericho)</td>
<td>5</td>
<td><em>L. tropica</em> (new Jericho)</td>
<td>0</td>
</tr>
<tr>
<td><em>L. tropica</em> (old Jericho)</td>
<td>10</td>
<td><em>L. braziliensis panamensis</em></td>
<td>0</td>
</tr>
<tr>
<td><em>L. tropica</em> (new Jericho)</td>
<td>2</td>
<td><em>L. braziliensis panamensis</em></td>
<td>1</td>
</tr>
</tbody>
</table>
known to be endemic for cutaneous and visceral leishmaniasis. This setting, of course, will provide a source of patients who will provide opportunities for further in vitro and in vivo studies.

The encouraging in vivo results thus far, as well as the possibility for field work in the endemic areas of leishmaniasis, should underscore the importance of the continued support of this work.
LITERATURE CITED


DISTRIBUTION LIST

12 Copies
Director (ATTN: SVM-UNZ-C)
Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington, DC 20012

4 Copies
USA-TEDC (SORD-RMS)
Fort Detrick
Frederick, MD 21701

12 Copies
Defense Technical Information Center (DTIC)
ATTN: DTIC-DDA
Cameron Station
Alexandria, VA 22314

1 Copy
Dean
School of Medicine
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

1 Copy
Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234