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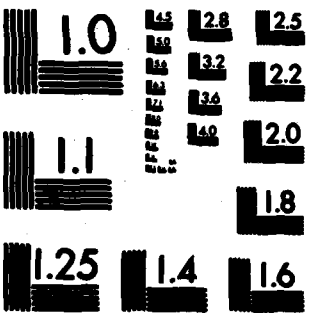

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REPORT #1

ENZYME MINI-TEST FOR FIELD IDENTIFICATION OF LEISHMANIA ISOLATES FROM U. S. MILITARY PERSONNEL

Annual Report

RICHARD D. KREUTZER

15 AUGUST 1983

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-83-C-3119

Youngstown State University
Yoingstown, Ohio 44555

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) ENZYME MINI-TEST FOR FIELD IDENTIFICATION OF LEISHMANIA ISOLATES FROM U. S. MILITARY PERSONNEL		5. TYPE OF REPORT & PERIOD COVERED 15 Mar 1983 - 15 Aug 1983 Annual Report 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Richard D. Kreutzer		8. CONTRACT OR GRANT NUMBER(s) DAMD17-83-C-3119
9. PERFORMING ORGANIZATION NAME AND ADDRESS Youngstown State University Youngstown, OH 44555		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick, Frederick, Maryland 21701-5012		12. REPORT DATE 15 August 1983 13. NUMBER OF PAGES 13
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) N/A		15. SECURITY CLASS. (of this report) N/A 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE N/A

16. DISTRIBUTION STATEMENT (of this Report)
Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)
Approved for public release; distribution unlimited.

18. SUPPLEMENTARY NOTES
N/A

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Leishmania; electrophoresis; identification

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
Certain WRAIR isolates have been characterized for up to 29 enzymes by cellulose acetate electrophoresis (CAE) and were identified as follows: 6 as Leishmania braziliensis braziliensis; 31 as L. b. panamensis; 3 as L. chagasi; 3 as L. mexicana mexicana; 1 as L. m. aristedesi; 6 as diffuse cutaneous leishmaniasis; 1 as L. donovani; 1 as L. m. pifanoi; 6 as not Leishmania; 4 unknown. There was allozyme polymorphism noted among the isolates in certain of these groups, but no attempt was made at this time to associate polymorphism with any specific parameter. Culturing began July, 1983 and attempts, as yet

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20. unsuccessful, have been made to simplify the procedures followed to prepare cultures for CAE. It appears from preliminary study that certain enzymes such as, phosphoglucoisomerase, GPI, mannose phosphate isomerase, MPI, and glutathione reductase, GSR, produce distinctly migrating bands for all new world isolates, and can therefore be used to accurately establish preliminary isolate identification. Preliminary studies on the enzyme systems GPI, MPI and GSR have shown it is feasible that buffer and stain components might be "packaged" which eliminates the need for a mettler type balance and pH meter.

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Summary

Work has progressed toward establishing a mini-test for rapid but accurate identifcation of Leishmania isolated from U. S. military personnel. Certain WRAIR isolates have been characterized for up to 29 enzymes by cellulose acetate electrophoresis (CAE) and were identified as follows: 6 as Leishmania braziliensis braziliensis; 31 as L. b. panamensis; 3 as L. chagasi; 3 as L. mexicana mexicana; 1 as L. m. aristedesi; 6 as diffuse cutaneous leishmaniasis; 1 as L. donovani; 1 as L. m. pifanoi; 6 as not Leishmania; 4 unknown. There was allozyme polymorphism noted among the isolates in certain of these groups, but no attempt was made at this time to associate polymorphism with any specific parameter. Culturing began July, 1983 and attempts, as yet unsuccessful, have been made to simplify the procedures followed to prepare cultures for CAE. It appears from preliminary study that certain enzymes such as, phosphoglucoisomerase, GPI, mannose phosphate isomerase, MPI, and glutathione reductase, GSR, produce distinctly migrating bands for all new world isolates, and can therefore be used to accurately establish preliminary isolate identification. Preliminary studies on the enzyme systems GPI, MPI and GSR have shown it is feasible that buffer and stain components might be "packaged" which eliminates the need for a mettler type balance and pH meter.

Body of Report

Problem, Background, Approach

This is a preliminary report of data collected 15 March 1983 - 15 August 1983.

It has been difficult in the past to obtain fast, accurate identification of Leishmania parasites from U. S. military personnel. The use of electrophoresis for such identification has been encouraging.[1] Cellulose acetate electrophoresis (CAE) has been used to identify WRAIR isolates; isolate pairs with enzyme profiles which were about 75% identical were considered samples from the same species/type and those which were significantly less than 75% identical were therefore samples from different species/types. There were five major groupings among these WRAIR isolates: braziliensis, mexicana, donovani, tropica, hertigi. These major groups could be divided further into subgroups, and three each were reported in the braziliensis and mexicana groups.

The isolates which were 75% identical were not always the same for all enzyme systems tested, therefore there was a certain amount of allozyme polymorphism among isolates in each group. In other organisms than Leishmania this polymorphism has been associated with parameters such as geographic distribution, etc.; then similar associations might likewise become evident in Leishmania once large numbers of isolates were characterized and their histories compared.

Of paramount importance was the development of a mini-test using CAE which would allow the clinician in the field to rapidly but accurately identify the species/type of Leishmania recently isolated from the military patient. This test should be designed so the smallest number of cells and enzymes and effort be needed for accurate identification.

Progress

Detailed CAE data on certain WRAIR isolates have been collected (Tables 1, 2, 3). The data on isolates completed in April (Table 1) and July (Table 2), 1983 have already been sent to Capt. McGreevy. Among the April isolate group were three L. chagasi or visceral types, WR 285, 317, 341; these were the first reported visceral cases from U. S. military personnel in Panama. In this same group another species/type was identified, L. mexicana aristedesi, which has an enzyme profile similar to the mexicana group but distinct from the other species/types, mexicana, amazonensis, DCL.

Also in the mexicana group of isozyme species/types is a type I'm calling diffuse cutaneous leishmaniasis, DCL. A number of WRAIR and NIH isolates from various geographical locals can be included in this group. Below is an abstract[2] which is to be published in the American Journal of Tropical Medicine and Hygiene in August, and the data presented at the society meeting in December, 1983. It is interesting to note that the one L. m. pifanoi which I have run is not as close to DCL as it is to L. m. mexicana.

ABSTRACT. NEW WORLD DIFFUSE CUTANEOUS LEISHMANIASIS: POSSIBLY ONE ENZYME TYPE. R. D. Kreutzer, N. Souraty and P. B. McGreevy. Youngstown State University, Youngstown, Ohio and Walter Reed Army Institute of Research, Washington, D. C.

Cases of diffuse cutaneous leishmaniasis (DCL) have been reported in patients from widely separated areas in the New World. Isozyme studies have reported that isolates with similar clinical manifestations also may have high levels of isozyme identity. Nine DCL isolates four from Venezuela and five from the Dominican Republic, an uta isolate from Peru and two unknown cutaneous isolates one from Panama and the other from Belize were characterized by extensive (up to 30 enzymes) isozyme analysis. They were found to be over 80% identical one to another, but the compiled group profile had only low levels of similarity with other New World Leishmania enzyme profiles. Certain of the isolates were identical for all enzymes tested, but the majority differed from one another for certain enzymes (EST, FUM, GOT, G6PD, MDH, PFK, PGM). Therefore

among these biochemically very similar isolates the Venezuela group and the Dominican Republic group had 90 to 100% intra-group identities, the Panama isolate was distinct from the others for at least two enzymes, the Peru, uta, isolate was distinct from all others for one enzyme and the Belize and Peru isolates were more genetically similar with each other than they were with the Venezuela, Dominican Republic or Panama isolates. Although the group profile of these isolates was distinct, it had a higher level (40%) of similarity with the mexicana profile than with the braziliensis or visceral enzyme profiles (less than 15%). These isolates many with similar clinical manifestations and all with high levels of isozyme identity represent a widely ranging Leishmania type which can be readily separated from other New World Leishmania types by enzyme analysis (possibly by study of only two enzyme systems - PGI and MPI). This project was sponsored in part by the U. S. Army Medical Research and Development Command, Contract #DAMD17-83-C-3119. The opinions or assertions herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of Defense.

I also tested isolates for Dr. Pete Jackson (Table 2). The WR 311 (L. donovani) isolate was compared with the two Crithidia isolates and WR 440 and 495 which were initially typed as Kenya L. donovani. These two Kenyan isolates are not L. donovani nor are they even similar to any other Leishmania species/type.

Certain isolates cultured in my laboratory and those recently sent to me by Capt. McGreevy are currently being identified (Table 3). Preliminary identifications are noted.

Mini-test

The data on New World isolates already collected (about 150 isolates) suggest that study of two (possibly three) enzyme profiles will allow accurate identification. These are phosphoglucoisomerase (GPI), mannos-phosphate isomerase (MPI) and glutathione reductase (GSR). All New World species/types produce identical but (each species type) differently migrating bands for MPI and GSR, and most species/types likewise for GPI.

I have attempted to change the buffer systems for these three enzymes so they can be pre-weighed and require only the addition of distilled water. This would eliminate the need for a mettler type balance and pH meter. Preliminary tests of these new buffers are encouraging. I intend to pursue this line and am confident that I should in the future be able to propose a mini-test which uses one or two enzymes the components of which can be pre-packaged.

Culturing

Culturing was started in my laboratory the second week in July, 1983, because I was not able to obtain the laminar flow hood until that time. Although I have cultured a few unknown isolates for identification (Table 3), most effort has been placed on obtaining control pellets. Each isolate initially is cultured on NNN with a Schneider's/FBS overlay and separately on Schneider's/FBS (70/30%). I have not as yet had any difficulty with culturing. Attempts, as yet unsuccessful, have been made to simplify the procedures followed to prepare cultures for CAE.

Polymorphism

Although I have noted polymorphism among the isolates already characterized, I have not as yet attempted to associate other parameters with the polymorphism. The levels (up to 25%) are similar to those previously reported.[1]

Discussion

I have included much of this section above, but because this is a preliminary report of data collected 15 March 1983 - 15 August 1983, I don't feel additional comment on results, conclusions and recommendations is appropriate at this time.

TABLE 1. WRAIR isolates characterized and identified and completed April, 1983

Lbb*	Lbp	Lc	Lm arist	DCL
063**	004	285	481	381
359	241	317		
	246	341		
	282			Unknown
	345			
	360			177
	390			316
	442			
	446			
	470			
	475			
	486			
	505			

*Lbb - Leishmania braziliensis braziliensis; Lbp - L. b. panamensis; Lc - L. chagasi; Lmm - L. mexicana mexicana; Lm arist - L. m. aristedesi; DCL - Diffuse cutaneous possibly L. m. pifanoi; Ld - old world isolate of L. donovani

**WRAIR numbers

TABLE 2. WRAIR isolates characterized and identified and completed July, 1983

Lbb*	Lbp	Lmm	Ld	DCL
294	003	524	311	140
410	111C	Castro		453
508	111LN			457
	132			527
	154A			
	176			Unknown
	179A			
	211			206
	232			281 close to Lmm
	322			
	487			
	525			
	Schoonmaker			

528 - L. m. pifanoi - close to Lmm and DCL

523 - Herpetomonas

Crithidia fasciculata

Crithidia aurelia

440 and 495 - Not Leishmania

*See legend in Table 1

TABLE 3. WRAIR isolates cultured at my laboratory - preliminary identifications

Lbp*	Lmn	DCL
491	347	348
492		
493		
526		

Cultured at WRAIR and received August 11

Lbp	Lbb
539	Bel-10

*See legend in Table 1

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[1]R. D. Kreutzer, M. E. Semko, L. D. Hendricks and N. Wright. 1983. Identification of Leishmania spp. by multiple isozyme analysis. Am. J. Trop. Med. Hyg., 32(4):703-715.

[2]R. D. Kreutzer, N. Souraty and P. B. McGreevy. New World diffuse leishmaniasis: possibly one enzyme type. Am. J. Trop. Med. Hyg. In press.

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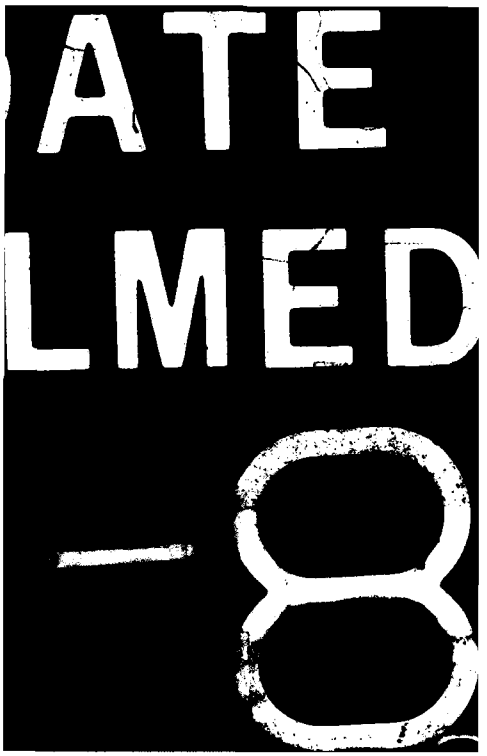
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