

PN-ABC-870

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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 9/20/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18
FROM: AID/SCI, Victoria Ose *VO*
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. C5-117
Second Annual Report

Attachment

PN-ABC-870

SECOND ANNUAL REPORT ON AID-CDR PROJECT C5-117

AID-CDR CONTRACT No. DPE-5544-G-SS-6020-00

DEVELOPMENT OF A VACCINE AGAINST CANINE LEISHMANIASIS

Dr. L.F. Schnur, Prof. P. Abranches, Dr. C. Jaffe, Prof. C. Greenblatt

Jerusalem, September, 1988.

INTRODUCTION

Over the last year, this project has gained greater momentum and seen several advances: the isolation and serological and enzymological characterization of Portuguese leishmanial strains from dogs and man; the serological diagnosis of canine leishmaniasis; the identification of parasite antigens recognized by canine serum antibodies; the purification of defined antigens that might serve in vaccination. Initial attempts to infect normal dogs were not successful and are cause for some concern. Much depends on our being able to create good, experimental leishmanial infections in dogs. By studying the dynamics of a 'normal' infection in naive dogs from its inception to its termination, i.e., the death of the dog, in terms of its parasitology, pathology, immunology, etc., we would have a base line against which one could measure alterations induced by 'vaccination' and be used as indicators of its efficacy. When we do succeed, it seems that some of the measuring of immune parameters will have to be done by sending ^{taken} samples/periodically from dogs to Israel, since the collaborating laboratory in Lisbon is insufficiently well-equipped, lacking a ^{and a CO₂ incubator} β -counter/. We hoped to remedy this by seeking additional funding from USAID in Portugal to help with the purchase of capital equipment.

Gabriella Gomes, a technical assistant to Professor Abranches, spent two months, February and March 1988, in Israel. Most of this period, six weeks, was spent with Dr. Jaffe at the Weizmann Institute learning how to measure immunological parameters of canine leishmanial infections and those of laboratory animal models of leishmaniasis available at the Weizmann Institute. She spent two weeks with Dr. Schnur at the Kuvim Centre learning basic parasitological and leishmanial strain typing techniques.

In March, 1988, Professor Greenblatt attended a public health meeting in Madrid, Spain, and used that opportunity to pay a two day courtesy visit to Professor Abranches.

Dr. Jaffe was to have visited Lisbon this September, but could not fulfil this commitment at this time. Owing to this and our current problem in obtaining adequate experimental infections in dogs, Dr. Schnur will visit Professor Abranches at the end of September, since he will be in Europe (Amsterdam) to attend the XIIth International Congress on Tropical Medicine and Malaria.

THE ISOLATION AND TYPING OF PORTUGUESE LEISHMANIAL STRAINS

A further four Portuguese leishmanial stocks collected and sent to Jerusalem for characterization were typed. Three were isolated from dogs from popliteal lymph nodes, and one was from the bone marrow of a human case of visceral leishmaniasis, an eight-year old female negro child. They were:

WHO-LRC REF	INTL. CODE	ORIGIN
LRC-L541	MCAN/PT/87/IMT 150	isolated from lymph node of dog caught in Lisbon on 9.11.87
LRC-L542	MHOM/PT/88/IMT 151	isolated from bone marrow of 8 yr old female on 2.2.88
LRC-L543	MCAN/PT/88/IMT 152	isolated from lymph node of dog caught at Vila Real on 12.2.88
* Arrived contaminated	MCAN/PT/88/IMT 153	isolated from lymph node of dog caught in Lisbon on 6.4.88

The three good strains were typed by our standard typing procedure (described in our first report). All three strains were alike in their excreted factor, which was subserotype B₂*, and in their 11 enzyme profile, which was identical to that of the WHO international reference strain of L.donovani infantum, MHOM/TN/80/IPT 1 (Montpellier zymodeme 1 = London zymodeme 49), and thus, like all the previously characterized Portuguese leishmanial strains; except for the second

* Gel diffusion of the used culture medium filtrate of the contaminated stock IMT 153 also showed it to contain sub-serotype B₂ excreted factor (EF).

line of IMT 143 (LRC-L528) described in the last report, that displayed distinct differences in the patterns of its NH and SOD (see appended abstract that will be published in the Society of Protozoologists Abstracts).

SERODIAGNOSIS OF CANINE LEISHMANIASIS, THE EVALUATION OF SERODIAGNOSTIC TESTS AND THE SEARCH FOR ANTIGENIC & IMMUNOGENIC COMPONENTS

In our last report we described briefly a direct ELISA (d-ELISA) developed in Israel at the Weizmann Institute that uses crude L.donovani membranes as antigen and its comparison with the standard indirect immunofluorescent test (IFAT) used in Lisbon by Professor Abranches' group. This study has been completed and is being prepared for publication.

Results obtained in Lisbon by IFAT, using whole L.donovani promastigotes as antigen, where serum titres ranged from $<1:8$ to $>1:8192$, $>1:64$ being considered positive, were compared with the Israeli d-ELISA using promastigote lysates and membranes and, now also with a competitive ELISA (c-ELISA) which measures the binding inhibition of two monoclonal antibodies, D-2 and D-13 (Jaffe and McMahon-Pratt, 1987). When the direct ELISA and the IFAT were compared (Figure 1, Table 1) no correlation between the titer determined by IFAT and the absolute absorbance in the d-ELISA was seen. This is probably owing to the fact that different parasite isolates were used as source of antigen preparation; the d-ELISA was carried out on antigen prepared from L.donovani donovani. However, 95.4% (21/22) of the IFAT positive samples and 80.1% of the IFAT negative samples were correctly identified by the d-ELISA. 4/21 IFAT negative sera had Sample/Negative absorbance ratios (P/N ratio) greater than 3, the predetermined negative cut off for the

d-ELISA. Of the four misidentified negative sera, 2/4 had IFAT titres of 1:64, suggesting that the IFAT cut off is not absolute, and that the d-ELISA is more sensitive than the IFAT when weak sera are assayed. These results suggest that both methods are suitable for diagnosis of canine visceral leishmaniasis in areas free from Chagas' disease.

Results from these assays were compared with the c-ELISA, Table 1. It was shown that only sera from patients with visceral leishmaniasis recognize the parasite antigens reacting with the two monoclonal antibodies, D-2 and D-13. No inhibition of monoclonal antibody binding to crude parasite homogenates by human chagasic sera or sera from patients with other diseases was observed. For this reason the c-ELISA is extremely useful in regions co-endemic for both diseases. Of the 22 IFAT positive samples, 21/22 sera inhibited D-2 and/or D-13 binding (> 30%) to parasite antigen, demonstrating that the 70 and 72 kd proteins recognized by these monoclonal antibodies are also immunogenic for dogs. No particular preference was noted and visceral leishmaniasis dog serum inhibition of both antibodies was observed. Using this assay, 6 false positives were detected and 3/6 had 1:64 titers by IFAT. Two of the latter three samples were also positive by d-ELISA.

The two antigens recognized by the monoclonal antibodies D-2 and D-13 were purified to biochemical homogeneity and used for diagnosis of human visceral leishmaniasis in a direct dot-blot ELISA on nitrocellulose paper (Zalis and Jaffe, 1987). A preliminary assay was carried out with the Portuguese dog sera and gave results identical to the c-ELISA. Attempts are underway to adapt these antigens for routine use on polyvinylchloride plates. Vaccination experiments using the pure antigens were begun in mice. The route

of administration and adjuvant in use are under examination. These studies will be extended to dogs over the next year.

Western blotting was used to identify those parasite antigens which react with infected dog sera (Figure 2). The same antigen was used for both the d-ELISA and the western blots. Proteins with molecular weights from 26.000 to 180.000 are recognized by the infected dog sera. Sera from all the dogs with visceral leishmaniasis examined (9) bound to proteins with molecular weights 26.000 and between 70-84.000. In the figure, the individual strips are arranged in increasing IFAT titer from left to right. No correlation between the strength of reaction on the blot and the IFAT titer is observed. However, if the strips are arranged according to increasing P/N ratio, some direct correlation between the d-ELISA value and number of bands visualized in the blot can be seen. The results in the western blot suggest that pure antigens of 26 and 70-84 kd should be useful in the development of a simple diagnostic assay. These proteins are highly antigenic and induce antibodies early in infection before titers become elevated. The fact that monoclonal antibodies D-2 and D-13 react with proteins around 70 kd which are recognized by all infected dogs reinforces the highly immunogenic nature of these proteins. Interestingly, serum 21 (Figure 2, lane e) which has a 1:64 IFAT titer and is strongly positive by d-ELISA shows reactions primarily with the 26 and 70-84 kd proteins.

Jaffe, C.L. and McMahon-Pratt, D. (1987) Serodiagnostic assay for visceral leishmaniasis employing monoclonal antibodies. Trans. Roy. Soc. Trop. Med. Hyg. 81, 587-594.

Zalis, M. and Jaffe, C.L. (1987) Routine dot-blot assay of multiple serum samples using a simple apparatus. J. Immunol. Methods 101, 261-264.

ATTEMPTS TO INFECT NORMAL DOGS

Two of the strains isolated from Portugese dogs and typed in Jerusalem (LRC-L530 =IMT 144 and LRC-L542=IMT 151) were inoculated into Syrian hamsters soon after isolation to check and maintain their infectivity. Heavy late log promastigotic inocula were used. Infections in hamsters were not equal. Some hamsters were quite heavily infected with splenic infections, others were only slightly infected and some did not become infected. Primary cultures of LRC-L530 were sent to Lisbon to use in experimentally infecting dogs. These were reinoculated into hamsters and were also grown in culture in NNN and Schneiders Drosophila medium supplemented with 20% foetal calf serum. Attempts to grow promastigotes have not gone well. This L.d.infantum grew very poorly in NNN and not at all in Schneiders medium. Thus, in the first attempt to infect two six-month old naive puppies with this strain, amastigotes isolated from hamster spleens were used. Each dog received 9.5×10^8 amastigotes inoculated intradermally-cum-subcutaneously. Professor Abranches decided against intravenous inoculation, since his amastigote preparations were not very free of hamster splenic tissue. These infections do not appear to have succeeded. Further attempts must be undertaken. Dr. Schnur will visit Professor Abranches to formulate new protocols for this.

PAPERS SUPPORTED BY THIS GRANT:

Abranches, P., Rachamim, N. and Jaffe, C.L. Immunological diagnosis of canine visceral leishmaniasis: comparison of three different methods. In preparation.

Schnur, L.F., Abranches, P. and Sulitzeanu, A. Enzymological variation in a strain of Leishmania donovani infantum from visceral leishmaniasis in an AIDS patient. Society of Protozoology Abstracts, in press.

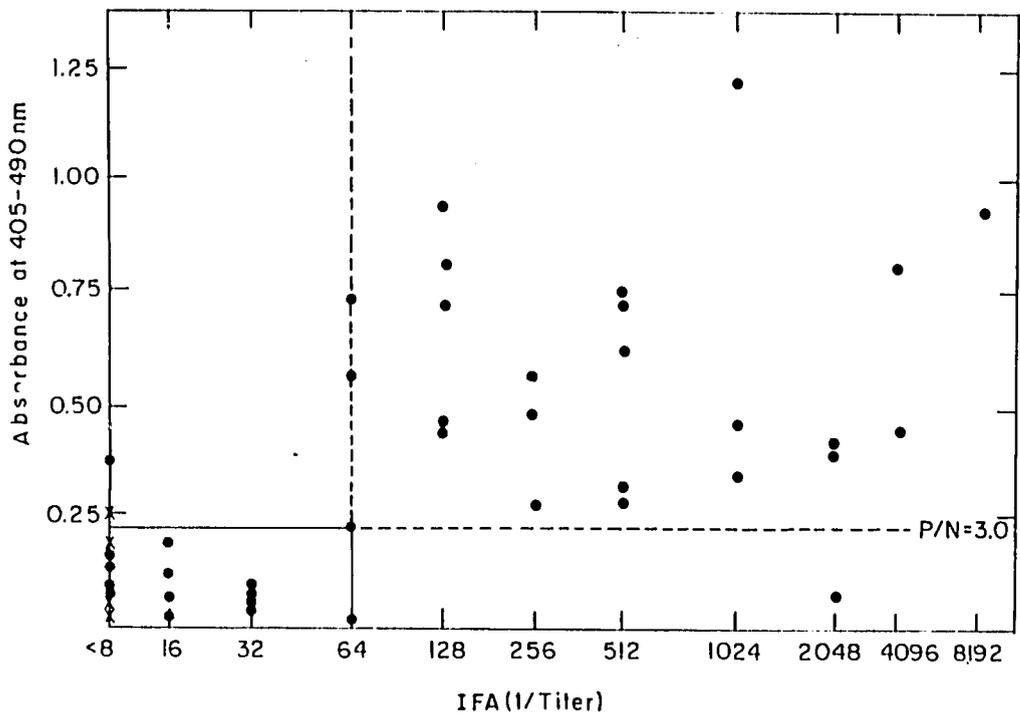


Figure 1. Comparison of IFA titers with optical absorbance obtained in a direct ELISA.

Table 1.

Sample	Direct ELISA		% Inhibition		
	(10 ⁻³ dilution) OD ₄₀₅	P/N RATIO	D2	D13	IFA titer
1	0.082	1.14	0.0	0.0	
2	0.157	2.18	11.5	33.3	
3	0.376	5.22 ^a	0.0	0.0	
4	0.145	2.01	0.0	0.0	Neg
5	0.090	1.25	0.0	0.0	
6	0.183	2.54	15.4	0.0	
7	0.032	0.43	0.0	0.0	
8	0.052	0.72	0.0	0.0	1/8
9	0.245	3.45 ^a	0.0	0.0	
10	0.187	2.59	65.4	100.0	
11	0.020	0.36	0.0	0.0	
12	0.121	1.68	0.0	0.0	1/16
13	0.064	0.90	0.0	0.0	
14	0.064	0.90	0.0	0.0	
15	0.055	0.76	0.0	0.0	
16	0.072	1.00	43.9	100.0	1/32
17	0.092	1.30	0.0	0.0	
18	0.014	0.20	80.5	100.0	
19	0.219	3.04 ^a	22.0	0.0	
20	0.551	7.70	70.0	100.0	1/64
21	0.707	9.82	78.0	93.0	
22	0.793	9.76	41.5	0.0	
23	0.403	6.00	4.0	81.0	
24	0.804	11.17	56.1	100.0	1/128
25	0.426	5.92	30.0	85.0	
26	0.923	12.82	80.4	0.0	
27	0.270	3.75 ^a	64.8	100.0	
28	0.461	6.40	0.0	50.0	1/256
29	0.396	5.50	5.4	100.0	
30	0.738	10.25	83.8	100.0	
31	0.615	8.54	37.8	0	
32	0.315	4.37	27.0	100.0	1/512
33	0.274	3.80 ^a	59.0	0.0	
34	0.721	10.01	73.0	100.0	
35	0.443	6.15	35.0	43.0	
36	1.199	16.65	100.0	100.0	1/1024
37	0.328	4.55	23.0	30.0	
38	0.386	5.36	45.9	100.0	
39	0.072	1.00 ^a	70.3	86.0	1/2048
40	0.393	5.46	0	0 ^b	
41	0.784	10.90	34.5	0	1/4096
42	0.432	6.00	56.8	86.0	1/4096
43	0.922	12.80	65.6	0.0	1/8102

^a Negative on Retest. ^b High Background could not assay.

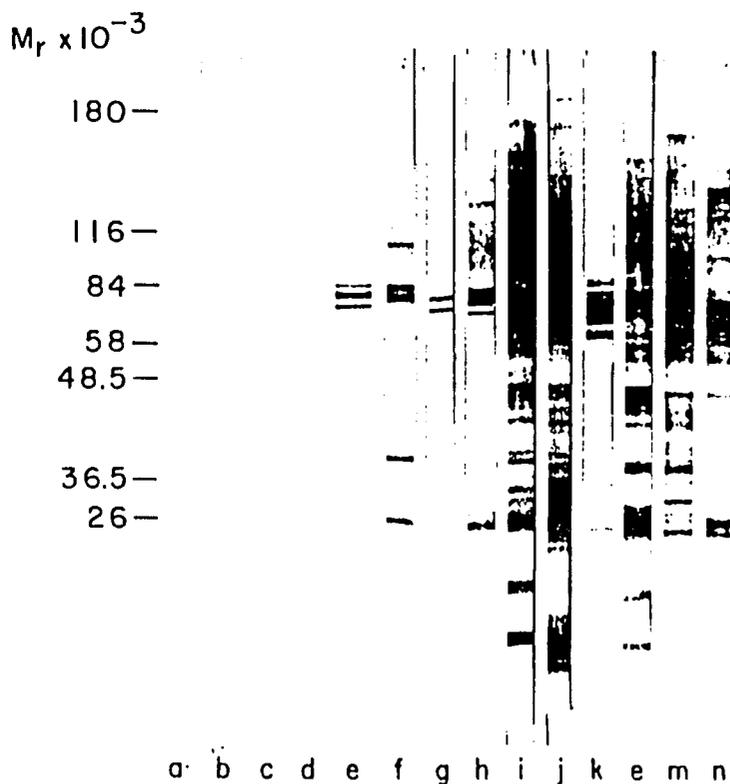


Figure 2. Leishmania donovani promastigote antigens recognized by Western blotting using sera from dogs with visceral leishmaniasis. The strips are organized with increasing serum titers by IFA from a - m. Lanes a - c had titers $<1:64$ and are considered negative. Lane n was blotted with serum obtained from a dog positively biopsied for visceral leishmaniasis in Israel. Serum dilutions of 1×10^{-4} were used.

ENZYMOLOGICAL VARIATION IN A STRAIN OF
LEISHMANIA DONOVANI INFANTUM FROM
VISCERAL LEISHMANIASIS IN AN AIDS PATIENT*

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Two lines of a leishmanial strain isolated from a Portuguese AIDS patient with visceral leishmaniasis (VL), and other parasitic, bacterial and viral infections (Antunes *et al.*, Trans. R. Soc. Trop. Med., 81, 595, 1986), were typed enzymologically by cellulose acetate electrophoresis and serologically by excreted factor (EF) typing. Both lines were identical with regard to EF subserotype which was B₂ and the electrophoretic mobility of 9 enzymes: MPI, GPI, MDH, 6PGD, PGM, PK, ALAT, ASAT, PEPD. They differed in their SOD and NH. One line was identical to the WHO international reference strain of L. d. infantum (MHOM/TN/80/IPT* the other also displayed all the enzymic components of the reference strain, but had an additional enzyme band in its SOD pattern and in its NH pattern. This is the first record of intrastrain variation of L. donovani s. l. Such intrastrain enzymological variation has been recorded in Israeli L. major strains (Greenblatt, *et al.*, in: Parasitology - Quo Vadit? ICOPA VI Handbook, p. 142, abstr. 261, 1986), where NH and 6PGD differed among strains and clones derived from them.

*Supported by the US-Israel CDR Programme contract No. DPE 5544-G-85-6020-00, Project C5-117.