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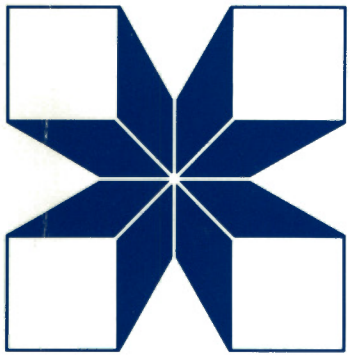

CANADA

ZOONOTIC AND PARASITIC DISEASES

PROCEEDINGS OF THE THIRD INTERNATIONAL AND PAN-ARAB SEMINAR HELD IN AMMAN, JORDAN, 17–20 OCTOBER, 1989

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This series includes meeting documents, internal reports, and preliminary technical documents that may later form the basis of a formal publication. A Manuscript Report is given a small distribution to a highly specialized audience.

La présente série est réservée aux documents issus de colloques, aux rapports internes et aux documents techniques susceptibles d'être publiés plus tard dans une série de publications plus soignées. D'un tirage restreint, le rapport manuscrit est destiné à un public très spécialisé.

Esta serie incluye ponencias de reuniones, informes internos y documentos técnicos que pueden posteriormente conformar la base de una publicación formal. El informe recibe distribución limitada entre una audiencia altamente especializada.

IDRC-MR263e
November 1990

ZOONOTIC AND PARASITIC DISEASES

Proceedings of the Third International and Pan-Arab Seminar held in Amman, Jordan, 17-20 October 1989

Edited by
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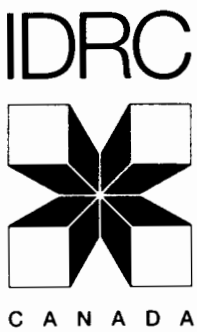
Cosponsored by
International Development Research Centre,
Ottawa, Canada
and The Higher Council for Science and Technology,
Amman, Jordan



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HISTOPATHOLOGICAL AND IMMUNOLOGICAL CHANGES IN EXPERIMENTAL LEISHMANIASIS

by

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Visceral leishmaniasis is a disseminated infection with Leishmania donovani. It has been described in four continents where both endemic zones and isolated cases can occur.(1)

The parasitic strain is important in determining the clinical manifestations of kala-azar in its pattern of transmission. It was found that pathogenicities of L. donovani of various geographic strains appear to differ.(2) Thus in the Near East, the Mediterranean basin and North Chinam Visceral leishmaniasis affects mainly rural children, and dogs are the main reservoir host "Leishmania infantum".(3)

In Egypt, rare cases have been reported since 1904. Some of these were important whereas others were probably authochtonus. Undoubted evidence of transmission in Egypt came from the focus of visceral leishmaniasis discovered in 1982 at El-Agamy, 30 km west of Alexandria, where more than 20 cases were diagnosed and treated. (4, 5, 6, 7)

The human isolates from this locality belong to the L. donovani complex and are more closely related to L. infantum, as evidenced by the isoenzyme pattern of 6-phosphogluconale dehydrogenase.(8, 9)

The aim of this work is to study the histopathological and some immunological changes which may take place in the liver of mice experimentally infected with L. donovani var. infantum (Egyptian Mediterranean strain).

Material and Methods

The Leishmania strain used in this study is Leishmania donovani (Leishmania infantum-Egyptian strain) isolated from an Egyptian child admitted to Shatby Pediatric Hospital. The case was a female 2.5 years old, from El-Agamy district, Alexandria, Egypt.

The complaint was intermittent fever. Physical examination revealed pallor, temperature 38°C, hepatosplenomegaly and enlarged cervical lymph nodes. Blood picture was:

- Hb 4bm/100ml.
- Total leucocytic count 2.500/mm^3.
- Polymorphonuclears 32%
- Eosinophils 0%.
- Basophils 0%.
- Monocytes 6%.
- Lymphocytes 62%.
- Platelets 28.000/mm^3.

The diagnosis was confirmed to be visceral leishmaniasis by bone marrow biopsy and positive serological tests (IHAT and IFAT).

Tanabe's medium (10, 11) was used for initial establishment and development of promastigotes from the amastigotes. It was used also for routine propagation of the strain. Subculture was done every 3 weeks.

Modified El-On's medium (12) was used for mass cultivation of promastigotes required for animal inoculation.

Seventy-five Swiss strain laboratory-bred albino mice were used. They were divided into two main groups:

* Fifty animals all infected with L. donovani promastigotes by intracardiac inoculation under anaesthesia, in a standard dose of 20 x 10^6 promastigotes per mouse. According to the duration of infection animals were subdivided into: group I-a, mice sacrificed one week after infection, groups I-b, I-c, I-d and I-e, sacrificed 2, 4, 8 and 12 weeks after infection respectively.

* Twenty-five animals were used as control, non-infected animals (group II).

After dissection of animals, liver was isolated and divided into two parts. One part was kept at -20°C for indirect fluorescent antibody technique(13), the other was kept in 10% formalin for the histopathological study by H&E and Masson's trichrome stain.(14)

Results

1) Histopathological results:

One week post-infection, liver showed perivascular cellular infiltration with granulomatous formation of lymphocytes, plasma cells and histiocytes (Fig. 15). Liver sinusoids were dilated, Kupffer cells were hyperplastic and prominent. Parasites would be detected inside the hepatocytes and Kupffer cells. No fibrosis was detected. By the end of the second week granulomas were found perivascular and intraparenchymal. Otherwise, no difference from the previous group was noted. After four weeks, granulomas increased in size, they were formed of plasma cells, histiocytes and frequent giant cells. In some animals vascular proliferation and hyalinization of the blood vessels wall was seen.

The parasite was detected in the cytoplasm of both liver and Kupffer cells and even intranuclear parasites could be seen. These nuclei showed early nuclear fragmentation. By the end of the eighth week, granulomas were frequently detected but they were all smaller in size than the early one. By the twelfth week, marked reduction in the size of the granuloma was seen. Hepatocytes were still showing few degenerative changes, but neither necrosis nor fibrosis was observed.

2) Immunofluorescence results:

The liver of normal non-infected animals were absolutely negative in the test.

One week after infection, at a titre of 1/32, fluorescence was observed around the hepatocytes, the dilated vessels and in areas showing granuloma, while at a higher dilution (titre of 1/64) selection of fluorescence occurred and became localized to the zone of granulomas and vessels. At a titre of 1/128, fluorescence appeared as a faint thin peripheral rim around the granulomas. Eight weeks after infection, fluorescence was observed in the same sites at a lower titre (1/4). Twelve weeks after infection the test was negative.

Discussion

In this study, the development of the granuloma was the characteristic histopathological lesion in the liver of all infected animals. Granulomas consisted mainly of lyphocytes,

plasma cells, histiocytes and frequent giant cells. L. donovani bodies were seen inside Kupffer cells as well as inside the hepatocytes.

Although it is well known that L. donovani is primarily a parasite of cells of mononuclear phagocytic series, it was also detected in the hepatic parenchymal cells.

A similar finding was previously reported in human Kalaazar and was documented by ultra-structural studies (15, 16, 17). Of utmost importance is the observation of L. donovani anastigotes inside the nucleus of some hepatocytes with early nuclear fragmentation. The reason is not clear and further investigations are recommended.

IFAT showed fluorescing deposits throughout the first four weeks which present the leishmanial antigen either free or in antigen-antibody complex. Eight weeks after infection fluorescence was observed at lower dilutions while it became negative after twelve weeks. These findings coincide with the histopathological findings where granulomas had healed and lesions resolved completely without residual damage.

It was found that during the course of visceral leishmaniasis there is a large amount of immunoglobulins at high titres, which are non specific as well as specific (18, 19). Amastigotes were also shown to liberate antigenic substances (20). Thus, the presence of L. donovani soluble antigen or antigens, corresponding antibodies, and the component of complement leads to the formation of circulating immune complexes (21). It is likely that immune complexes were initially deposited in the hepatic tissue. It was reported by Farsh et al. (22), that infected macrophages have leishmania antigen on their surfaces, and presumably immune complexes can form on them (23). Considering this information, it can be suggested that deposition of immune complexes in the detected sites is likely to be in part due to the circulating antigen-antibody complex, and also to be locally formed complex in situ.

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