They also reported progressive involution in the splenic white pulp.

The hamster inoculated with 10⁷ amastigotes of L. (L.) chagasi presents a uniform evolution of the disease which kills the animals from the 60th day onward. It has proved to be a good model for histopathological studies (Duarte et al. 1978, 1988; Duarte & Corbett 1984).

In this study we analyse the sequential histopathological changes in hamsters experimentally infected with L. (L.) chagasi in order to evaluate the involvement of lymphoid organs in this experimental model of visceral leishmaniasis.

Material and methods

Hamsters (Mesocricetus auratus), 2 months old and weighing 80–90g, were inoculated with L. (L.) chagasi (MHOM/BR/72/LD46) isolated from a human case in Mantena, Minas Gerais, Brazil, by Dr W. Mayrink of the Federal University of Minas Gerais, Brazil. An intraperitoneal inoculum of previously infected spleen homogenate with 10⁷ amas-
tigotes was administered to groups of seven animals which were killed on days 7, 15, 30, 45 and 60. Control groups of three animals were inoculated with normal spleen homogenate and saline solution (NaCl 0.09%).

The parasite load of the spleen was calculated by determining the number of amastigotes found per 1000 nuclei of the cells x organ’s weight (mg) x 2 x 10⁵ in spleen smears fixed in methanol and stained by Giemsa’s method (Stauber 1958).

Fragments from thymus, spleen and lymph nodes were processed for histopathology and stained by haematoxylin–eosin. Giemsa, Wilder’s silver reticulin stain and by methyl green pyronin solution in order to identify plasma cells. A morphometric method using Weibel’s graticule with 100 points was used for sequential quantitative analyses. The Kruskall–Wallis non-parametric test was used for statistical analyses.

Results

From the 30th day of the infection, leishmanial amastigotes were detected in smears of spleen. The progression of parasite load is shown in Fig. 1 together with the variation in volume of the spleen white pulp and lymphoid follicles.

Sequential histopathological changes

Group 1 (7th day). Spleen white pulp (Fig. 2a) with normal periarteriolar lymph sheath (Fig. 4a) was separated from the lymphoid follicles by a well defined marginal sinus as usually seen in rodents (Fig. 2b). The red pulp showed congestion only. The lymph nodes were normal (Fig. 3).

Group 2 (15th day). The main change seen in the white pulp of the spleen was reactivity of the marginal centres of the lymphoid follicles, where reticular cells and mitosis were conspicuous.

The periarteriolar lymph sheath and marginal sinus were preserved. The red pulp showed a mild increase in the numbers of

![Graph](image_url)
plasma cells and macrophages. The lymph nodes showed activity of the germinal centres of the lymphoid follicles only.

**Group 3 (30th day).** There were numerous active follicular germinal centres with many mitoses in the spleen. The marginal sinus was only partially preserved. By comparison with controls, the periairteriolar sheath had a lower density and cellular pleomorphism and macrophages and plasma cells were present among the lymphocytes (Fig. 4b). The red pulp was enlarged with increased numbers of macrophages and plasma cells. Macrophages containing parasites were identified in some, but not all, animals. The lymph nodes showed hyperplasia of the follicular germinal centres. The lymphoid follicle marginal zone showed increased cell density, mostly pyroninophilic cells representing active lymphocytes and plasma cells. In the paracortical zone, macrophages and plasma cells were seen together with the lymphocytes.

**Group 4 (45th day).** The splenic white pulp did not change in volume, but there was a decrease in cell density and lymphocytes were present as well as macrophages, plasma cells and reticular cells (Fig. 4c). The follicular germinal centres, which were still hyperplastic, showed an increase in the perilollicular zone where plasma cells were also seen. The marginal sinus was partly interrupted (Fig. 5). There was an intense proliferation of macrophages mostly containing parasites which formed nodules within the red pulp.

The lymph nodes were still somewhat hyperplastic (Fig. 6) but the marginal zones also contained pyroninophilic cells and macrophages. The paracortical zone contained a few lymphocytes and macrophages, some of them with parasites. The parasitism varied from low to high in animals within the same group. In the highly parasitized animals, there were groups of macrophages full of parasites.

**Group 5 (60th day).** The spleen changes were more marked, with the white pulp contain-
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Fig. 2. Spleen white pulp. a. Normal appearance. HE. × 250; b. A well defined marginal sinus. HE. × 500.
Fig. 3. Lymph node with normal lymphoid follicle. HE. × 500.
paracortical zone and the B lymphocytes in
cortical areas mainly in the lymphoid foli-
cles. In this experiment the evaluation of the
white pulp of the spleen showed increase in
activity of the lymphoid follicle from the 15th
to the 45th day of the infection, which is
compatible with the already known hyper-
gammaglobulinaemia. However, even with
decrease of the germinal centre of the lymph-
oid follicles at the 45th and 60th day, there
was an increase in B lymphocytes and
even transformation to plasma cells as indicated
by the pyroninophilia of these cells.
The marginal sinus, clearly visible at the 7th
and 15th days, partially disappeared in the
30th day group and could not be detected at
the 45th and 60th days. The T lymphocyte
zones at the 30th day showed a lower
 cellular density and cellular pleomorphism
with macrophages and plasma cells present
among lymphocytes. This last group showed
few lymphocytes and a lower cellular density
and most of the cells were macrophages with
parasites, plasma cells and reticular cells.
The lymph nodes showed a similar time
date of changes in both the B and T
lymphocytes zones, apart from the lymphoid
follicle reactivity which started early.
The quantitative evaluation of the white
pulp showed a significant increase at the
30th day which was related to an increase in
lymphoid follicles; there was a return to the
same initial mean volume by the 60th day.
However, there was marked selective de-
crease of T lymphocytes and even with low
 cellular density there was an increase of
plasma cells, parasitized macrophages and
reticular cells.
Regressive changes in the spleen and
lymph nodes were reported previously as
a general feature of the histopathology of
visceral leishmaniasis (Melency 1925; Bhaskara-Menon 1939; Veress et al. 1974).
Veress et al. (1977) reported a morpho-
metric analysis of human visceral leishma-
niasis spleen which demonstrated a signifi-
cant decrease of the white pulp and no
germinal centres. They also described lower
 cellular density with few lymphocytes and
the presence of parasitized macrophages and
plasma cells in the white pulp. Similar T
lymphocytic changes were also seen in
lymph nodes. These authors also described
necrosis, fibrosis, lymphoid follicles and
extracellular hyalin deposits which they
interpreted as precipitated antigen–antibody
complexes due to excess of antigens. They
also noted the decrease of T lymphocytes
zones and related this to a reduction in the
immunocellular response. In this work no
necrosis, fibrosis or evident hyalin deposits
were observed. However, in our experience,
amyloid is present in the kidneys and in
blood vessel walls in these phases of the
disease.
The relationship between lymphoid follicle
activity and the evolution of the disease was
described in Brazilian human visceral leish-
maniasis (VL) by Carvalho et al. (1985) who
divided the VI. patients into two groups, one
with hyperplasia or normal follicles and the
other with atrophic lymphoid follicles which
was related to the duration of disease. The
results presented here confirm his hypoth-
eses.
The depressed immunocellular response
reported in the active phase of the disease
(Rees et al. 1981) and the decrease in
number of T lymphocytes (Rezai et al. 1978;
Aikat et al. 1979; Musumeci et al. 1981;
Carvalho et al. 1985; Koech et al. 1987) is
also compatible with the lower cellular
density and pleomorphism found in the T
lymphocyte zones in the spleen and lymph
nodes.
The hyperplasia and parasitism of the
phagocytic mononuclear system was the
most striking change in the red pulp of spleen
at the 45th and 60th day. Before that, there
were great variations between animals in the
same group. These changes were reported in
the earliest histopathological observations of
VL either in hamsters (Hu 1933) or in man
(Hu 1936). These data demonstrate a highly
proliferated and parasitized phagocytic
mononuclear system and confirm the
accepted characteristic histopathological
changes.
Fig. 4. Peritumoral sheath. a. Normal cellularity (7th day). HE, x 500; b. Mild decrease of cellularity and cellular pleomorphism (30th day). HE, x 500; c. Low cellular density and cellular pleomorphism (45th day). HE, x 500; d. Lymphocyte depletion with low cellular density and cellular pleomorphism (60th day). HE, x 500.
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Fig. 7. Spleen white pulp showing marked lymphocytic depletion and macrophages with parasites (60th day). HE × 500.
Fig. 8. Spleen red pulp with marked hyperplasia of the phagocytic mononuclear system and a high degree of parasitism (60th day). HE. × 500.
Fig. 9. Lymph node with pyroninophilic cells in the outer limit of the lymphoid follicle (6th day). Methyl green-Pyronin. × 250.
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Fig. 10. Lymph node paracortical zone with lymphocyte depletion (60th day). HE, ×287.5.
Epithelioid granulomas which were occasionally noted in previous studies (Bell et al. 1958; Jehan et al. 1982; Gutierrez et al. 1984) were not seen in this experiment. Similarly, extracellular hyalin deposits (Andrade & Andrade 1966; Veress et al. 1974, 1977) were not evident in this material.

Sequential histopathology thus demonstrates a dynamic response of the lymphoid organs which parallels the immunological changes usually described. The hypergammaglobulinemia seems to be the result of hyperplasia of the lymphoid follicles followed by hyperactivity of lymphocytes or their transformation to plasma cells. The compromised T lymphocyte-dependent immune response was related to selective and progressive lymphocyte depletion in the T dependent zones in 60th spleen and lymph nodes.

A progressive increase in the phagocytic mononuclear system activity was also visible and is characteristic of visceral leishmaniasis.

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