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Intestinal amyloidosis in hamsters with visceral leishmaniasis

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Summary. Thirty hamsters about 10 weeks old were inoculated intraperitoneally with *Leishmania donovani* amastigotes and were serially killed after 15, 30, 45, 60, 75 and 85–90 days. Both the small and large intestines were examined grossly, and the histopathology was assessed by light and electron microscopy. The lamina propria and the submucosa of the whole length of the intestinal tract showed a progressive deposition of amyloid, selectively identified by optical and ultrastructural techniques. The presence of amyloid fibrils in the cytoplasm of plasma cells suggests that appearance of intestinal amyloidosis during visceral leishmaniasis may be the result of a pathological dysfunction of these cells. In addition to these deposits, the presence of inflammatory infiltrates containing lymphocytes, plasmocytes and macrophages confirmed the establishment of leishmaniasis. In the end-stages of the infection both vacuolar degeneration of the epithelial cells lining the lumen of the intestine and a moderate hyperplasia of lymphatic nodules was observed.

Keywords: leishmaniasis, amyloid, intestine, hamster

Visceral leishmaniasis is a protozoal disease which affects man, dogs and other mammals and is caused by the organism *Leishmania donovani*. The disease has been described in four continents (Europe, Asia, Africa and America) where both endemic zones and isolated cases can occur (WHO 1984).

Intestinal lesions both in man (Chadli & Philippe 1961; Veress *et al.* 1973; Ravisse 1978; Mauel & Behin 1982) and in dogs (Lennox *et al.* 1972; Anderson *et al.* 1980; Rodriguez *et al.* 1981; Keenan *et al.* 1984) have been reported to occur during visceral leishmaniasis. However, previous reports have presented only special morphological aspects of the lesions such as the degree of intestinal parasitism (Lennox *et al.* 1984; Keenan *et al.* 1984) or the nature of the inflammatory phenomena in the intestinal tract (Veress *et al.* 1973; Mauel & Behin 1982; Keenan *et al.* 1984).

The present report describes experimentally induced histopathological changes occurring in the intestine of hamsters following inoculation of *Leishmania donovani*.

Materials and methods

Thirty-six Syrian golden hamsters (*Mesocricetus auratus*), 18 males and 18 females, about 10 weeks old and with a mean weight of 100 g, were fed a commercial diet supplemented with lettuce, carrots and sunflower seeds. Drinking water was supplied *ad libitum*.

They were divided into two groups: Group A, 30 animals, were inoculated i.p. with 1.0 ml of infective material containing 2×10^7 amastigotes. The inoculum was prepared from a spleen homogenate obtained from a hamster infected with *Leishmania donovani*. The total parasite count was determined using the same method as described in a previous study (González *et al.* 1983). The hamsters were serially killed at 15, 30, 45, 60, 75 and 85–90 days after inoculation. Group B, 6 animals, were inoculated i.p. with 1.0 ml of healthy hamster spleen homogenate.

Tissue samples for light microscopy study were fixed in 10% formalin, dehydrated, embedded in Histosec (Merck) and cut at 5 μm. The sections obtained were stained with hematoxylin and eosin, Gallego's trichrome, periodic acid-Schiff (PAS) and Congo red. The Congo red stained sections were viewed with polarized and fluorescent light. Samples for transmission electron microscopy were fixed in 3% Millonig-buffered glutaraldehyde, pH 7.3, and post-fixed in 1% osmium tetroxide. The samples were dehydrated through graded alcohols and embedded in epon-araldite. Thin sections were cut with an ultratome, stained with 2% aqueous uranyl acetate and lead citrate and examined in a JEOL 100 B electron microscope at 80 kV.

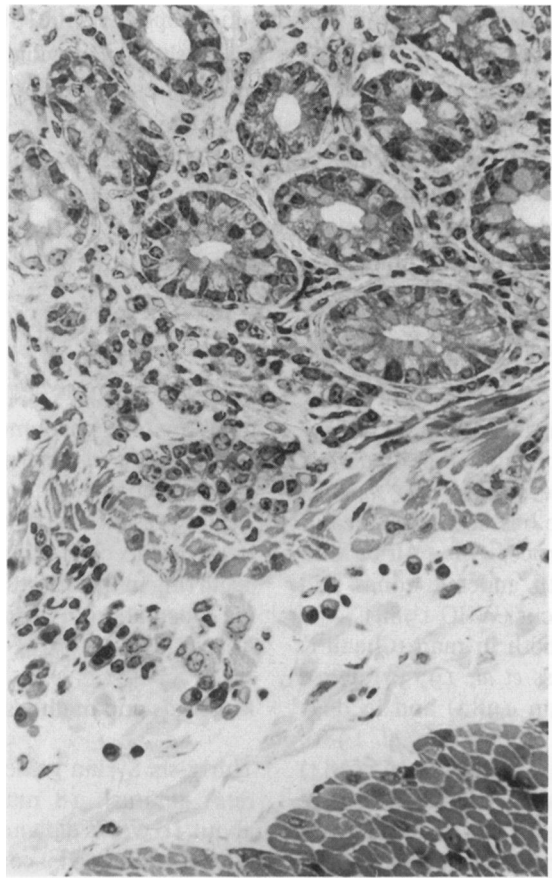

Fig. 1. Colon, 75 days after inoculation. Lympho-histio-plasmacytic inflammatory infiltrates in the lamina propria and submucosa. Macrophages in submucosa showing intracytoplasmic amastigotes. Methylene blue (1 μm section) × 312.

Results

No significant gross pathology was observed in the parasitized animals' intestines by comparison with controls.

Histopathological study

Animals killed between 15 and 60 days after inoculation showed only a gradual increase of a lympho-histio-plasmacytic inflammatory infiltrate in the lamina propria of the mucosa; this was more abundant in the small intestine than in the caecum. Adjacent to this infiltrate were a few isolated eosinophilic polymorphonuclear leukocytes and mast cells. In the initial phases of the disease, macrophages containing leishmania were not seen.

After 75 days the total length of the intestinal tract was inflamed with diffuse infiltrates which were more prominent in the lamina propria than in the submucosa (Fig. 1). Some macrophages, mainly in the colon and rectum, carried 1 or 2 *Leishmania donovani* amastigotes in their cytoplasm.

A notable finding at this stage was the accumulation of an amorphous, eosinophilic and acellular material in the lamina propria and submucosa which was positive to Congo red and had yellow-green birefringence under polarized light. This was identified as amyloid and ultrastructurally appeared

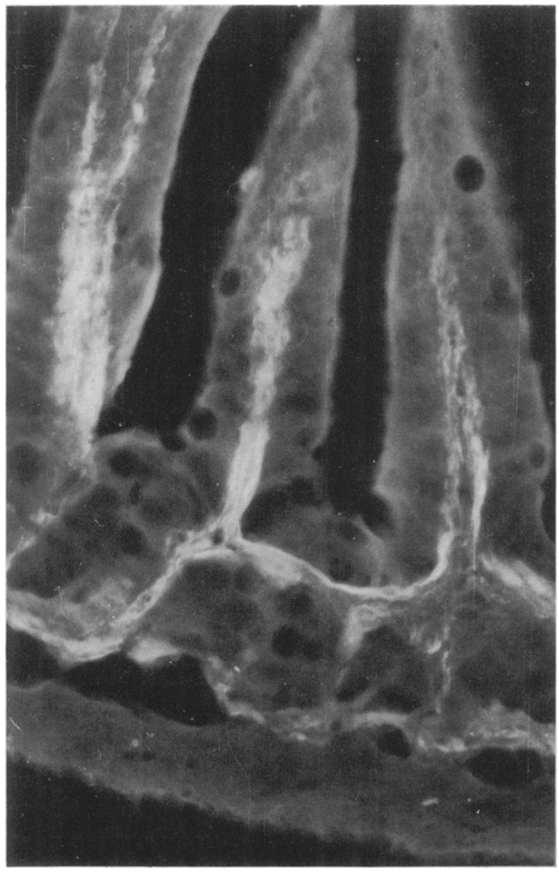

Fig. 2. Duodenum, 85 days after inoculation. Severe amyloidosis in lamina propria and submucosa. Congo red with fluorescence × 312.

fibrillar with characteristic thin transversally striated fibrils arranged randomly.

Some columnar epithelial cells, particularly in the small intestine, exhibited signs of mitochondrial tumefaction, i.e. swelling of the mitochondria, with rupture of the cristae to form spherical vesicles.

In the end-phase of the disease, at 85–90 days after inoculation, an increase of amyloid accumulation was observed by Congo red staining, which involved the entire length of the small intestine (Fig. 2). These extensive deposits of amyloid located in the lamina propria and submucosa, in some instances separated the crypts of Lieberkühn. These fibrils of amyloid were shown by electron microscopy to be extracellular and to be located in the blood vessel walls.

Some plasma cells, mainly in the periphery and among the amyloid deposits, had characteristic intracytoplasmic Russell bodies which appeared as voluminous spherical or oval acidophilic formations.. Ultrastructurally these appeared to be large rounded expansions of the granular endoplasmic reticulum (Fig. 3) containing protein deposits, possibly representing newly synthesized immunoglobulins.

Occasionally plasma cells containing large cytoplasmic accumulations of fibrillar substance resembling amyloid were observed (Fig. 4). The extracellular amyloid fibrils appeared to arise from these cells (Fig. 5).

Adjacent to these amyloid deposits a pronounced round cell inflammatory infiltrate was seen with a predominance of plasma cells and with lymphocytes and histiocytes.

Variable numbers of amastigotes within macrophages were found along the entire length of the intestinal tract. The numbers of parasites in the colon and rectum (maximum of 10 amastigotes per cell) (Fig. 6) were higher than in the different segments of the small intestine (maximum of four amasti-

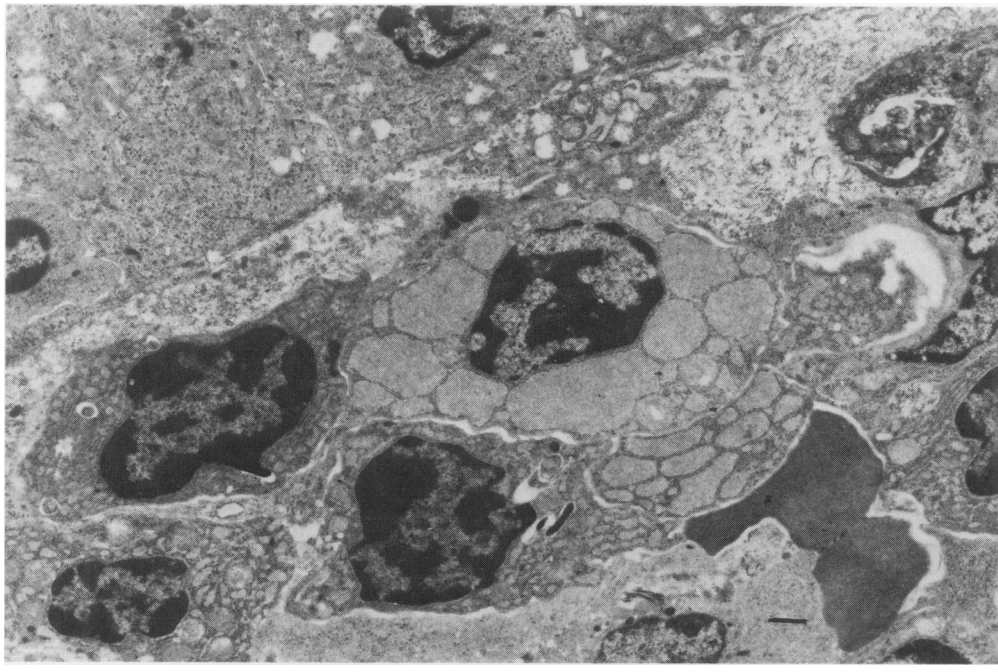

Fig. 3. Ileum, 85 days post-inoculation. Plasma cell with ample expansions of the granular endoplasmic reticulum. × 5000. Bar = 1 μm.

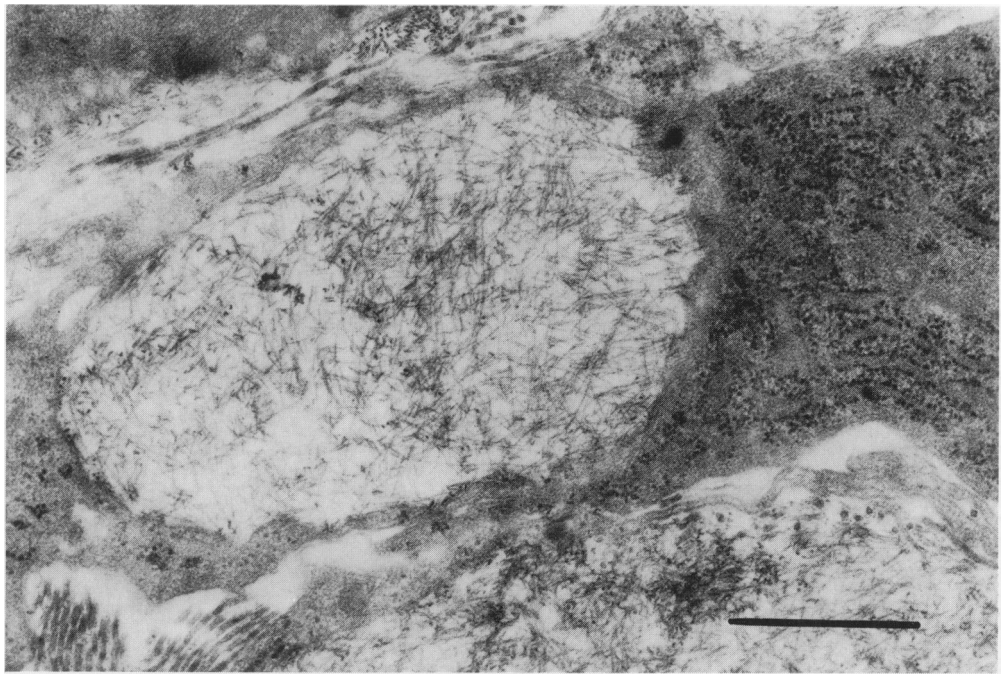

Fig. 4. Ileum, 85 days after inoculation. Plasma cell with intracytoplasmic deposits of amyloid fibrils. × 25 000. Bar = 1 μm.

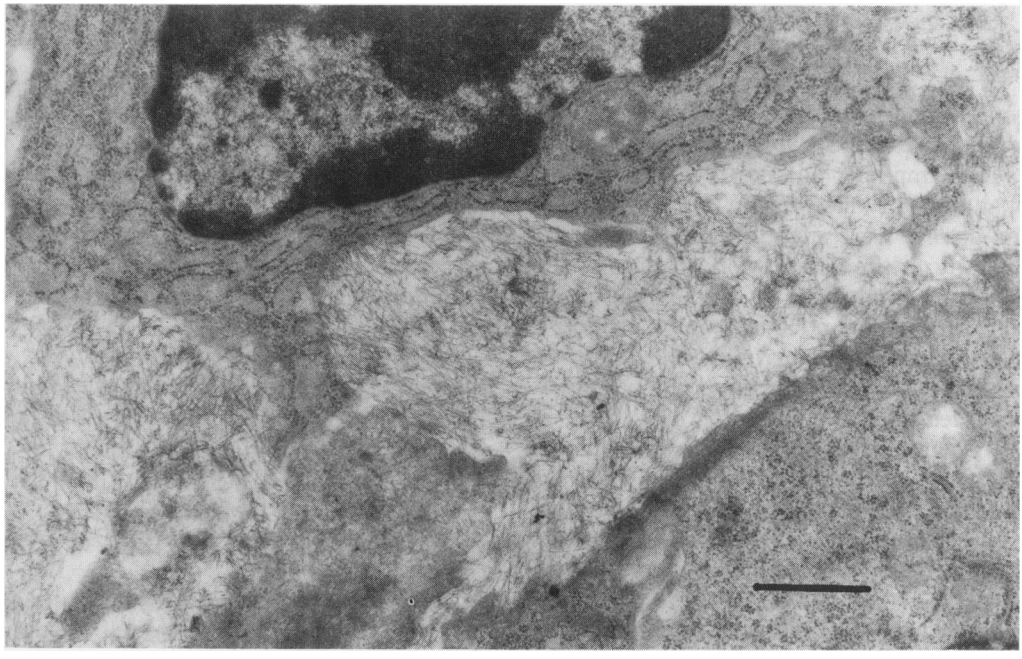

Fig. 5. Jejunum, 85 days after inoculation. Extracellular amyloid fibrils appear to emerge from within a plasma cell. × 15 500. Bar = 1 μm.

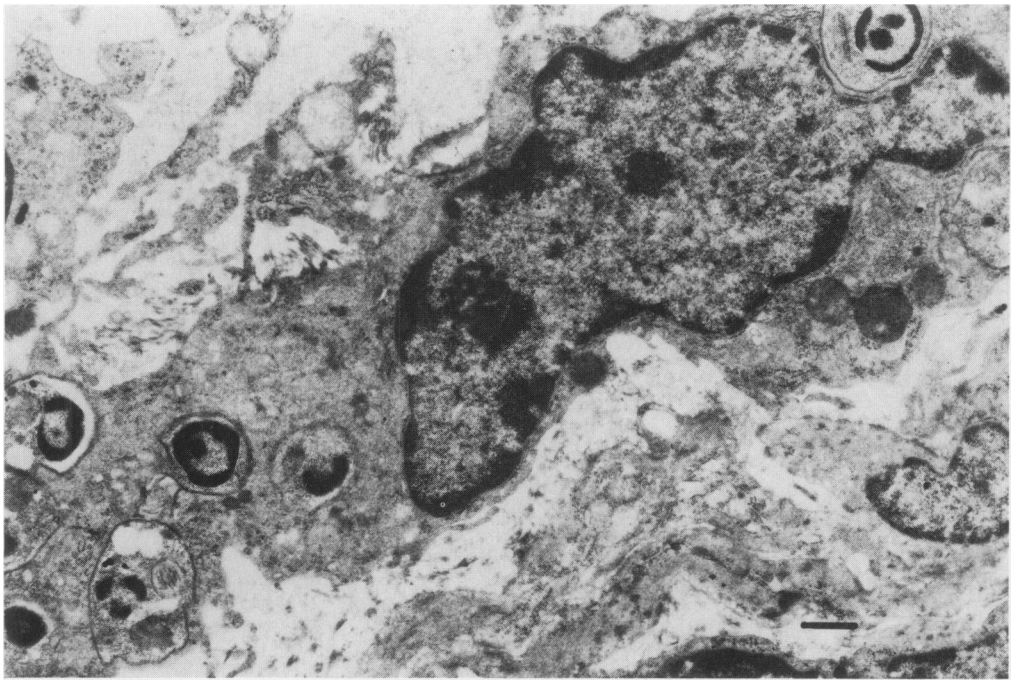

Fig. 6. Rectum, 85 days after inoculation. *Leishmania donovani* amastigotes contained in the cytoplasm of a macrophage. × 7700. Bar 1 μm.

gotes per cell). Macrophages with intracellular parasites were scarce in the caecum.

The lymphatic nodules (Peyer's patches) of all animals killed during the late stages of the disease were slightly hyperplastic, particularly in the ileum. Morphologically the lymphatic nodules had large germinative centres with lymphoblasts, mitotic figures, plasmocytes and macrophages containing parasites surrounded peripherally with lymphocytes.

By comparison with control animals, the columnar epithelial cells showed vacuolar degeneration in the final stages of the disease, seen histologically as small cytoplasmic vacuoles (Fig. 7) and ultrastructurally by swelling of the mitochondria and a slight enlargement of the cisternae of the Golgi complex and endoplasmic reticulum. The microvilli were not significantly affected.

Discussion

The present study demonstrates that experimental infection of hamsters with *Leishmania donovani* causes intestinal amyloidosis characterized by the deposit of amyloid in the lamina propria and submucosa of the intestinal tract.

Amyloidosis as a complication of visceral leishmaniasis has rarely been reported either in man (Andrade & Andrade 1966; Andrade & Iabuki 1972) or in dogs (Corbeil *et al.* 1976; George *et al.* 1976), but has been noted frequently in hamsters (Gellhorn *et al.* 1946; Hinglais *et al.* 1964; Abruzzo 1971; González *et al.* 1983).

Amyloid is characterized by a mesh of rigid and non-ramified fibrillar proteins with a thickness ranging between 80 and 100 Å (Glenner & Terry 1973). X-ray crystallogra-

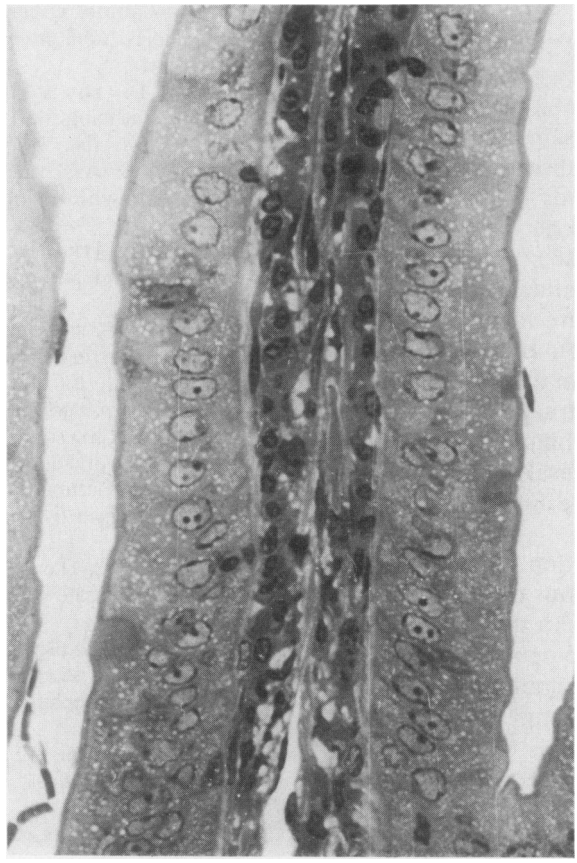

Fig. 7. Jejunum, 85 days after inoculation. Intestinal villi with small vesicles appearing in the cytoplasm of the simple columnar epithelium cells. Methylene blue (1 μm section) $\times$ 500.

phy has shown these proteins to be formed by polypeptide chains arranged as β-pleated sheets, a stable molecular conformation which makes them extremely insoluble and totally resistant to normal proteolytic enzymes (Tyzard 1982). Consequently accumulation of amyloid within tissues is generally irreversible and leads to progressive tissue destruction.

The origin of amyloid and its pathogenesis are still controversial. This study confirms the ultrastructural findings of Corbeil *et al.* (1976), who described the presence of amyloid fibrils in the cytoplasm of plasma cells which appeared to give rise to extracellular amyloid deposits, in a dog with visceral leishmaniasis. Our observations support Corbeil's suggestion that amyloid synthesis during visceral leishmaniasis could be due to a dysfunction of the plasma cells involving a switch to amyloid production instead of immunoglobulin synthesis (Corbeil *et al.* 1976).

Associated with the deposits of amyloid were progressive and diffuse lympho-histioplasmacytic inflammatory infiltrates (Daneshbod 1972; Keenan *et al.* 1984; Longstaffe & Guy 1985), which were moderate in the submucosa but more intense in the lamina propria; these were found throughout the entire intestinal tract.

The reports of macrophages carrying

amastigotes in the human (Daneshbod 1972; Veress *et al.* 1973) and canine intestine (Lennox *et al.* 1972; Anderson *et al.* 1980; Keenan *et al.* 1984) were confirmed in this study on hamsters; in this species during the end-stage of the disease the number of leishmanias in the colon and rectum (maximum of 10 parasites per cell) was greater than in other intestinal segments.

Some simple columnar epithelial cells of the small intestine were vacuolated from 75 days after inoculation onwards and gradually increased to finally affect the whole intestinal tract. Contrary to other reports (Veress *et al.* 1973; Philippe 1961; Mauel & Behin 1982) no mucosal necrosis or extensive ulceration of the intestinal tract was observed.

Experimental infection of hamsters with *Leishmania donovani* thus causes progressive intestinal amyloidosis in the lamina propria and submucosa accompanied by lymphohistio-plasmacytic inflammatory infiltration and degenerative changes of the simple columnar epithelium.

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