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# Morphology of the spleen and lymph nodes in fatal visceral leishmaniasis

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**Summary.** Histological appearances of the spleen and lymph nodes were analysed in twenty fatal cases of human visceral leishmaniasis from the Sudan. Marked atrophy of the splenic white pulp was associated with necrosis and fibrosis of thymus-dependent areas, accumulation of parasite-containing histiocytes and plasma cell hyperplasia. Depletion of small lymphocytes in the paracortical areas of the lymph nodes was accompanied by proliferation of plasma cells and histiocytes in the paracortex. Depletion of small lymphocytes in thymus-dependent regions of lymph nodes and spleen is viewed as arising from immune suppression associated with antigen overloading or other factors, which may impair those aspects of lymphocyte–macrophage cooperation that are presumably necessary to kill the invading parasites.

## INTRODUCTION

The study of immunological responses in different forms of leishmaniasis is of past and present interest. Thus in endemic areas of East Africa it has long been customary to inoculate children with materials taken from leishmanial ulcers to prevent the development of scars due to natural infection (Manson, 1914). In Kenya, immunity is said to last for at least 10 years following recovery from kala-azar (Manson-Bahr, 1959, 1961); and cellular immunity is viewed as a main defence mechanism against the leishmanial parasite (Adler, 1963; Bryceson, Bray, Wolstencroft & Dumonde, 1970; Stauber, 1970; Heyneman, 1971; Bray, 1972; Woo, 1974) as in certain other tropical, helminthic and fungal diseases (Turk, 1969, 1970; Turk & Waters, 1968, 1971; Keller & Keist, 1972; Krupp, 1974; Purtilo, Meyers & Connor, 1974; Carrada-Bravo, 1975).

In leishmaniasis, immunological and morphological studies have generally been undertaken in animals and in human cutaneous forms of the infection (Bryceson, 1969, 1970; Bryceson *et al.*, 1970; Heyneman, 1971). To our best knowledge, the morphological interpretation of changes in the spleen and lymph nodes in human kala-azar (Meleney, 1925) has not yet been referred to modern immunological concepts. This paper describes the examination of twenty fatal cases of visceral leishmaniasis and reports a depletion of small lymphocytes in thymus-dependent areas both in spleen and lymph nodes accompanied by an abundance of parasite-containing histiocytes and hyperplasia of plasma cells.

## MATERIALS AND METHODS

Spleens and lymph nodes were obtained from twenty post-mortem cases of visceral leishmaniasis examined at the Pathology Department, University of Khar-

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toum. Clinical and other morbid anatomical data were obtained from the records. Paraffin-embedded sections of the spleen and lymph nodes were stained with haematoxylin and eosin, Giemsa, Leishman, PAS and reticulin stains for light microscopical investigation; and with Congo red (Romhanyi, 1971) for polarization microscopy.

Morphometric analysis of splenic sections was carried out on haematoxylin-eosin stained sections from two blocks of each case. The area of the whole section was first determined by projection of the slide on to the screen of a Reichert–Visopan microscope equipped with millimeter paper. After having drawn the borders of each section the number of square millimeters was counted and finally the area of the section and the white pulp area were expressed in square microns. The area of the Malpighian corpuscle was evaluated by subtracting the projected area of the central artery from that of the total white pulp area. For comparative purposes a ratio of white pulp over total spleen area (WP/S) was calculated and referred to a unit spleen area of $1 \times 10^6$ $\mu m^2$. A control group of sections from lymph node and spleen was obtained from fifteen subjects who died without a history of disease (e.g. car accident; murder) and were selected from the same age group as those with leishmaniasis. The total number of individual white pulp areas measured were 962 and 574 in the kala-azar and in the control groups, respectively.

## RESULTS

In the leishmaniasis group all twenty patients were males; the average age was 25 years (range: 16–45 years); leucopenia was regularly observed (average white blood cell count: 4300/mm$^3$) but relative lymphocytosis was absent in sixteen cases. In all patients the spleen was considerably enlarged (average weight 860 g). The immediate cause of death was sepsis (including two cases with secondary fungal lung infection), bronchopneumonia and miscellaneous conditions such as uremia, haemorrhagic shock and amoebiasis in 11, 4, 2, 2 and 1 patients, respectively. In five of the patients leishmaniasis had been diagnosed prior to death and had been treated for periods of 7–16 days.

Morphometric analysis of the spleen in kala-azar revealed significant reduction ($p = 0{\cdot}02$) in the size of the white pulp areas ($10{\cdot}72 \pm 6{\cdot}52 \times 10^3$ $\mu m^2$) as compared to that of the control group ($29{\cdot}38 \pm 5{\cdot}7 \times 10^3$ $\mu m^2$). In the control spleens histology showed densely packed lymphocytes around the central arteries of the Malpighian corpuscles, whereas in none of the twenty cases of kala-azar studied were any germinal centres seen and the white pulp itself was loosened and disorganized (Fig. 1). In six out of the twenty cases central necrosis was found destroying the normal architecture of the white pulp (Fig. 2). The number of lymphocytes was invariably very low in all white pulps examined; and occasionally, the lymphocytes were virtually missing, being replaced by plasma cells and parasite-containing histiocytes (Fig. 3). Fibrosis of the white pulp was sometimes observed (Fig 4). A striking feature was the accumulation of an eosinophilic, PAS-positive material in the extracellular space of the follicles. This material did not show birefringence under polarized light. In the red pulp, large numbers of plasma cells and parasitized histiocytes were seen as well as proliferation of the sinus endothelial cells. In the cytoplasm of several histiocytes, fragments of red blood corpuscles and haemosiderin pigment were found.

In the lymph nodes of kala-azar patients there was a PAS-positive substance deposited in the germinal centres of the primary follicles similar to that described in the spleen (Fig. 5). The paracortical areas appeared pale at low power view due to the proliferation of parasite-containing histiocytes with occasional plasma cells and large lymphoid cells replacing the missing small lymphocytes (Fig. 6). Epithelioid cells were absent. The medullary cords were packed with plasma cells and histiocytes. In eight out of twenty cases the histiocytes contained fragments of erythrocytes.

## DISCUSSION

During the last decade considerable progress has been made in interpreting the histopathology of various tropical diseases accompanied by abnormal immune status. Thus Turk and his colleagues showed that lepromatous leprosy was regularly accompanied by lymphocyte depletion in the paracortex of lymph nodes, whereas no such paracortical depletion was seen in the tuberculoid form of the disease (Turk & Waters, 1968; Turk, 1969). Similar results were obtained in experimental leprosy (Hangen, Skjorten & Closs, 1975; Pfak, Gaugas, Rees &

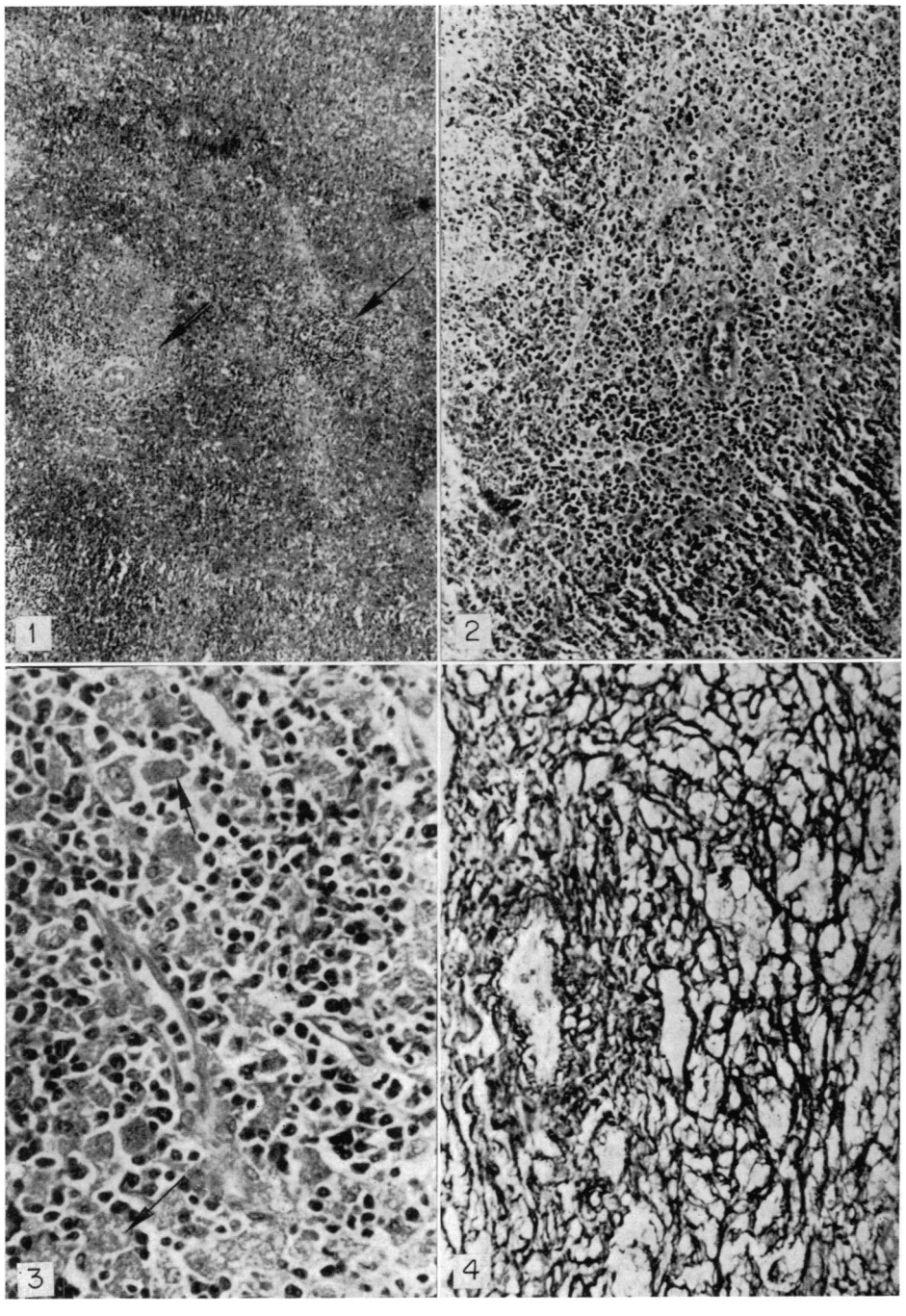


**Figure 1.** The Malpighian corpuscles are small (arrow) and their architecture is disorganized (double arrow) (H & E, ×60).
**Figure 2.** The number of lymphocytes diminished and large areas show shadows and remnants of necrotic cells (H & E, ×100).
**Figure 3.** Detail of a Malpighian corpuscle. The lymphocytes are replaced by plasma cells and parasitized histiocytes (arrow) (H & E, ×220).
**Figure 4.** Silver impregnation reveals marked fibrosis in the Malpighian corpuscle (Silver impregnation, ×180).

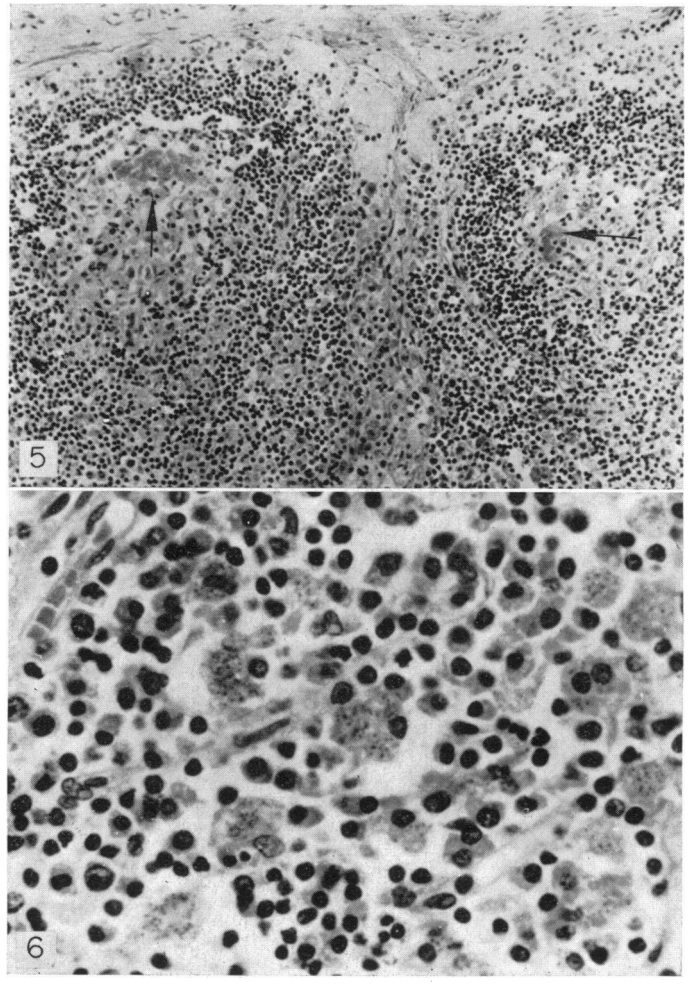


**Figure 5.** Detail of a lymph node. In the primary follicles eosinophilic materials are deposited (arrow) (H & E, × 100).
**Figure 6.** In the paracortical area of a lymph node the small lymphocytes are partly replaced by plasma cells and parasitized histiocytes (H & E, × 300).

Allison, 1970). In experimental immunology morphometric methods have been used to relate the white pulp areas of the spleen with immunological responsiveness (Graber & Corvazier, 1960; Feher & Nagy, 1972; Hard, 1974) but such morphometric methods have not previously been applied to the study of human spleen in tropical diseases which are known to be accompanied by immunological abnormality.

In the present study, morphometric analysis of spleen white pulp areas, together with the lymph node morphology were suggestive of a profound disturbance in cell-mediated immunity in fatal kala-azar. Our investigations revealed marked reduction in size of the white pulps due to a loss of small lymphocytes, with necrosis and fibrosis of the central areas of the follicles. This process was accompanied by the proliferation of parasite-containing histiocytes and plasma cells both in the white and red pulps of the spleen and in the paracortical areas of the lymph nodes. These findings extend the observations of Meleney (1925) who described splenic follicular atrophy in kala-zaar accompanied by the accumulation of an intrafollicular eosinophilic substance, which we and others have also observed (Andrade & Andrade,

1966; Veress, Malik, Satir & El Hassan, 1974). In experimental kala-azar, Stauber (1958, 1970) described atrophy of the splenic follicles due to a loss of small lymphocytes and to an increased number of macrophages and plasma cells; and human kala-azar is viewed as associated with suppression of specific cell-mediated immunity (Bryceson *et al.*, 1970; Turk, 1970).

Histological depletion of thymus-dependent regions in kala-azar might be caused by two mechanisms. The first one might be the appearance of an antilymphocytic antibody causing selective necrosis observed in the splenic follicles. This suggestion arises from the findings of Bryceson & Turk (1971) who produced paracortical depletion in the regional lymph nodes of guinea pigs by the injection of anti-lymphocytic serum into animals bearing experimental infections with *Leishmania enriettii*. The possible pathogenetic role of antilymphocytic antibodies has been suggested in syphilis (Levene, Turk, Wright & Grimble, 1969; Turk, 1970) and in systemic lupus erythematosus (Horwitz & Cousar, 1975). A second mechanism might be the overloading of the body by leishmanial antigen as described for lepromatous leprosy where there is a deficiency of specific cell-mediated immunity (Turk, 1969). In diffuse cutaneous leishmaniasis, Bryceson (1970) suggested that cellular anergy was caused by the over-production of a soluble leishmanial antigen ('tolerogen') which would specifically interfere with the cellular immune response. The morphological manifestation indicating such 'antigen-overloading' in kala-azar might be the accumulation of the intrafollicular hyaline substance. In experimental immunization, van Rooijen (1973, 1975) showed that labelled antigen was trapped by the splenic follicles; and Cooper, Haq & Baquell (1969) concluded that intrafollicular hyaline deposits were due to antigen-antibody complexes precipitated by excess antigen. In kala-azar a further immunological abnormality is suggested by erythrophagocytosis observed in the spleen and lymph nodes; this would indicate pathological destruction of erythrocytes and would support Woodruff's (1972) theory of the autoimmune nature of anaemia in kala-azar.

Our histological observations support the view that cooperation between (T)-lymphocytes and macrophages is important for effective killing of the invading *Leishmania*, as in other related diseases (Bryceson, 1969; Turk & Waters, 1971; Farah, Samra & Nuwayri-Salti, 1975). The abundance of parasite-containing histiocytes in these fatal cases indicated the ability of these cells to phagocytose but not to kill *Leishmania*; and a similar phenomenon has been described in lepromatous leprosy (Turk & Waters, 1971; Hangen *et al.*, 1975). This finding might be explained either by lymphocyte depletion or by interference by excess antibody with macrophage membrane function (see Watson, Sljivic & Brown, 1975). Our observations raise the desirability of carefully investigating the immunological status of patients with kala-azar, before, during and after treatment.

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