

The Ultrastructural Morphology of Human Cutaneous Leishmaniasis of Low Parasite Load

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The ultrastructural morphology of the inflammatory infiltrate in cutaneous leishmaniasis with low parasite load from seven patients was studied. Cells of the mononuclear phagocyte system (macrophages and epithelioid cells), plasma cells, and lymphocytes formed the infiltrate. Disseminated shrinkage necrosis or apoptotic as well as coagulative necrosis with loss of the plasma membrane of single macrophages occurred. An intimate contact was observed between macrophages and lymphocytes. The relationship of the morphological alterations to the persistence of the chronic inflammatory reaction and the possible explanations of the macrophage necrosis are discussed. *Key words: Macrophage; Necrosis.* (Received January 16, 1984.)

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Leishmania are obligate intracellular protozoa, that reside in macrophages. Depending on the species of the organism three forms of the disease occur in man: visceral, mucocutaneous, and cutaneous leishmaniasis. The latter is caused by different strains in different geographical regions. In the Middle East *L. major* produces the classical oriental sore and a variety of other clinical forms including muco-cutaneous leishmaniasis (1). The lesion heals spontaneously or with treatment but in some patients it persists for many months or years and may become refractory to treatment. This is particularly true for the recidiva form which shows signs of central healing but recurs and spreads at the margin (2). The diffuse form of the disease which is described in Ethiopia and South America produces a non-healing lesion that continues for years and shows many parasites.

The reasons for the persistence of the infection in some cases are poorly understood. Cell-mediated immunity appears to be important for the elimination of the parasite (3) but it appears that the excessive cell-mediated reactivity is responsible in some way for the persistence of the recidiva form. This preliminary communication describes the occurrence of apoptosis, an immunologically induced cell necrosis (4) in chronic cutaneous leishmaniasis that failed to heal despite a low parasite load. It is proposed that persistence of leishmanial antigen in the macrophages or macrophage plasma membrane receptors cross-reacting with leishmanial antigen can prolong the inflammatory reaction and delay healing.

PATIENTS AND METHODS

Patients were seen in Hufuf oasis in the Eastern Province of the Kingdom of Saudi Arabia. There were six males and one female, aged between 3 and 45 years. They had no treatment and showed no signs of healing. 4 mm punch biopsy of the lesions was performed under local anaesthesia. One half was fixed in a formaldehyde mercuric chloride-acetic acid mixture. Paraffin sections were stained with haematoxylin and eosin, and Giemsa and examined for parasite load and cellular reaction. Smears from the cut surface of the fresh biopsy were fixed in methanol and stained with Giemsa for

parasites. The material for electron microscopy was fixed in 4% glutaraldehyde, 4°C for one hour, washed in phosphate buffer pH 7.2, postfixed in 1% OsO₄ and embedded in Epon. Sections were cut on an LKB Ultratome III. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a Zeiss EM-9 electron microscope. The electron micrographs were taken on Gevaert Scientia 23D56 P3AH films. 1 µm sections were stained with toluidine blue for light microscopy.

RESULTS

The parasite load was 1+ or 2+ on the Ridley-scale (2), in which the maximum was 5+ representing a heavy load of amastigotes in all parts of the section.

The composition of the inflammatory infiltrate was basically similar in all cases examined. 1 µm thick sections showed very few parasitized histiocytes in one case only, whereas no parasites were observed in the thick sections of the other 6 cases though some parasites were always seen in the paraffin sections or smears. The inflammatory infiltrate was composed of cells of the mononuclear phagocyte system, plasma cells, and lymphocytes. Intermingled with these cells there were scattered, small, very dark, compressed cells without clearly defined nuclei (Fig. 1).

Electron microscopy revealed that the cells of the mononuclear phagocyte system were activated macrophages and epithelioid cells. The former ones were smaller and elongated cells, their cytoplasm had long, slender projections and the hyaloplasm was electron dense, darker than that of the epithelioid cells. These activated macrophages also contained numerous cytoplasmic vacuoles. The large, polygonal and pale epithelioid cells had bean-shaped nuclei with finely distributed chromatin. Numerous mitochondria, polyribosomes, and lysosomes were a constant feature of these cells (Fig. 2). Several lymphocytes showed the signs of activation: they were larger, had short cytoplasmic projections and an increased number of mitochondria and endoplasmic reticulum. A close contact between the plasma membrane of the lymphocytes and the macrophages was a common finding.

The scattered, small, very dark cells showed a condensed, very electron dense cytoplasm which occasionally showed shadows of the cell organelles. The nuclei were pyknotic with marked condensation of the chromatin and sometimes the occurrence of paler, irregular areas within the condensed chromatin (Fig. 3). Other macrophages showed the signs of coagulative necrosis: oedematous degeneration or extensive vacuolization of the cytoplasm, fragmentation or dissolution of the nuclei, and partial or complete loss of the plasma membrane.

DISCUSSION

The mechanisms of parasite elimination and the persistence of the inflammatory process, hence the clinical disease, despite a low parasite load are still poorly understood. It was believed that the most important mechanism for the elimination of the parasites was necrosis of the parasite-loaded macrophages and not the development of delayed hypersensitivity (2, 5). Another possible mechanism, at least in some geographical areas, may involve lymphokine activated macrophages. Veress et al. (6) have shown that parasites were being destroyed in the cytoplasm of activated macrophage in patients with mucosal leishmaniasis. Intracellular digestion of *Leishmania* parasites and their elimination was described in connection with the development of skin-test hypersensitivity of the delayed type (7).

Two cell types of the mononuclear phagocyte system were seen in the inflammatory infiltrate of low parasite load in our cases. One was the activated macrophage with many cytoplasmic vesicles, long cell projections, and well developed endoplasmic reticulum. The other one was the epithelioid cell with very abundant, pale cytoplasm. These cells

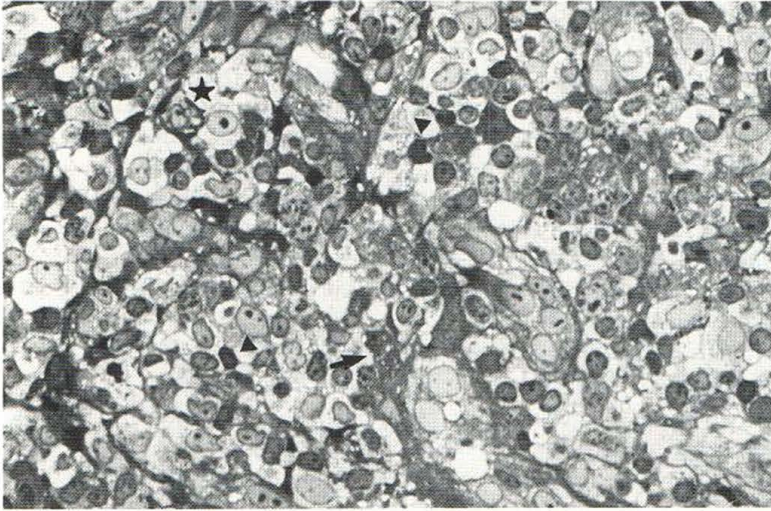


Fig. 1. Densely packed infiltrate with large, pale epithelioid cells (star) and darker macrophages (arrow) as well as shrunken, compressed cells without well defined nuclei (arrowheads). $\times 160$.



Fig. 2. Showing large epithelioid cells (*E*) and macrophages (*M*). $\times 1800$.

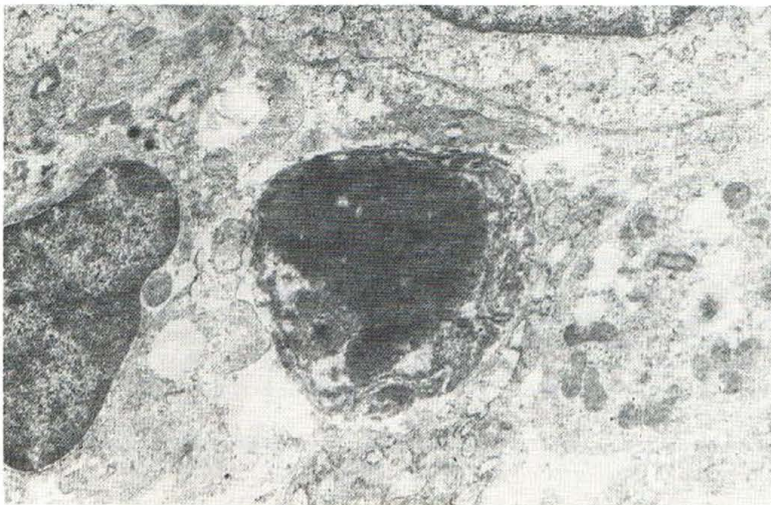


Fig. 3. Apoptotic body with condensed, electron dense chromatin, irregular electron lucent areas, and dark cytoplasm without recognizable organelles. $\times 4900$.

represent different stages of macrophage development with different functional activities as was shown in experimental models (8, 9, 10). We could find in these cases a special type of cell necrosis: the necrotized cells showed a marked condensation of the nuclear chromatin and that of the cytoplasm. These cells eventually disintegrated into smaller fragments consisting of cytoplasm and nuclear material, possibly appearing as haematoxyphil bodies in the light microscope, a fairly common finding in cutaneous leishmaniasis (2). These features of cell necrosis are those of apoptosis (4) which is caused by killer T-lymphocytes as was shown in vitro experiments (11). Apart from apoptosis, coagulative necrosis with the loss of plasma membrane was also seen in our cases. Bryceson et al. (12) proposed that the loss of plasma membrane of the necrotizing macrophages in experimental leishmaniasis was due to immunological mechanism.

In our cases there was an intimate contact observed between activated lymphocytes and macrophages. It is known that leishmanial antigen can be present in the macrophages (12, 13, 14, 15). The antigen presentation is thought to be essential in the activation of lymphocytes (16). On the other hand, antigen-carrying macrophages or cells which have membrane receptors cross-reacting with leishmanial antigen, as shown on the plasma membrane of human red blood cells (17), can be the target cells of activated killer lymphocytes or specific antibodies. Hence, one can speculate that in these cases of chronic cutaneous leishmaniasis of low parasite load, when the treatment with antileishmanial drugs is often unsatisfactory (El Hassan, personal observation), the persistence of defected macrophages having either leishmanial antigen or cross-reacting membrane receptors, and not the parasites themselves are responsible for unsuccessful treatment. It is interesting in this connection to cite two publications reporting the healing of persistent cutaneous leishmaniasis after the application of transfer factor (18) or BCG and cord factor (19) which stimulate the host's immune response but not antileishmanial drugs.

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