A CYTOLOGICAL STUDY OF MACROPHAGES IN CUTANEOUS LEISHMANIASIS

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ABSTRACT

Exfoliative cytology smears from the lesions of 179 patients with cutaneous leishmaniasis due to Leishmania tropica were studied with specific reference to cellular reactions and their effect on the parasite. Aggregates of the parasite (so-called Leishman bodies) were present within macrophages and in some fibroblasts. The nature of the inflammatory cells present in the smears was correlated with the number of Leishman bodies contained within them and the percentages of small lymphocytes, neutrophils and type I macrophages present. It is postulated that aggregates of activated macrophages (designated types II and III) and the Leishman milieu (sticky matrix) protect the Leishman parasites from being eradicated by the inflammatory and immune reactions.

KEY WORDS: Cutaneous Leishmaniasis, Cytology, Cellular reactions, Cellular immunity.

INTRODUCTION

Leishmaniasis is a disease of major importance caused by species of the intracellular Leishmania parasite. It is endemic in 82 countries on four continents and has an estimated global prevalence of 12 million cases annually. Transmitted by bites of the sand-fly, the organism invades histiocytes at the site of the bite and multiplies within the macrophages of the skin, oro-nasal mucosa and reticulo-endothelial system. Accumulations of the amastigote phase of the organism within the histiocytes constitute the characteristic Leishmania bodies which are diagnostic of the disease.

Although many sophisticated in vivo and in vitro studies have been performed to elucidate the mode of replication and transmission of the organism there is much still to be learnt about the cellular and immunological responses to cutaneous leishmaniasis.

The clinical spectrum of the disease seen in Iran comprises localised "dry"[1] and "wet"[2] types of leishmaniasis (LCL), localised leishmania lymphadenitis (LLL) [3] and visceral leishmaniasis (VL)[4]. In the semi-tropical Kerman province of south-east Iran, close to the central desert region, leishmaniasis is the second most common parasitic disease. Patients seen in Kerman city present most frequently with the dry type of LCL and these formed the population of the study reported here.

MATERIALS AND METHODS

One hundred and seventy nine patients with the dry type of leishmaniasis were seen at the Dermatology department of Kerman hospital during the period 1992-1993. Their ages ranged from one to sixty years; there were slightly more males (55%) than females; all the lesions were on exposed uncovered skin and had been present for periods from one month to three years.

Smears were made from granulation tissue at the edge of the skin ulcers using a sterile lancet, smearing the material directly onto glass slides, airdrying and staining according to the Wright-Giemsa method. These smears were examined microscopically using a 40X objective by two independent observers who also made differential counts of the various types of inflammatory cells and the cells containing Leishman bodies.

The macrophages were classified into three types. Type I macrophages (inactivated monocytes) had bean-shaped nuclei, cytoplasmic granules, and finely vacuolated cytoplasm. Type II (activated) macrophages had larger, oval or elongated nuclei with a fine chromatin pattern and abundant pale cytoplasm. Type III ("epithelioid") macrophages had oval or round, vesicular nuclei containing prominent nucleoli and abundant dense cytoplasm resembling

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that of epithelial cells. Two hundred cells in each smear were counted and the results expressed as percentages. The amount of the cytoplasm occupied by the parasite was recorded using an arbitrary system scored from 0 - 5 (see Table 1). Spearman’s rank-order coefficient was used for analysis of the data.

RESULTS

Leishman bodies were identified in all three types of macrophage and were also noted within fibroblasts (Figure 1). Typically, when the macrophages containing Leishman bodies were surrounded by neutrophils, small lymphocytes and inactive histiocytes, degenerative changes were seen in the Leishman bodies as illustrated in Figures 2 and 3. The organisms showed hydropic swelling and the kinetoplast was lost. When the former inflammatory cells were scanty, anastomosing sheets of activated macrophages were present in a background of proteinaceous matrix forming the so-called “Leishmanin milieu”. In these cases aggregates of parasites (parasitophores) were observed floating in the milieu, having been discharged from the cytoplasm of the adjacent macrophages leaving empty spaces and resulting in apparently injured macrophages (Figures 4 and 5). The discharged organisms in the matrix lacked degenerative changes. In some cases it seemed that organisms had been able to transfer between adjacent macrophages through the interconnecting cytoplasmic processes. (Figure 6).

Differential counts of the inflammatory cells and Leishman bodies are summarised in Table 1. These counts showed an inverse relationship between the number of neutrophils, small lymphocytes and inactive macrophages and the number of Leishman bodies. There appeared to be no correlation between the cellular pattern of the smears, the age of the patient, the site of involvement or the duration of the lesions.

Figure 1: Leishman bodies within a fibroblast in a cytological scrape preparation from the lesion (Wright-Giemsa x 400).

Figures 2: Leishman amastigotes showing degenerative changes when surrounded by neutrophils and small lymphocytes. Note the hydropic degeneration and loss of the kinetoplast (Wright-Giemsa x 1000).

Figures 3: Leishman amastigotes showing degenerative changes when surrounded by neutrophils and small lymphocytes. Note the hydropic degeneration and loss of the kinetoplast (Wright-Giemsa x 1000).

Figure 4: Well preserved Leishman bodies within the cytoplasm of type II macrophages (Wright-Giemsa x 400).
Table 1: The changing cellular pattern of the inflammatory infiltrate and the number of Leishman bodies in skin lesions of different duration.

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KEY: L.B = Leishman bodies, scored arbitrarily as follows: 0 = absent; 1 = occupying < 5% of cell volume; 2 = 5 - 25%; 3 = 25 - 50%; 4 = 50 - 75%; 5 = 75 - 100%

Figures for the inflammatory cells are average percentages. (*) In lesions of less than 18 months duration, type III macrophages present mostly as multinucleated giant (epithelioid) cells.

DISCUSSION

The involvement of dermal fibroblasts observed in this study is not appreciated widely but is supported by previous ultrastructural studies in closely related species of leishmania [7,8]. The cellular immune response to the parasite appears to be a major factor in limiting the spread of the disease.

The cytological study showed an inverse relationship between the number of Leishman bodies and some components of the cellular reaction to the parasite. In particular, the presence of numerous neutrophils, small lymphocytes, and type I macrophages correlated with smaller numbers of Leishman bodies. Furthermore, morphological changes suggesting a destructive effect on the organism were observed in these cases. These observations support the importance of cell-mediated immunity in the reaction of the body to this parasitic infection and the more sophisticated studies by other workers.
which suggest that cellular immunity comprises the major component of the eradication phenomenon in leishmaniasis\textsuperscript{[10].} In contrast, when large numbers of activated Type II and III macrophages were present in the smears, Leishman bodies were numerous and did not show degeneration. It seems that the organism is relatively protected when it is present within the lysosomal bodies of these macrophages. It has been shown in ultrastructural studies using L. mexicana that the leishmania amastigote utilizes components of the lysosome, is resistant to lysosomal hydrolases and circumvents the macrophage’s antigen-presenting capabilities\textsuperscript{[11].} The parasite damages the histiocytes resulting in the release of growth factors such as transforming growth factor-beta which induce the proliferation of activated macrophages and fibroblasts especially in the early stages of the disease\textsuperscript{[12].} We confirmed the observation of others working on the lesions of L. braziliensis guyanensis\textsuperscript{[9]} that the presence of plasma cells, large lymphocytes, mast cells and eosinophils within the infiltrate was not associated with significant destructive effects as judged by the morphological appearance of the organism in the smears.

Acknowledgments

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REFERENCES