Matrix Remodelling and Fibroblast Phenotype in Early Lesions of Human Cutaneous Leishmaniasis

P. Esterre, J. P. Dedet, S. Guerret, M. Chevallier, C. Frenay and J. A. Grimaud
Parasitology Laboratory, Institut Pasteur de Guyane française, Cayenne, French Guiana; Ultrastructural Pathology Laboratory, Institut Pasteur de Lyon, Lyon, France; Instituto Boliviano de Biología de Altura, Embajada de Francia, La Paz, Bolivia

SUMMARY

The connective matrix participates directly in early pathological events observed in the cutaneous lesion of leishmaniasis, due to Leishmania braziliensis guyanensis. A sample of 19 skin biopsies was examined by light and electron microscopy, in order to identify the matrix components (collagen isotypes I to IV, elastin and membrane associated proteins) of the dermal infiltrate, and the pattern of organization of the reparative connective matrix.

An extensive remodelling process of apparently parasite-independent nature involves different fibroblast sub-populations. The original organization of this immune-mediated lesion offers a rare opportunity to study in situ the local inflammatory mediators inducing the activation of fibroblasts and macrophages.

Introduction

Cutaneous leishmaniasis granulomas are focal dermo-epidermal lesions characterized by the persistence of parasites in situ. Release of antigenic substances from living and/or destroyed parasites induces simultaneous activation of local cells and recruitment of exogenous mononuclear cells and fibroblasts.

Little attention has been paid to the early stages of granuloma formation. It is widely accepted that dermal connective tissue cells and extracellular macromolecules are involved in the reparative process of skin alterations. Since the connective matrix participates directly in the early pathological changes observed in the leishmaniasis skin lesion, it seemed of importance to investigate the particularities of establishment and maintenance of connective tissue reaction and repair. The aim of the present work is to study these aspects of pathology in a type of American cutaneous leishmaniasis, due to Leishmania braziliensis (L. b.) guyanensis, which we have already investigated from an immunopathological viewpoint.8,13,14.

Material and Methods

1. Clinical Data

All leishmaniasis patients seen at the Institut Pasteur de Guyane française between November 1986 and March 1987, whose consent to biopsy was obtained, were included in the study.

The lesions selected were acute cutaneous lesions containing amastigotes, and without superinfections. Smears, NNN medium cultures and inoculations into the dorsal part of the foot in hamsters were carried out in all cases, following a protocol previously described. The biopsies were performed by using a 3 mm punch-biopsy (Stefel lab., FRG) after local anaesthesia by lidocaine (Roger Bellon lab., France).

The biopsies were collected from 18 patients infected with cutaneous leishmaniasis. Personal, epidemiological and biological data were collected for all of them (Table 1). All were young male adults (mean age: 23.2 ± 5.1 years) of European (14 cases), Creole (3) or cross-bred (1) origin. In all cases the infection was recent (mean duration of disease: 3.2 ± 4.7 weeks) and untreated at the time of the biopsy. Seventeen cases were primary infections and two cases were secondary leishmaniasis infections acquired more than six months after complete cure of a first attack. The patients presented between 1 and 18 lesions (mean number of lesions per patient: 4.5 ± 4.5).

© 1991 by Gustav Fischer Verlag, Stuttgart
Table 1. Characteristics of biopsied lesions (N = 19)

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Amastigote density</th>
<th>Isolated strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary infection</td>
<td>Secondary infection</td>
<td>Ulcerative lesion</td>
</tr>
<tr>
<td>17</td>
<td>2</td>
<td>18</td>
</tr>
</tbody>
</table>

1 The amastigote density, calculated on smears, is expressed as number of parasites at 100× magnification. 2 Further information on isoenzyme characterisation is given in ref. 6.

All the lesions biopsied contained amastigotes, as exhibited in smears and/or cultures. The data obtained from amastigote measurement (mean diameter: 3.56 ± 0.21 μ) culture growth and infection in hamsters were compatible with *L. b. guyanensis* species. In twelve cases, the isolates were characterized by isoenzyme electrophoresis and shown to be indistinguishable from *L. b. guyanensis* MHOM/BR/M4147 reference strain.

2. Laboratory Techniques

Each biopsy was cut longitudinally into four pieces, which were fixed as follows: a) (4% glutaraldehyde in cacodylate buffer followed by 1% osmium tetroxyde with embedding in epoxy resin (standard electron microscopy technique); b) (2 pieces) 4% formaldehyde in cacodylate buffer for immunolabelling (pre-embedding technique) or indirect immunofluorescence in light microscopy; c) aqueous Bourns' fluid for classical histopathological examination with Hematoxylin and eosin (HE) staining.

Semi-thin sections of epoxy-resin-embedded material were cut before ultramicrotomy to localize the limits of the infiltrate identified by histology (Fig. 2).

For immunochemistry, antibodies were used to detect desmin and smooth muscle actin, and the following extracellular antigens: collagen isotypes I, pro-III, III, IV and α2CB (3.5), elastin, laminin and fibronectin.

Results

1. Granuloma Architecture and Host-Parasite Interactions

By light microscopy, an inflammatory infiltrate, classically referred to as "lymphoplasm-histiocytic", was observed extending from superficial to lower dermis (Fig. 1). Two major zones were identified: a) the central zone of the infiltrate, where polymorphonuclear necrotic cells and debris were predominant; and b) a peripheral zone, consisting of macrophages, lymphocytes, and rounded, oval or spindled cells, which by electron microscopy (see below) could be identified as fibroblasts and myofibroblasts. In this peripheral zone, the infiltrate itself could be distinguished from a "peri-infiltrate zone", marking the boundaries of normal dermis.
Even though all the smears were positive for amastigotes, with a mean density of 3.4 ± 4.1 per field at 100 × magnification, only 30% of the HE stained paraffin-embedded biopsy material showed the parasites. This scarcity of parasites was confirmed by electron microscopy (see below) in that amastigotes were found in only 5 cases and in these only very infrequently (Fig. 5).

Extending our preliminary observations\(^5,14\) that the inflammatory cell infiltrate (Fig. 6) is principally made of T cell lymphocytes (but with moderate numbers of macrophages and a few plasma cells), we now present data showing that this inflammatory infiltrate is associated with a significant remodelling of the matrix components (collagen isotypes and associated proteins-fibronectin, proteoglycans and elastin).

---

**2. Cell-Matrix Interactions in the Infiltrate**

### 2.1 The Granuloma Matrix

By immunofluorescence (Table 2), connective matrix components of the granuloma were found to include interstitial collagens types I and III, the former only found in the peri-infiltrate zone (Fig. 3); basement membrane-associated proteins (collagen IV (Fig. 4), laminin, fibro-

---

**Table 2. Matrix and cytoskeletal proteins expressed in the granuloma (indirect immunofluorescence technique)**

<table>
<thead>
<tr>
<th></th>
<th>Normal Dermis</th>
<th>Perilesional Matrix</th>
<th>Inflammatory Infiltrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Extracellular Matrix Proteins:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen isotype I</td>
<td>++</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>Isotype III/pro-III</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Isotype IV</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>α5CB (3.5): I</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Laminin</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Elastin</td>
<td>++</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td><strong>Cytoskeletal Proteins (fibroblasts):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-actin</td>
<td>0(^1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Desmin</td>
<td>0(^2)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^1\) Positivity limited to the blood vessels periphery. \(^2\) Positivity limited to the muscular skin adnexae.
Fig. 5. Three extracellular amastigotes (arrowheads) in the peri-infiltrate zone. Note the irregular pattern of the connective matrix, × 3250.

Fig. 6. The inflammatory infiltrate is principally composed of macrophages and lymphocytes, sometimes with a plasmacyte morphology (arrowhead), × 6300.
nectin); and elastin. Weak but positive staining was also shown for α2-CB (3.5), which has been demonstrated to be a tissular marker of type I collagen degradation\textsuperscript{2,17}, around and within the inflammatory granuloma. Staining for anti-α actin and anti-desmin was always negative in inflammatory cells, but blood vessels and muscular skin adnexae served as positive internal controls.

Ultrastructurally, compared with the normal adjacent dermis, the extracellular matrix of the infiltrate and peri-infiltrate zones showed a disorganized pattern of deposition (Fig. 5). The bundles of fibrils and microfibrils were smaller, and isolated microfibrils, mixed with irregular, amorphous dense material associated with basement membrane elements, were frequently observed. Elastin-like deposits, indistinguishable from amorphous dense osmiophilic material, were also present.

2.2 Phenotypic Expression of Fibroblasts

Rounded or ovoid myofibroblasts were characteristic of the infiltrate zone. They exhibited a continuous or focal pericellular basement membrane, a well-developed Golgi and abundant rough endoplasmic reticulum. Microfilaments were present, organized in bundles with focal densities. These filament-bundles were readily seen in longitudinal section, following the direction of cytoplasmic expansions. Intercellular gap-junctions were occasionally seen between myofibroblasts. No evidence of a polarized pattern among these cells was detected.

3. Matrix Degradation

3.1 Extracellular Degradation

The peri-infiltrate zone had an extracellular matrix consisting of dense collagen bundles organized in a regular network, and showed many fibroblasts and microvessels. The latter were easily identified by regular basement membranes around endothelial and pericytic cells. The peri-infiltrate zone was distinct from the normal dermis in having an oedematous interstitial network of loose collagen bundles organized in microfibrillar aggregates. This area was characterized by alternating zones of extracellular matrix degradation and synthesis to judge by the ultrastructure of the spindled cells present (see below). The process of extracellular degradation of collagen fibrils was particularly evident, with typical collagen hyperfibrils\textsuperscript{11} observed in the vicinity of fibroblasts.

3.2 Cellular Components of Matrix Degradation

Internalization and deggregation of matrix components by fibroblasts was evidenced by large lysosomal vacuoles showing entire fibrils (Fig. 7). These vacuoles contained finely granular and somewhat disorganized material in addition to fibrils.

Less frequently, infiltrating macrophages also contained electron dense vacuoles containing fibrillar material lacking banding patterns. Macrophages without such a "phagocytic" pattern were also observed in the same area.

Fig. 7. A fibroblast of the peri-infiltrate zone, showing in the upper part internalized collagen fibrils (arrowheads). This pattern is consistent with a "fibroblast" phenotype, × 10 400.

Discussion

The patient group studied is highly homogeneous and representative of the cutaneous leishmaniasis type occurring in French Guiana, whose clinical and epidemiological aspects have been reported elsewhere\textsuperscript{1}. All patients were infected by \textit{L. b. guyanensis}, the species responsible for 96.7% of the human lesions found in this country\textsuperscript{8}.

This ultrastructural study shows a notable discrepancy between the scarcity of parasites inside the granuloma, as seen in biopsy sections, and the number of parasites in the superficial parts of the edges, as shown on smears. Such an observation has already been made in the same form of leishmaniasis, by light microscopy with immunoenzymatic staining\textsuperscript{8,14}.

Classically, in wound healing immature or modified fibroblasts are recruited during the accumulation of
inflammatory cells and an important element in this process is the synthesis of collagen, particularly in some circumstances type III. It can be assumed that in the early inflammatory lesion of leishmaniasis, recruitment and activation of fibroblasts is of major importance. It must be emphasised that the myofibroblasts, which predominate in the periphery of the infiltrate, are desmin and α-actin negative indicating probably a local origin instead of a differentiation from smooth muscle cells.

The present investigation in leishmanial granuloma also demonstrates a remodelling of the matrix, not only in comparison with the normal surrounding dermis, but also with respect to the time course of the granuloma, where matrix remodelling terminates in the generation of a mature phase representing a cicatricial type of repair (Fig. 8). This remodelling includes both synthetic and degradative aspects. Several well-defined structural and qualitative changes occur in the matrix, compared with normal dermis. There is a loosening and disorganisation of the fibrillar texture of the matrix, and a reduction of some components, especially certain basement membrane components. This is partly interpretable as degradation, as the appearance of α1 CB (3.5), an extracellular matrix protein which has been associated with type I collagen degradation.

This duality of synthesis and degradation is reflected in the unusual phenotype of the fibroblasts. They contain abundant rough endoplasmic reticulum, indicative of matrix synthesis, and also possess what we have interpreted as vacuoles containing internalized matrix components, principally collagen fibrils. It suggests degradation that these vacuoles also contained disorganized-looking material in addition to fibrils. The process of internalization and degradation of matrix components by fibroblasts has been well documented both in vitro and in vivo. The term “fibroblast” has been applied to the cells participating in these processes and this seems to be an appropriate designation in the present context (see Fig. 7). The fact that individual cells show a duality of both synthesis and degradation emphasised the likelihood that the fibroelastic phenotype is a functional and possibly transient or reversible state in the fibroblast cycle.

The remodelling process, of apparently parasite-independent nature, not only involves fibroblasts, but also, judging by their contents of internalised matrix, the macrophages. Taking into account the fact that remodelling is found in association with the infiltrate and what is known of the process of chronic inflammation, it seems likely that fibroblast activation is a stage in a complex interaction between the mononuclear cells of the infiltrate. The original organization of this dermal granuloma, which from the early beginning combines an acute inflammatory infiltrate together with a significant reparative process, offers a rare opportunity to study in situ phenotypically distinct fibroblasts sub-populations. An exciting field of investigation remains open: which factors locally induce fibroblast activation and/or phenotype differentiation? Details of precise mechanisms have to be elucidated but it

Fig. 8. A dense connective matrix is observed in the periphery of the lesion, with numerous bundles of fibrils mixed with amorphous dense material (arrowhead), × 3250.
may be speculated that effector cells (fibroblasts with, to a lesser degree, tissular macrophages) might be activated by a monokine (probably IL-1) produced by lymphokine-activated macrophages. Further investigations on such mechanisms are warranted in the different clinical forms of cutaneous leishmaniasis, and particularly in the early steps of the modulation of the cutaneous immune response by parasites.

Acknowledgements

The authors would like to thank all the physicians of the Military Health Services in French Guiana, who provided them with the clinical material for this study; and Brian Eyden (Manchester Univ., U.K.) for his review of the manuscript.

References

20 Ten Cate AR, Deporter DA (1975) The degradative role of the fibroblast in the remodelling and turnover of the collagen in soft connective tissue. Anat Rec 182: 1–5

Received August 15, 1990 · Accepted in revised form November 7, 1990

Key words: Extracellular matrix – Fibroblast – Skin – Cutaneous leishmaniasis

Dr. P. Esterre, Pathologie Cellulaire (CNRS URA 602), Institut Pasteur de Lyon, Av. Tony Garnier, F-69007, Lyon, France