Wade histoid Leprosy: histological and immunohistochemical analysis

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Accepted for publication 1 October 2013

Summary Histoid leprosy is a rare multibacillary form that presents with disseminated papule-nodular cutaneous lesions. To study the inflammatory infiltrate of the histoid form and to compare it with other lepromatous forms, we performed histological and immunohistochemical analysis on skin biopsies. Fifteen patients were included for histopathological analysis (10 histoid and five lepromatous) via the haematoxylin-eosin and Ziehl-Neelsen-Faraco stains. Thus, immunohistochemical techniques using immunoperoxidase assay were performed for: anti-BCG, anti-\textit{M. leprae}, anti-CD8, anti-CD3, anti-CD20, anti-S100, anti-CD1a, anti-CD68 and anti-vimentin. Spindle cells were present in all histoid patients. A pseudocapsule was observed in half of both studied forms. A comparison using the Ziehl-Neelsen-Faraco stain to evaluate anti-BCG and anti-\textit{M. leprae} showed no major differences. The CD3$^+$ cells were more pronounced in the histoid form than the lepromatous form. There was greater immunoreactivity toward CD8$^+$ cells in the histoid form, as well as the CD20$^+$ cell count. A similar count of S100$^+$ cells in the epidermis of both leprosy forms was observed. There was a slight increase of dendritic cells in the histoid patients in the superficial and deep dermis. For CD1a marker, we observed expression in the epidermis and superficial dermis in both forms. A diffuse and intense infiltrate of CD68$^+$ cells was also observed in the histoid and lepromatous forms. The high positivity for vimentin did not allow for a positive cell count. We concluded that the activation of both the cellular and humoral response is more pronounced in the histoid form because the T and B cells showed greater infiltration
than those in the lepromatous form. The activation of dendritic and Langerhans cells is similar in both forms. The spindle cells likely belong to the macrophage population, thus maintaining phagocytic ability. The quantities of pseudocapsules and bacilli are similar and cannot serve as criteria for diagnosis.

Introduction

Histoid leprosy was described by Wade \(^1\) in 1963 as a rare subtype of lepromatous leprosy (multibacillary form) caused by an acid-fast bacillus, (AFB) \(\textit{Mycobacterium leprae}\). Clinically, it is characterised by hard and shiny papules or nodules that are (especially on palpation) similar to keloids. This form primarily affects the back, buttocks, face and extremities. This event can occur in patients who were: 1. previously submitted to irregular treatment for lepromatous leprosy; 2. treated regularly but without a good response (indicating treatment failure); or 3. infected by resistant strains (primary resistance to dapsone (diamino-diphenyl sulfone, or DDS) is also described in some cases). \(^1\)–\(^4\) The disease may also arise \textit{de novo} in some cases. The tissue infiltration is composed of foamy, polygonal and vacuolated histiocytes \(^2\) in which the presence of spindle-shaped cells resemble some histiocytomas or nodular subepidermal fibrosis. \(^1\) A well-delimited lesion surrounded by a pseudocapsule is also observed. Histoid leprosy also presents a large number of solid-staining acid-fast bacilli. \(^3\)–\(^4\)

The primary histopathological difference between histoid and lepromatous leprosy is the presence of a spindle cell infiltrate. Developing the histopathological aspects and the process of evaluating the cellular population in the inflammatory infiltrate could help in diagnosing the histoid form of this disease.

The aim of this study was to characterise the tissue inflammatory infiltrate in histoid leprosy. To this end, we performed a histopathological and immunohistochemical study comparing histoid and lepromatous leprosy.

Patients and Methods

This was a retrospective study using patients with histoid or lepromatous leprosy diagnoses. These patients attended the Department of Dermatology, Escola Paulista de Medicina/ Federal University of São Paulo, from January 1998 to December 2011. We included patients who fulfilled both the clinical and histological criteria for either the histoid or the lepromatous form of leprosy. None of the patients had received any specific treatment. For routine investigation, skin fragments were obtained via 5-mm punch biopsy and were fixed in formalin (10%) and embedded in paraffin. Fifteen untreated patients were studied. Ten were histoid patients (eight male and two female; median 27 years old), and five were lepromatous patients (four male and one female; median 49 years old). The primary limitations of this study were that some skin fragments were not available, and we could not include the same number of patients in the two studied groups.

HISTOPATHOLOGY

The skin fragments were cut in 5 to 7 \(\mu\)-thick sections and stained with a Haematoxylin-eosin (HE) stain, as well as a Ziehl-Neelsen-Faraco (ZNF) stain for acid fast bacilli (AFB).
IMMUNOHISTOLOGY

Serial sections were also subjected to immunoperoxidase assay via the third generation polymer technique with 3,3’-diaminobenzidine (DAB) as the chromogenic substrate. The counterstaining was performed with Harry’s haematoxylin. The staining intensity was evaluated using a semiquantitative method that was analysed by two dermatopathologists. The anti-BCG (dilution 1:100,000, Dako Denmark, Glostrup, Denmark) and rabbit polyclonal anti-
\( M.\text{leprae} \) (dilution 1:6,000, developed at the Tropical Medicine Institute, São Paulo, Brazil) antibodies were used to demonstrate the infectious agent in the infiltrate and to increase the sensitivity of bacilli detection. Anti-CD8 (dilution 1:200, Dako Denmark, Glostrup, Denmark) and anti-CD3 (dilution 1:300, Dako Denmark, Glostrup, Denmark) antibodies were used to demonstrate T cell infiltrate and especially CD8+ (suppressor) cells. The anti-CD20 antibody (dilution 1:2,000, Dako Denmark, Glostrup, Denmark) shows B cell infiltrate. This cell produces high levels of antibodies in multibacillary patients. Anti-vimentin (dilution 1:500, Dako Denmark, Glostrup, Denmark) antibody was included in this panel because vimentin could be expressed by fibroblasts. Anti-CD68 (dilution 1:20,000, Dako Denmark, Glostrup, Denmark) antibody was used to identify the epithelioid cells and macrophages in tuberculoid and lepromatous leprosy, respectively. Dendritic cells were identified using anti-S100 (dilution 1:2,000, Dako, Denmark) antibodies, and antigen-presenting Langerhans cells were identified via anti-CD1a antibodies (Dilution 1:1, Dako Denmark, Glostrup, Denmark).

ANALYSIS

For histological and immunohistochemical analysis, two investigators (DAMC and MMSSE) evaluated five fields using an optical microscope (Olympus®, Japan), and the average was calculated for each parameter. The ZNF stain, anti-BCG and anti-
\( M.\text{leprae} \) were classified using the following: 1+ 25% of the macrophages containing bacilli; 2+ 50% of the macrophages containing bacilli; 3+ 75% of the macrophages containing bacilli; and 4+ all macrophages containing bacilli. For anti-CD3, CD8, CD20, CD1a, S100, CD68 and vimentin, the cell count was performed using five observed fields.

INSTITUTIONAL REVIEW BOARD

This study was approved by the Ethics Committee in Research of UNIFESP-HSP in 2008 (# 0882/08).

Results

HISTOPATHOLOGY

The haematoxylin-eosin stain showed an intense inflammatory infiltrate in all histoid patients. None of the patients presented an inflammatory infiltrate constituted by typical Virchow’s or foamy cells. The histoid patients presented a representative number of typical spindle cells. We observed a pseudocapsule in five of the 10 histoid cases. Among the lepromatous patients, a pseudocapsule was also observed in 3/5 of the cases. All lepromatous cases showed an
inflammatory infiltrate consisting of Virchow’s or foamy cells. However, none presented typical spindle cells in their infiltrates.

**Bacilli Presence Determination**

Using the ZNF stain, both histoid and lepromatous patients presented bacilli in cutaneous lesions. Four of the 10 histoid patients were strongly (4+) positive for the presence of bacilli, and only one lepromatous patient presented a strongly (4+) positive result.

Using the average obtained from counting the immunostained cells (semiquantitative method), the comparison of the three techniques [anti-BCG (average 3.4+ for the histoid form and 3.2+ for the lepromatous form); anti-*M. leprae* (average 3.1+ for the histoid form and 2.8+ for the lepromatous form) and the ZNF stain (average for histoid form 3.4+ and for lepromatous 3.2+)]; showed that there was no major difference when using the three techniques to compare leprosy varieties.

**Immunohistochemistry**

The following findings are related to the cells present in the middle dermis; the inflammatory infiltrate was more intense in this layer. The cell counting average of the five fields (cells per field) and general averages for each antibody used in the panel of cell markers are presented in Table 1.

The mononuclear cell population (CD3+ cells) was more pronounced in the histoid form (average of 41.4 cpf – cells per field) (Figure 1a) compared with the lepromatous form (average 14.7 cpf).

<table>
<thead>
<tr>
<th>Leprosy form</th>
<th>CD3</th>
<th>CD8</th>
<th>CD20</th>
<th>S100/EP</th>
<th>S100/SDe</th>
<th>S100/DDe</th>
<th>CD1a/EP</th>
<th>CD1a/SDe</th>
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<tr>
<td>H1</td>
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<td>23.4</td>
<td>0.8</td>
<td>8.0</td>
<td>8.0</td>
<td>0.0</td>
<td>1.4</td>
<td>0.0</td>
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<tr>
<td>H2</td>
<td>26.8</td>
<td>15.8</td>
<td>6.0</td>
<td>8.4</td>
<td>4.6</td>
<td>4.8</td>
<td>7.0</td>
<td>2.8</td>
</tr>
<tr>
<td>H3</td>
<td>54.0</td>
<td>39.4</td>
<td>1.8</td>
<td>0.0</td>
<td>3.4</td>
<td>1.2</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>H4</td>
<td>29.6</td>
<td>21.8</td>
<td>2.8</td>
<td>5.2</td>
<td>4.4</td>
<td>0.0</td>
<td>4.8</td>
<td>0.0</td>
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<td>H5</td>
<td>35.2</td>
<td>32.4</td>
<td>11.4</td>
<td>5.2</td>
<td>4.4</td>
<td>8.8</td>
<td>6.4</td>
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<td>46.4</td>
<td>53.2</td>
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</tr>
<tr>
<td>H8</td>
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<td>22.4</td>
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<td>2.2</td>
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<td>H9</td>
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<td>3.8</td>
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<td>48.9</td>
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<td>Average ± SD</td>
<td>41.4 ± 10.3</td>
<td>30.8 ± 12.5</td>
<td>3.8 ± 3.6</td>
<td>48 ± 24</td>
<td>48 ± 15</td>
<td>28 ± 29</td>
<td>44 ± 22</td>
<td>19 ± 17</td>
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<tr>
<td>L1</td>
<td>3.0</td>
<td>0.8</td>
<td>0.0</td>
<td>5.0</td>
<td>3.6</td>
<td>0.8</td>
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<tr>
<td>L2</td>
<td>15.4</td>
<td>8.6</td>
<td>0.2</td>
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<td>8.0</td>
<td>0.7</td>
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<td>0.8</td>
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<tr>
<td>L4</td>
<td>22.0</td>
<td>1.4</td>
<td>0.8</td>
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<td>0.6</td>
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<tr>
<td>L5</td>
<td>18.7</td>
<td>17.2</td>
<td>1.8</td>
<td>5.8</td>
<td>9.6</td>
<td>7.9</td>
<td>6.2</td>
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</tr>
<tr>
<td>Average ± SD</td>
<td>14.7 ± 2.2</td>
<td>9.4 ± 2.0</td>
<td>0.7 ± 0.7</td>
<td>48 ± 8.0</td>
<td>40 ± 3.2</td>
<td>2.1 ± 3.2</td>
<td>5.6 ± 1.3</td>
<td>1.7 ± 2.0</td>
</tr>
</tbody>
</table>

Legend: H = histoid patient; L = lepromatous patient; EP = epidermis; SDe = superficial dermis; DDe = deep dermis; SD = standard deviation.
Figure 1. Immunohistochemical expression in histoid leprosy for anti-CD3 (a), anti-CD8 (b), anti-CD20 (c), anti-S100/epidermis (d), anti-S100/superficial dermis (e), anti-S100/deep dermis (f), anti-CD1a/epidermis (g) and anti-CD1a/superficial dermis (h). Original magnification ×400.
Regarding the subpopulation of T lymphocytes, there was a greater immunoreactivity in the CD8+ cells in the histoid patients (average 30.8 CD8+ cpf) (Figure 1b) compared with the lepromatous patients (average 9.4 CD8+ cpf) (Table 1). The lymphocyte count of the CD20+ cells (pan B) was higher in the histoid patients (average of 3.8 cpf) compared with the lepromatous patients (average of 0.7 cpf) (Figure 1c) (Table 1).

Five fields were examined using the anti-S100 (dendritic cells in the epidermis, dermis superficial and deep dermis) and anti-CD1a (Langerhans cells in the epidermis and dermis) antibodies. The averages are shown in Table 1. There was the same number of S100+ cells in the epidermis of both leprosy forms (average of 4.8 cpf for each). There was a slight increase of dendritic cells in the superficial dermis (including papillary and reticular) of the histoid (average of 4.8 cpf) compared with lepromatous patients (average 4.0 cpf). However, for the deep dermis, we observed an overall decrease in dendritic cells for both forms compared with the epidermis and superficial dermis of the histoid patients (average 2.8 cpf for histoid; 2.1 cpf for lepromatous) (Figures 1d, 1e, 1f).

Regarding the morphometric results of the CD1a marker expressed in the epidermis and superficial dermis (Table 1), the superficial dermis showed a decrease of CD1a+ cells (Langerhans cells) compared with the epidermis. The positivity in the epidermis was higher in lepromatous patients (average 5.6 cpf). The deep dermis showed no Langerhans cells (Figures 1g, 1h).

There was a diffuse and intense immunohistochemical expression for anti-vimentin in all leprosy patients, sparing only areas with normal collagen fibres. The high positivity did not allow a positive cell count.

There was a diffuse and intense immunohistochemical expression of inflammatory cells (CD68+ cells) in both leprosy patient types, indicating a predominance of histiocytes in the cellular infiltrate. Due to the high positivity obtained with this marking antibody, it was not possible to count the cells. However, we noticed that both Virchow cells with a vacuolated aspect and spindle storiform cells (present in the histoid form) were positive for CD68 (Figures 2a, 2b).

**Figure 2.** Immunohistochemical expression in histoid leprosy for anti-CD68, staining foamy and spindle cells. Original magnification (a) ×40 and (b) ×400.
Discussion

The histoid form has been considered a sub-type of lepromatous leprosy. Histological examination often does not confirm the clinical diagnosis and vice versa. Sometimes, the same patient presents concomitant clinical and histological lesions of both the lepromatous and histoid forms. Concerning diagnostic difficulty, we decided to study histological and immunohistochemical parameters that could be useful in histoid form determination and tissue immune response characterisation.

The histopathological description of lepromatous leprosy indicates a polar histiocytic granuloma with persistent macrophages. These macrophages do not undergo metamorphosis (transforming into epithelioid and giant Langerhans cells), as occurs in tuberculoid leprosy. Macrophages of lepromatous leprosy are also known as Virchow or foamy cells. These cells represent macrophage degeneration with nuclear pyknosis, which is described as a ‘crumpled sheet’ with droplets of fat around it. These macrophages contain a large number of bacilli disposed in globi. The Ziehl-Neelsen-Faraco staining reveals a large number of AFB, which can sometimes assume a curved shape to facilitate fitting inside the macrophages. The AFB can be located (besides in the macrophages) in eccrine glands, nerves, Schwann cells and the vascular endothelium.

In histoid leprosy, macrophages have a spindle aspect, disposing themselves in bundles and containing long, well-stained and aligned bacilli (along the longer cell axis). The cells are arranged in an interconnected structure, sometimes resembling a fibrous histiocytic tumour. In this form, the lesion has well-defined limits and can be surrounded by a pseudocapsule. The infiltrate consists of spindle histiocytes arranged along Virchow cells. The amount of AFB in the histoid form is large compared with the lepromatous form. The bacilli form globi, which is typical of infection by \textit{M. leprae}.

In the present study, none of the forms presented tuberculoid granuloma, demonstrating that the histoid form has some characteristics of the multibacillary pole of the disease. Parameters previously described as the formation of a pseudocapsule and a large number of AFB in histoid patients did not differentiate the lepromatous form. However, the presence of spindle cells with a storiform arrangement was an important feature in the histoid form that was not present in the lepromatous form. Kalla \textit{et al.} also described the presence of spindle cells in 100\% of histoid leprosy cases. We also noticed that some patients were clinically classified as having lepromatous leprosy despite presenting a typical histoid histopathology. Other patients had a histopathology similar to lepromatous leprosy in some lesions but histoid leprosy in other lesions. As suggested by Mansfield in 1969, “(...) histoid lesions may represent a rapidly growing mass of lepromatous tissue, and the vacuolar cytoplasmic change may represent a return to a more normal growth pattern”. Thus, these findings demonstrate that the histoid form could be considered to be on a spectrum in multibacillary leprosy and not a distinct form.

Comparing the three methods (Ziehl-Neelsen-Faraco stain, immunohistochemistry for anti-BCG and anti-\textit{M. leprae}) for the evaluation and quantification of bacilli in leprosy tissue, we found no advantages in using immunohistochemical techniques for either the non-specific identification (BCG antibody) or specific identification (polyclonal antibody anti-\textit{M. leprae}) of the bacillus. Regarding the comparison between the two leprosy forms, we noticed no difference between the histoid and lepromatous forms, as reported in the literature. Therefore, both can be considered multibacillary, and the current methods available for evaluating the bacillus in tissue cannot be used to differentiate the two forms.
Both the lymphoid and myeloid cell lineages share a relationship during the immune response. However, in mycobacterial infections, this relationship is predominantly cell-mediated, and the antibodies produced do not perform a protective role against the agent. The characterisation of the cell population present in the inflammatory infiltrate in the histoid form may elucidate some aspects of immune tissue response. Data from the literature report that the expression of CD4+ and CD8+ cells is similar in the paucibacillary (tuberculoid) and multibacillary (histoid) forms (10). However, fewer CD8+ cells have also been reported in the tuberculoid and histoid forms than the lepromatous form.10–12 The results obtained in this study are divergent with those because we observed that the cell population of infiltrated mononuclear cells was more pronounced in the histoid form compared with the lepromatous form, suggesting an intense immune activation. Via morphometric analysis, the infiltration of CD8+ and pan T cells was higher in the histoid patients than the lepromatous patients.

A small amount of CD20+ (pan B) cells was observed in both leprosy forms, as is described in the literature;10,11,13 however, contrary to previous findings,10,13 we found that the infiltration of CD20+ cells was higher in the histoid patients. This finding corroborates those of other authors who claim that humoral immunity is considerably increased in histoid leprosy.11,14,15 B lymphocytes are associated with an increased production of antibodies in multibacillary forms,16 stimulated by Th2 response.

Cuevas-Santos et al. showed that macrophages from multibacillary lesions express S100 protein intensely and constantly.17 In contrast, lesions of paucibacillary forms have few cells immunoreactive for S100 protein.13 Cuevas-Santos et al. analysed three histoid leprosy patients and found that infiltrates were negative for S100 protein.17

Dendritic cells are known to be antigen-presenting cells. The protein S100 marker stains these cells as well as melanocytes, which also have a dendritic aspect in the epidermis. In our current study, no difference in the quantification of S100+ cells was observed between the epidermises of the leprosy patient types. Regarding the analysis of the superficial and deep dermis, there was a slight increase of dendritic cells in the histoid patients. The infiltration of dendritic cells was greater in the superficial portions such as the epidermis and superficial dermis. The same results were not observed by Cuevas-Santos et al. who described a decrease in the number of dendritic cells in the epidermis.17

The CD1a marker for Langerhans cells (antigen presenting cells) was positive in superficial portions of the skin such as the epidermis and superficial dermis. An immunoreaction was absent in the deep dermis. These cells were concentrated in the epidermis, though some cells migrated into the dermis and showed an intense local immune activity.

Using anti-CD68 antibody, we demonstrated positivity both in the vacuolated macrophages and in the spindle cells present in histoid leprosy. The spindle cells and Virchow cells likely share a common origin and are actually macrophages that have acquired another morphology. As demonstrated using an electron microscopy, this cell type in the histoid infiltrate belongs to the mononuclear phagocyte population.13 There are fewer organelles in the spindle cell cytoplasm, suggesting a low phagocytic function.16 The intensity of the reaction for anti-CD68 was high in both the histoid and in lepromatous forms and showed a large macrophage infiltrate.

Both the vacuolated macrophages and spindle cells intensely expressed vimentin. Although described as a marker for mesenchymal cells (such as fibroblasts), this antibody reacts with different cells, thus demonstrating itself as non-specific. Therefore, we could not determine whether the spindle cells were a type of fibroblast.
In summary, there is a slightly different tissue immune response between the histoid and lepromatous forms. Both the cellular and humoral responses are higher in the histoid form; the infiltrate of T and B cells is greater compared with those of the lepromatous form (although not significantly). There is also an activation of dendritic and Langerhans cells primarily in the epidermis and superficial dermis. Spindle cells likely belong to a macrophage population that maintains its phagocytic ability but not its ability to eliminate bacilli. Though the differences were not remarkable, we can conclude that the histoid and lepromatous forms are both part of the leprosy spectrum. They show few differences in immunological response but clear differences regarding histopathological aspects. The presence of pseudocapsule and the number of bacilli in the tissues of lepromatous and histoid patients are similar and thus cannot serve as diagnostic criteria; however, the presence of spindle cells indicates a histoid leprosy diagnosis.

Acknowledgements

We are thankful to Neuza Kasumi Shirata and Lidia Midori Kimura, from Pathology Center, Instituto Adolfo Lutz, São Paulo, Brazil, for their technical assistance in immunohistochemical techniques. We thank to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazilian Ministry of Science and Technology, for providing a scholarship for the first author.

Funding sources: a scholarship was provided for the first author by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazilian Ministry of Science and Technology.

Conflict of interest: none declared.

Ethics committee approval: Number 0882/08, Escola Paulista de Medicina/UNIFESP.

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