Nodular Leprosy of Childhood and Tuberculoid Leprosy: A Comparative, Morphologic, Immunopathologic and Quantitative Study of Skin Tissue Reaction

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ABSTRACT

Nodular leprosy of childhood (NL) is a benign clinical variant of tuberculoid leprosy that affects breast-feeding infants and children that remained in a highly infected environment. The lesions resolve with complete healing and NL has been considered a manifestation of allergy and congenital immunity to *Mycobacteria leprae*. We studied the tissue reaction, *Mycobacterial* antigen frequency, and the lymphocyte subsets (CD45RO+, CD4+, CD8+, B, NK), dendritic cells (epidermal CD1a+ cells and S100+ dermal dendrocytes), and macrophages in skin lesions of a clinically well characterized NL group (N = 11). Results were compared to children (N = 23) and adults (N = 24) with classical tuberculoid leprosy.

NL lesion histopathology was characterized by dense granulomatous inflammatory reaction, with a greater number of confluent tubercles when compared to the other groups. Neural compromise was seen in all biopsies. The frequency of *Mycobacterium* antigen was similar in all groups. The population of CD45RO+, CD4+ and CD8+ T lymphocytes, natural killer cells, B lymphocytes, CD1a+ epidermal cells, and macrophages of NL lesions did not differ from the other groups. The number of S100+ dermal dendritic cells of the NL group was smaller than that of the adult group, although it did not differ from the other group of children. Except for the confluent tubercules, our data could not disclose any other difference in the tissue reaction of NL, in spite of its peculiar clinical features and evolution when compared with the classical tuberculoid leprosy. The localization of NL lesions may be the result of the intimate skin contact with lepromatous parents or relatives, in areas such as cheeks, arms, buttocks, and limbs, and the inoculation of *M. leprae* into skin may strongly stimulate cell mediated immunity (CMI) against the bacilli. These circumstances might explain the good CMI response leading to high resistance, stability, and auto-resolution of nodular leprosy of childhood.

RÉSUMÉ

La lèpre nodulaire de l’enfance (LN) est une variante clinique bénigne de la lèpre tuberculoid qui affecte les enfants en âge d’allaitement et des enfants qui sont restés dans un environnement à forte pression infectieuse. Les lésions cicatrisent complètement et la LN a été de ce fait considérée comme une manifestation d’allergie et d’immunité congénitale aux *M. leprae*. Nous avons étudié la réaction tissulaire in situ, la fréquence de détection des antigènes mycobactériens, les sous populations lymphocytaires CD45RO+, CD4+, CD8+, B et NK, ainsi que les cellules dendritiques (incluant les cellules épidermiques CD1a+ et les dendrocytes dermiques S100+) et les macrophages des lésions de la peau d’un groupe bien caractérisé de LN (N = 11). Les résultats ont été comparés à un groupe d’enfants (N = 23) et d’adultes (N = 24) souffrant de lèvre tuberculoi de classique.

Les lésions histologiques de NL était composées d’une dense réaction inflammatoire granulomateuse et d’un plus grand nombre de tubercules confluents que ceux des autres groupes. Les lésions neuromales ont été observées dans toutes les biopsies. La fréquence des antigènes anti-mycobactériens a été semblable dans tous les groupes. Les populations des lymphocytes T CD45RO+, CD4+, et CD8+, des cellules NK, des lymphocytes B, des cel-
La lepra nodular infantil (NL) es una variante clínica benigna de la lepra tuberculoiide que afecta a los lactantes y a los niños que permanecen en regiones altamente infectadas. Las lesiones evolucionan con curación completa y la NL se ha llegado a considerar como una manifestación de alergia e inmunidad congénita contra *Mycobacterium leprae*. En este trabajo se estudió la reacción tisular, la frecuencia de antígenos micobacterianos, y la presencia de poblaciones de linfocitos (CD45RO+, CD4+, CD8+, B, NK), de células dendríticas (CD1a+ epidérmicas y dendrocitos dérmicos S100+), y de macrófagos en lesiones de NL.

Histopatológicamente las lesiones NL estuvieron caracterizadas por la presencia de una densa reacción inflamatoria granulomatosa, con mayor número de granulomas confluentes que el observado en los otros grupos. En las biopsias de todos los casos se observó afectación nerviosa. La frecuencia de antígenos micobacterianos fue similar en todos los grupos. Las poblaciones de linfocitos T CD45RO+, CD4+ y CD8+, células NK, linfocitos B, células epidérmicas CD1a+ y macrofágos en lesiones NL no difirieron de los otros grupos. El número de células dendríticas S100+ en el grupo NL fue menor que en los adultos TT pero fue similar al encontrado en niños TT. En comparación con las lesiones de la lepra TT, excepto por los granulomas confluentes, nuestros datos no revelan ninguna otra diferencia en la reacción tisular NL no obstante sus características clínicas peculiares y su evolución benigna. La localización de las lesiones NL puede ser el resultado del contacto íntimo de la piel con los progenitores o familiares lepromatosos, sobre todo en áreas expuestas como las mejillas, brazos, glúteos y piernas, donde la inoculación directa de *M. leprae* en la piel podría estimular fuertemente la inmunidad celular contra el bacilo, circunstancia que podría explicar la alta resistencia, la estabilidad, y la auto-resolución de la lepra nodular en los niños.
Souza Campos (1937) considered NL lesions to be manifestations of allergy and immunity (30). Nolasco and Lara reported complete healing in most cases, indicating a high degree of resistance. The children observed had remained in a highly infected environment throughout their illness without presenting new signs of disease (22).

In spite of its peculiar clinical evolution, the histopathologic features of skin lesions are described as similar to that observed in classical tuberculoid leprosy, showing well formed epithelioid cell granulomata in the dermis (22, 23, 26, 30).

Appropriate cellular mediated immunity is essential for host protection against *Mycobacterium leprae* infection (3, 9, 10, 18, 28). Immunohistochemical techniques allow the evaluation of phenotypes of cellular elements in tissue inflammatory infiltrates (10, 19, 33), enhancing immunopathologic observations of the host-parasite relationship.

Since there are no data on immunopathologic aspects of the tissue reaction that could be related to the peculiar clinical evolution of NL, we described and quantified the cellular elements related to cell mediated immunity, as well as *Mycobacterium* antigens, in skin biopsies of NL, comparing these results to those observed in classical tuberculoid leprosy of children and adults.

**MATERIALS AND METHODS**

Skin biopsies were obtained from 11 patients with clinical diagnosis of NL, and from 23 children and 24 adult patients with classical tuberculoid leprosy.

Eleven infants and children (3 males and 8 females) constituted the NL group (group 1), with ages ranging from 19 months to 9 yrs (mean of 3.61 yrs). The tuberculoid leprosy children group (group 2) was composed of 23 patients (9 males and 14 females), with ages ranging from 2 to 14 years of age (mean of 8.63 yrs). The adult group (group 3) included 24 patients (11 male and 13 female) with ages ranging from 19 to 69 yrs (mean of 40.33 yrs). The Mitsuda skin test was positive in all patients.

Nine NL patients were born to lepromatous mothers, and the remaining had close relatives (one father and one aunt) with lepromatous disease. We could access their complete clinical records and follow up (mean duration of 5.9 yrs), and all patients presented an auto-resolutive course of disease without specific treatment. Only one patient received chaulmoogra oil treatment. All of them resolved without progression to other disease forms or deformities.

Five µm tissue sections obtained from each skin specimen were submitted to routine histological procedures and stained with hematoxylin-eosine and Fite-Faraco technique for AFB. The histopathologic features analyzed included the inflammatory infiltrate pattern, nerve involvement, and epidermal changes.

In order to demonstrate T lymphocytes (Pan T cells, CD4 and CD8 lymphocytes), natural killer cells, B lymphocytes, macrophages, Langerhans cells, nerves, and mycobacteria antigens, 5 µm histologic sections were placed on glass slides coated with 3-aminopropyltriethoxysilane (Sigma A3648) and submitted to immunohistochemical staining techniques with strept-ABC complex/HRP (Dako 0492), according to Hsu, *et al.* (1981) (8). Monoclonal antibodies used were anti-CD45RO (Dako M742) at a dilution of 1:100, anti-CD4 (Dako M834) at a dilution of 1:100, anti-CD8 (Dako M7103) at a dilution of 1:50, anti-CD56 (Becton-Dickison HNK1) at a dilution of 1:20, and anti-CD1a (Immunotech 1590) at a dilution of 1:20, and polyclonal antibody rabbit anti-Mycobacterium bovis (Dako B124) at a dilution of 1:60,000. Reactions using anti-CD4, anti-CD8, and anti-CD1a antibodies were performed with the Catalyzed Signal Amplification (CSA) method (Dako K1500) (11). Reactions using anti-CD68 and anti-CD56 antibodies were performed after antigen heat retrieval in citrate buffer 0.01 M pH 6.0 in a micro-oven and pressure cooker, respectively. The reactions were developed with chromogen 3,3'-diaminobenzidine (Sigma) and counterstained with Mayer’s hematoxylin.

Lymph node fragments and lepromatous leprosy skin biopsies were used as positive controls for the immunohistochemical techniques with the anti-cellular and anti-BCG antibodies, respectively. Negative controls were obtained by omitting the primary antibodies for each reaction; they were replaced...
The number of T lymphocytes (CD45RO+ cells), CD4+ and CD8+ T lymphocytes, natural killer cells (CD56+ cells), S100-protein positive dendritic dermal cells, and CD68 positive cells were obtained by counting the number of stained cells in nine 0.0625 mm² randomized microscopic fields.

Quantification of epidermal Langerhans cells (CD1a positive cells) was obtained by evaluation of CD1a+ epidermal expression. It was obtained by using a ×10 ocular with a grid with 100 hits (i.e., points of crosses of two lines) and a ×40 objective. The fraction of area of epidermis positive for CD1a antigen was obtained by counting the number of hits over CD1a positive reaction and the total number of hits over the entire epidermis (excluding the cornified epidermal layer) of each specimen. The ratio of the number of hits over CD1a positive reaction/total number of hits over the epidermis corresponded to the epidermal fraction of area occupied by Langerhans cells².

Bacillary antigens, as well as AFB, were verified in the whole dermis of each skin fragment stained with anti-Mycobacterium bovis antibody and stained with Fite-Faraco technique.

Numerical data obtained for each group were compared by non-parametric statistical tests of Kruskal-Wallis and Mann-Whitney. The frequency of observation of confluent granulomas, Mycobacterial antigen, and AFB among the three groups was compared by the Fisher’s exact test. The level of significance was set at p ≤0.05.

RESULTS

All three groups studied exhibited the same histopathologic features. It was characterized by compact epithelioid tubercles surrounded by lymphocytes and few plasma cells. Langhans giant cells were seen in almost all specimens. Inflammatory reaction obscured the dermal-epidermal interface. Epithelioid granulomas were elongated and involved superficial and deep dermal vessels, cutaneous adnexal structures, small dermal nerves, and arrector pili muscles. Confluent granulomas were seen in 8/11 biopsies from group 1 (Fig. 1), 5/23 from group 2, and 5/24 from group 3. The number of skin specimens showing confluent granulomas in group 1 was significantly higher than those seen in groups 2 and 3 (p <0.05).

Nerve lesions were better disclosed by S100 protein reaction, which revealed nerve fragments within granulomas (Fig. 2) in all groups studied.

T lymphocytes (CD45RO+ cells) were the predominant small mononuclear cells of the dermal infiltrate, and were observed within the whole granuloma formation. CD4+ T lymphocytes exhibited the same distribution as CD45RO+ T lymphocytes, while CD8+ T lymphocytes showed a tendency to surround the epithelioid tubercles. Few T lymphocytes of either phenotype were observed within the basal and para-basal layers of epidermis. Natural killer cells revealed by anti-CD56 antibody were large round cells distributed sparsely within granulomata. B lymphocytes were scarce and arranged in small clusters around epithelioid tubercles. Compact clusters of epithelioid cells as well as macrophages...
placed among the other inflammatory cells were strongly stained by anti-CD68 antibody. S100 protein positive dermal dendritic cells (dermal Langerhans cells) were seen at the periphery and around granulomas.

Epidermal Langerhans cells were better shown by anti-CD1a antibody. They were regularly distributed among keratinocytes and exhibited long and anastomosing dendrites in the three groups (Fig. 3).

AFB were seen in 18.18% of group 1 specimens, 8.69% of group 2, and 16.66% of group 3. The anti-Mycobacterium bovis immune reaction revealed Mycobacterial antigen in 72.72% of group 1 specimens, 60.86% of group 2, and 58.33% of group 3. There was no difference in the frequency of AFB nor mycobacterial antigens between the three groups studied.

The number of T lymphocytes (CD45RO positive cells) and its subsets (CD4+ and CD8+ T lymphocytes), natural killer cells, B lymphocytes, macrophages, and epidermal Langerhans cells population of group 1 did not differ from the other two groups. Group 3 showed a higher number of T lymphocytes and epidermal Langerhans cells when compared to group 2 (p <0.001 and p <0.05, respectively). The number of dermal dendritic cells revealed by anti-S100 protein antibody in group 1 and group 2 biopsies were smaller than that in group 3 (p <0.05 and p <0.001, respectively). Results of quantification of these different immunolabeled cells are presented in Tables 1 and 2.

**DISCUSSION**

NL was first described by Souza Campos (30). It is considered a benign form of tuberculous leprosy that affects breast-feeding children and infants that remained in a highly infected environment. Lesions resolve usually to complete healing, and NL is considered to be a manifestation of allergy and congenital immunity to M. leprae (31). Other authors that referred to spontaneous healing in childhood leprosy (13, 14, 17) confirm the essentially benign character of papulonodular leprosy.

The epidemiological and clinical presentation of patients in group 1 were similar to that related by former authors (15, 16, 17, 22, 30). Mean age of patients was 3.61 years. All cases presented positive Mitsuda reaction, 9 of them were born to lepromatous mothers, and 2 were living with close relatives who had lepromatous disease. All patients with nodular lesions resolved to healing without disease progression or deformities.

Histopathology of NL lesions was characterized by dense granulomatous inflammatory reaction with confluent tubercles.
Confluent granulomas were more frequently seen in group 1 skin specimens than in groups 2 and 3. Probably the nodular aspect of these lesions was a macroscopic translation of this microscopic feature. Neural injury was present in all biopsies from group 1.

According to Nolasco and Lara (22), nodular leprosy lesions exhibit predominantly tuberculoid histology. These authors stated that tuberculoid foci were usually not as well defined as those seen in tuberculoid lesions of older children and adults. They also reported that, in skin lesions, some of the nerve twigs may exhibit tuberculoid type of infiltration, as well as thickened cellular and laminated epineurium.

In all three groups studied neural damage was better demonstrated by S100 protein immune staining, which disclosed nerves fragments inside granulomas. S100 protein staining was considered to be superior to hematoxylin and eosin staining in identifying dermal nerve destruction in leprosy (6,21,32).
spite of neural damage seen in skin lesions, patients with nodular leprosy resolve without peripheral nervous system injury such as neuritis, amyotrophy, paralysis, or trophic lesions (1, 4).

AFB and *Mycobacterium* antigen frequency in lesions were similar in the three groups studied. Immunohistochemistry sensitivity to detect bacterial antigen was greater than that of Fite-Feraco staining. We observed AFB in 18.18% of NL biopsies and *Mycobacterium* antigen in 72.72% of the specimens. Nolasco and Lara (13) demonstrated AFB in nearly 75% of their cases, although Souza Campos (30) remarked on the scarcity or absence of AFB in the lesions of nodular leprosy.

The development of an effective immune response to invading pathogen is achieved by the complex interaction of a variety of immuno-competent cells including T cells, B cells, natural killer cells, and macrophages (7). T lymphocytes play a key role in managing the immune response against *M. leprae* as it avoids dissemination of bacteria and, consequently, of disease (3, 24). T-cell-dependent immunity to *M. leprae* is high in healthy exposed individuals and in tuberculoid leprosy patients (24). In NL, as in the other two groups studied, we observed T lymphocytes and CD4+ lymphocytes within epithelioid granulomas. CD8+ T lymphocytes were seen surrounding epithelioid tubercles. The distribution of T lymphocytes and their subsets was the same as described by Modlin, *et al.* (19) in tuberculoid leprosy lesions. The authors correlated the intimate admixture of CD4+ T lymphocytes and epithelioid histiocytes to a possible co-operation in promoting effective immune response. They also speculated that CD8+ T lymphocytes at the periphery of granulomas might contribute to bacilli confinement within circumscribed granulomas.

In our study, CD45RO+, CD4+ and CD8+ lymphocytes, natural killer cell, B lymphocyte, and macrophage populations in NL lesions did not differ from tuberculoid leprosy groups. The role of B lymphocytes in leprosy is not totally clear, but in tuberculoid lesions its population is higher than in lepromatous leprosy lesions (5).

Dendritic cells, monocytes, and natural killer cells play a role in shaping the nature of the T cell response to a pathogen (7). We could not demonstrate any difference in the natural killer cell population, as well as of

### Table 1

Comparison of the number of labeled cells/mm² in skin lesions of nodular leprosy of childhood (group 1), tuberculoid leprosy in children (group 2), and tuberculoid leprosy of adults (group 3).

<table>
<thead>
<tr>
<th>Labeled cell</th>
<th>Group 1 (N = 11)</th>
<th>Group 2 (N = 23)</th>
<th>Group 3 (N = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45RO+</td>
<td>mean ± S.D.</td>
<td>2137.86 ± 566.11</td>
<td>1972.96 ± 727.98</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>1941.33</td>
<td>1946.72</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>1427.52–3272.96</td>
<td>968.96–3558.72</td>
</tr>
<tr>
<td>CD4+</td>
<td>mean ± S.D.</td>
<td>1391.27 ± 419.64</td>
<td>1120.96 ± 409.53</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>1290.67</td>
<td>1077.28</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>816.00–2020.16</td>
<td>74.72–2186.72</td>
</tr>
<tr>
<td>CD8+</td>
<td>mean ± S.D.</td>
<td>672.42 ± 450.25</td>
<td>725.28 ± 680.38</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>563.56</td>
<td>465.76</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>38.40–1384.96</td>
<td>10.72–2625.76</td>
</tr>
<tr>
<td>CD20+</td>
<td>mean ± S.D.</td>
<td>15.68 ± 25.88</td>
<td>26.72 ± 52.63</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>5.33</td>
<td>7.04</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>0–85.28</td>
<td>0–232.96</td>
</tr>
<tr>
<td>CD56+</td>
<td>mean ± S.D.</td>
<td>74.51 ± 49.98</td>
<td>59.04 ± 46.86</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>69.33</td>
<td>46.24</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>0–156.48</td>
<td>1.76–174.24</td>
</tr>
<tr>
<td>CD68+</td>
<td>mean ± S.D.</td>
<td>841.70 ± 235.60</td>
<td>705.60 ± 351.43</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>712.96</td>
<td>671.21</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>439.04–1207.04</td>
<td>64.00–1130.72</td>
</tr>
<tr>
<td>S100+</td>
<td>mean ± S.D.</td>
<td>25.21 ± 24.46</td>
<td>20.02 ± 25.58</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>17.78</td>
<td>10.67</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>0–81.78</td>
<td>0–108.44</td>
</tr>
</tbody>
</table>

*aStatistically significant when compared group 3 to group 2 (p <0.001) (Mann-Whitney test).

*bStatistically significant when compared group 3 to groups 1 and 2 (p <0.001; p <0.05) (Kruskal-Wallis test).

S.D. = standard deviation.
It has been suggested that the clinical spectrum of leprosy is incomplete in the 0–14 years age group, and a discrepancy between clinical and histologic parameters has been reported in these patients (12, 25). Nodular leprosy may be an exception of children response against *M. leprae* correlated with its clinical evolution and tissue reaction, represented by well organized epithelioid granulomas.

There are many factors influencing the development of leprosy. The clinical spectrum is related to the ability of the patient’s cell mediated immune response (CMI) in destroying *M. leprae*. An innate immunity has been proposed for some of the infected individuals; however, for the majority of the patients CMI seems to be the predominant factor. The transmission of infection is still a point of discussion. It has been suggested that the entry point of *M. leprae* may be important to the immune response. An encounter at skin and draining lymph nodes (peripheral compartment) stimulates CMI (20). Localization of NL lesions are described as those areas of closest contact with lepromatous parents or relatives, such as cheeks, arms, buttocks, and limbs (13, 22, 30), suggesting that the inoculation of *M. leprae* into the skin in this group of patients may strongly stimulate CMI against the bacilli. These circumstances might explain a good CMI response leading to high resistance, stability, and auto-resolution of nodular leprosy of childhood.

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