Immunohistochemical Profile of Multicentric Reticulohistiocytosis

Flávio Barbosa Luz, MD, PhD; Antonio Pedro Gaspar, MD; Marcia Ramos-e-Silva, MD, PhD; Eliene Carvalho da Fonseca, MS; Enoi Guedes Villar, MD; Andrea Rodrigues Cordovil Pires, MD; Neide Kalil-Gaspar, MD, PhD


Abstract and Introduction

Abstract

Background: There is enough evidence to support the knowledge that multicentric reticulohistiocytosis (MR) is a histiocytic proliferative disorder; however, the type of histiocytes involved is not well established.

Objective and Methods: To study the nature of cells present in MR lesions by studying the immunohistochemical profile of three new cases and reviewing 23 cases reported in the literature.

Results: MR histiocytic cells are positive for vimentin, CD68, and CD45, negative for S-100 protein, CD34, and XIIIa factor, and weak reactors for thrombomodulin. Small activated histiocytes are MAC387 positive. Lymphocytes, mainly CD4 + cells, are found in MR infiltrates.

Conclusions: The MR immunophenotypic pattern does not suggest a type I or type II dendrocyte or a Langerhans cell origin. On the other hand, it points to a different cell derived from the monocyte-macrophage line. CD4 + cells may be responsible for activating the proliferation of histiocytic cells. Small histiocytic MAC387 + cells are likely to become the MR multinucleated giant cells.

Introduction

Multicentric reticulohistiocytosis (MR) is a rare proliferative disorder of histiocytes, characterized by typical cutaneous and synovial lesions and a unique histopathologic change: a predominantly histiocytic infiltration distinguished by the presence of some multinucleated cells within an eosinophilic, periodic acid-Schiff-positive, and diastase-resistant material. Overall, 164 MR cases were reported in the three great series[1-3] that intended to include all published cases worldwide until 1999.

The immunohistochemical (IHC) study of MR aimed to identify the differentiation of proliferated histiocytic cells inducing MR lesions and to be one more source of information to be used for diagnostic confirmation. In this paper, the term histiocyte will be used to refer to all types of bone marrow-derived macrophages and immune-related dendritic cells, as proposed by researchers.[4]

In this article, we discuss three new cases (Table 1) in addition to the MR cases reported in the literature (23 cases) for which IHC data were available. IHC studies from different laboratories over many years are not completely comparable; however, we believe this work could help the understanding of the IHC profile of MR.

Materials and Methods

Since 1977, 23 MR cases for which IHC data are available have been published in the literature. To them we added three new cases tested for CD4, CD34, CD45, CD68, factor XIIIa, S-100, vimentin, MAC387, thrombomodulin, α1-antitrypsin, and lysozyme.

Samples collected from our MR cases were processed in accordance with the routine histology, immunohistochemistry, and hybridization procedures adopted by the Pathology Sector of the University Hospital Antônio Pedro.

Techniques used to enhance epitope exposure (antigenic recovery) varied with the primary antibody used, as well as the dilution and origin (Table 2). For slides on which a double marking was done for CD68 and MAC387, the first was revealed with diaminobenzidine and the second with fast red.
Case Reports

Case A

A 53-year-old white woman, first seen in May 1990, had a 6-month history of disseminated itching and numerous nodules over the hands, face, and arms. She also complained of diffuse joint pain for the past month.

On examination, many grouped, shiny, red, rounded papules were present on the face, ears, hands, arms, trunk, and abdomen. Firmly attached fibrous nodules were seen on the wrists and tiny white papules on the palate. Laboratory data were normal except for an erythrocyte sedimentation rate of 78 mm. X-ray exams were normal.

Case B

A 52-year-old white woman, first observed in 1990, had disseminated itching and had been treated with terfenadine without response. One year later, papules and nodules arose over her hands, wrists, face, and elbows. In 1992, these lesions became painful, and symmetrical polyarthralgias of fingers, hands, knees, elbows, shoulders, and feet developed.

On examination, the following findings were observed: nonconfluent, small, rounded papules at the center of the face and tongue; pink flat plaques, 1 cm in diameter, on her forehead; yellowish flat plaques on both upper eyelids; round pink papules on the pinnae of the ear and gums; vermicular red lesions bordering the nostrils; firm reddish-gray nodules ranging from 0.3 cm to 2 cm in diameter over the joints of the hands, wrists, and elbows; and grouped papules bordering the thumb nails (coral beads signs).

Osteopenia and juxta-articular erosions were found on her hands (mainly on distal interphalangeal joints), knees, feet, and proximal femurs. The remaining clinical and laboratory findings were normal.

Case C

A 62-year-old black man had a 5-year history of painful joint swelling and a 1-year history of skin lesions on his face, scalp, ears, nape, hands, back, and abdomen. Lesions were nonconfluently grouped erythematous papulonodules, red papules bordering the nails (coral beads) and nostrils, and a firm attached red nodule on his right shoulder. Hands and knees were warm and swollen with limited joint movement.

X-ray studies revealed a typical destructive erosive arthritis. Laboratory findings were normal, except for the presence of 20,300 leukocytes per field and an erythrocyte sedimentation rate of 62 mm.

Histopathology findings were typical of MR in the three cases.

Results

The CD68 marker was uniformly positive in MR infiltrates. In these three patients, this staining was extremely strong and without background. The monocyte-macrophage marker MAC387 was positive in six out of seven tested cases; in four of those six cases, it was positive only in small nonactivated histiocytes within the infiltrate. Double staining for CD68 and MAC387 was performed in case A and revealed not only small nonactivated MAC387+ histiocytes and multinucleated and oncocytic CD68+ cells, but also double-marked intermediate-sized histiocytes (Figure 1). Lysozyme, a lysosomal enzyme, was present in 13 out of 14 tested cases. The $\alpha_{1}$-anti-trypsin, an inhibitory protein of another lysosomal enzyme, trypsin, was positive in four out of nine cases and the $\alpha_{1}$-antichymotrypsin in six out of 11 cases. The marker thrombomodulin was weakly positive in cases A, B, and C. Neither XIIIa factor (dermal dendrocyte type I) nor CD34 (dermal dendrocyte type II) was found in histiocytic cells in any tested cases. S-100 (Langerhans or indeterminate cells) were negative in 11, positive in two, and weakly or variably expressed in four out of 17 samples of 16 cases (Figure 2). CD1a (Langerhans cell) positivity has been observed in
three out of eight cases. HLA-DR (antigen-presenting cells) and HAM56 were always positive.

**Figure 1.** Case A. Double staining for CD68 (brown) and MAC387 (pink). Arrow head = small nonactivated MAC387+ histiocytes; narrow arrow = intermediate-size double marked histiocytes; large empty arrow = giant multinucleated CD68+ cell (original magnification x400).
Figure 2. Case B. S-100. Arrow head = Langerhans cell; narrow arrow = melanocyte; large empty arrow = indeterminate cell; large black arrow = histiocyte S-100+ (original magnification x400).

A strong staining was noted for leukocyte common antigen (commonly known as CD45) in seven out of eight cases: a peripheral pattern was described in six of them (Figure 3).
Pan T-cell marker CD3 has been observed in nonhistiocytic cells within the infiltrate in five out of six cases (Figure 4), but in two cases (one in the literature[6] and case B) this was also present in MR multinucleated giant cells. CD4 was always present in lymphocytes within the infiltrate and CD8 was positive in four out of six cases, although it was weak in one case.[6] All cases tested for B-cell markers were negative.
Some cytokines could be shown in the MR infiltrate: interleukin-1β (synthesized by macrophages) and PDGF-β (platelet-derived growth factor). Despite the fact that interferon-γ is produced by T-cells and natural killers to activate macrophages and increase the expression of major histocompatibility complex, it was not found in MR infiltrates.

**Discussion**

In 1990, one group of researchers\cite{7} confirmed the macrophage origin of MR by detailed immunophenotyping. To the best of our knowledge, the most complete attempt to immunohistochemically characterize MR lesions was made in 1995.\cite{8} It was found that cytoplasm strongly stained with the monocyte-macrophage marker CD68 in the four cases of MR; the pan-T marker CD3 and CD45 were positive in three; and the pan-B marker CD20, Xllla factor (dermal dendrocyte cells), and S-100 protein (Langerhans and indeterminate cells) stains were uniformly negative. They concluded that MR dermal infiltration appears to be due to cells of monocyte-macrophage origin (CD68+, CD45+) with variable expression of T-cell-restricted (CD3) and T-cell-associated (CD43 and CD45RO) antigens. The lack of staining with S-100 and factor Xllla in the four cases of MR suggested that cells are not of Langerhans-cell or dermal dendrocyte origin. They also concluded that the meaning of T-cell related antigen expression is unclear but is unlikely to indicate a T-cell origin of the infiltrating cells in MR.

According to our results and those available in the literature, the strong and constant marking by the anti-CD68 antibody can be considered an essential criterion for MR characterization. CD68 antibody detects a 110 kDa glycoprotein, related to lysosomal granules and highly specific to the cells of monocyctic macrophagic origin;\cite{9} however, its presence is clearly correlated with the abundance of lysosomes, as observed in the electronic microscopy study of the MR.

The marked positiveness for the lysozyme found in our cases and described in the literature supports the hypothesis that MR results from the growth of cells originating from the monocyte-macrophage line.\cite{10} The variable expression of α1-antitrypsin, a proteolytic enzyme, seems to reflect the irregularity of lysosomal activity in MR.

The fact that the MAC387 antigen was consistently present in the cases tested may indicate that these cells play an accessory role in MR pathogenesis or represent an initial stage of maturation of the disease-causing cells. One group of researchers\cite{11} had already observed a similar pattern of markers for MAC387 and presumed that it was found only in the infiltrate nonactivated histiocytes.

The presence of double-marked cells for CD68 and MAC387 in case A supports the hypothesis that variations in the phenotypic expression of the MR cells exist, probably as a result of their maturation process. The natural history of an MR lesion presumes the transformation of small histiocytes into oncocytes, and of the latter into giant cells. The observation of small MAC387+ histiocytes, large oncocyte histiocytes, giant CD68+ cells, and intermediated size histiocytes with cytoplasms expressing both antigens allows us to suggest that maturation of these cells must be followed by a change in their antigenic expression. Taking into account that this maturation results in loss of the phagocytic activity of cells\cite{12} and that the cell MAC387 is found in activated granulocytes and macrophages,\cite{13,14} one can presume that small activated MAC387+ histiocytes, resulting from some unknown stimulus, grow and suffer changes until being converted into giant CD68+ cells filled with hypofunctioning lysosomes.

The epidermal changes observed by us and others\cite{15} correspond to MAC387 expression in epidermal cells covering the infiltrates in cases A and B. The MAC387 antibody reacts against L1 antigen, a protein produced by epithelial and myelomonocytic cells expressed under inflammatory conditions.\cite{16} The presence of this antibody in the epidermis over MR infiltrates seems to be an epidermal reaction against dermal infiltration, suggesting that substances produced in infiltrates stimulate L1 expression in the epidermis.

The human leukocyte antigen-D related expression occurred in all cases for which it was tested, as reported in the literature. If one takes into account that these findings reflect that macrophages are able to interact with T lymphocytes and that they are found in MR, one can presume that substances released from these cells are able to induce histiocytic growth in MR. A significant increase in MAC387+ cells can result from a stimulus by interferon-γ.\cite{13}
The low reactivity to CD1 and S-100 allows one to rule out a Langerhans or undetermined cell origin in MR; however, reactivity to these antigens found in histiocytic cells from the dermis of some patients leads us to consider a role for these cells (especially the undetermined ones) because of their dermal location in the MR pathogenesis.

Except for endothelial cells, the anti-CD34 antibodies (myeloid progenitor cells, capillary endothelium, and type II dermal dendrocytes) and factor XIIIa (vascular endothelium and type I dermal dendrocytes) did not mark any of the histiocytic cells from MR lesions.

A constant immunolabeling for vimentin in MR cells in all but one case supports their mesenchymal origin. Where the primary antibody CD45 was used, a constant immunolabeling was also seen in lymphocytes. The amount and distribution of the positive CD45 and CD3 lymphocytes are apparently equal. Therefore, we can conclude that the majority of lymphocytes present in MR infiltrates are T cells. This observation is corroborated by the literature: out of six cases tested for CD4 and CD8, four were positive for CD8 and all cases were positive for CD4.

There is no evidence that MR cells have a B origin or that they take part in the MR pathogenesis since all the cases tested for lymphocytes B markers CD19, CD20, CD21, and CD22 were negative.

In case B, markers with a lace work aspect for CD45 (as if contiguous to membrane markers), observed within some giant cells, seem to confirm the observation made by other researchers in electronic microscopy studies that complex interdigitations exist between the cytoplasmic membranes of giant cells, possibly meaning that fusion between the histiocytes is not complete, at least in part of them.

The presence of the leukocyte common antigen (CD45) in histiocytes in which it was investigated (in our cases and in seven out of the eight published cases) supports the myelocytic nature of these cells.

We are not informed of prior publications in which the antibody thrombomodulin, an anticoagulant recently demonstrated in dermal dendrocytes from normal and psoriatic skin and annular granuloma, has been tested in MR lesions. The meaning of its weak expression found in some MR giant cells is unknown. These cells might play a secondary role in the MR pathogenesis. The absence of an increased number of positive thrombomodulin dendrocytes in infiltrates seems to indicate that they do not take part in the genesis of this disease.

One author group observed that solitary reticulohistiocytoma and xanthogranuloma present common histopathological aspects and XIIIa factor expression in giant cells. They concluded that these two diseases can take part in the same nosologic spectrum. Perrin et al. observed the XIIIa factor expression in MR, which suggests a dermal dendrocytic branching for the cells causing this disease. On the other hand, in accordance with our casuistry and that of others, MR cells did not express XIIIa factor. Another group considered that the positive results obtained by Perrin et al. for the XIIIa factor could derive from material freezing and the low dilution of the primary antibody. Our results are consistent with those researchers who discard the theory that MR arises from dermal dendrocytes, in contrast with xanthogranuloma. These results suggest that MR and the solitary reticulohistiocytoma are unrelated diseases, the latter probably being a variation of xanthogranuloma and other lesions resulting from type I dermal dendrocytes, such as dermatofibroma or another unique nosologic entity. The factor XIIIa, due to its ability to mark type I dermal dendrocytes, must be used more extensively in the non-Langerhans histiocytes IHC investigation panels.

The MS-1 antigen, specific to sinusoidal endothelial cells and dendritic perivascular macrophages in normal human organs, seems to be promising in the study of histiocytosis and was present in all non-Langerhans histiocyte cases studied by one research group, whereas it remained constantly negative in cases of Langerhans histiocytosis and noninfectious granulomata studied by the same authors.

Conclusions

The basic MR IHC profile consists of immunoreactivity to CD68, MAC387 (in small histiocytes), and lysozyme, and of lack of expression of the XIIIa factor, CD34, CD1a and S-100 protein, despite the fact

that the latter two are able to have a weak expression in some cases. Although evidence of phagocyte activities in MR giant cells is lacking, such an immunophenotypic profile strongly suggests a macrophage origin for these cells.

Immunolabeling for the CD68 antigen can be considered an essential criteria for MR characterization. This finding, together with the nonreactivity to factor XIIIa, CD34, and S-100 makes the MR differential diagnosis easier. The great majority of lymphocytes in MR infiltrates present a T-cell behavior, as confirmed by the presence of CD3. Evidence of B-lymphocyte and granulocyte participation is lacking. The thrombomodulin expression in the membrane of some MR giant cells deserves further investigation.

Table 1. Panel of Markers Used in Cases A, B, and C

<table>
<thead>
<tr>
<th>Marker</th>
<th>Immunohistochemical Profile - Multicentric Reticulohistiocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-100</td>
<td>Melanocytes, Langerhans cells, undetermined cells, interdigitating cells, and Schwann cells</td>
</tr>
<tr>
<td>Factor XIIIa</td>
<td>Endothelial cells and type I dermal dendrocytes</td>
</tr>
<tr>
<td>CD3</td>
<td>T lymphocytes</td>
</tr>
<tr>
<td>CD34</td>
<td>Stem cells, mast cells, endothelial cells, and type II dermal dendrocytes</td>
</tr>
<tr>
<td>CD45</td>
<td>Common leukocyte antigen</td>
</tr>
<tr>
<td>CD68</td>
<td>Macrophages</td>
</tr>
<tr>
<td>MAC387</td>
<td>Immature macrophage</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Cells with lysozymes, usually macrophages</td>
</tr>
<tr>
<td>α1-antitrypsin</td>
<td>Cells with trypsin, usually macrophages or mast cells</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Cells of mesenchymal origin</td>
</tr>
<tr>
<td>Thrombomodulin</td>
<td>Anticoagulant present in mesothelioma cells and in some dermal dendrocytes</td>
</tr>
</tbody>
</table>

Table 2. Dilution, Origin, and Technique for Antigenic Recovery
References


![Image of a table with antibody information]


Acknowledgements

The authors would like to thank Manuel Luis Moraes, MD; José Alvimar Ferreira, MD; Carlos Alberto Basilio-de-Oliveira, MD, PhD; and Simone Tavares Velloso, MD, for allowing us to use their records and biopsy specimens.

Reprint Address

Flavio Barbosa Luz, MD, PhD, Rua Desembargador Izidro, 28/606, 20.521-160, Rio de Janeiro, Brazil. E-mail: flavioluz@dermatologista.net.

Flávio Barbosa Luz, MD, PhD, Antonio Pedro Gaspar, MD , Marcia Ramos-e-Silva, MD, PhD, Eliene Carvalho da Fonseca, MS, Enoi Guedes Villar, MD, Andrea Rodrigues Cordovil Pires, MD, Neide Kalil-Gaspar, MD, PhD

Dermatology Sector, Universidade Federal Fluminense, Niterói, Brazil; Dermatology Sector, Universidade Federal do Rio de Janeiro, Brazil; Pathology Sector, Universidade Federal Fluminense, Niterói, Brazil.