Histiocytic Necrotizing Lymphadenitis (Kikuchi-Fujimoto Disease)

Lesional Cells Exhibit an Immature Dendritic Cell Phenotype

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**Abstract**

Histiocytic necrotizing lymphadenitis (HNL) is a rare benign disorder characterized histologically by nodal lesions composed of histiocytes, lymphoid cells, and so-called plasmacytoid T cells/plasmacytoid monocytes, with associated karyorrhexis. It has been proposed that plasmacytoid monocytes represent immature myeloid and lymphoid (plasmacytoid) early-committed dendritic cells (DCs). Monoclonal antibodies are now available for the detection of myeloid (CD1c [BDCA-1]+) and plasmacytoid (CD303 [BDCA-2]+) dendritic cells. With an extensive panel of antibodies to immature and mature DCs and interferon-α (IFN-α), cryostat section studies of 6 cases of HNL revealed that the morphologically distinctive mononuclear cells in lesional areas consisted of 2 populations of immature DCs: myeloid DCs immuno reactive for CD1c with coexpression of myeloid antigens CD13 and CD33 and plasmacytoid DCs immuno reactive for CD303 and CD123. These cells were CD68+, strongly expressed the IFN-α inducible protein MxA, and were nonreactive for fascin, a mature DC marker.

Histiocytic necrotizing lymphadenitis (HNL; Kikuchi-Fujimoto disease) is a benign and self-limited disease with a female predominance affecting preferentially the Asian population. HNL usually manifests as isolated cervical lymphadenopathy accompanied by fever and night sweats. The etiology is unknown; however, viral and/or autoimmune pathogenesis is suspected. Some studies suggest that HNL and systemic lupus erythematosus (SLE) share pathogenetic events based on histologic overlap. In some patients with HNL, SLE has subsequently developed.1,2

HNL is characterized morphologically by nodal paracortical and cortical patchy lesions with karyorrhectic nuclear debris and associated mononuclear cell response composed of histiocytes, lymphoid cells, and a striking population of mononuclear cells with plasmacytoid morphology. Plasma cells and neutrophils are not a feature. Depending on the time course, karyorrhexis or a mononuclear reaction with variable cellular composition may predominate. The morphologically distinct plasmacytoid mononuclear cells of HNL are of intermediate size with polarized, often eccentric nuclei with dispersed chromatin, absent nucleoli, and amphophilic cytoplasm without a prominent Golgi area. In the literature, different terms have been used for this cell population, including plasmacytoid T cells,3 plasmacytoid monocytes,4,5 natural interferon (IFN)-α–producing cells,8 type 2 predendritic cells,9 and plasmacytoid dendritic cells (PDCs).10-12 It is known that these cells produce IFN-α, differentiate in vitro into antigen-presenting DCs, and are part of a composite group of myeloid and lymphoid early-committed immature DCs.13

Monoclonal antibodies are now available for the detection of these specific cell types.10 By flow cytometry, myeloid DC (MDCs), a major subpopulation of human
peripheral blood DCs, are positive for CD1c (blood dendritic cell antigen [BDCA]-1), CD13, and CD33 and are CD11chigh
and CD123low. PDCs are positive for CD303 (BDCA-2) and CD123high, are negative for CD13 and CD33, and produce IFN-α when triggered by antigens. MxA (myxovirus A)
protein is a surrogate marker for IFN-α in tissue sections and may be detected immunohistochemically. Both of these early dendritic subtypes are negative for fascin, a marker of mature
DCs.14,15 The goal of this study was to characterize the lesional cells in HNL as they relate to the DC system.

Materials and Methods

We retrieved 6 cases of HNL with available archival frozen tissues from the pathology files of the Brigham and
Women’s Hospital, Boston, MA. The patients included 3 females and 3 males with a mean age of 26 years (range,
17-33 years). The patients’ ethnicity was Korean, Caucasian, Guatemalan, Haitian (1 each), and unknown (2). All patients
had cervical lymphadenopathy, and 2 also had axillary lymphadenopathy. All but 1 patient had documented fever
of a few weeks’ duration. An infectious disease workup was negative as reported in the patients’ hospital records. Biopsy
specimens of 5 cervical nodes and 1 axillary node were available for studies.

Immunoperoxidase studies were performed manually
on paraformaldehyde- or acetone-fixed cryostat sections according to routine protocols using an EnVision+
(DAKO, Carpinteria, CA) or PowerVision (ImmunoVision Technologies, Daly City, CA) detection system. Antibodies
used are summarized in Table I. Briefly, following fixation, slides were placed in 0.05 mol/L of tris(hydroxymethyl)
aminomethane (Tris) buffer (pH 7.6) with 3% porcine serum,
then, slides were placed in 0.05 mol/L of tris(hydroxymethyl) hydrochloride (DAB+, DAKO) as the chromogen (except for fascin). Slides were counterstained with methyl green,
dehydrated, and cover slipped. Fascin was detected by using a 3-step immuno–alkaline phosphatase technique with naph-
thol AS-MX phosphate as the substrate and new fuchsin as the chromogen. Following incubation with antibody to
fascin, slides were incubated sequentially with rabbit anti-
mouse immunoglobulins antibody (DAKO; 1:150 dilution,
15 minutes) and alkaline phosphatase–conjugated goat anti-
rabbit immunoglobulin detection reagent (ImmunoVision Technologies; 30 minutes). Negative control slides were
prepared by substituting Tris buffer for the antibody in sequential cryostat sections.

Results

Morphologic Features

The morphologic findings observed in all 6 cases exam-
ined were similar and included paracortical patchy lesions
composed of mononuclear cells and karyorrhectic nuclear
debris Image 1A and Image 1B. Although the cellular
composition of the lesions varied depending on the age of
the lesion, karyorrhectic debris and numerous mononuclear
cells could be identified in all lesions, some of which were
extensive. The latter included phagocytic (tangible body mac-
rophages) and nonphagocytic (crescentic) mononuclear cells,
as well as foamy cells, plasmacytoid mononuclear cells, and small and larger lymphocytes. In 1 case, the lesional cells extended into the perinodal fibroadipose tissue.

**Immunohistochemical Features**

The 6 cases of HNL revealed relatively similar distribution patterns for the immunohistochemical markers evaluated in sequential sections. In the negative control studies, cells with endogenous peroxidase activity were absent or rare, corroborating an absence of neutrophils (which typically exhibit strong peroxidase activity in cryostat sections) in these lesions.

Reactivity for CD1c was strongly and diffusely detected in approximately 70% to 80% of the lesional cells **Image 2A** and **Image 2B**. Interdigitating DCs and an extranodal population of lesional cells were also immunoreactive.

The CD303 immunoreactivity **Image 3A** and **Image 3B** was diffuse and of variable intensity and was observed in nearly all lesional cells and in interdigitating DCs. Many of the lesional cells showed strong cytoplasmic

**Image 1** Lymph node, cryostat section. **A**, At low power, patchy pale lesions of histiocytic necrotizing lymphadenitis are seen in the paracortical area of the node (H&E, ×50). **B**, At higher magnification, the lesions are composed of mononuclear cells with round to irregular nuclei with associated characteristic karyorrhectic debris. Plasmacytoid dendritic cells are present (arrow) (H&E, ×1,000).

**Image 2** Immunoperoxidase studies. **A**, About 70%-80% of the lesional cells are reactive for the myeloid dendritic cell marker CD1c (BDCA-1, blood dendritic cell antigen-1) (×50). **B**, At higher magnification, frequent mononuclear cells, including phagocytic forms, as well as ingested apoptotic bodies, appear positive for CD1c. A subset of cells appears nonreactive (×1,000).
CD123 (interleukin [IL]-3-R) immunoreactivity, including different mononuclear subtypes and the plasmacytoid forms. Staining intensity for CD123 was more prominent at the peripheral aspect of the lesions. We noted fewer cells and/or decreased staining intensity in areas corresponding to the CD1c+ population **Image 4A** and **Image 4B**, likely reflecting the variation in CD123 expression for MDCs (exhibit CD123 low antigen density by flow cytometry) compared with PDCs (exhibit CD123 high antigen density by flow cytometry). Thus, most CD123 reactivity corresponded to areas containing CD303+ cells. Endothelial cells were also strongly immunoreactive for CD123, particularly the high endothelial venules. In addition, the CD123 antibody highlighted a meshwork of paracortical DCs, dividing the paracortical lymphocytes into trabeculae 3 to 5 cells thick.

The patterns of CD1c, CD303, and CD123 immunoreactivity in the lesional cells were overlapping but not identical (compare Images 2, 3, and 4). Lesional cells also revealed strong cytoplasmic expression of the IFN-α inducible protein.

**Image 3** Immunoperoxidase studies. **A**, A majority of the lesional cells are positive for CD303 (BDCA-2, blood dendritic cell antigen-2), a marker for plasmacytoid dendritic cells (×50). **B**, At higher magnification, mononuclear cells, including some with more abundant cytoplasm and eccentric nuclei, are CD303+ and exhibit a delicate staining pattern of variable intensity. Ingested apoptotic bodies are nonreactive (×1,000).

**Image 4** Immunoperoxidase studies. **A**, Reactivity for CD123 (interleukin 3R) is observed for the majority of the lesional cells with accentuation at the peripheral aspect of the lesions and a decrease in the central areas (×50). **B**, Higher magnification of the reactive cells demonstrates frequent cells with abundant cytoplasm and eccentric nuclei (CD123, ×1,000).
MxA (Image 5A) and Image 5B. In addition, cytoplasmic MxA immunoreactivity of varying intensity was seen in the majority of paracortical lymphocytes, in DCs, and in endothelial cells.

Lesional cells were not immunoreactive for fascin, a marker for mature DCs (Image 6). However, strong cytoplasmic immunoreactivity for fascin was observed in patchy aggregates of DCs in the paracortex, in the vicinity of but discrete from the lesions (Image 6), apparently mainly representing interdigitating DCs.

CD68 (KP1 clone) (Image 7) positivity highlighted a population of lesional cells (about 70%-100%), depending on the age of the lesion, and included crescentic and phagocytic histiocytes, plasmacytoid mononuclear cells, and interdigitating DCs. Preliminary cryostat section studies demonstrated that reactivity for CD68 using the PG-M1 clone of the antibody was weaker than that observed using clone KP1; therefore, the latter clone was selected for our studies.

Reactivity for the pan–T-cell marker CD3 was observed for the majority of paracortical lymphoid cells surrounding

**Image 5A** Immunoperoxidase studies. A, Interferon-α-inducible protein myxovirus A (MxA) is uniformly present in cells throughout the lesions (×50). B, Higher magnification of the MxA+ cells (×1,000).

**Image 6** Immuno–alkaline phosphatase study. Lesional cells are negative for the mature dendritic cell marker fascin; however, interdigitating dendritic cells located outside the lesions are positive for fascin (red) (×50).

**Image 7** Immunoperoxidase study. The majority of the lesional cells are reactive for CD68 (KP1 clone), as are scattered histiocytes (×50).
the lesions and for a minor population (10%-20%) of cells within the lesions (not shown). Strong CD4 immunoreactivity was present in a major subpopulation of paracortical T cells, with moderate reactivity noted for a large proportion of lesional cells, a finding that is not unanticipated given that histiocytes, as well as MDCs and PDCs, may express this marker Image 8. The very early lesions appeared to contain few CD4+ cells. In the more developed lesions, the staining pattern for CD4 largely corresponded to the composite patterns seen for CD1c, CD303, and CD123. Strong CD8 immunoreactivity was observed in varied proportion in scattered cells in the paracortex at the periphery of the lesions, with scattered cells present within the lesions (5%-10% of lesional cells) Image 9. Notably, the major CD4+ cell population appeared to be CD8−.

CD13 immunoreactivity was observed in varying proportions of the lesional cells, up to a majority of the cells, predominantly with a central location in the early lesions and more extensively in the later lesions Image 10 and topographically corresponded to the CD1c+ cells (compare with Image 2). In addition, scattered DCs were also immunoreactive for CD13. Similarly, CD33 immunoreactivity (not shown) was noted for a large proportion of the lesional cells. Aggregates of CD19+ B cells were present in the paracortex at the periphery of the lesions and in residual follicular areas, with very few cells within the lesions (not shown). The results for the various immunohistochemical studies in sequential cryostat sections are illustrated in Images 2 through 10 and summarized in Table 2.

Discussion

HNL is a benign disorder of unknown cause with characteristic clinical findings and affected lymph nodes showing lesions with karyorrhectic nuclear debris and proliferating mononuclear cells, including histiocytes, lymphoid cells, and plasmacytoid T cells/plasmacytoid monocytes (CD68+). In the original reports by Kikuchi16 and Fujimoto et al,17 these proliferating cells are described as histiocytes and reticular cells. Based on morphologic and phenotypic properties such as plasmacytoid features and associated T-cell-helper/inducer phenotype, Feller et al3 named this subpopulation plasmacytoid T-cells. Facchetti et al4,5 provided immunohistochemical evidence of monocytic derivation for these cells, renaming them plasmacytoid monocytes and proposing their function as antigen-presenting cells.

Morphologically, these cells are of intermediate size with an ample amount of eosinophilic cytoplasm, an eccentric nucleus, and variable nucleolus and can be identified in various anatomic locations like lymph nodes, tonsils, bone marrow, thymus, and spleen in normal and pathologic conditions.7 Proposals regarding origin and function of these so-called plasmacytoid monocytes/T cells emerged with identification of CD68+ lineage-negative peripheral blood cells with dendritic precursor characteristics resembling DCs found in tissues.9,18 In general, DCs and precursors are HLA-DR+ and lineage-negative, bone marrow–derived stem cells, which enter the bloodstream and migrate spontaneously to lymph nodes and other peripheral sites.19,20 They constitute approximately 2% of peripheral blood mononuclear cells.18 These cells are essential antigen-presenting...
cells initiating primary T-cell response and subsequent interactions involving CD4 and CD8 T lymphocytes, natural killer cells, and B cells. It has been demonstrated that the precursor DCs consist of 2 subsets with separate differentiation pathways: MDCs, resembling myeloid-derived cells, and lymphoid (plasmacytoid) DCs, resembling tissue plasmacytoid monocytes/T cells.\textsuperscript{9,13,18,20} Herein we use the term plasmacytoid DCs for cells previously designated as plasmacytoid monocytes/T cells.

The CD11c\textsuperscript{high} and CD123\textsuperscript{low} MDCs represent a major subset of DCs in peripheral blood, are CD1c\textsuperscript{+}, and exhibit monocyteid morphologic features. The MDCs develop into mature DCs in culture after stimulation with granulocyte-macrophage colony-stimulating factor, IL-4, and tumor necrosis factor \(\alpha\).\textsuperscript{22,23} CD1c is an excellent flow cytometric marker for identification of MDCs;\textsuperscript{10} however, limited tissue studies are available. In our cryostat section studies, CD1c was immunoreactive in up to 80% of the lesional cells in HNL and appeared to be coexpressed on some CD303\textsuperscript{+} presumptive PDCs.

PDCs are HLA-DR\textsuperscript{+}, CD68\textsuperscript{bright}, CD123\textsuperscript{+}, and lineage-negative because they lack reactivity for CD3 (T cells), CD19 (B cells), CD16 (natural killer cells), myeloid markers (myeloperoxidase, CD33, CD13, CD11b, and CD11c), and monocytic markers (CD14 and lysozyme). In blood, they constitute only a minor subpopulation of immature DCs.\textsuperscript{10}

In tissues, PDCs concentrate around and within the lumina of high endothelial venules and aggregate loosely within the lymph node parenchyma.\textsuperscript{6} By molecular methods, PDCs have been shown to have lymphoid markers, including high levels of immunoglobulin light chain transcripts.\textsuperscript{22} After ligand stimulation, such as virus or IL-3, they become a major producer of IFN-\(\alpha\) and are known as natural IFN–producing cells.\textsuperscript{7,13,18,24} In tissue sections, this capacity can be demonstrated by a strong immunoreactivity for the IFN-\(\alpha\) surrogate protein MxA. The natural ability to produce IFN-\(\alpha\) diminishes within 24 hours, the time required for maturation into antigen-presenting DCs under the influence of autocrine IL-3 secretion. The functional switch from natural IFN–producing cells to antigen-presenting DCs is viewed as a major link between innate and adaptive immunity.\textsuperscript{25} PDCs are thought to be essential for tolerance or autoimmunity and allergic phenomena.\textsuperscript{23,26,27}

SLE is an autoimmune disease with nodal changes showing morphologic overlap with HNL. Abnormal activation of PDCs has been proposed to have a major pathogenic role in the development of this disease.\textsuperscript{2} In keeping with this hypothesis, patients with SLE show sustained elevation of IFN-\(\alpha\) in blood, decreased circulating PDCs, and increased abundant IFN–producing PDCs in affected organs like skin.\textsuperscript{8,27} Ronnblom and Alm\textsuperscript{2} proposed that anti-dsDNA antibodies, DNA complexes, and material released by

**Table 2**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Myeloid DCs</th>
<th>Plasmacytoid DCs</th>
<th>Lesional Cells in HNL</th>
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<tr>
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<td>CD203 (BDCA-2)</td>
<td>–\textsuperscript{†}</td>
<td>+</td>
<td>+ (subset)</td>
</tr>
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<td>CD141 (BDCA-3)</td>
<td>Low\textsuperscript{‡}</td>
<td>Low\textsuperscript{‡}</td>
<td>ND</td>
</tr>
<tr>
<td>CD304 (BDCA-4)</td>
<td>–</td>
<td>+\textsuperscript{†}</td>
<td>ND</td>
</tr>
<tr>
<td>CD123</td>
<td>Dim \textsuperscript{‡}</td>
<td>Bright +\textsuperscript{‡}</td>
<td>+</td>
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<tr>
<td>CD3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
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<td>+</td>
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<td>CD7</td>
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<td>CD8</td>
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<td>ND</td>
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<tr>
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<td>–</td>
<td>+ (subset)\textsuperscript{‡}</td>
</tr>
<tr>
<td>CD33</td>
<td>+</td>
<td>–</td>
<td>+ (subset)\textsuperscript{‡}</td>
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<tr>
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<td>Bright +\textsuperscript{‡}</td>
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<tr>
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<td>–</td>
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</tr>
<tr>
<td>MxA</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD68</td>
<td>+</td>
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</tr>
</tbody>
</table>

*BDCA, blood dendritic cell antigen; DCs, dendritic cells; HNL, histiocytic necrotizing lymphadenitis; MxA, myxovirus A; ND, not determined; +, positive; †, negative.

\textsuperscript{†} Subset +.

\textsuperscript{‡} Subset +.

\textsuperscript{§} Subset circulating plasmacytoid DCs positive; not confirmed in tissue sections.

\textsuperscript{¶} Pre–T-cell antigen receptor \(\alpha\) chain.

\textsuperscript{*} Corresponds to CD1c\textsuperscript{+} cells.
apoptotic cells induce IFN-α production by PDCs, which, in certain genetically predisposed people, continues as a vicious cycle of PDC activation. Similarly, PDC activation could have a pathogenetic role in HNL but in a milder form leading to a self-limited disorder.

The characterization of CD303 by Dzionek et al\textsuperscript{10,11} permits positive identification of PDCs in blood and tissues and represents an important step toward understanding their function. CD303 is a type II C lectin with signaling receptor properties mediating inhibition of IFN-α.\textsuperscript{12} Facchetti et al\textsuperscript{7} evaluated CD303 and CD123 by immunohistochemical studies and found these markers to be helpful in identification of PDCs in normal tissues and in various disorders. Our studies demonstrated that abundant immature (fascin-negative) PDCs, positive for CD303 (Image 3) and CD123 (Image 4), are actively producing IFN-α, as evidenced by the strong MxA immunoreactivity (Image 5), and are present in all lesions of HNL independent of their age. A population of MDCs expressing CD1c (Image 2) and CD13 (Image 10) is also present in these lesions, and some cells appear to coexpress PDC and MDC markers (compare Images 2 and 3). The latter observation raises the possibility that PDCs and MDCs may not only coexist within the lesions, but also may evolve into each other. This finding is consistent with the hypothesis proposed by Comeau et al\textsuperscript{19} that, rather than forming a distinct lineage, the PDCs might represent a population of immature DCs undergoing in vivo fate conversion from a lymphoid to an MDC type.

It is unclear at this point if primary microbial activation stimulates focal proliferation of PDCs in peripheral sites like skin or lymph nodes or, rather, promotes their continuous influx from the blood. In situ differentiation from a local or circulation-derived extramedullary CD34+ progenitor is another possibility. In accord with the perception that immature PDCs are not effective in triggering a T-cell response, we observed only scattered CD3 positive effector T cells within the lesions. It is interesting that the apoptotic bodies identified within the phagocytic CD1c+ DCs were also positive for CD1c (Image 2B). This finding suggests an autophagocytic process of self-elimination among DCs in accord with the findings of Brière et al\textsuperscript{22} who observed the capacity of PDCs to undergo spontaneous apoptosis in culture in the absence of survival factors. This is in contrast with earlier studies by Felgar et al\textsuperscript{28} and Ohshima et al\textsuperscript{29} who considered the nuclear debris of HNL to be apoptotic bodies of dying cytotoxic T lymphocytes representing exuberant T cell–mediated immune response to presumed microbial agents in genetically predisposed people.\textsuperscript{30}

Although HNL is an infrequent disease, the study of lesional cells in HNL can provide useful insights into the biology of DCs. DCs have a central immunoregulatory function, which has been studied extensively in vitro. Monoclonal antibodies to CD1c and CD303 have been used for the identification of MDCs and PDCs, mainly in flow cytometric or cryostat section studies, which limits their applicability. For paraffin sections, CD123 has been recently used as a marker for detection of PDCs.\textsuperscript{31,32} This cryostat section study is the first to show that the lesional cells of HNL are composed mainly of immature DCs, including MDCs and PDCs, which appear reactive for MxA protein, a surrogate marker for IFN-α, with features raising the possibility of a transition between the 2 types of immature DCs. Based on our results and the findings in other studies, the terms plasmacytoid T cells and plasmacytoid monocytes are no longer acceptable designations for the lesional cells of HNL, which actually represent immature DCs and include PDCs and MDCs. This study is the first to demonstrate the presence of both of these cells in HNL using antibodies to CD1c and CD303. Their role in possible immune or autoimmune phenomena associated with HNL remains to be elucidated.

References


