Myeloperoxidase Expression by Histiocytes in Kikuchi's and Kikuchi-Like Lymphadenopathy

Stefano A. Pileri,* Fabio Facchetti,[†]
Stefano Ascani,* Elena Sabattini,*
Simonetta Poggi,* Milena Piccoli,*
Damiano Rondelli,* Federica Vergoni,[†]
Pier Luigi Zinzani,* Pier Paolo Piccaluga,*
Brunangelo Falini,[‡] and Peter G. Isaacson[§]

From the Pathology and Clinical Units,* Institute of Hematology and Clinical Oncology "L. & A. Seràgnoli," Bologna University, Bologna, Italy; the Institute of Pathologic Anatomy and Histopathology,[†] Brescia University, Brescia, Italy; the Hematopathology Laboratory,[‡] Institute of Hematology, Perugia University, Perugia, Italy; and the Histopathology Department,[§] University College London, London, United Kingdom

Forty-five examples of Kikuchi's lymphadenitis (KL), 5 Kikuchi-like lupus erythematosus lymphadenopathies, 25 nonnecrotizing lymphadenitides (5 toxoplasmic, 5 sarcoid-like, 6 dermatopathic, 4 suppurative, 3 tubercular, 2 with sinus histiocytosis), 4 examples of hyaline-vascular Castleman disease (CD), 2 plasmacytoid monocyte tumors (PM-Ts), and 61 accessory cell neoplasms were studied by a panel of antibodies, including the PG-M1 (against a macrophage-restricted CD68 epitope) and a polyclonal anti-myeloperoxidase (MPO). In KL and Kikuchi-like lupus erythematosus lymphadenopathies, 25 to 75% of CD68⁺ histiocytes co-expressed MPO. This did not occur in nonnecrotizing lymphadenitides and accessory cell neoplasms. MPO⁺/CD68⁺ elements corresponded to nonphagocytosing mononuclear cells and some crescentic macrophages and phagocytosing histiocytes. Typical PMs were MPO⁻/CD68⁺ in all cases, including CD and PM-T. Our observations suggest that in KL and KL-like lymphadenopathies: 1) MPO⁺/CD68⁺ blood monocytes might be attracted into tissues because of the lack or paucity of granulocytes and the need of MPO for oxidative processes; 2) PMs are more likely to be involved in the cytotoxic immune reaction than in phagocytic phenomena; 3) the peculiar phenotype of the histiocytic component can be usefully used for the differentiation from malignant lymphoma and PM-T. (*Am J Pathol* 2001, 159:915-924)

In 1972, Kikuchi¹ and Fujimoto and colleagues² independently described an unusual and idiopathic form of benign lymphadenitis that they, respectively, called "lymph-

adenitis showing reticulum cell hyperplasia with nuclear debris and phagocytosis" and "cervical subacute necrotizing lymphadenitis." In 1982, Pileri and colleagues³ first reported this condition outside Japan and termed it "histiocytic necrotizing lymphadenitis without granulocytic infiltration," aiming to differentiate it from other forms of necrotizing lymphadenitis with variable amounts of granulocytes, such as those occurring in systemic lupus erythematosus (LE), bacterial and viral infections, and thrombosis of the afferent veins. Since then, several other cases, generically referred to as Kikuchi's lymphadenitis (KL), have been observed in Western countries.⁴⁻¹¹

Although, the etiology of the lesion has still to be clearly established, an infectious cause is suggested by the many associations with Epstein-Barr virus,^{12,13} human herpesvirus (HHV)6,^{14,15} HHV8,¹⁶ parvovirus B19,¹⁷ *Toxoplasma gondii*,¹⁸ and *Yersinia enterocolitica*.⁵ Kikuchi's disease has also been described in HIV-¹⁹ and HTLV-1-positive patients.²⁰ Some authors favor an autoimmune mechanism, and interestingly KL has also been described in patients affected by connective tissue diseases.²¹⁻²⁶

Clinically, KL is usually characterized by cervical adenopathy/ies, mainly occurring in young women, and sometimes associated with fever and transitory leukopenia. KL shows a benign clinical course with resolution in few months, either spontaneously or after antibiotic therapy. Rare reports exist of multicentric organ involvement, which can be occasionally fatal,²⁷ and extranodal locations, mainly in the skin.^{28,29}

Morphologically, the lesion affects the cortical and paracortical areas of the node with foci that have "clear" appearance at low magnification and are composed of varying amounts of histiocytes,³⁰ small- to medium-sized lymphocytes and immunoblasts, abundant karyorrhectic and granular eosinophilic debris, and possible overt coagulative necrosis. By definition, neutrophils are absent or sparse. On the basis of the proportion of the different cytological components, Kuo³¹ has recently proposed three different histological subtypes of KL: proliferative, necrotizing, and xanthomatous. The diagnosis of KL is

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Address reprint requests to Professor Stefano A. Pileri, Unità Operativa di Anatomia Patologica ed Ematopatologia, Istituto di Ematologia ed Oncologia Medica "L. e A. Seràgnoli," Università di Bologna, Policlinico S. Orsola, Via Massarenti 9, 40138 Bologna, Italia. E-mail: pileri@almadns.unibo.it.

generally not difficult, although early lesions lacking overt necrosis can be misdiagnosed as malignant lymphoma, because of the presence of abundant immunoblasts.^{32,33} At immunohistochemistry, the histiocytic component is characterized by the expression of the CD68 antigen, whereas the lymphoid component carries a T-cell phenotype with a prevalence of CD8⁺ cytotoxic cells.^{9,31,34–36} Plasmacytoid monocytes (PMs) are thought to be an important cell component of early stages of KL,^{3,5,8–10,29–32,34,37–39} and their identification is considered helpful for diagnosis.³¹ PMs are medium-sized cells with eccentric nuclei and clumped chromatin, which are regularly found in the pulp of reactive lymph nodes, where they occur either as large aggregates or as isolated, dispersed cells. They are closely related to high endothelial venules. Although their phenotype has been clearly defined (including positivity for CD4, CD31, CD36, CD43, CD68, CD74, and CLA/HECA452),^{39–51} the exact nature of their relationship to the myelomonocytic cell lineage still remains debated. Recent reports suggest that PMs secrete large amounts of type I interferon and drive a potent Th1 T-cell polarization;^{52–54} in addition, they express the interleukin-3 receptor α and CD40, and on stimulation with interleukin-3 and CD40-ligand differentiate into dendritic cells.^{51–55}

In this study we report the occurrence of large numbers of histiocytes expressing MPO in Kikuchi's and Kikuchi-like lymphadenitis, and discuss the nature of this peculiar histiocyte, its relationship to other cell types occurring in KL, and its diagnostic relevance.

Materials and Methods

Case Selection and Histological and Immunohistological Methods

Formalin-fixed, paraffin-embedded lymph node blocks of 45 Italian KL patients were retrieved from the files of the Unit of Pathological Anatomy and Hematopathology of Bologna University. The age and sex of the patients, as well as the site of lymphadenopathy were known in all instances. Three- μ m-thick sections were cut from the paraffin blocks and stained with hematoxylin and eosin (H&E), Giemsa, periodic acid-Schiff (with and without diastase digestion), and Gomori silver impregnation for

reticulin fibers. Further sections were cut, coated on naturally charged slides, stored at 56°C for at least 2 hours, and then rinsed in water through repeated washes in Histoclear (National Diagnostics, Atlanta, GA) and graded alcohols. These sections were used for immunohistochemistry, which was performed by applying the following antibodies: PG-M1/CD68 (Prof. B. Falini, Perugia University, Italy), polyclonal anti-myeloperoxidase (MPO) (DAKO AS, Denmark), Qbend10/CD34 (Menarini, Italy), L26/CD20 (DAKO), JCB117/CD79a (Prof. D.Y. Mason, Oxford University, United Kingdom), polyclonal anti-CD3 (DAKO), OPD4/CD45R0 (DAKO), 114B/CD8 (Prof. D.Y. Mason), Ber-H2/CD30 (Prof. B. Falini), Mib-1 (DAKO), TIA-1 (Coulter Immunology, FL, USA), G2b-7/Granzyme-B (Kemiya Biochemical Company, WA, USA), and CLA/HECA425 (Pharmingen, San Diego, CA). The antibodies were dispensed on a TechMate 500 immunostainer and detected by the alkaline phosphatase-anti alkaline phosphatase (APAAP) technique or the streptavidin-biotin-peroxidase complexes (SABC) method.⁵⁶ Antigen retrieval was performed according to previously reported experience.⁵⁷ In particular, sections were placed in 1 mmol/L of ethylenediaminetetraacetic acid-NOH solution (pH 8.0), microwaved for 5 minutes at 900 W twice and then left at room temperature for 20 minutes. In all instances, positive and negative controls were used to assess the reliability of the results, as previously described.⁵⁷ The results were graded in a semiquantitative manner as follows: +, positivity in 75 to 100% of the cells evaluated; +/-, positivity in 50 to 75% of the cells evaluated; -/+, positivity in 25 to 50% of the cells evaluated; rare, positivity in 10 to 25% of the cells evaluated; exceptional, positivity in 1 to 10% of the cells evaluated; and -, negativity in all of the cells evaluated. The intensity of the staining and the pattern of positivity (nuclear, cytoplasmic diffuse, dot-like, granular, membrane-bound, and so forth) were also recorded.

In seven cases, double staining for CLA/HECA452 (used as a PM marker)⁵⁸ and MPO was performed. In particular, the rat anti-human CLA/HECA425 and rabbit anti-human MPO antibodies were respectively revealed by the immunoalkaline phosphatase (chromogen: Fast Red or nitro blue tetrazolium) and the immunoperoxidase

Figure 1. KL proliferative type. The lesion shows a large amount of histiocytes intermingled with small lymphocytes, immunoblasts, and apoptotic bodies (Giemsa staining; original magnification, $\times 400$). **Inset:** Multiple lesions in the cortex and paracortex that stain grayish at Giemsa and sharply contrast with the deeply basophilic-spared portions of the node (original magnification, $\times 50$).

Figure 2. KL proliferative type. The lymphoid component mainly corresponds to CD3⁺ peripheral T cells (**a**), which mostly express CD8 phenotype (**b**) and carry cytotoxic markers (**c**), as shown by the positivity at the determination of Granzyme B (APAAP technique; Gill's hematoxylin counterstaining; original magnifications, $\times 400$).

Figure 3. KL proliferative type. CD68-staining pattern in the same case as Figure 1 (APAAP technique; Gill's hematoxylin counterstaining; original magnification, $\times 300$).

Figure 4. KL proliferative type. In the same case the positivity for MPO primarily corresponds to that for CD68. **Inset:** Exceptional granulocytes express neutrophilic elastase (APAAP technique; Gill's hematoxylin counterstaining; original magnification, $\times 300$).

Figure 5. KL-necrotizing type. The pattern is characterized by phenomena of tissue necrosis, a rich histiocytic component and numerous apoptotic bodies (H&E; original magnification, $\times 400$).

Figure 6. KL-necrotizing type. The histiocytic component stains for CD68 (APAAP technique; Gill's hematoxylin counterstaining; original magnification, $\times 300$).

Figure 7. KL-necrotizing type. In the same case the positivity for MPO primarily corresponds to that for CD68. **Inset:** Despite tissue necrosis, there are rare granulocytes that express neutrophilic elastase (APAAP technique; Gill's hematoxylin counterstaining; original magnification, $\times 300$).

Figure 8. KL proliferative type. The morphological spectrum of MPO⁺ elements varies from mononuclear cells to crescentic and phagocytosing (**arrows**) histiocytes (SABC technique; hematoxylin counterstaining; original magnification, $\times 800$).

Figure 9. A plasmacytoid monocyte cluster at some distance from the lesions is negative for MPO (APAAP technique; Gill's hematoxylin counterstaining; original magnification, $\times 250$). **Inset:** Cytological details of the cluster at Giemsa staining; note the presence of apoptotic bodies (original magnification, $\times 400$).

techniques (chromogen: diaminobenzidine or amino-ethyl-carbazole).

The specificity of the MPO polyclonal antibody was tested in the course of previously reported studies^{59,60} and in >1000 examples of acute leukemia, malignant lymphoma, and nonhematopoietic tumors tested in the laboratories of the senior authors of this paper (BF, FF, PGI, SAP). Because no improper stains have ever been recorded, the antibody has been adopted as an operational key marker by the Italian Co-operative Study Group on Acute Leukemias and is used daily for the diagnosis of these diseases in Italy.

Comparisons were made with 5 LE lymphadenopathies, 23 nonnecrotizing lymphadenitides (5 toxoplasmic, 5 sarcoid-like, 6 dermatopathic, 4 suppurative, 3 tubercular), 2 reactive lymphadenopathies with huge mature sinus histiocytosis, 4 examples of hyaline-vascular Castleman disease (CD), and 2 PM tumors (PMTs), retrieved from the files of the Unit of Pathological Anatomy and Hematopathology of Bologna University, the Histopathology Department of the University College London, or the Institute of Pathological Anatomy and Histopathology of Brescia University. All of the specimens were treated and immunohistochemically studied as above. In addition, the series of tumors derived from histiocytes and accessory dendritic cells (61 cases) recently studied by the International Lymphoma Study Group⁶¹ was examined as a further control.

Fluorescence-Activated Cell Sorting (FACS) Analysis Method

Blood samples from two normal individuals were obtained after informed consent for FACS analysis. This was performed according to previously reported procedures.⁶² The following mouse monoclonal antibodies (mAbs) were used: anti-human MPO conjugated with fluorescein isothiocyanate (clone MPO-7, DAKO) and anti-human CD14 conjugated with phycoerythrin (clone TÜK4, DAKO). The rabbit anti-human MPO-unconjugated polyclonal antibody used for immunohistochemistry was tested in parallel by indirect immunofluorescence and a secondary swine anti-rabbit immunoglobulin/fluorescein isothiocyanate (DAKO). Adequate isotype controls were always applied. Briefly, appropriate amounts of conjugated mAbs or unconjugated polyclonal antibody were added to 100 ml of whole blood and incubated for 15 minutes at room temperature. Red cells were then lysed. For indirect immunofluorescence experiments, the incubation with the primary antibody was followed by the application of the secondary antibody for 15 minutes at room temperature. After washing the samples twice, 10 to 20 × 10⁴ events were acquired on a FACScalibur instrument (Becton-Dickinson, Mountain View, CA) and analyzed by CellQuest software. Monocytes were gated using linear side scatter and forward scatter characteristics to exclude small lymphocytes as well as neutrophils.

Results

Thirty-three patients with KL were females and 12 were males (male:female ratio, 1:2.75). Their age ranged from 16 to 51 years, the mean and median values being 29.2 and 33.5 years, respectively. All cases presented with laterocervical adenopathy, which was occasionally associated with swelling of the axillary nodes (three cases) or with adenopathies in the supraclavicular region (two cases) or axillary and inguinal sites (two cases). Follow-up data were available for 30 patients, the length of observation ranging from 1 to 12 years; at the time of writing, all these patients are alive and well, without signs or symptoms either of KL or of collagen vascular disease, infection, deep venous thrombosis, or malignancy.

Morphology

At low magnification, the normal lymph node architecture was variably effaced because of the presence of patchy circumscribed or confluent lesions that were located in the cortex and paracortex (Figure 1). On Giemsa staining, these lesions were grayish, strongly contrasting with the deeply basophilic spared areas (Figure 1). The latter were characterized by sparse follicles and expansion of the T zone, which contained variable amounts of immunoblasts, as well as by occasional clusters of PMs and scattered macrophages and dendritic cells producing a mottled appearance.

At closer examination (Table 1), 18 and 27 cases were, respectively, classified as proliferative and necrotizing lesions, according to Kuo³¹ (Figures 1 and 5). No xanthomatous forms³¹ were recorded. In all of the specimens examined, neutrophils were absent or rare, whereas apoptotic bodies were easily detected. In particular, the histiocytic component—the composition of which did not significantly vary with the proliferative or necrotizing nature of the adenopathy—consisted of: 1) nonphagocytosing mononuclear elements showing a moderate amount of cytoplasm, weakly stained by Giemsa, and a round-oval or deeply indented nucleus with dispersed chromatin; 2) variable numbers of morphologically recognizable PMs with slightly basophilic cytoplasm and eccentric nuclei with clumped chromatin; 3) crescentic macrophages;³⁰ and 4) phagocytosing histiocytes with a large rim of clear cytoplasm containing abundant nuclear debris.

Immunohistochemistry

Lymphoid Component

Within the lesions, lymphoid cells showed mainly T-cell phenotype, as revealed by their CD3 expression (Figure 2a). With the exception of a single case, only rare CD79a⁺ elements were scattered throughout. Most T lymphocytes carried the CD8 molecule (Figure 2b); with one exception, the number of OPD4⁺ elements was extremely low. These findings did not significantly vary between the proliferative and necrotizing subgroups.³¹ The only difference observed between the two histological

Table 1. Immunophenotypic Findings Observed in 45 Examples of KL

Reference number	Subtype	Histiocytic component			PM		
		CD68	MPO	NE	CD68	MPO	NE
E842/88	P	+	+/-	-	+	-	-
E1922/89	N	+	+/-	-	+	-	-
E1948/89	N	+	+/-	-	+	-	-
E176/91	P	+	-/+	-	+	-	-
E358/91	P	+	-/+	-	+	-	-
E741/92	P	+	-/+	-	+	-	-
E1648/92	N	+	+/-	-	+	-	-
E5463/92	N	+	+/-	-	+	-	-
E83/93	N	+	-/+	-	+	-	-
E4576/93	N	+	-/+	-	+	-	-
E4851/93	N	+	-/+	-	+	-	-
16602/93	N	+	-/+	-	+	-	-
17122/93	N	+	-/+	-	+	-	-
17287/93	P	+	+/-	-	+	-	-
E391/94	N	+	-/+	-	+	-	-
E1914/94	P	+	-/+	-	+	-	-
E1688/95	N	+	+/-	-	+	-	-
E2118/95	P	+	-/+	-	+	-	-
E3091/95	N	+	+/-	-	+	-	-
E4841/95	N	+	-/+	-	+	-	-
12529/95	P	+	+/-	-	+	-	-
E5239/96	P	+	+/-	-	+	-	-
E925/97	N	+	+/-	-	+	-	-
E1173/97	N	+	-/+	ND	+	-	ND
E1174/97	N	+	+/-	-	+	-	-
E1276/97	N	+	-/+	-	+	-	-
E1789/97	P	+	-/+	-	+	-	-
E1982/97	P	+	-/+	-	+	-	-
E2651/97	N	+	+/-	-	+	-	-
E3168/97	P	+	+/-	-	+	-	-
E3169/97	N	+	+/-	NEV	+	-	NEV
E3170/97	N	+	NEV	-	+	-	-
E3618/97	N	+	+/-	-	+	-	-
E4990/97	N	+	+/-	-	+	-	-
E1115/98	N	+	-/+	ND	+	-	ND
E2214/98	N	+	-/+	ND	+	-	ND
E3915/98	N	+	+/-	-	+	-	-
E4230/98	P	+	-/+	-	+	-	-
E4748/98	P	+	+/-	-	+	-	-
E5637/98	P	+	+	-	+	-	-
E2992/99	P	+	+	-	+	-	-
E1138/00	P	+	+/-	-	+	-	-
E1773/00	P	+	+/-	-	+	-	-
E3158/00	N	+	+	-	+	-	-
E3835/00	N	+	+/-	-	+	-	-

Abbreviations: +, positivity in 75 to 100% of the cells evaluated; +/-, positivity in 50 to 75% of the cells evaluated; -/+, positivity in 25 to 50% of the cells evaluated; rare, positivity in 10 to 25% of the cells evaluated; exceptional, positivity in 1 to 10% of the cells evaluated; -, negativity in all the cells evaluated; PM, plasmacytoid monocytes; CD, cluster of differentiation; NE, neutrophilic elastase; MPO, myeloperoxidase; ND, not done due to the lack of material available; NEV, not evaluable, even though the staining was repeated twice.

subtypes consisted in the slightly higher content of CD3⁺ T lymphocytes in the proliferative form. The activation marker CD30 was determined in 40 samples: it was usually expressed by 10% or less of T cells, with the exception of eight cases that showed values ranging from 25 to 55%. A variable number of T lymphocytes (10 to 80%) displayed a distinct granular cytoplasmic positivity at the determination of the cytotoxic markers TIA-1 and Granzyme B (Figure 2c), the staining of which ran in parallel. No differences in terms of CD30, cytotoxic markers, and Ki-67 antigen expression were found between the two histological subtypes defined according to the criteria of Kuo.³¹

Histiocytic Component

The PG-M1 mAb—raised against a fixation-resistant epitope of the CD68 molecule, specific to monocytes and macrophages⁵⁰—confirmed the amount and distribution of histiocytes seen at conventional light microscopy within the pathological foci (Table 1; Figures 3 and 6). The positivity was always restricted to these elements, and further demonstrated their cytological variability. Interestingly, in all of the cases tested, the polyclonal antibody anti-MPO stained from 25 to 75% of the CD68-positive cells, as assessed in serial sections, irrespective of the proliferative or necrotizing nature of the lesion

(Table 1 and Figures 4 and 7).³¹ In particular, positivity corresponded to the vast majority of nonphagocytosing mononuclear cells and a proportion of crescentic macrophages and phagocytosing histiocytes (Figure 8). In contrast, morphologically recognizable PMs both within the lesions and at some distance from them turned out to be MPO-negative (Table 1; Figure 9). Double staining gave further confirmation of the latter finding, showing that CLA/HECA425-positive PMs were regularly MPO-negative (Figure 10). Interestingly, variable numbers of MPO histiocytes were identified around high endothelial venules or within PM clusters in areas of the lymph node not directly involved by the proliferative or necrotizing foci (Figure 11).

The antibody anti-neutrophilic elastase stained none of the histiocytes co-expressing MPO and CD68 (Table 1): it revealed rare scattered granulocytes in 15 cases (Figures 4 and 7). No elements were stained by the Qbend10 mAb, CD34-positivity being restricted to vessel endothelia. The Mib-1 antibody gave virtually negative results with the histiocytic component.

Control Groups

The five cases of Kikuchi-like LE lymphadenopathy revealed morphological changes corresponding to those observed in the necrotizing form of KL; the only differences consisted in areas with more extensive tissue necrosis (Figure 12) and slightly higher numbers of plasma cells and granulocytes in LE. In addition, the numbers of PM admixed with the lesions and of PM aggregates in the spared lymph node were usually less numerous than those found in KL. On immunohistochemistry, most histiocytes showed co-expression of CD68 and MPO (Figures 13 and 14).

In the 25 examples of reactive lymphadenitis unrelated to KL, the histiocytic population revealed regular expression of CD68, but negativity for MPO, with the exception of occasional macrophages in granulomatous-suppurative lymphadenitis. Occasional clusters of PMs, more readily found in toxoplasma lymphadenitis, were clearly CD68⁺ and MPO⁻, as were those observed in the examples of CDs (Figures 15, 16, and 17) and PMTs tested (Figures 18, 19, and 20). Finally, expression of MPO was never observed in the 61 tumors of histiocytes and accessory dendritic cells studied by the International Lymphoma Study Group.⁶¹

FACS Analysis

When gating the monocyte fraction, a double staining for CD14 and MPO was observed, irrespective of the anti-MPO antibody used (Figures 21, 22, and 23). In particular, the polyclonal antibody showed the same reactivity and specificity as the fluorescein isothiocyanate-conjugated reference monoclonal antibody.

Discussion

Since the first description of KL in 1972,^{1,2} several attempts have been made to understand its etiology and pathogenesis. Although the identification of causative agent(s) is still a matter of some debate,¹²⁻²⁶ most studies agree on the fact that KL is probably a cell-mediated phenomenon sustained by activated cytotoxic CD8⁺ T lymphocytes.³⁴⁻³⁶ The latter cells might be at the same time the cause and victims of the cell-death process that occurs in KL.³⁴⁻³⁶ Our immunohistochemical findings showing that most CD8⁺ T lymphocytes do express TIA-1 and Granzyme B are consistent with this hypothesis. However, although positivity for cytotoxic markers provides an explanation for the large amounts of apoptotic cells and nuclear debris typically observed in KL, it does not clarify all of the pathogenetic mechanisms of the process. In particular, it does not explain why granulocytic infiltration is strikingly absent in KL lesions, despite the prominent necrotizing features.

Our study shows that the phenotypic profile of the histiocytic component of KL is much more complex than was previously thought. In particular, we observed that most histiocytes in KL co-express CD68 and MPO. This result was totally unexpected, because we used the PG-M1 monoclonal antibody that detects a CD68 epitope that is restricted to the monocyte/macrophage lineage and is absent in all steps of myeloid differentiation.⁵⁰ Indeed, unlike the anti-CD68 KP1 antibody, PG-M1 is useful for the differential diagnosis between M0-M3 and M4-M5 acute leukemias, which are PG-M1⁻ and PG-M1⁺, respectively.^{50,59,60}

A similar cell composition was also identified in the examined cases of KL-like (KL-L) lymphadenitis associated with LE, in which the histiocytes within the necrotic foci co-expressed CD68/PG-M1 and MPO. This observation further supports previous data indicating that KL and

Figure 10. Plasmacytoid monocytes expressing CLA/HECA425 (in red) are completely negative for MPO; the latter molecule is carried by histiocytic elements (in brown) (double staining; original magnification, ×600).

Figure 11. KL. A cluster of MPO⁺ histiocytes around a high endothelium venule at distance from cortical lesions (SABC technique; hematoxylin counterstaining; original magnification, ×500).

Figure 12. Kikuchi-like LE lymphadenitis. A focus with prominent necrotizing features is shown (H&E; original magnification, ×500).

Figure 13-14. Kikuchi-like LE lymphadenitis. The same histiocytic elements co-express CD68 (**Figure 13**) and MPO (**Figure 14**) (SABC technique; hematoxylin counterstaining; original magnification, ×600).

Figure 15. Castleman disease. A plasmacytoid monocyte cluster located in the **bottom left** corner (Giemsa; original magnification, ×100).

Figure 16. Castleman disease. Plasmacytoid monocytes strongly express CD68 (APAAP technique; Gill's hematoxylin counterstaining; original magnification, ×300).

Figure 17. Castleman disease. Plasmacytoid monocytes lack MPO (note some granulocytes in the surrounding, which represent the internal control of the reaction) (APAAP technique; Gill's hematoxylin counterstaining; original magnification, ×250).

Figure 18. Plasmacytoid monocyte tumor. Cytological details at high magnification (H&E; original magnification, ×400).

Figure 19. Plasmacytoid monocyte tumor. Neoplastic cells express CD68 (SABC technique; hematoxylin counterstaining; original magnification, ×400).

Figure 20. Plasmacytoid monocyte tumor. Double staining for CD45RA and MPO shows the negativity of tumoral plasmacytoid monocytes for the latter molecule (note the staining of some granulocytes as an internal control) (SABC technique; hematoxylin counterstaining; original magnification, ×500).

Figure 21. Peripheral blood monocytes gated on a linear forward and side scatter.
Figure 22. CD14-positive peripheral blood monocytes co-expressed MPO, when the reference monoclonal antibody is applied.
Figure 23. Identical results were obtained using the anti-MPO polyclonal antibody.

KL-L conditions share important morphological and immunophenotypical features, and probably constitute a single cellular process.²²⁻²⁶ It could be argued that the results observed in KL and LE might theoretically be because of a cross-reactivity of the antibody against MPO; however, this possibility is quite remote because the specificity of the reagent was supported by proper internal positive and negative controls, as well as by

FACS-analysis experiments and previously reported data.^{59,60} The latter showed that circulating monocytes express MPO, and this expression was independent of the antibody used.

Our phenotypic and morphological findings both suggest that the histiocytes of KL and KL-L conditions might primarily correspond to CD68⁺/MPO⁺ peripheral blood monocytes accumulating in the lymph node. This hypothesis is supported by the recent observation that the MPO system of human monocytes can substitute neutrophilic MPO for the production of advanced glycation end products at sites of inflammation.⁶³ Thus, peripheral blood monocytes (MPO⁺/CD68⁺) might be attracted into tissues during particular inflammatory processes—such as those occurring in Kikuchi's and Kikuchi-like lymphadenopathies—that show lack or paucity of granulocytes, but nevertheless require MPO. The peculiarity of the immune reaction occurring in KL is further supported by the observation that the CD68⁺/MPO⁺ tissue histiocytes are not identified either in nonspecific reactive lymphadenitis or in several granulomatous lymphadenitides (whether or not associated with necrosis and tissue neutrophilia) and in histiocyte and accessory cell tumors.⁶¹

The data obtained in this study do not support the widely reported concept that PMs represent a main cellular component in KL.^{3,5,8-10,29-32,34,37-39} In fact, PM clusters were frequently noticed in the lymph node parenchyma spared by the lesions. They were usually sparse within both the proliferative and necrotic lesions, and were easily recognizable by their morphology and expression of CLA, along with their negativity for MPO. Therefore, PMs seem to correspond to a minor component of KL. Their importance has probably been overemphasized in the past because of their morphological similarities with circulating monocytes attracted within the necrotizing lesions.

What role, if any, PMs play in the immunological reaction that characterizes KL remains to be established. Evidence is emerging that PMs are neither terminally differentiated elements⁶⁴ nor precursors of phagocytosing histiocytes. They seem to correspond to the natural type I interferon producing cells,^{51-55,65} originating in the bone marrow and migrating to peripheral organs, where they eventually differentiate into dendritic cells or die, depending on microenvironmental stimuli.⁵¹⁻⁵⁵ We can speculate that PMs may play a role in KL and KL-L LE by accumulating in the lymph nodes and secreting large amounts of type I interferon,^{66,67} thus activating a Th1 T-cell reaction and a cytotoxic immune response,⁶⁸ which typically occurs in this condition.^{34,36,69}

On clinical grounds, recognition of the peculiar phenotype of the histiocytic component of KL should have important diagnostic implications. Indeed knowledge of the distinctive KL phenotype can be used to avoid a misdiagnosis of malignant lymphoma in patients bearing the proliferative form of KL, an unfortunate error that has been repeatedly reported in the literature.^{32,33} In addition, this knowledge should be relevant for the differentiation of KL from PMT, chloroma, or nodal colonization by acute nonlymphoid leukemia.⁴⁰ The exclusion of PMT is straightforward, because in contrast to the histiocytic

component of KL, PMT does not express MPO. However, differential diagnosis with a myeloid tumor, which is obviously MPO⁺, may be somewhat more demanding: it should be resolved using the knowledge that the histiocytic component of KL has a bland morphology, is basically quiescent, is admixed with abundant cytotoxic T lymphocytes and apoptotic bodies, and does not express neutrophilic elastase, TdT, or CD34.^{59,60}

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References

- Kikuchi M: Lymphadenitis showing focal reticulum cell hyperplasia with nuclear debris and phagocytosis. *Nippon Ketsueki Gakkai Zasshi* 1972, 35:379–380
- Fujimoto Y, Kozima Y, Yamaguchi K: Cervical subacute necrotizing lymphadenitis. A new clinicopathological entity. *Naika* 1972, 20:920–927
- Pileri S, Kikuchi M, Helbron D, Lennert K: Histiocytic necrotizing lymphadenitis without granulocytic infiltration. *Virchows Arch Pathol Anat* 1982, 395:257–271
- Turner RR, Martin J, Dorfman RF: Necrotizing lymphadenitis. A study of 30 cases. *Am J Surg Pathol* 1983, 7:115–123
- Feller AC, Lennert K, Stein H, Bruhn H-D, Wuthe H-H: Immunohistology and etiology of histiocytic necrotizing lymphadenitis: report of three instructive cases. *Histopathology* 1983, 7:825–839
- Ali MH, Horton LWL: Necrotizing lymphadenitis without granulocytic infiltration (Kikuchi's disease). *J Clin Pathol* 1985, 38:1252–1257
- Chan JKC, Saw D: Histiocytic necrotizing lymphadenitis (Kikuchi's disease). A clinicopathologic study of 9 cases. *Pathology* 1986, 18:22–28
- Dorfman RF: Histiocytic necrotizing lymphadenitis of Kikuchi and Fujimoto. *Arch Pathol Lab Med* 1987, 111:1026–1029
- Rivano MT, Falini B, Stein H, Canino S, Ciani C, Gerdes J, Ribacchi R, Gobbi M, Pileri S: Histiocytic necrotizing lymphadenitis without granulocytic infiltration (Kikuchi's lymphadenitis). Morphological and immunohistochemical study of eight cases. *Histopathology* 1987, 11:1013–1027
- Dorfman RF, Berry GJ: Kikuchi's histiocytic necrotizing lymphadenitis: an analysis of 108 cases with emphasis on differential diagnosis. *Semin Diagn Pathol* 1988, 5:329–345
- Lorand Metzger I, Vassallo J, Mori S: Histiocytic necrotizing lymphadenitis in Brazil: report of a case and review of the literature. *Pathol Int* 1994, 44:548–550
- Anagnostopoulos I, Hummel M, Korbjuhn P, Papadaki T, Anagnostou D, Stein H: Epstein-Barr virus in Kikuchi-Fujimoto disease. *Lancet* 1993, 341:893
- Cho KJ, Lee SS, Khang SK: Histiocytic necrotizing lymphadenitis. A clinicopathologic study of 45 cases with in situ hybridization for Epstein-Barr virus and hepatitis B virus. *J Korean Med Sci* 1996, 11:409–414
- Eizuru Y, Minematu T, Minamishima Y, Kikuchi M, Yamanishi K, Takahashi M, Kurata T: Human herpes virus 6 in lymph node. *Lancet* 1989, 40
- Sumiyoshi Y, Kikuchi M, Ohshima K, Joneda S, Kobari S, Takeshita M, Eizuru Y, Minamishima Y: Human herpesvirus-6 genomes in histiocytic necrotizing lymphadenitis (Kikuchi's disease) and other forms of lymphadenitis. *Am J Clin Pathol* 1993, 99:609–614
- Huh J, Kang GH, Gong G, Kim SS, Ro JY, Kim CW: Kaposi's sarcoma-associated herpesvirus in Kikuchi's disease. *Hum Pathol* 1998, 29:1091–1096
- Yufu Y, Matsumoto M, Miyamura T, Nishimura J, Nawata H, Ohshima K: Parvovirus B19-associated haemophagocytic syndrome with lymphadenopathy resembling histiocytic necrotizing lymphadenitis (Kikuchi's disease). *Br J Haematol* 1997, 96:868–871
- Kikuchi M, Yoshizumi T, Nakamura H: Necrotizing lymphadenitis: possible acute toxoplasmic infection. *Virchows Arch A Pathol Anat Histopathol* 1977, 19:99–113
- Pileri SA, Sabattini E, Costigliola P, Poggi S, Ricchi E, Tumietto F, Chiodo F: Kikuchi's lymphadenitis in HIV infection: a case report. *AIDS* 1991, 5:459–461
- Bataille V, Harland CC, Behrens J, Cook MG, Holden CA: Kikuchi disease (histiocytic necrotizing lymphadenitis) in association with HTLV1. *Br J Dermatol* 1997, 136:610–612
- Imamura M, Ueno H, Matsumura A, Kamijima H, Suzuki T, Kikuchi K, Onoe T: An ultrastructural study of subacute necrotizing lymphadenitis. *Am J Pathol* 1982, 107:292–299
- Ohta A, Mastumoto Y, Ohta T, Kaneoka H, Yamaguchi M: Still's disease associated with necrotizing lymphadenitis (Kikuchi's disease). Report of 3 cases. *J Rheumatol* 1988, 15:981–983
- Lyberatos C: Two more cases of Still's disease and Kikuchi's. *J Rheumatol* 1990, 17:568–569
- Tumiat B, Bellelli A, Portoli I, Prandi S: Kikuchi's disease in systemic lupus erythematosus: an independent or dependent event? *Clin Rheumatol* 1991, 10:90–93
- el Ramahi KR, Karrar A, Ali MA: Kikuchi disease and its association with systemic lupus erythematosus. *Lupus* 1994, 5:406–411
- Gourley I, Bell AL, Biggart D: Kikuchi's disease as a presenting feature of mixed connective tissue disease. *Clin Rheumatol* 1995, 14:104–107
- Chan JKC, Wong K-C, Ng C-S: A fatal case of multicentric Kikuchi's histiocytic necrotizing lymphadenitis. *Cancer* 1989, 63:1856–1862
- Sumiyoshi Y, Kikuchi M, Takeshita M, Yoneda S, Kobari S, Ohshima K: Immunohistological study of skin involvement in Kikuchi's disease. *Virchows Arch B Cell Pathol* 1992, 62:263–269
- Spies J, Foucar K, Thompson CT, LeBoit PE: The histopathology of cutaneous lesions of Kikuchi's disease (necrotizing lymphadenitis). A report of five cases. *Am J Surg Pathol* 1999, 23:1040–1047
- Tsang WYW, Chan JKC, Ng CS: Kikuchi's lymphadenitis. A morphologic analysis of 75 cases with special reference to unusual features. *Am J Surg Pathol* 1994, 18:219–231
- Kuo T-T: Kikuchi's disease (histiocytic necrotizing lymphadenitis). A clinicopathologic study of 79 cases with an analysis of histologic subtypes, immunohistology and DNA ploidy. *Am J Surg Pathol* 1995, 19:798–809
- Chamulak GA, Brynes RK, Nathwani BN: Kikuchi-Fujimoto disease mimicking malignant lymphoma. *Am J Surg Pathol* 1990, 14:514–523
- Menasce LP, Banerjee SS, Edmondson D, Harris M: Histiocytic necrotizing lymphadenitis (Kikuchi-Fujimoto disease): continuing diagnostic difficulties. *Histopathology* 1998, 33:248–254
- Takakuwa T, Ohnuma S, Koike J, Hoshikawa M, Koizumi H: Involvement of cell-mediated killing in apoptosis in histiocytic necrotizing lymphadenitis (Kikuchi-Fujimoto disease). *Histopathology* 1996, 28:41–48
- Felgar RE, Furth EE, Wasik MA, Gluckman SJ, Salhany KE: Histiocytic necrotizing lymphadenitis (Kikuchi's disease): in situ end-labeling, immunohistochemical, and serologic evidence supporting cytotoxic lymphocyte-mediated apoptotic cell death. *Mod Pathol* 1997, 10:231–241
- Ohshima K, Shimazaki K, Kuma T, Suzumiya J, Kanda M, Kikuchi M: Perforin and Fas pathways of cytotoxic T-cells in histiocytic necrotizing lymphadenitis. *Histopathology* 1998, 33:471–478
- Facchetti F, de Wolf-Peters C, van den Oord JJ, de Vos R, Desmet VJ: Plasmacytoid monocytes (so-called plasmacytoid T-cells) in Kikuchi's lymphadenitis. An immunohistologic study. *Am J Clin Pathol* 1989, 92:42–50
- Hansmann M-L, Kikuchi M, Wacker HH, Radzun HJ, Nathwani BN, Hesse K, Parwaresch MR: Immunohistochemical monitoring of plasmacytoid cells in lymph node sections of Kikuchi-Fujimoto disease by a new pan-macrophage antibody Ki-M1P. *Hum Pathol* 1992, 23:676–680
- Sumiyoshi Y, Kikuchi M, Takeshita M, Ohshima K, Masuda Y, Parwaresch MR: Immunohistologic studies of Kikuchi's disease. *Hum Pathol* 1993, 24:1114–1119
- Warneke RA, Weiss LM, Chan JKC, Cleary ML, Dorfman RF: Tumors of

- the lymph nodes and spleen. Atlas of Tumor Pathology, 3rd series, fascicle 14. Washington DC, Armed Forces Institute of Pathology 1994, pp 375–378
41. Müller-Hermelink HK, Steinman G, Stein H, Lennert K: Malignant lymphoma of plasmacytoid T-cells. Morphologic and immunologic studies characterizing a special type of T-cell. *Am J Surg Pathol* 1983, 7:849–862
42. Horny HP, Feller AC, Horst HA, Lennert K: Immunohistology of plasmacytoid T-cells: marker analysis indicates a unique phenotype of this enigmatic cells. *Hum Pathol* 1986, 18:28–32
43. Parwaresch MR, Radzun HJ, Kreipe H, Hansmann ML, Barth J: Monocyte/macrophage-reactive monoclonal antibody Ki-M6 recognizes an intracytoplasmic antigen. *Am J Pathol* 1986, 124:141–151
44. Kreipe H, Radzun HJ, Parwaresch MR, Haislip A, Hansmann ML: Ki-M7 monoclonal antibody specific for myelomonocytic cell lineage and macrophage in human. *J Histochem Cytochem* 1987, 35:1117–1126
45. Poppema S, Hollema H, Visser L, Vos H: Monoclonal antibodies (MT1, MT2, MB1, Mb2, MB3) reactive with leukocyte subsets in paraffin-embedded tissue sections. *Am J Pathol* 1987, 127:418–429
46. Facchetti F, De Wolf-Peeters C, Mason DY, Pulford K, van den Oord JJ, Desmet VJ: Plasmacytoid T-cells. Immunohistochemical evidence for their monocyte macrophage origin. *Am J Pathol* 1988, 133:15–21
47. Facchetti F, De Wolf-Peeters C, van den Oord JJ, De Vos R, Desmet VJ: Plasmacytoid T-cells: a cell population normally present in the reactive lymph node: an immunohistochemical and electronmicroscopic study. *Hum Pathol* 1988, 19:1085–1092
48. Pulford KA, Rigney EM, Micklem KJ, Jones M, Stross WP, Getter KC, Mason DY: KP1: a monoclonal antibody detecting a new monocyte/macrophage associated antigen in routinely processed tissue sections. *J Clin Pathol* 1989, 42:414–421
49. Koo CH, Mason DY, Miller R, Ben-Ezra J, Sheibani K, Rappaport H: Additional evidence that “plasmacytoid T-cell lymphoma” associated with chronic myeloproliferative disorders is of macrophage/monocyte origin. *Am J Clin Pathol* 1990, 93:822–827
50. Falini B, Flenghi L, Pileri SA, Gambacorta M, Bigerna B, Dürkop H, Eitelbach F, Thiele J, Cavaliere A, Martelli MF, Poggi S, Sabatini E: PG-M1: a new monoclonal antibody directed against a fixative-resistant epitope on the macrophage-restricted form of the CD68 molecule. *Am J Pathol* 1993, 142:1359–1372
51. Facchetti F, Candiago E, Vermi W: Plasmacytoid monocyte express IL-3 receptor alpha and differentiate into dendritic cells. *Histopathology* 1999, 35:88–89
52. Cella M, Jarossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, Colonna M: Plasmacytoid monocytes migrate to inflamed lymph nodes and produce high levels of type I interferon. *Nat Med* 1999, 5:919–922
53. Cella M, Facchetti F, Lanzavecchia A, Colonna M: Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent Th1 polarization. *Nature Immunol* 2000, 1:305–310
54. Kadowaki N, Antonenko S, Yiu-Nam Lau J, Liu Y-J: Natural interferon α/β -producing cells link innate and adaptive immunity. *J Exp Med* 2000, 192:219–225
55. Grouard G, Risoan MC, Filgueira L, Durand I, Banchereau J, Liu YJ: The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J Exp Med* 1997, 185:1101–1111
56. Sabatini E, Bisgaard K, Ascani S, Poggi S, Piccoli M, Ceccarelli C, Pieri F, Fraternali-Orcioni G, Briskomatis A, Pileri SA: Envision Plus a new immunohistochemical method of choice for diagnostics and research. Critical comparison with the APAAP, ChemMate, CSA, LABC, and SABC techniques. *J Clin Pathol* 1998, 51:506–511
57. Pileri SA, Roncador G, Ceccarelli C, Piccoli M, Briskomatis A, Sabatini E, Ascani S, Santini D, Piccaluga PP, Leone O, Damiani S, Ercolelli C, Sandri F, Pieri F, Leoncini L, Falini B: Antigen retrieval techniques in immunohistochemistry: comparison among different methods. *J Pathol* 1997, 183:116–123
58. Facchetti F, De Wolf-Peeters C, Van den Oord JJ, Meijer CJLM, Pals ST, Desmet VJ: Anti-high endothelial venule monoclonal antibody HECA-452 recognizes plasmacytoid T cells and delineates an “extranodular” compartment in the reactive lymph node. *Immunol Lett* 1989, 20:277–282
59. Pileri SA, Ascani S, Piccoli M, Poggi S, Fraternali-Orcioni G, Sabatini E, Milani M, Falini B: Immunophenotyping of acute leukemias in paraffin sections: a powerful tool. *Br J Haematol* 1999, 105:394–401
60. Breccia M, Petti MC, Fraternali-Orcioni G, Monarca B, Latagliata R, D’Elia GM, Mandelli F, Pileri SA: Granulocytic sarcoma with breast and skin presentation: a report of a case successfully treated by local radiation and systemic chemotherapy. *Acta Haematol* 2000, 104:34–37
61. Pileri SA, Grogan TM, Harris NL, Weiss LM, Warnke RA, Chan JKC, Banks PM, Campo EG, Delsol G, De Wolf-Peeters C, Falini B, Dalla Favera R, Gascoyne RD, Gatter KC, Gaulard P, Issacson PG, Jaffe ES, Kluin P, Knowles DM, Mason DY, Mori S, Müller-Hermelink HK, Piris MA, Ralfkiaer E, Stein H, Su I-J: Tumors of histiocytes and accessory dendritic cells. A proposed classification from the International Lymphoma Study Group based on comprehensive evaluation of 61 cases. *Histopathology*, (in press)
62. Rondelli D, Lemoli RM, Ratta M, Fogli M, Re F, Curti A, Arpinati M, Tura S: Rapid induction of CD40 on a subset of granulocyte colony-stimulating factor-mobilized CD34(+) blood cells identifies myeloid committed progenitors and permits selection of nonimmunogenic CD40(–) progenitor cells. *Blood* 1999, 94:2293–2300
63. Anderson MM, Requena JR, Crowley JR, Thorpe SR, Heinecke JW: The myeloperoxidase system of human phagocytes generates N^ε-(carboxymethyl)lysine on proteins: a mechanism for producing advanced glycation end products at sites of inflammation. *J Clin Invest* 1999, 104:103–113
64. Lennert K: Malignant lymphomas other than Hodgkin’s disease. New York, Springer-Verlag, 1978, pp 31–33
65. Siegal FP, Kadowaki N, Shodell M, Fitzgerald Bocarsly PA, Shah K, Ho S, Antonenko S, Liu YJ: The nature of the principal type 1 interferon-producing cells in human blood. *Science* 1999, 284:1835–1837
66. Sumiyoshi Y, Kikuchi M, Takeshita M, Ohshima K, Masuda Y: Alpha-interferon in Kikuchi’s disease. *Virchows Arch B Cell Pathol* 1991, 61:201–207
67. Vallin H, Blomberg S, Alm GV, Cederblad B, Ronnblom L: Patients with systemic lupus erythematosus (SLE) have a circulating inducer of interferon-alpha (IFN-alpha) production acting on leucocytes resembling immature dendritic cells. *Clin Exp Immunol* 1999, 115:196–202
68. Fitzgerald-Bocarsly P: Human natural interferon- α producing cells. *Pharmac Ther* 1993, 60:39–62
69. Facchetti F, Vermi W, Fiorentini S, Chilosi M, Caruso A, Duse M, Notarangelo LD, Badolato R: Expression of inducible nitric oxide synthase in human granulomas and histiocytic reactions. *Am J Pathol* 1999, 154:145–152