Immature Sinus Histiocytes

Their Identification as a Novel B-Cell Population

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The true nature of cells of "immature sinus histiocytosis" (ISH) is uncertain because they lack the typical features of normal histiocytes when analyzed by enzyme cytochemistry or electron microscopy. In the present study the antigenic profile of ISH cells has been analyzed by immunohistologic techniques in six cases of Piringer's lymphadenitis with the use of a large panel of monoclonal and polyclonal antibodies reactive with the major cell types of the hematolymphoid system. The results obtained indicate that ISH cells consistently lack markers found on cells of the monocyte/macrophage series, myeloid cells, interdigitating reticulum cells, follicular dendritic reticulum cells, T cells, or Ki-1-positive cells. They constantly express B-cell antigens and HLA-DR and (on a variable proportion of cells) surface immunoglobulin. The application of antibodies reactive with different B-cell subsets showed that the cells of ISH do not correspond to any previously described B-cell population, eg, pre-B cells, germinal center cells, follicular mantle lymphocytes, or marginal zone cells. Furthermore, ISH cells and germinal center cells are found in association with clearly different cell types. These findings indicate that ISH cells represent a B-cell population at a previously undescribed differentiation stage, occurring only under certain circumstances (eg, in toxoplasmosis or AIDS). It is proposed that the term "immature sinus histiocytosis" be replaced by "B-cell sinus reaction." (Am J Pathol 1984, 117:44–52)

IN STUDIES of Piringer's lymphadenitis and early stages of Hodgkin's disease, one of the authors[1–4] observed broadened sinuses densely packed with morphologically unusual small to medium-sized cells. The term "sinus histiocytosis" was coined for this hitherto undescribed histologic appearance.[1–3] Because the enzyme cytochemical pattern of these cells differed from that of classic histiocytes, however, the term was subsequently changed to "immature sinus histiocytosis" (ISH).[4]

It has since been found that ISH is a constant feature of Piringer's lymphadenitis,[2,3] a reactive condition which is most frequently secondary to lymph node toxoplasmosis. It may also occur in infectious mononucleosis,[2,5] rubella, acquired immune deficiency syndrome (AIDS), and other types of lymphadenitis, and has been observed in a very few cases of Hodgkin's disease.[1,3]

Supported by the Deutsche Forschungsgemeinschaft SFB 111, Project CL1, and the Leukaemia Research Fund.

Accepted for publication May 11, 1984.

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Since the original description by Lennert, other authors have also reported ISH occurring in toxoplasmosis,[6-8] but classified the cells present in the sinuses as "monocytoid cells." In the description of sinus reactions in various types of lymphadenitis, only Symmers[9] gave special consideration to the phenomenon of ISH.

On enzyme-histochemical staining, the cells of ISH are negative for acid phosphatase and nonspecific esterase; ie, they lack the features of "mature" histiocytes. Furthermore, they are negative on immunohistochemical staining for lysozyme, α_1-antitrypsin, α_1-antichymotrypsin, and all cytoplasmic immunoglobulin (CIg) classes.

On electron microscopy, the cells of ISH show more similarity to lymphocytes than to histiocytes,[10] but it has not been possible to classify them with certainty by this means.

The development of modern immunohistochemical techniques based on the use of monoclonal antibodies has provided the opportunity of resolving the nature of ISH cells. In the present report we describe the results of analyzing 6 cases of Piringer's lymphadenitis with these techniques.

Table 1 — Polyclonal and Monoclonal Antibodies Used for the Immunohistologic Labeling of Biopsy Specimens From Patients With Piringer's Lymphadenitis

Antibody	Source/Reference
Anti-α_1-antitrypsin	DAKO Immunoglobulins
Anti-α_1-antichymotrypsin	DAKO
OKM1	Ortho Diagnostic Systems
Anti-Human Monocyte 1	Bethesda Research Laboratory (BRL)
Anti-Human Monocyte 2	BRL
Anti-lysozyme	DAKO
R4/23	Authors' laboratories, 15
DAKO-DRC1	DAKO
T-ALL2	BRL
OKT11	Ortho
Lyt-3	New England Nuclear (NEN)
UCHT1	16
TÜ14	Authors' laboratories
TÜ93	Authors' laboratories
DAKO-T2	DAKO
TÜ71	Authors' laboratories
Anti-Leu-1	Becton Dickinson
DAKO-T1	DAKO
Anti-Leu-3a	Becton Dickinson
DAKO-T4	DAKO
OKT4	Ortho
TÜ102	Authors' laboratories
DAKO-T8	DAKO
OKT8	Ortho
Ki-1	Authors' laboratories, 17
DAKO-IgM	DAKO
DAKO-IgD	DAKO
Anti-IgG	BRL
Anti-IgA	BRL
Anti-κ	BRL
Anti-λ	BRL
To15	Authors' laboratories
DAKO-pan B	DAKO
F8-11-13	18
B1	Coulter
B4	19
TÜ35	Authors' laboratories, 20
L243	Becton Dickinson
DAKO-HLA-DR	DAKO
TÜ22	Authors' laboratories, 20
VIL-A1	21
TÜ1	Authors' laboratories, 22
TÜ15	Authors' laboratories, 20
TÜ66	Authors' laboratories
TÜ108	Authors' laboratories
Anti-Leu-7	Becton Dickinson

Materials and Methods

Fresh Biopsy Material

Fresh, unfixed biopsy specimens were obtained from the hospitals of the University of Kiel Medical School. The specimens were put into soft plastic tubes, covered with saline, and frozen in liquid nitrogen. The frozen specimens were stored at −80 C until sectioning. A portion of each fresh tissue sample was fixed in formol saline and processed for conventional histologic examination.

Antibodies and Immunohistologic Reagents

The monoclonal and polyclonal antibodies against human tissue constituents used in this study, along with their source, are listed in Table 1. The specificity of these antibodies is described in Table 2. Peroxidase-conjugated rabbit anti-mouse immunogobulin was purchased from DAKO Immunoglobulins (Copenhagen, Denmark) and peroxidase-conjugated goat anti-rabbit IgG from Medac or from Dianova (both of Hamburg, West Germany).

Immunoperoxidase Staining

The method used in the present investigation has already been described in detail in several other articles.[11-13]

Results

Morphology of ISH

In Giemsa-stained sections the cells of ISH showed pleomorphic nuclei, sometimes with one or more indentations (Figure 1). Occasionally, U-shaped depressions of the nuclear membrane were seen instead of notchlike indentations (Figure 2). Chromatin was moderately coarse and was surrounded by moderately basophilic "nuclear sap." One or two nucleoli were generally recognizable; they were small to medium-sized, definitely basophilic, and centrally located. Cytoplasm was relatively scanty and pale grayish blue on Giemsa staining. Mitotic figures were rarely found.

Table 2—Comparison of the Antigenic Profile of Cells of ISH With That of the Most Important Known Cell Types of the Lymphoid Tissue as Detected by Immunoperoxidase Staining of Frozen or Paraffin Tissue Sections of 6 Cases of Piringer's Lymphadenitis

Reagent	Specificity	ISH	Sinus histiocytes	Extrasinus histiocytes	Epithelioid cells	IRC	FDRC	T cells	B cells
Anti-lysozyme	Lysozyme	–	–/(+)	++	++	–	–	–	–
Anti-α_1-antitrypsin	α_1-Antitrypsin	–	++	++	++	–	–	–	–
Anti-α_1-antichymotrypsin	α_1-Antichymotrypsin	–	++	++	++	–	–	–	–
OKM1	Monocytes, macrophages, neutrophils	–	+	+	+	–	(+)/+	–	–
Anti-human monocyte 1	Macrophages	–	++	++	++	–	+	–	–
Anti-human monocyte 2	Macrophages	–	++	++	++	–	(+)/+	–	–
T-ALL2	IRC, cortical thymocytes	–	–	–	–	++	–	–	–
R4/23, DAKO-DRC1	FDRC strongly, MZC weakly	–	–	–	–	–	++	–	–
OKT11, Lyt-3	Sheep erythrocyte receptor	–	–	–	–	–	–	++	–
UCHT1	19000 T-lineage-specific, mitogenic	–	–	–	–	–	–	++	–
TÜ14, TÜ93, DAKO-T2	Majority of T cells plus thymic blasts	–	–	–	–	–	–	++	–
TÜ71, Anti-Leu-1, DAKO-T1	T cells plus cells of B-CLL and centrocytic lymphoma	–	–	–	–	–	–	++	–
Anti-Leu-3a, DAKO-T4, OKT4	Inducer/helper T cells, macrophages	–	+	+	+	–	–	67% +	–
TÜ102, OKT8, DAKO-T8	Cytotoxic/suppressor T cells	–	–	–	–	–	–	33% +	–
Ki-1	Hodgkin and Sternberg-Reed cells and normal populations around follicles	–	–	–	–	–	–	–	–
DAKO-IgM	μ heavy Ig chain	0–10% +	–/(+)	–	–	–	++	–	++
Anti-IgG	γ heavy Ig chain	0–100% +	+	–	–	–	++	–	–
Anti-IgA	α heavy Ig chain	0–70% +	+	–	–	–	++	–	Few ++
Anti-κ	κ light Ig chain	0–60% +	+	–	–	–	++	–	60–70% +
Anti-λ	λ light Ig chain	0–30%	+	–	–	–	++	–	30–40% +
To15, DAKO-pan B	All B cells	100% ++	–	–	–	–	–	–	++
F8-11-13	All B cells, subset of T cells	100% ++	–	–	–	–	–	33% ++	++
B1	B cells, epithelioid histiocytes	100% +	–	–	+	–	(+)	–	+
B4	All B cells	100% ++	–	–	–	–	–	–	++
TÜ66	Vessels, smooth muscles	100% ++	–	–	–	–	–	–	–/(+)
TÜ108	B and T cells except germinal center cells	100%	–	–	–	NA	–	+	+*
TÜ35, L243, DAKO-HLA-DR	HLA-DR	100% ++	–	++	++	+++	–/(+)	–	++
TÜ22	HLA-DC molecules	100% ++	–	++	++	+++	–/(+)	–	++

ISH, immature sinus histiocytosis; IRC, interdigitating reticulum cells; FDRC, follicular dendritic reticulum cells; MZC, marginal zone cells; CLL, chronic lymphocytic leukemia.
* Except germinal center cells.

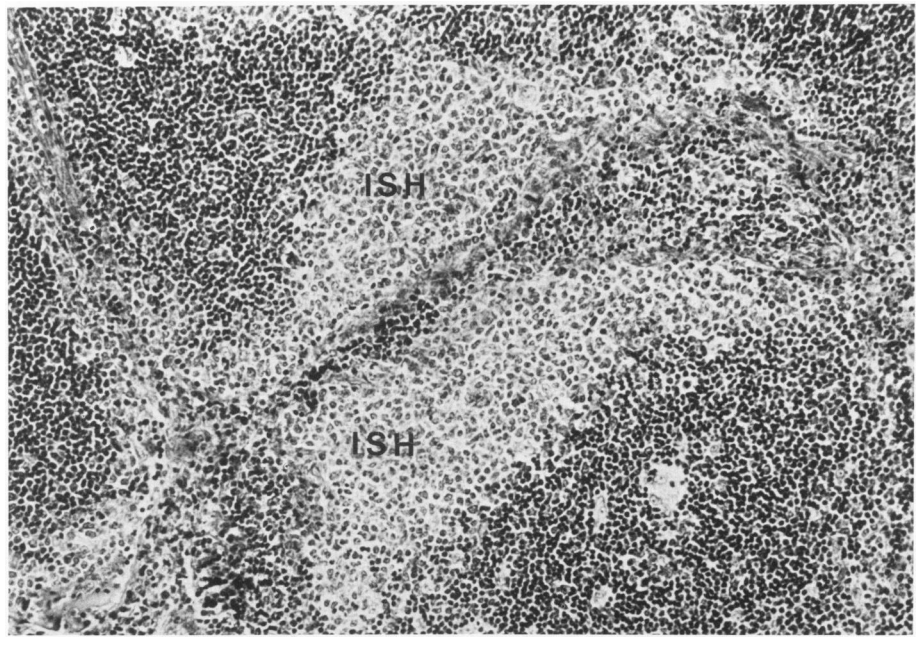

Figure 1— Low-power view of immature sinus histiocytosis (ISH) in Piringer's lymphadenitis (toxoplasmosis). (Giemsa, ×135)

Antigen Profile of ISH

The results obtained in analyzing 6 cases of Piringer's lymphadenitis are summarised in Table 2. ISH cells were negative for SIg in 3 cases (Figure 3) and positive in the 3 remaining cases (Table 2). The latter finding suggested that ISH cells might be B-cell-derived. This was substantiated by labeling sections with pan-B-cell-reactive antibodies (To15, B1, B4, and F8-11-13); ISH cells in all 6 cases were strongly positive with these antibodies (Figure 4). In keeping with their B-cell nature was the strong reactivity of ISH cells with antibodies against HLA-DR and HLA-DC. ISH cells were constantly negative for markers found on monocytes/macrophages (Figure 5), myeloid cells (Figure 6), T cells (Figure 7), null cells, Ki-1-positive cells, interdigitating reticulum

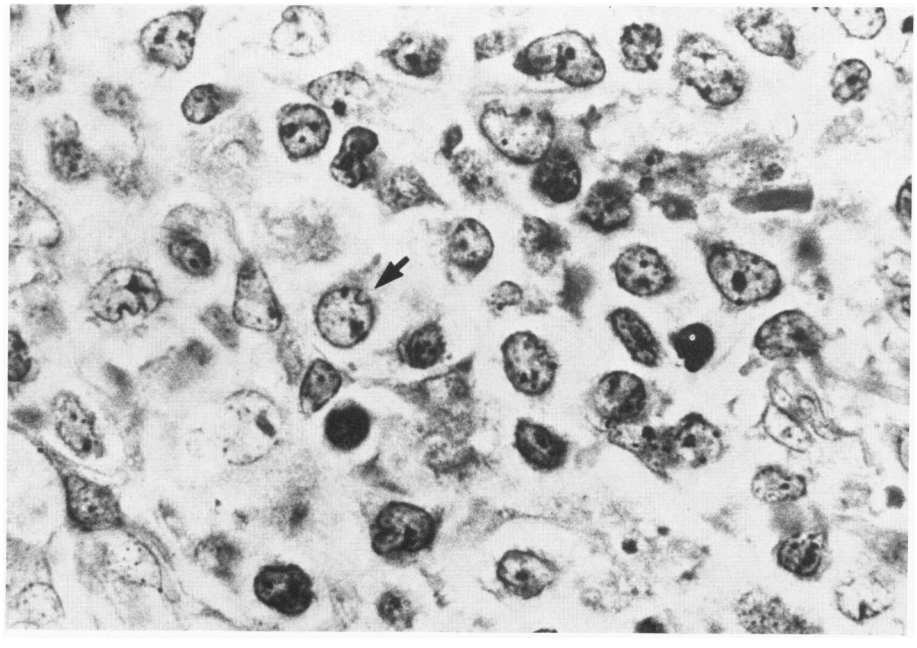

Figure 2— High-power view of immature sinus histiocytes. Occasionally, these cells show a U-shaped indentation (*arrow*) in the nuclear membrane. (Giemsa, ×1024)

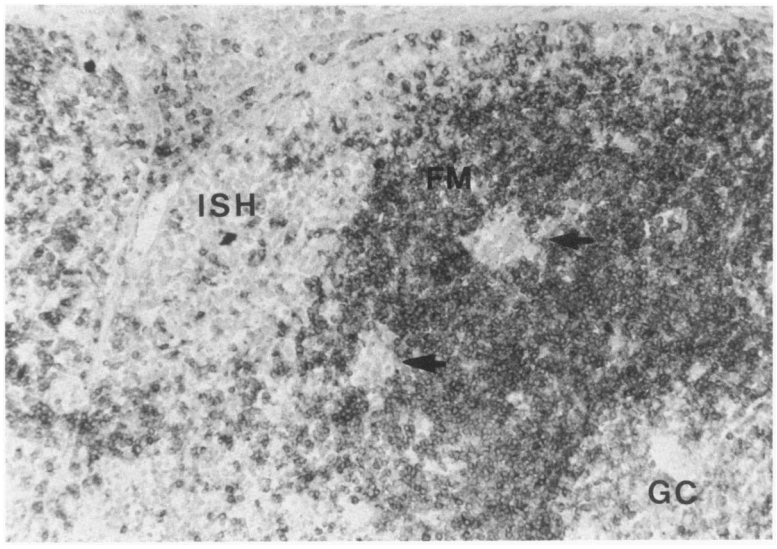

Figure 3 – Frozen section of a lymph node with Piringer's lymphadenitis stained for IgM. Follicular mantle lymphocytes are strongly stained, whereas the immature sinus histiocytes (*ISH*) (at least the majority) are negative. Two epithelioid cell granulomas (*arrows*) are also negative. *GC*, germinal center.

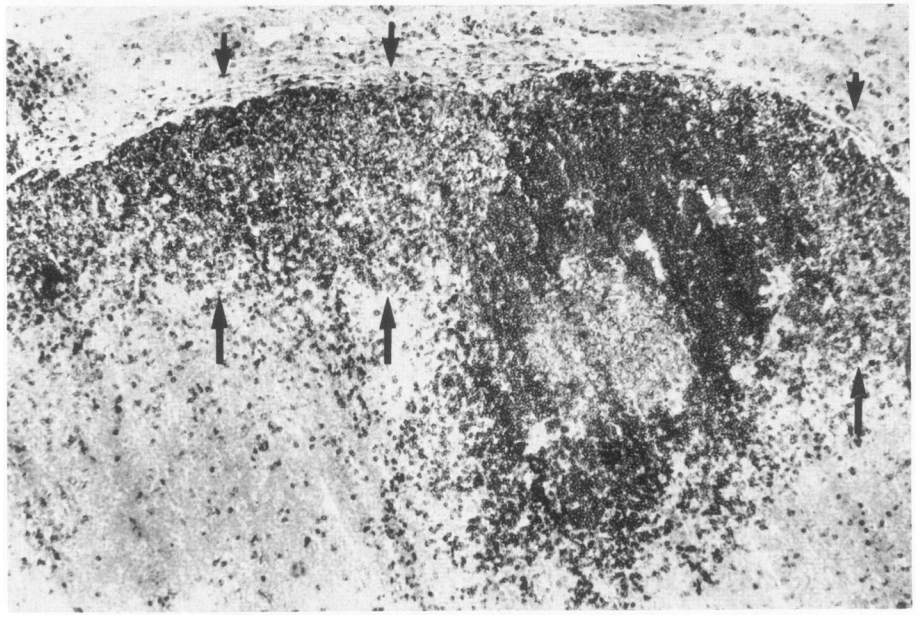

Figure 4 – Frozen section of the same case as shown in Figure 3, stained with the pan-B-cell antibody To15. All B cells are positive, including the immature sinus histiocytes (*arrows*).

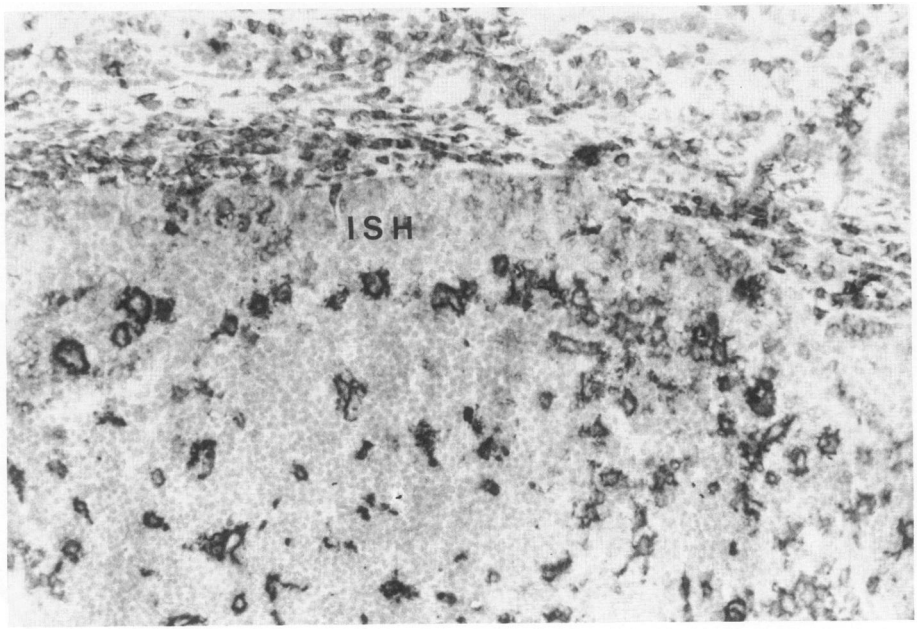

Figure 5 – Frozen section of the same case as shown in Figure 3, stained with the monoclonal antibody anti-human monocyte 2. Macrophages are stained, whereas the lymphoid cells, including immature sinus histiocytes (*ISH*), are negative.

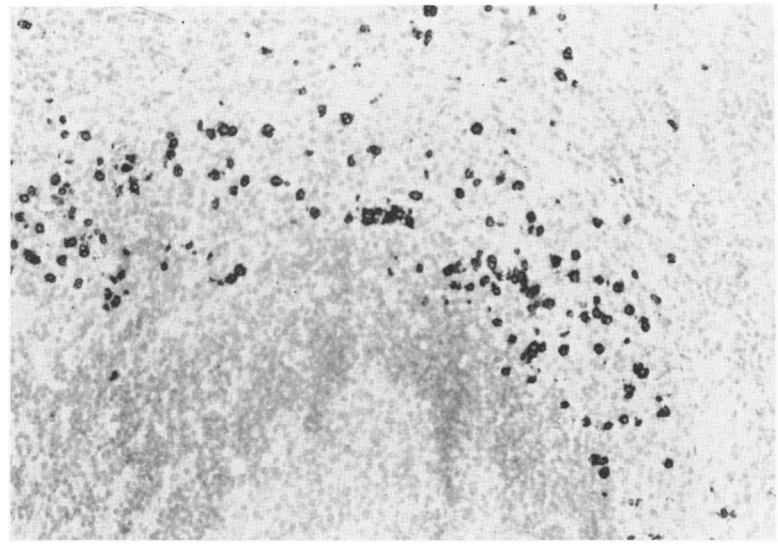

Figure 6 – Frozen section of the same case as shown in Figure 3, stained with the anti-granulocyte antibody 3C4. The neutrophils among the immature histiocytes are strongly stained, whereas all other cells are negative.

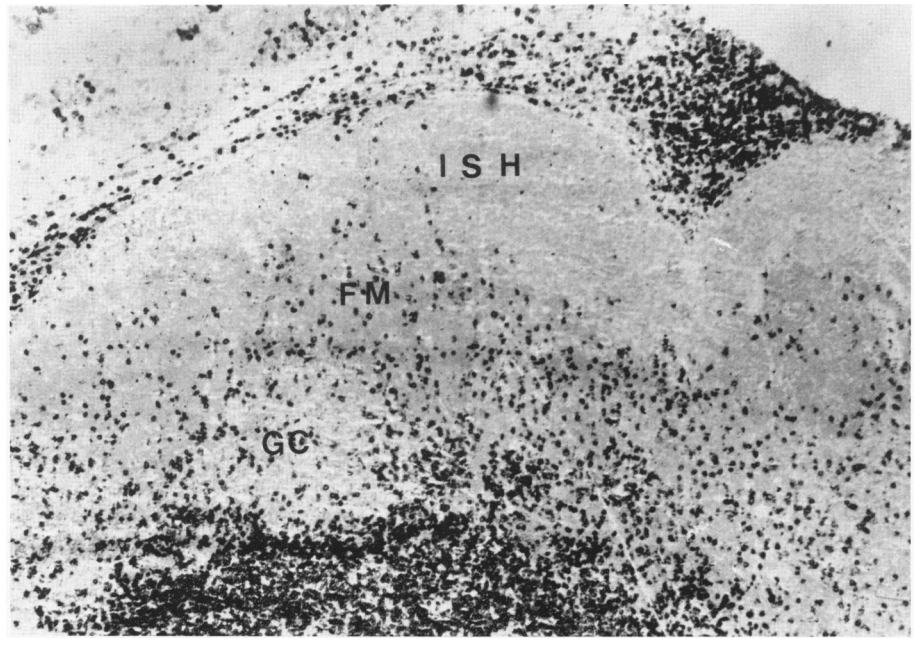

Figure 7 – Frozen section of the same case as shown in Figure 3, stained with the monoclonal anti-T cell antibody UCHT1. Immature sinus histiocytes (*ISH*) are negative. *FM*, follicular mantle; *GC*, germinal center.

Table 3 – Differences in Antigen Profile Between Cells of ISH, Pre-B Cells, Germinal Center Cells (GCC), Follicular Mantle Lymphocytes (FML), and Marginal Zone Cells (MZC)

Reagent	Specificity	ISH	Pre-B cells	GCC	FML	MZC
DAKO-IgD	δ chain	0–10% +	–	0–5% +	90% +	All +, but weakly
VIL-A1	CALLA	None	All +	Probably 100% +	None	None
TÜ1	FML, DRC	None	–	None or few +	Nearly all +	None or few +
R4/23 DAKO-DRC1	DRC, MZC	None	–	–	–	All +, but weakly
TÜ15	GCC, some T cells, and macrophages	None	?	Nearly all +	None	None
TÜ66	Vessels, smooth muscles	100% +	?	None	None	None
TÜ108	All lymphoid cells except GCC	100% +	+	None	100% +	100% +

DRC, dendritic reticulum cells.

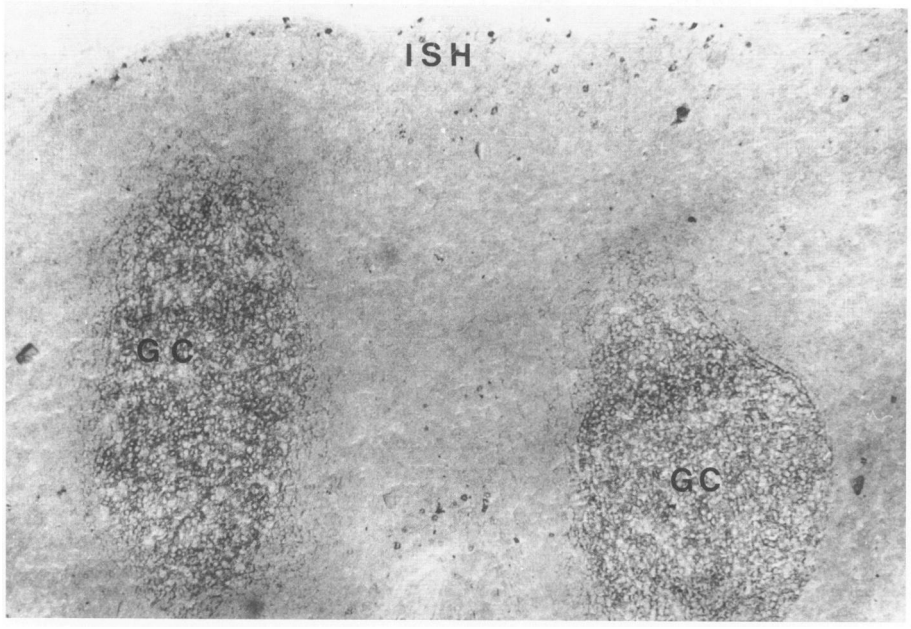

Figure 8—Frozen section of the same case as shown in Figure 3, stained with the monoclonal anti-CALLA antibody Vil-A1. All cells, including immature sinus histiocytes (*ISH*), are negative except germinal center (*GC*) cells and neutrophils among the *ISH*s.

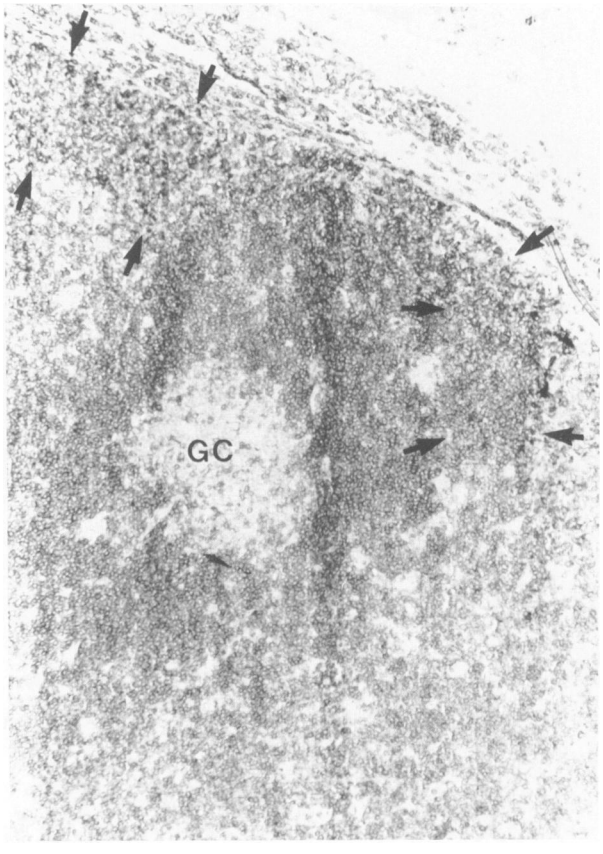

Figure 9—Frozen section of the same case as shown in Figure 3, stained with the monoclonal antibody Tu108 (a reagent which recognizes all lymphoid cells with the exception of germinal center cells). All lymphoid cells are positive, including immature sinus histiocytes (*arrows*), except germinal center (*GC*) cells.

cells, and follicular dendritic reticulum cells (for details of the antibodies used to detect these cell populations see Table 2).

Reactivity of Immature Sinus Histiocytes With Antibodies to B-Cell Subpopulations

As shown in Table 3 and Figures 8 and 9, ISH cells differ clearly in their antigenic profile from pre-B cells, germinal center cells, follicular mantle lymphocytes, and marginal zone cells.

Cell Types Found in Association With ISH Cells

Table 4 shows that ISH cells and germinal center cells are associated with clearly different cell types, the former cells being intermingled with neutrophils (Figure 6), whereas germinal center cells are associated with dendritic reticulum cells, helper T-cells, and anti-Leu-7–positive cells (putative natural killer cells). Furthermore, epithelioid cell clusters are often found in germinal centers in Piringer's lymphadenitis, whereas these cells were not detectable in association with ISH cells.

Discussion

The term "immature sinus histiocytosis" has always been hypothetical. It was by no means possible to prove that the cells had any relationship to histiocytes. The term was chosen merely because of a certain cytologic similarity to monocytes. Cytochemical studies subse-

Table 4—Presence of Various Cell Types in ISH and Germinal Centers (GC) in Piringer's Lymphadenitis

	ISH	GC
Constantly contains Anti-Leu-3a + T cells	No	Yes
Constantly contains Anti-Leu-7 + (natural killer) cells	No	Yes
Constantly contains follicular dendritic reticulum cells	No	Yes
Contains macrophages	Often	Yes
Often contains epithelioid cell clusters	No	Yes
Usually contains neutrophils	Yes	No

quently showed that the cells are not "mature" histiocytes, ie, cells of "sinus histiocytosis" of Anglo-American literature. Numerous morphologic differences between ISH and SH have also been demonstrated.[2,3,9] The most important differences are the following:

ISH occurs only in marginal sinuses and peritrabecular sinuses but not in medullary sinuses. In contrast, SH is usually most pronounced in the medullary region.

ISH usually shows a markedly focal arrangement, whereas SH mainly exhibits a uniform distribution in the lymph node.

The demarcation between the sinuses and the surrounding lymphoid tissue in ISH is less clear-cut than in SH.

The cells of ISH lie much closer together than do those of SH. They are often interspersed with a few neutrophils.

In contrast to the cells of ISH (see Morphology of ISH), SH cells have somewhat larger, usually round or oval nuclei with very fine dustlike chromatin and usually solitary large violet-stained nucleoli. On the whole, the nucleus looks much paler. Cytoplasm is more abundant and weakly reddish violet with Giemsa staining.

Very few fibers can be seen in ISH by silver impregnation techniques, and any preexisting fibers are pushed apart by the cells. The fibrous border of the sinus wall is also often infiltrated by ISH cells, giving the impression that the cells migrate into the sinuses from the adjacent lymphoid tissue. In SH, the reticular fibers of the sinuses are markedly increased in number if the lesion has existed for any length of time.

Electron-microscopic investigations have not provided any evidence for the histiocytic nature of ISH cells.

The nuclear volume of ISH cells is somewhat larger than that of SH cells. The average size of ISH cell nuclei is about twice that of lymphocyte nuclei, whereas SH cell nuclei are about one and a half times as large as lymphocyte nuclei.[4]

Previous studies using immunologic techniques have shown that the cells of ISH and SH differ in the expression of α_1-antichymotrypsin, ISH being negative and SH positive.[14] The present investigation showed that monocyte/macrophage markers cannot be demonstrated in ISH. The constant presence of four different B-cell-associated antigens (To15, B1, B4, F8-11-13) indicates instead that ISH cells are B cells, although they are usually devoid of SIg and CIg.

The finding that ISH cells are B cells raises the question of which B-cell subpopulation they belong to. From the morphologic point of view, the B cells that ISH cells resemble most closely are germinal center cells of the centrocyte type (although even this similarity is limited). They differ very clearly in phenotype, however, from germinal center cells (and also from all other recognized B-cell types, eg, mantle zone cells, marginal zone cells, pre-B cells). Furthermore, ISH cells are usually found in physical association with neutrophils, which are not present in germinal centers.

Taken together, the results obtained indicate that ISH cells represent a B-cell population at an as yet undescribed differentiation stage, which occurs only under certain conditions of lymphoid tissue stimulation, eg, in toxoplasmosis, AIDS, etc. Because "immature sinus histiocytosis" is clearly no longer an appropriate term, we suggest that the term be replaced by "B-cell sinus reaction."

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