Immature Sinus Histiocytosis

Light- and Electron-Microscopic Features, Immunologic Phenotype, and Relationship With Marginal Zone Lymphocytes

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The light-microscopic, ultrastructural, and immunohistochemical features of immature sinus histiocytosis were studied in 10 lymph nodes with the histologic picture of toxoplasmic lymphadenitis and compared with the features of lymphoid cells present in the marginal zone of the splenic white pulp. Areas of immature sinus histiocytosis consisted largely of medium-sized lymphoid cells with markedly irregular nuclei and abundant pale cytoplasm. Using a panel of monoclonal antibodies, the predominating lymphoid cells were found to carry the B-cell phenotype $B1^+Ba1^-sIgM^+sIgD^-OKIa1^+$. Admixed were variable numbers of larger, blastic lymphoid cells, small lymphocytes, histiocytic elements, and polymorphonuclear granulocytes. The marginal zone of the splenic white pulp was composed of a similar mixture of cells, and marginal-zone lymphocytes demonstrated an analogous immunohistochemical phenotype. Our results indicate that immature sinus histiocytes are B-lymphoid cells that are closely related to marginal zone lymphocytes. As such, immature sinus histiocytes may have a role similar to that of marginal-zone lymphocytes, which have been claimed to transport antigens or immune complexes toward the follicular center or to serve as precursors of plasma cells. We suggest that immature sinus histiocytosis represents an abnormal expansion of the marginal zone, normally present at the sinusoidal pole of lymphoid follicles. The reason for this marginal-zone hyperplasia, recognized as immature sinus histiocytosis in a variety of reactive lymph node conditions, may be a maturation arrest in the normal development of immature sinus histiocytes into small, $sIgM^+$ $sIgD^+$ lymphocytes. (Am J Pathol 1985, 118:266–277)

IMMATURE sinus histiocytosis is a poorly understood pattern of reactive lymph node change that occurs in a number of reactive lymphadenopathies. In its most characteristic form, subcapsular and/or medullary sinuses are focally distended and filled with cells that have variously been called small macrophages,[1] sinus histiocytes,[2] monocytoid cells,[3,4] histiomonocytoid cells,[5] and lymphoblasts.[6] In a study of 113 cases of epithelioid cell lymphadenitis, Lennert[7] drew attention to this particular sinoidal cell population, differentiated it from nonspecific sinus histiocytosis by the presence of nonspecific esterase and acid phosphatase activity in the latter, and introduced the term "unripe" or "immature sinus histiocytosis" (ISH).[7] Subsequent electron-microscopic investigations in man[8] and rabbits[9] suggested that ISH cells are not histiocytes but rather a special variety of lymphocytes.

Despite its common occurrence in various reactive lymph node conditions, the origin and function of ISH is largely unknown. For this reason, we conducted a light- and electron-microscopic and immunohistochemical study of 10 reactive lymph nodes in which ISH was observed. During the course of this study, we noticed a striking histologic resemblance between ISH cells and the lymphoid cells that occupy the marginal zone (MZ) of the splenic white pulp. This similarity proved to exist also on electron-microscopic and immunohistochemical levels. A putative role for ISH, based on data concerning the role of MZ lymphocytes, is offered.

Materials and Methods

Ten lymph nodes, removed for diagnostic purposes and showing histologically the characteristic features

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of toxoplasmic lymphadenitis,[1-3] formed the basis of this study. Splenic tissue was obtained from two patients who underwent surgery for gastric ulcer. The white pulp was well developed and demonstrated many secondary lymphoid follicles surrounded by a prominent MZ (activation stage IVB according to Lukes[10]).

Representative parts of each specimen were processed for light microscopy, immunohistochemistry, and electron microscopy. For light microscopy, samples were fixed in Bouin's solution and/or B5 fixative, embedded in paraffin, and stained with hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), and reticulin stains. For immunohistochemistry, samples were rapidly frozen in liquid nitrogen-cooled isopentane and stored at −75 C until used. For electron microscopy, small samples were fixed in glutaraldehyde for 1 hour, postfixed in OsO_4, and embedded in Epon. One-micron-thick sections were used for identification of areas of ISH or splenic MZ which subsequently were selected for ultrathin sectioning.

For the immunohistochemical demonstration of surface antigens, an indirect immunoperoxidase procedure was performed. Five-micron cryostat sections were dried overnight at room temperature and fixed in absolute acetone. Without prior washing, the monoclonal antibodies listed in Table 1 were applied. After 30 minutes' incubation, sections were washed in three changes of phosphate-buffered saline (PBS), pH 7.6, for 15 minutes. Peroxidase-conjugated rabbit anti-mouse Ig (Dakopatts, Copenhagen, Denmark) was applied for 30 minutes at room temperature, followed by a 15-minute wash in three changes of PBS, pH 7.6. The reaction product was developed with the use of 3-amino-9-ethylcarbazole and hydrogen peroxide.[20] Sections were briefly counterstained with Harris' hematoxylin and mounted in DPX. Helper/inducer T cells were demonstrated by a simultaneous application of two monoclonal antibodies, as described previously.[21]

For the immunohistochemical demonstration of cytoplasmic (c) immunoglobulins, the unlabeled peroxidase–antiperoxidase method according to Sternberger[22] was used with paraffin-embedded material. Dewaxed, rehydrated sections were incubated with polyclonal, monospecific rabbit anti-human Ig antisera (Dakopatts) for 30 minutes at room temperature, washed in three changes of PBS, pH 7.6, for 15 minutes, and subsequently incubated with swine anti-rabbit Ig (Dakopatts), diluted 1:20 and containing 10% normal human serum (NHS). After a second wash in three changes of PBS, pH 7.6, sections were treated with peroxidase–rabbit anti-peroxidase complex (Dakopatts), diluted 1:50 and containing 10% NHS, for 30 minutes. After a brief wash in PBS, pH 7.6, the reaction product was developed with the use of 3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, Mo) and hydrogen peroxide.[23] Sections were counterstained with Harris' hematoxylin and mounted.

Controls, which were invariably negative, included

Table 1—Details of Monoclonal Antibodies Used in the Present Study

Monoclonal antibody	Specificity	Source	Reference
OKIal	Common framework of HLADR or Ia-like antigens	Ortho Pharmaceutical Corp. (Raritan, NJ)	11
BA1	Peripheral blood B-lymphocytes; granulocytes	Hybritech (La Jolla, Calif)	12
B1	All mature B cells	Coulter Electronics, Inc. (Hialeah, Fla)	13
Anti-kappa	Kappa light chains	Becton-Dickinson (Sunnyvale, Calif)	
Anti-lambda	Lambda light chains	Becton-Dickinson	
Anti-IgM	Mu heavy chains	Dakopatts (Copenhagen, Denmark)	
Anti-IgG	Gamma heavy chains	Becton-Dickinson	
Anti-IgD	Delta heavy chains	Dakopatts	
OKT4	Helper/inducer T cells	Ortho Pharmaceutical	14
Leu3a	Helper/inducer T cells	Becton-Dickinson	15
OKT8	Suppressor/cytotoxic T cells	Ortho Pharmaceutical	16
OKT9	Transferrin receptor (some activated and proliferating cells; some thymocytes; some histiocytes/macrophages; follicular center cells)	Ortho Pharmaceutical	17
OKT10	Precursor cells; activated lymphocytes; cortical thymocytes; circulating null cells; plasma cells; follicular center cells	Ortho Pharmaceutical	16
OKM1	Monocytes; granulocytes; some NK cells	Ortho Pharmaceutical	18
Leu7	K/NK cells	Becton-Dickinson	19

substitution of primary mouse monoclonal antibody with nonimmune supernatant of P3X63NS1 myeloma culture (Cappel Laboratories, Cochranville, Pa) and omission of peroxidase-conjugated secondary antibody.

Results

Light-microscopic Findings

Lymph Nodes

All lymph nodes were enlarged and demonstrated a rather uniform histologic picture. In each case, a florid reactive follicular hyperplasia was observed, consisting of large, often irregularly shaped lymphoid follicles surrounded by a poorly developed lymphocytic corona. Follicular centers showed polarization and a "starry-sky" appearance due to large numbers of tingible-body macrophages and contained numerous mitotic figures. Many small clusters of epithelioid histiocytes with abundant slightly eosinophilic cytoplasm and an oval, vesicular nucleus were present in the follicular and interfollicular area. These congeries of epithelioid histiocytes tended to encroach upon lymphoid follicles and were sometimes present within follicular centers. Giant cells of Langhans type, granulomas, and caseation necrosis were not observed. The expanded paracortical area contained an increased number of high endothelial venules, filled with lymphocytes. The medullary cords contained an increased number of plasma cells and few neutrophilic polymorphonuclear granulocytes; immunoblasts and eosinophils were not conspicuous. All lymph nodes demonstrated permeation of the inflammatory infiltrate into the capsule and perinodal tissue; in two cases, lymph node imprints revealed the presence of *Toxoplasma gondii* cysts.

In all lymph nodes, a variable degree of ISH was observed, ranging from a few circumscribed nests in the marginal sinus to extensive areas filling up the marginal sinus and extending into the medullary sinuses. In many cases, pale areas composed of ISH cells surrounded hyperplastic lymphoid follicles (Figure 1). Small hemorrhagic foci and fresh necrotic areas were found within subcapsular areas of ISH in two cases. On light microscopy, foci of ISH consisted of a mixture of cell types. The predominant cell population showed an irregular, medium-sized nucleus with finely dispersed chromatin and inconspicuous nucleoli (Figure 1, inset). The nuclear membrane displayed grooves and infoldings, resulting in a cleaved or, occasionally, convoluted appearance. Their pale-staining cytoplasm was abundant. Less frequently, larger and more rounded cells with less cytoplasm and a vesicular nucleus containing a promi-

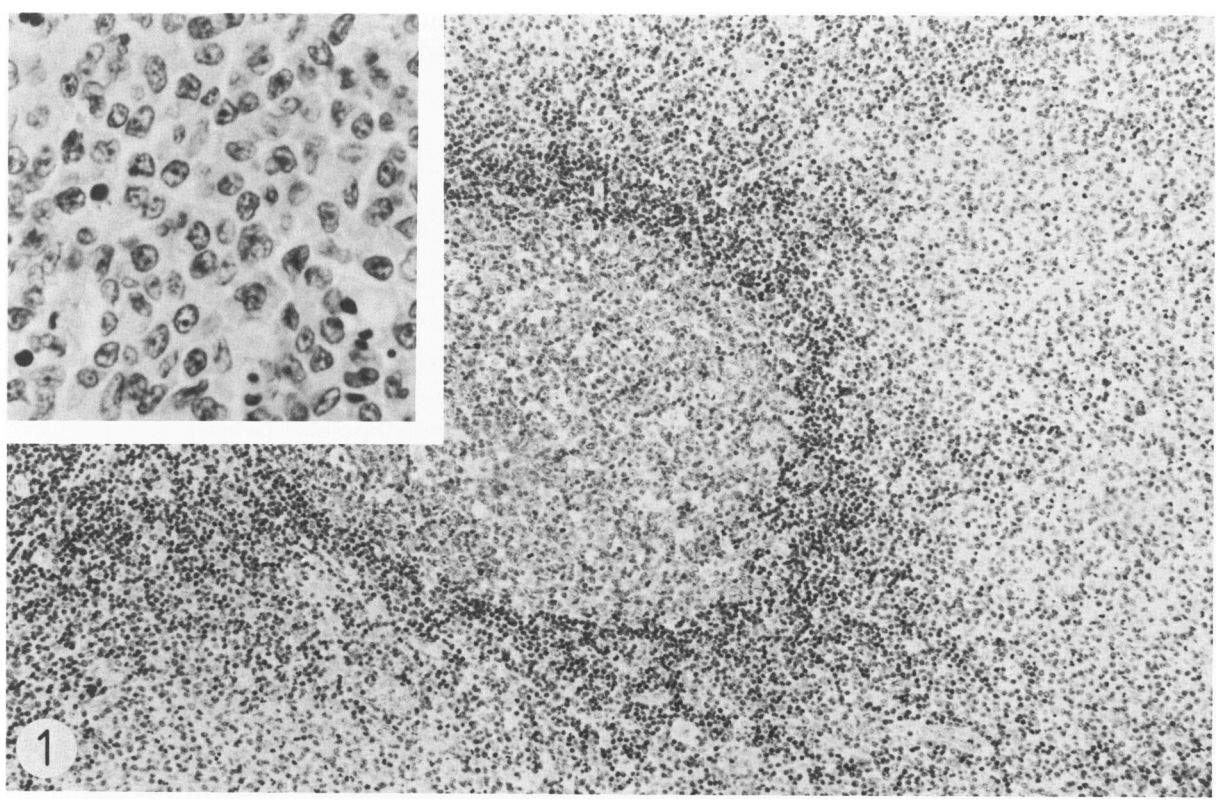


Figure 1—Lymph node, toxoplasmic lymphadenitis. A prominent lymphoid follicle is surrounded by a pale area of ISH. (H&E, ×125) **Inset**—Cytologic detail of ISH, showing a predominant population of medium-sized lymphoid cells with markedly irregular nuclei and pale cytoplasm. (H&E, ×375)

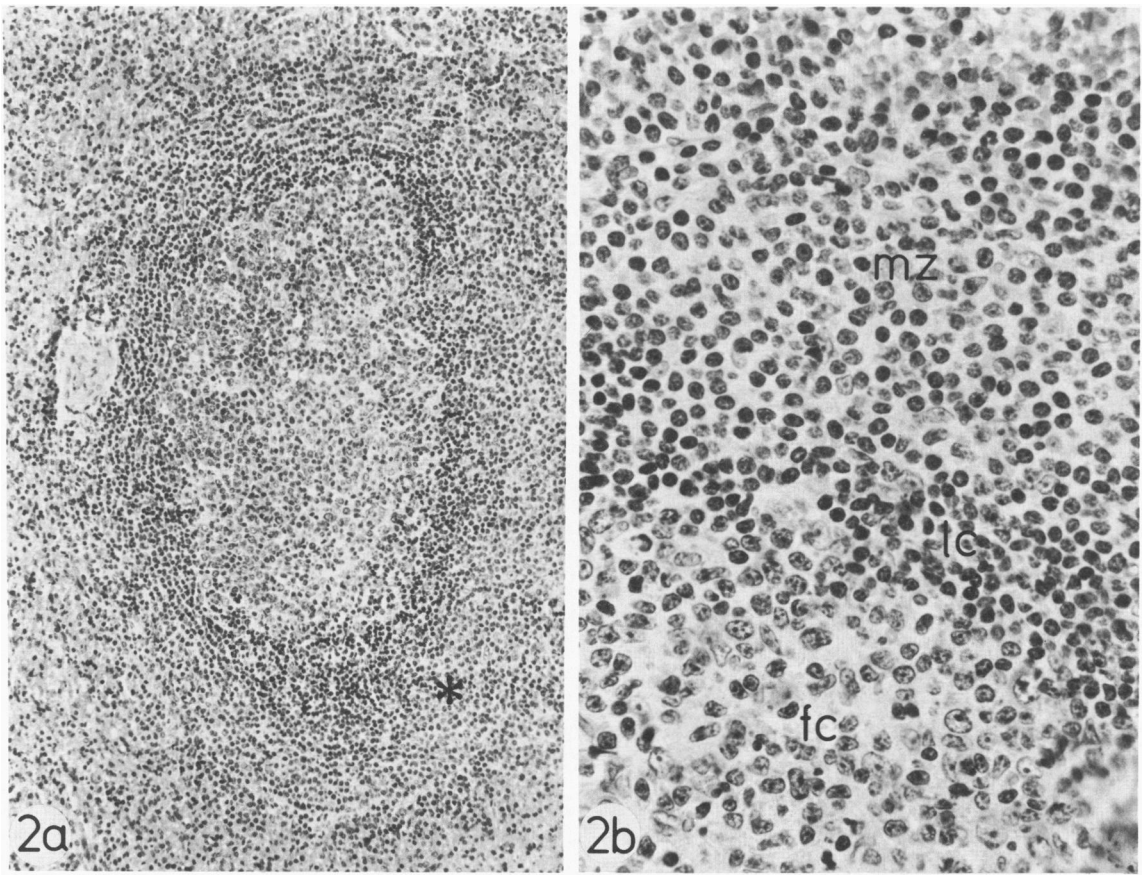


Figure 2—Activated splenic white pulp. **a**—A prominent lymphoid follicle is surrounded by a broad marginal zone (*asterisk*). (H&E, ×100) **b**—Detail of cellular composition of marginal zone (*mz*, top), lymphocytic corona (*lc*, center) and follicular center (*fc*, bottom). (H&E, ×350)

nent nucleolus, were observed. Intermingled with these cells, some plasma cells, small round lymphocytes, neutrophilic polymorphonuclear granulocytes, and a variable number of large histiocytic elements were found. The latter cells possessed an oval, vesicular nucleus with small, eosinophilic nucleoli, and contained phagocytosed material in their cytoplasm. Finally, pyknotic cell debris, occasional red blood cells, and a few small blood vessels were found in areas of ISH. Around these areas, a variable number of plasma cells was observed. In those cases in which foci of ISH were situated adjacent to hyperplastic lymphoid follicles, these foci were partly surrounded by a narrow rim of small lymphocytes, which in turn appeared continuous with the lymphocytic corona surrounding the lymphoid follicle.

Spleen

Both splenic specimens demonstrated no major abnormalities on macroscopic examination. On light microscopy, the white pulp was well developed; periarteriolar lymphocyte sheets (PALS) and adjacent secondary follicles could readily be seen. Surrounding these white pulp compartments, a prominent MZ was observed in which a mixture of cell types was found. In addition to some small round lymphocytes, some histiocytic elements, and many scattered neutrophilic polymorphonuclear granulocytes, a predominant medium-sized lymphoid population was observed (Figure 2). The majority of these lymphocytes contained a centrally placed, irregularly outlined nucleus with dispersed chromatin and inconspicuous nucleoli, surrounded by abundant clear cytoplasm. A minority of the lymphocytes were somewhat larger, possessed a more rounded, vesicular nucleus, with one or two prominent nucleoli, and contained less cytoplasm.

Electron-Microscopic Findings

Areas of ISH as well as the marginal zone of the splenic white pulp appeared to be composed of a similar mixture of cell types. Phagocytic histiocytes, neutrophilic polymorphonuclear granulocytes, and small round lymphocytes could easily be recognized and were admixed with a predominant "lymphoid" cell population (Figures 3 and 4). Although some variability was found in the latter population, all morphologic vari-

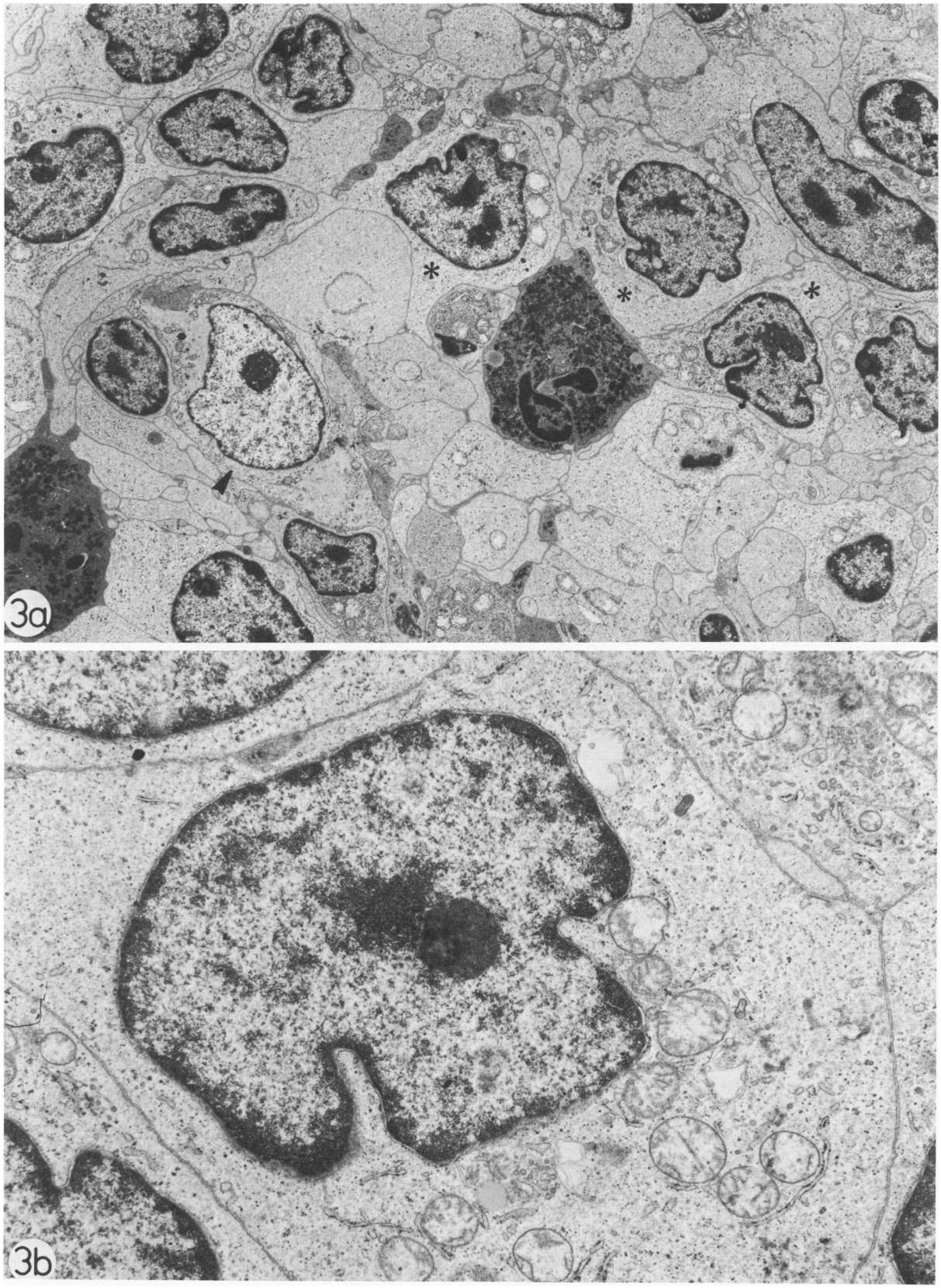


Figure 3—Electron micrograph of an area of ISH. **a**—Lymphoid cells are medium-sized and have abundant cytoplasm and an irregularly outlined nucleus with inconspicuous nucleoli (*asterisk*). Admixed are some polymorphonuclear granulocytes and a phagocytic histiocyte (*arrow*). (×3680) **b**—Detail of lymphoid cell, showing an irregular nucleus with a thick rim of heterochromatin and polarization of cell organelles toward the nuclear indentation. (×12,800)

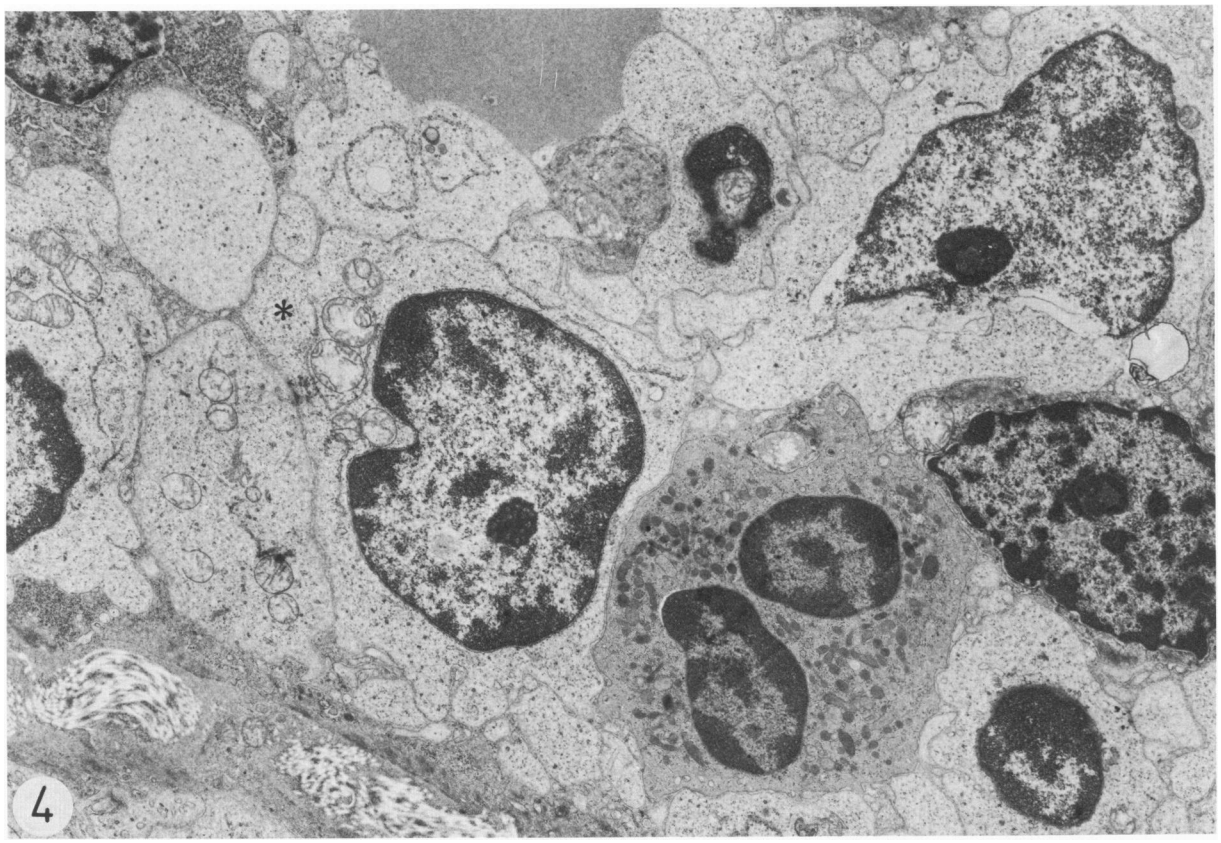


Figure 4—Electron micrograph of splenic marginal zone. The predominant lymphoid population has an irregular nuclear outline and abundant cytoplasm (*asterisk*). Some polarization of cell organelles is apparent. (×7250)

ants appeared to belong to one and the same cell type that displayed the following characteristic features. All cells showed abundant, irregularly shaped cytoplasm with a variable number of cytoplasmic extensions and a more or less pronounced irregular outline of the nucleus with a moderately thick rim of heterochromatin. In the less irregular nuclei, one prominent nucleolus was observed, whereas the markedly irregular nuclei contained one or two small, inconspicuous nucleoli. Parallel to this nuclear irregularity, a variable degree of polarization of cell organelles was observed (Figure 3b). Round to oval, clear mitochondria, some strands of rough endoplasmic reticulum, and some vesicles of the smooth endoplasmic reticulum were found at the indented pool of the nucleus. In this area, a pronounced Golgi apparatus was found, as well as one or more dark granules resembling dense-core vesicles.[24] Occasionally, fat droplets or a centrosome was observed.

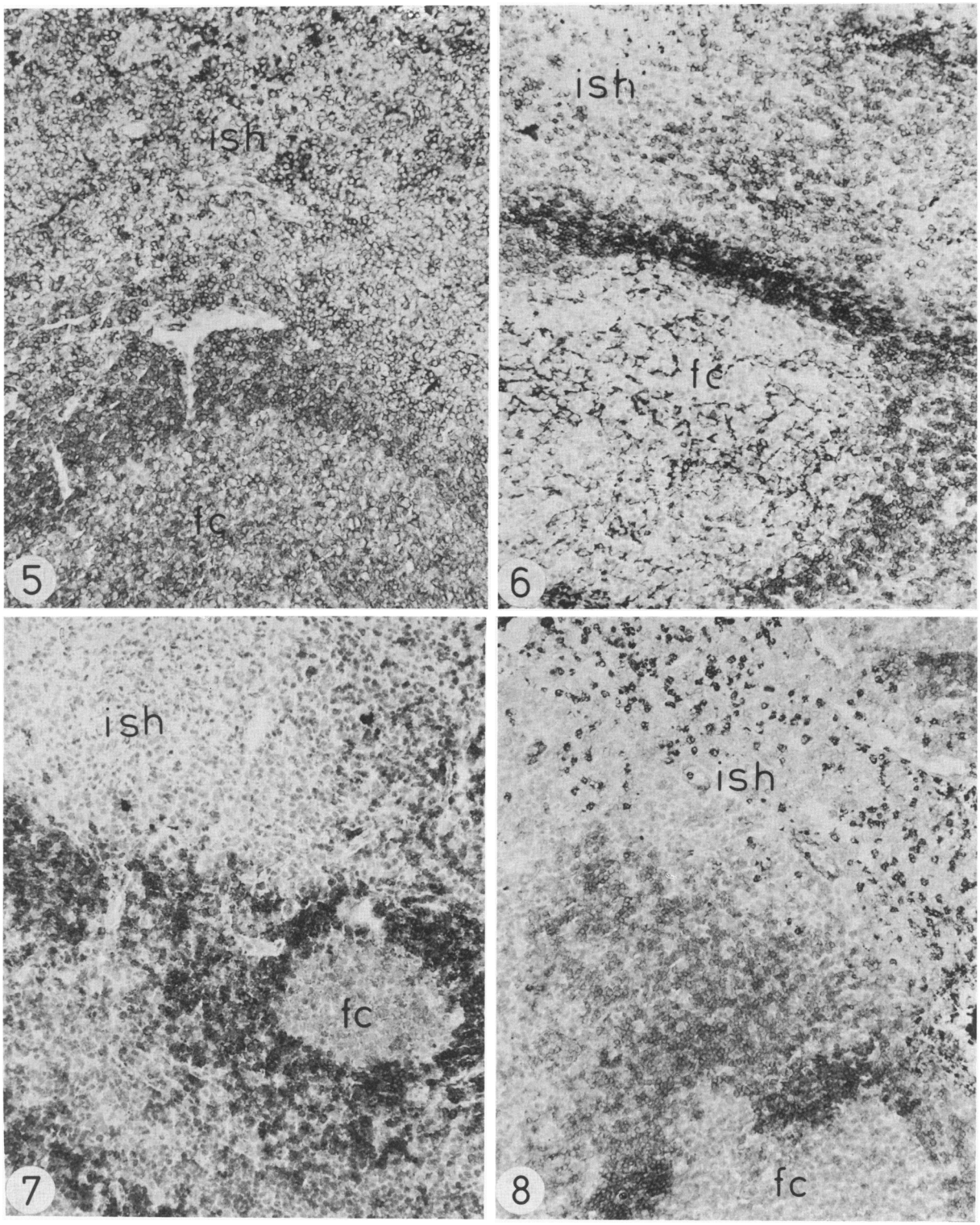
Immunohistochemical Findings

Lymph Nodes

The reactivity of the lymphoid parenchyma with antibodies to lymphocytes and monocytes/macrophages was comparable to previously reported results in reactive lymph nodes[25-31] and will therefore not be repeated here. An unexpected and not previously reported finding was the strong cytoplasmic reactivity of epithelioid histiocytes with monoclonal anti-transferrin receptor antibody OKT9.

Foci of ISH could easily be recognized in frozen tissue sections by virtue of their weak intensity of staining with hematoxylin and their particular topographic localization.

The majority of cells in areas of ISH demonstrated membranous staining with OKIal (Figure 5). The predominant cell population in areas of ISH showed surface reactivity with monoclonal antibodies B1 and anti-IgM (Figure 6), as well as with antibodies directed to both types of light chains; kappa appeared to be the predominant type of light chain expressed at the surface of ISH cells. All other surface markers, including BA1, anti-IgD (Figure 7), and anti-IgG, were negative. In all cases studies, BA1 and OKM1 only visualized a variable number of densely stained, scattered polymorphonuclear granulocytes (Figure 8). Some OKT4$^+$ Leu3a$^+$ helper/inducer T cells were observed and seemed to be situated predominantly at the outer margins of



Figures 5–8—Lymph node, stained for OKIal (**5**), IgM (**6**), IgD (**7**), and BA1 (**8**). **Figure 5**—Membranous positivity for OKIal is observed in the follicular center (*fc*), lymphocytic corona, and area of immature sinushistiocytosis (*ish*). **Figure 6**—IgM is present in a network pattern in the follicular center, as well as on the surface of lymphocytes in the lymphocytic corona; the majority of cells in the area of ISH are $sIgM^+$. **Figure 7**—Cells constituting the lymphocytic corona and extending around the area of ISH are $sIgD^+$. No reactivity for IgD is observed in the follicular center or in the area of ISH. **Figure 8**—The majority of cells in the lymphocytic corona react with BA1; scattered immunoreactive polymorphonuclear granulocytes are present in an otherwise unreactive area of ISH. (Indirect immunoperoxidase, hematoxylin counterstain, × 100)

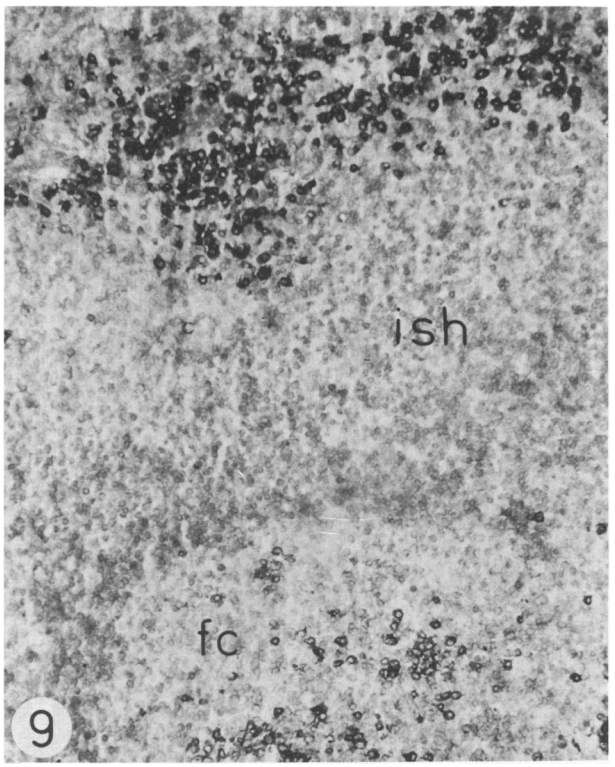


Figure 9—Lymph node, stained for OKT10. Immunoreactive cells are present in the follicular center (*fc*); a rim of OKT10$^+$ plasma cells surrounds the area of immature sinushistiocytosis (*ish*). (Indirect immunoperoxidase, hematoxylin counterstain, × 100)

areas of ISH; very few or no OKT8$^+$ and Leu7$^+$ cells were present. Reactivity with monoclonal antibody OKT9 was observed in some histiocytic elements. In those instances in which foci of ISH were situated nearby secondary follicles, a narrow rim of small B1$^+$BA1$^+$sIgM$^+$sIgD$^+$ lymphocytes was found around those foci of ISH (Figure 7). Finally, OKT10$^+$ plasma cells were observed predominantly around areas of ISH in some cases (Figure 9) and in the medullary cords of most cases. IgG was the predominant type of heavy chain, detected in the cytoplasm of these plasma cells.

Spleen

The reactivity of the splenic red and white pulp with the antibodies tested was comparable to previously reported results in normal splenic tissue.[28,31] For the purpose of this report, only the reactivity of MZ lymphocytes will be described in detail.

MZ lymphocytes were OKIa1$^+$ (Figure 10), the majority of them bearing IgM (Figure 11) and a small minority bearing IgD (Figure 12) at the surface. sIgD$^+$ MZ lymphocytes were found especially at the inner border of the MZ, near the lymphocytic corona of secondary follicles. Both types of light chains were observed. MZ lymphocytes were, in addition, B1$^+$ (Figure 13); monoclonal antibody BA1, however failed to label the surface of MZ lymphocytes, but revealed a concentric pattern of dendritic processes throughout the MZ and most pronounced around the PALS (Figure 14).

Discussion

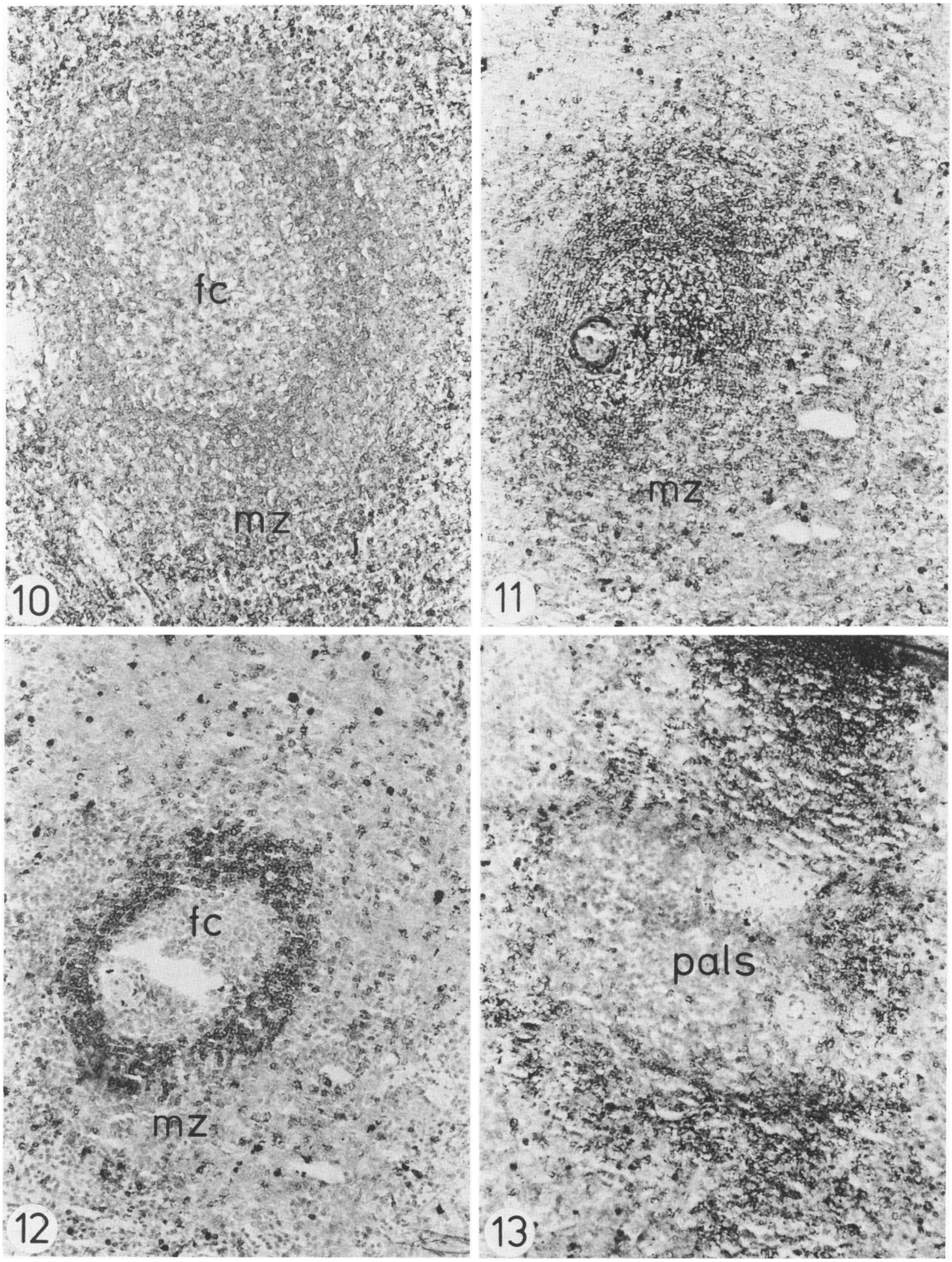
Our results indicate that foci of ISH, on the one hand, and the splenic MZ, on the other, are similar with respect to their histologic, immunohistochemical, and electron-microscopic features.

On morphology, a striking resemblance in the cellular composition of areas of ISH and the MZ of the splenic white pulp was observed. Both areas were composed predominantly of intermediate-sized lymphoid elements, characterized by an irregular nucleus with finely dispersed chromatin and inconspicuous nucleoli, and a large amount of pale cytoplasm.

The MZ is a normal component of the white pulp and surrounds the follicles and PALS. The MZ can be distinguished from the lymphocytic corona by its paler appearance on routine histologic sections and by its characteristic cell content, ie, a mixture of lymphocytes, granulocytes, and macrophages.[32] Among the lymphoid elements in the MZ, intermediate-sized cells with abundant clear cytoplasm predominate.[32]

In both areas, we found this predominant lymphoid cell population to carry the B-lineage surface phenotype B1$^+$BA1$^-$sIgM$^+$sIgD$^-$Ia$^+$ and to be admixed with variable numbers of OKM1$^+$BA1$^+$ polymorphonuclear granulocytes, few OKT9$^+$ histiocytic elements, and scattered OKT4$^+$Leu3a$^+$ helper/inducer T cells.

Immunohistochemical studies have demonstrated that the MZ belongs to the B-dependent area of the splenic white pulp. Immunophenotyping in human spleens has revealed surface C3b-receptors[31,33] and has demonstrated the expression of sIgM but not sIgD on MZ lymphocytes.[31] Furthermore, MZ lymphocytes carry receptors for the Fc-portion of IgG as well as Ia-like molecules at the surface.[28,33] Similar results have been obtained in rats.[34,35] MZ lymphocytes represent a distinct lineage of B cells with a distinct migration pattern and differentiation pathway,[34,36] because they originate as an independent lineage from recirculating precursors which have never expressed sIgD.[37] After a maturation period of some 3 weeks within follicular centers of the gut-associated lymphoid tissue or of the splenic white pulp, these lymphoid cells would reside in the MZ and fail to recirculate.[38] Their ontogenetic relationship with follicular center cells, previously demonstrated in autoradiographic studies in rabbits,[38] is confirmed in the present study. Both follicular center cells and MZ lymphocytes share the phenotype B1$^+$BA1$^-$sIgM$^+$sIgD$^-$Ia$^+$; follicular center cells, in addi-



Figures 10–13—Activated splenic white pulp, stained for OKIal (**10**), IgM (**11**), IgD (**12**), and B1 (**13**). Compare with Figures 5–8. **Figure 10**—Membranous positivity for OKIal is observed in the follicular center (*fc*), lymphocytic corona, and marginal zone (*mz*). **Figure 11**—IgM is present in a network pattern in the follicular center, as well as on the surface of lymphocytes in the lymphocytic corona; a large number of cells in the marginal zone are $sIgM^+$. **Figure 12**—A narrow rim of $sIgD^+$ cells is formed by the lymphocytic corona. No reactivity for IgD is observed in the follicular center or the marginal zone. **Figure 13**—The majority of cells in the marginal zone here surrounding a nonimmunoreactive periarteriolar lymphocyte sheath (*pals*), react with B1. (Indirect immunoperoxidase, hematoxylin counterstain, ×100)

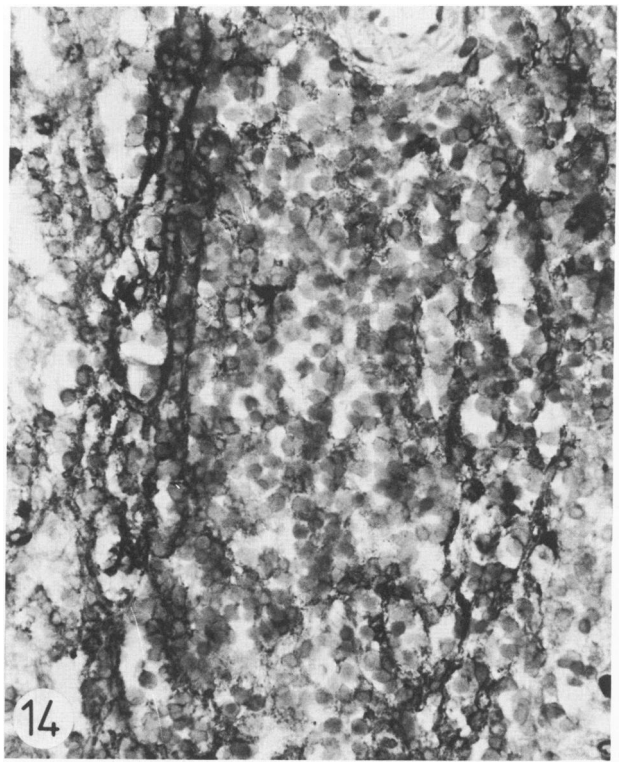


Figure 14—Splenic white pulp, stained for BA1. At the outer region of the periarteriolar lymphocyte sheath, ie, the marginal zone, a dendritic pattern for BA1 is observed. (Indirect immunoperoxidase, hematoxylin counterstain, ×250)

tion, may express sIgG, which is absent from MZ lymphocytes.[26,27] The nonreactivity of MZ lymphocytes with monoclonal antibody BA1 parallels the absence of sIgD and is in line with the general finding that BA1 in tissue sections is expressed by $sIgM^+sIgD^+$ B-lymphocytes, ie, those present in the lymphocytic corona surrounding lymphoid follicles.[30,31] In contrast, monoclonal antibody BA1 revealed a concentric pattern of dendritic processes in between MZ-lymphocytes in the splenic white pulp. These $BA1^+$ dendritic cells may represent accessory dendritic cells which have recently been shown to react with BA1.[31]

On electron microscopy, the similarity between cells constituting areas of ISH and those present in the MZ is further confirmed by our findings. The predominant cell population in both areas corresponded ultrastructurally to lymphoid elements with abundant cytoplasm, an irregularly outlined nucleus with a characteristic heterochromatin pattern, and a few cell organelles, which were more or less concentrated beneath the nuclear indentation. Moreover, in both areas a somewhat larger cell type was seen, characterized by a less irregularly shaped nucleus and a more prominent nucleolus. These findings agree well with previously reported studies on the ultrastructural features of ISH in man[8] and rabbits.[9]

With respect to the splenic MZ, most electron-microscopic studies have focused on the microvasculature[39] and nonlymphoid cells in this area,[40,41] and an adequate description of the lymphoid cells in the MZ is lacking.

The MZ is most characteristically present around the white pulp in the spleen. However, several studies have indicated that a similar compartment is present in lymph nodes. In their study of the popliteal lymph nodes of rabbits, Nieuwenhuis and Keuning[42] described a marginal zone in the outer cortex between marginal sinus and lymphoid follicles. Using immunohistochemical techniques, Stein et al[25] demonstrated caps of $sIgM^+sIgD^-Ia^+$ MZ lymphocytes at the sinoidal pole of lymphoid follicles in a variety of reactive lymph node conditions.

Relying on these data and on the similarity we observed between areas of ISH and the MZ of the splenic white pulp, we suggest that ISH represents an abnormal expansion of the normally present perifollicular MZ. In that respect, a putative role analogous to the role of the splenic MZ may be ascribed to ISH.

Two main functions have been related to MZ lymphocytes, ie, antigen transport toward follicular centers, on the one hand, and differentiation into antibody-forming cells, on the other. With respect to their role in antigen transport, experimental studies have revealed that injected antigens[43,44] and immune complexes[45,46] first accumulate in the MZ of the spleen. Noncirculating MZ lymphocytes have been claimed to be responsible for the transport of the nonphagocytozed residue toward the follicular center.[43-47] The presence of sIg, complement receptors, and Fc receptors render MZ lymphocytes well equipped to bind various antigenic particles and immune complexes. MZ lymphocytes in the rat spleen have also been considered to play a pivotal role in the generation of antibody-forming cells[37] and to represent the morphologic counterparts of the medium-sized to large $sIgM^+sIgD^-Ia^+$ preprogenitor B cells in the mouse spleen.[48] These B cells are in an intermediate stage of differentiation and respond to antigens via a two-stage nonspecific, then specific pattern.[49] In a first T-independent stage, $sIgM^+sIgD^-$ preprogenitors proliferate in response to nonspecific antigens, particularly macrophage stimulants, resulting in the generation of small, $sIgM^+sIgD^+$ direct progenitors which have lost the receptor for the nonspecific stimuli that triggered the preprogenitor subset, and which require specific stimulation for further development into antibody-forming cells.[50]

ISH is most frequently observed in, and represents one of the diagnostic criteria of toxoplasmic lymphadenitis.[1-4] Masihi and Werner[51] have demonstrated by immunoadherence that spontaneous rosette formation with *T gondii* antigens occurs mainly by sIg^+ B cells.

We suggest that these cells correspond to the $sIgM^+sIgD^-Ia^+$ ISH. As such, ISH may come in contact with the antigenic particles or antigen-antibody complexes, bind them to their surface, cross the discontinuous parenchymal sinus wall, and enter the lymphoid follicle. Since *T gondii* is a potent stimulator of macrophages through sensitized T-lymphocyte-derived soluble mediators,[52,53] a macrophage-mediated triggering of ISH similar to the murine model[49,50] may occur and result in the generation of a clone of small $sIgM^+sIgD^+$ lymphocytes, which we observed to surround some foci of ISH. Under the stimulatory influences of paracortical $OKT4^+Leu3a^+$ helper T cells, the latter population may subsequently give rise to antibody-forming cells, represented by the $OKT10^+cIg^+$ plasma cells found around areas of ISH.

In conclusion, we have demonstrated that the cellular composition of areas of ISH is light-microscopically, ultrastructurally, and immunohistochemically similar to that of the MZ surrounding the splenic white pulp. Because an MZ is found in lymph nodes also, we suggest that ISH represents an abnormal expansion of this compartment. Further studies on isolated lymphoid cells derived from areas of ISH are required to delineate more precisely their function in antigen transport and generation of antibody-forming cells.

Addendum

Since the submission of the manuscript, two studies concerning the immunohistochemical characterization of ISH have been published.[54,55]

Sheibani et al[54] found ISH to carry the phenotype $B1^+BA1^+OKT11^-$ and demonstrated polyclonal immunoglobulin light chains at their surface. They found that ISH lacked cytoplasmic lysozyme and showed no positive reaction with peanut agglutinin. They concluded that ISH cells were B-lymphocytes and proposed the term "monocytoid B-lymphocytes."[54]

De Almeida et al[55] found ISH to represent $B1^+sIgG^+OKT1^-OKT3^-OKM1^-$ lymphocytes which only occasionally expressed sIgM. They suggested that ISH cells represent post-antigen-stimulated B-lymphocytes, possibly produced in the follicular center, and proposed the term "large sinus lymphocytes."[55]

Our study as well as the former two studies[54,55] prove the B-cell origin of ISH. However, we did not observe BA1-immunoreactivity nor appreciable IgG expression at the surface of ISH. These differences in surface phenotype are not in contradiction to our hypothesis on the origin of ISH if these discrepancies are the result of subtle changes in surface marker and isotype expression ocurring upon differentiation of ISH into more mature mantle zone or antibody-forming cells.

References

1. Stansfeld AG: The histological diagnosis of toxoplasmic lymphadenitis. J Clin Pathol 1961, 14:565–573
2. Gray GFJr, Kimball AC, Kean BH: The postero-cervical lymph node in toxoplasmosis. Am J Pathol 1972, 69:349–358
3. Dorfman RF, Remington JS: Value of lymph-node biopsy in the diagnosis of acute acquired toxoplasmosis. N Engl J Med 1973, 289:878–881
4. Miettinen M: Histological differential diagnosis between lymph node toxoplasmosis and other benign lymph node hyperplasias. Histopathology 1981, 5:205–216
5. Garin Y, Audouin J, Diebold J: La lymphadénite toxoplasmique: Aspect histopathologiques et étude en immunofluorescence (Toxoplasma lymphadenitis: Histopathological appearance and immunofluorescent study). Arch Anat Cytol Pathol 1977, 25:221–226
6. Appel BN, Mendelow H, Pasqual HN: Acquired toxoplasma lymphadenitis. Oral Surg 1979, 47:529–532
7. Lennert K: Diagnose und Ätiologie der Piringerschen Lymphadenitis. Verh Dtsch Ges Path 1959, 42:203–208
8. Mori Y, Lennert K: Electron Microscopic Atlas of Lymph Node: Cytology and Pathology. Berlin, Heidelberg, New York, Springer Verlag, 1969, pp 22–24
9. Ràcz P, Tenner-Ràcz K, Myrvik QN, Shannon BT, Love SH: Sinus reactions in the hilar lymph node complex of rabbits undergoing a pulmonary cell-mediated immune response: Sinus macrophage clumping reaction, sinus lymphocytosis, and immature sinus histiocytosis. J Reticuloendothel Soc 1978, 24:499–525
10. Lukes RJ: The pathology of the white pulp of the spleen, Die Milz (The Spleen). Edited by K Lennert, D Harms. Berlin, Heidelberg, New York, Springer Verlag, 1970, pp 130–138
11. Reinherz EL, Kung PC, Pesando JM: Ia determinants on human T-cell subsets defined by monoclonal antibody: Activation stimuli required for expression. J Exp Med 1979, 150:1472–1482
12. Abramson CS, Kersey JH, LeBien TW: A monoclonal antibody (BA-1) reactive with cells of human B lymphocyte lineage. J Immunol 1981, 126:83–88
13. Nadler LM, Stashenko P, Ritz J, Hardy R, Pesando JM, Schlossman SF: A unique cell surface antigen identifying lymphoid malignancies of B cell origin. J Clin Invest 1981, 67:134–140
14. Reinherz EL, Schlossman SF: Monoclonal antibodies defining distinctive human T cell surface antigens. Science 1979, 206:347–349
15. Ledbetter JA, Evans RL, Lipinski M, Cunningham-Rundles C, Good RA, Herzenberg LA: Evolutional conservation of surface molecules that distinguish T lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. J Exp Med 1981, 153: 310–323
16. Reinherz EL, Kung PC, Goldstein G, Levey RH, Schlossman SF: Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. Proc Natl Acad Sci USA 1980, 77:1588–1592
17. Goding JW, Burns GF: Monoclonal antibody OKT9 recognizes the receptor for transferrin on human acute lymphocytic leukemia cells. J Immunol 1981, 127: 1256–1258
18. Breard J, Reinherz EL, Kung PC, Goldstein G, Schlossman SF: A monoclonal antibody reactive with human peripheral blood monocytes. J Immunol 1980, 124: 1943–1948
19. Abo T, Balch CM: A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). J Immunol 1981, 127:1024–1029.
20. Graham RC Jr, Lundholm U, Karnovsky MJ: Cytochem-

ical demonstration of peroxidase activity with 3-amino-9-ethyl-carbazole. J Histochem Cytochem 1965, 13: 150–152
21. Van den Oord JJ, De Wolf-Peeters C, Vanstapel MJ, Desmet VJ: Improved immunohistochemical visualization of helper/inducer T cells by the simultaneous application of two non-crossblocking monoclonal antibodies. Stain Technol (In Press)
22. Sternberger LA: The unlabeled antibody enzyme method, Immunohistochemistry. Englewood Cliffs, NJ, Prentice Hall, 1974, pp 129–171
23. Graham RC, Karnovsky JJ: The early stages of absorption of injected horseradish peroxidase in the proximal tubule of the mouse kidney: Ultrastructural cytochemistry by a new technique. J Histochem Cytochem 1966, 14:291–302
24. Mori Y, Lennert K: Electron microscopic Atlas of Lymph Node: Cytology and Pathology. Berlin, Heidelberg New York, Springer Verlag, 1969, pp 132–133
25. Stein H, Bonk A, Tolksdorf G, Lennert K, Rodt H, Gerdes J: Immunohistologic analysis of the organization of normal lymphoid tissue and non-Hodgkin's lymphomas. J Histochem Cytochem 1980, 28:746–760
26. Poppema S, Bhan AK, Reinherz EL, McCluskey RT, Schlossman SF: Distribution of T cell subsets in human lymph nodes. J Exp Med 1981, 153:30–41
27. Bhan AK, Nadler LM, Stashenko P, McCluskey RT, Schlossman SF: Stages of B cell differentiation in human lymphoid tissue. J Exp Med 1981, 154:737–749
28. Hsu S-M, Cossman J, Jaffe ES: Lymphocyte subsets in normal human lymphoid tissues. Am J Clin Pathol 1983, 80:21–30
29. Si L, Whiteside TL: Tissue distribution of human NK cells studied with anti-Leu-7 monoclonal antibody. J Immunol 1983, 130:2149–2155
30. Hsu S-M, Jaffe ES: Phenotypic expression of B-lymphocytes. 1. Identification with monoclonal antibodies in normal lymphoid tissues. Am J Pathol 1984, 114:387–395
31. Van der Valk P, van der Loo EM, Jansen J, Daha MR, Meijer CJLM: Analysis of lymphoid and dendritic cells in human lymph node, tonsil and spleen. Virchows Arch (Cell Pathol) 1984, 45:169–185
32. Millikin PD: The nodular white pulp of the human spleen. Arch Pathol 1969, 87:247–258
33. Jaffe ES, Shevach EM, Sussman EH, Frank M, Green I, Berard CW: Membrane receptor sites for the identification of lymphoreticular cells in benign and malignant conditions. Br J Cancer 1975, 31 (Suppl II):107–120
34. Kumararatne DS, Bazin H, MacLennan ICM: Marginal zones: the major B cell compartment of rat spleens. Eur J Immunol 1981, 11:858–864
35. Dijkstra CD, Döpp EA: Ontogenetic development of T- and B-lymphocytes and non-lymphoid cells in the white pulp of the rat spleen. Cell Tissue Res 1983, 229:351–363
36. Kumararatne DS, MacLennan ICM: Cells of the marginal zone of the spleen are lymphocytes derived from recirculating precursors. Eur J Immunol 1981, 11:865–869
37. Bazin H, Gray D, Platteau B, MacLennan ICM: Distinct delta$^+$ and delta$^-$ B-lymphocyte lineages in the rat. Ann NY Acad Sci 1982, 399:157–174
38. Nieuwenhuis P, van Nouhuijs CE, Eggens JH, Keuning FJ: Germinal centres and the origin of the B-cell system: I. Germinal centres in the rabbit appendix. Immunology 1974, 26:497–507
39. Burke JS, Simon GT: Electron microscopy of the spleen: I. Anatomy and microcirculation. Am J Pathol 1970, 58:127–155
40. Veerman AJP, van Ewijk W: White pulp compartments in the spleen of rats and mice: A light and electron microscopic study of lymphoid and non-lymphoid cell types in T- and B-areas. Cell Tissue Res 1975, 156:417–441
41. Blue J, Weiss L: Species variation in the structure and function of the marginal zone: An electron microscope study of cat spleen. Am J Anat 1981, 161:169–187
42. Nieuwenhuis P, Keuning FJ: Germinal centres and the origin of the B-cell system: II. Germinal centres in the rabbit spleen and popliteal lymph nodes. Immunology 1974, 26:509–519
43. Nossal GJV, Austin CM, Pye J, Mitchell J: Antigens in Immunity: XII. Antigen trapping in the spleen. Int Arch Allergy 1966, 29:368–383
44. Mitchell J, Abbot A: Antigens in immunity: XVI. A light and electron microscope study of antigen localization in the rat spleen. Immunology 1971, 21:207–224
45. Brown JC, Harris G, Papamichail M, Slijivic VS, Holborow E: The localization of aggregated human gamma-globulin in the spleens of normal mice. Immunology 1973, 24:955–968
46. Van Rooijen N: Mechanism of follicular antigen trapping: Migration of antigen-antibody complexes from marginal zone towards follicle centres. Immunology 1973, 25:847–852
47. Veerman AJP, van Rooijen N: Lymphocyte capping and lymphocyte migration as associated events in the in vivo antigen trapping process: An electron-microscopic autoradiographic study in the spleen of mice. Cell Tissue Res 1975, 161:211–217
48. Nossal GJV, Shortman K, Howard M, Pike BL: Current problem areas in the study of B lymphocyte differentiation. Immunol Rev 1977, 37:187–209
49. Shortman K, Howard M, Teale J, Baker J: Antigen-initiated B lymphocyte differentiation: XVI. Primary and secondary adoptive responses involve two sequential stages, antigen nonspecific then antigen specific, in the generation of specific AFC. J Immunol 1979, 122: 2465–2472
50. Layton JE, Baker J, Bartlett PF, Shortman K: Antigen-initiated B lymphocyte differentiation: XVIII. Preprogenitor B cells that give primary adoptive responses are s-IgM$^+$IgD$^-$Ia$^+$. J Immunol 1981, 126:1227–1233
51. Masihi KN, Werner H: Types of cells involved in antigen-stimulated and spontaneous rosette formation with Toxoplasma gondii. J Immunol 1978, 121:2056–2059
52. Anderson SE, Bautista S, Remington JS: Induction of resistance to toxoplasma gondii in human macrophages by soluble lymphocyte products. J Immunol 1976, 117:381–387
53. McLeod R, Remington JS: Influence of infection with Toxoplasma on macrophage function, and role of macrophages in resistance to Toxoplasma. Am J Trop Med Hyg 1977, 26:170–186
54. Sheibani K, Fritz RM, Winberg CD, Burke JS, Rappaport H: "Monocytoid" Cells in Reactive Follicular Hyperplasia with and without Multifocal Histiocytic Reactions: An immunohistochemical study of 21 cases including suspected cases of toxoplasmic lymphadenitis. Am J Clin Pathol 1984, 81:453–458
55. De Almeida PC, Harris NL, Bhan AK: Characterization of immature sinus histiocytes (monocytoid cells) in reactive lymph nodes by use of monoclonal antibodies. Hum Pathol 1984, 15:330–335

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