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FURTHER DELINEATION OF THE IMMUNO-ARCHITECTURE OF THE HUMAN SPLEEN

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ABSTRACT:

Using a battery of monoclonal antibodies directed at B and T cell antigens, we delineate the immunotopography of the human spleen. The tissue section immunohistochemical methods employed demonstrate the complexity of white pulp reactivity with both light and heavy chain immunoglobulin expression appearing polyclonal. Zonal expression of heavy chains suggests an anatomic basis for the known sequence of heavy chain switching due to immunoglobulin gene rearrangement. Localization of universal B-cell antigen, B_1, delineates the entire B-cell zone whereas antibodies directed at universal T antigens (e.g. Leu 1,4,9) delineate the T cell zone. Leu 14 demonstrates striking B-zone staining in normal spleen and in hairy cell leukemia, suggesting that leukemic cells in this disease arise from Leu 14$^+$ B cells. Leu 2a staining occurs not only on lymphoid cells but probably also on sinusoidal endothelial lining cells.

In a previous study, we established basic B and T-cell lymphoid neighborhoods in the human spleen (1). By examining additional B and T-cell antigens, we now further define intrasplenic compartmentalization of heavy chain immunoglobulin expression and universal B-cell antigens (B_1 and Leu 14), along with topographic expression of common ALL (CALLA) and dendritic reticulum cell (DRC) antigens. Additional observations on a few newly described T-cell antigens are also included (e.g., Leu 9). The illustrations emphasize the utility of serial section immunohistochemistry for demonstrating expression of multiple antigens at a single splenic tissue site.

MATERIALS AND METHODS

Three grossly and microscopically normal human spleens were studied immunohistochemically. Each was removed during staging laparotomy for Hodgkin's disease. Portions of resected spleen were snap frozen in OCT compound (Miles Laboratory, Naperville, ILL.) at −150°C in isopentane quenched in liquid nitrogen and stored at −70°C. As previously described (1,2), tissue section analysis was performed using indirect immunohistochemistry with the following reagents: mouse anti-human monoclonal antibodies (see below) for the first stage; biotin conjugated $F(ab')_2$ goat anti-mouse IgG (Tago, Burlingame, CA.) as the second stage; avidin-D conjugated horseradish peroxidase (Vector Lab., Burlingame, CA.) as the third stage; diaminobenzidine tetrahydrochloride (DAB) with H_2O_2 as the final detection agent.

The first stage included monoclonal antibodies to the following B-cell antigens: CALLA (common ALL antigen), B_1 (pan-B cell antigen), and B_2 (C_3d receptor; Coulter Immunology, Hialeah, FL.), immunoglobulins (kappa, lambda, mu, gamma, alpha, delta; Leu 14 (B-cell reagent) and HLA-DR (I_a) (all obtained from Becton-Dickinson, Mountainview, CA.). Also evaluated were mouse monoclonal antibodies to T-cell antigens (Leu 1, 4, 9 pan-T antigens); Leu 2a (T-suppressor/cytotoxic antigen); Leu 3a (T-helper antigen), Leu 5

(E-rosette receptor), Leu 6 (immature thymic antigen); Leu 7 (natural killer antigen) (Becton-Dickinson). These reagents were applied to all three spleens. Mouse ascitic fluid (Bethesda Res. Lab., Gaithersburg, MD.) was applied to one slide in each run, instead of the primary antibody, as a negative control.

RESULTS

A. Immunotopographic localization of multiple B and T-cell antigens within a single white pulp site:

Fig. 1 demonstrates the complex topography of B and T-cell antigenic expression at a single white pulp site using serial sections and a battery of monoclonal antibodies directed at a single T-cell and multiple B-cell antigens. A polyclonal pattern of both light and heavy chain immunoglobulin (Ig) expression is visible with kappa-bearing cells consistently outnumbering lambda-bearing cells. Both cell types, however, are primarily located in the mantle zone of the germinal center and adjacent marginal zone, with a few scattered in the red pulp and periarteriolar lymphoid sheath (PALS). While kappa and lambda-bearing cells are also detectable in the germinal center itself, this site is mainly characterized by "intercellular" or "dendritic" deposition of immunoglobulin (I_g). A similar germinal center intercellular pattern of Ig expression is seen with IgM and IgG. Cellular (i.e., surface) IgG expression is scant and largely limited to scattered cells within the red pulp; the mantle zone shows a notable lack of cellular IgG. There is substantial expression of cellular IgM in both the mantle zone and in the adjacent marginal zone. I_gD occurs in a strict cellular staining pattern most notably within the mantle zone, but also scattered throughout the red pulp. IgA expression appears scant, but in other loci within the spleen there is stronger germinal center expression (Fig. 2).

B_1 antigen localizes throughout the entire B-cell compartment in each of the three spleens (Fig. 1). B_1^+ cells are evident in the germinal center, mantle zone, and the adjacent marginal zone, but infrequent in the PALS. This "universal" antigen identifies B-cells over a wide range of differentiation and B_1 positivity demonstrates the substantial compartmentalization of B-cells in the splenic white pulp (compare with L_1^+ T-cell compartment in the PALS region)(see below).

Anti-B_2 reveals striking localization of this B-cell antigen in both the germinal center and mantle zone. Although B_2^+ cells are visible in the red pulp, they are rarely seen in the PALS. Ia expression is noted throughout both the B-cell compartment and red pulp, and to a lesser extent in the PALS. This widespread distribution reflects the occurrence of this antigen on most B-cells, monocytes and histiocytes as well as "activated" T-cells.

Anti-Leu 1 in contrast detects a "universal" T-cell antigen. Leu 1^+ cells are especially notable in the PALS, but they also appear scattered in the red pulp and germinal center. Additional assessment with Leu 2a and Leu 3a (not shown) indicates that T-cells in the PALS are predominantly of T-helper (Leu $3a^+$) type with a minority component of T-suppressor/cytotoxic cells (Leu $2a^+$. The overall ratio is approximately 3 or 4:1 of T-helper/T-suppressor cytotoxic cells. Note the substantial compartmentalization of T- and B- cells in the Leu 1^+ PALS region and B_1^+/B_2^+ B-cell zone, repectively.
monoclonal antibodies further discloses prominent Leu 4 and Leu 9 (pan-T or universal T-cell antigen) expression in the PALS with scattering in the red pulp. This finding corresponds with the distribution of Leu 1 in the PALS (see above). There also are rare scattered Leu 6^+ cells and scattered natural killer cells (Leu 7^+) in the red pulp and Leu 7^+ cells clustered in germinal centers as previously reported (1). Common ALL antigen staining is scattered in some germinal centers with both a cellular and dendritic pattern.

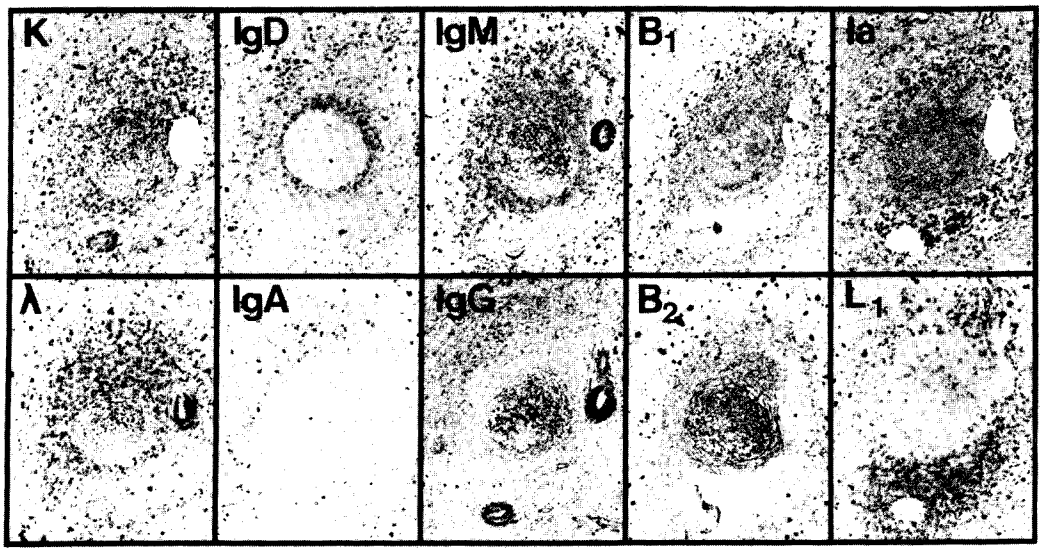

Fig. 1: Complex localization of multiple B and T cell antigens within a single white pulp site. L1 = Leu 1. Magnificaton 120x. See text for details.

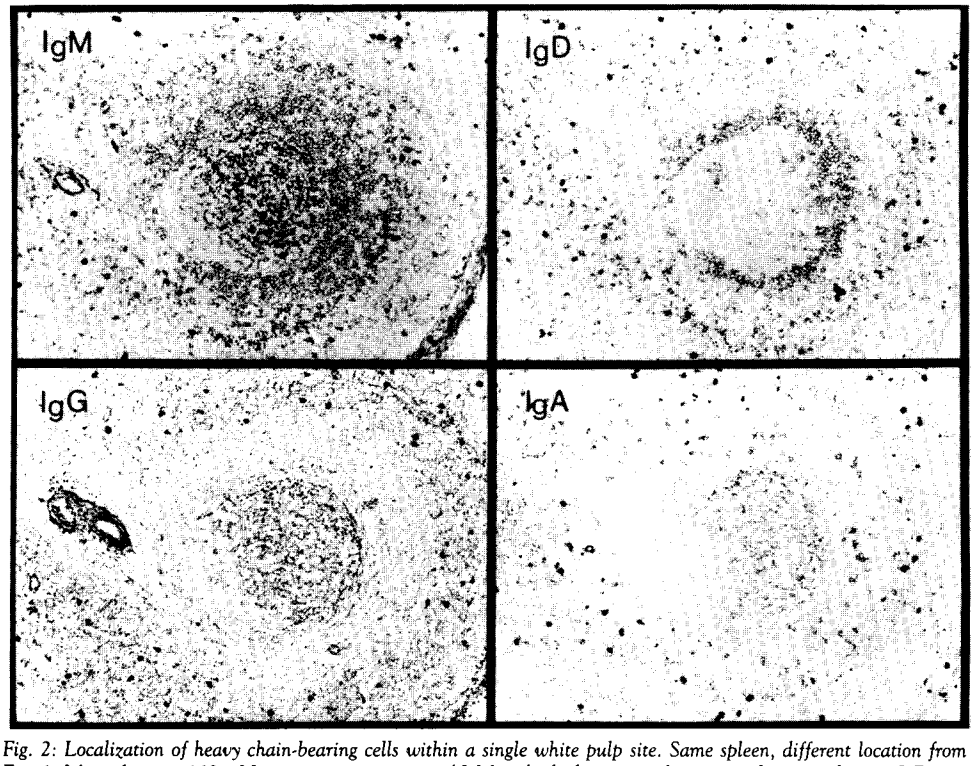

Fig. 2: Localization of heavy chain-bearing cells within a single white pulp site. Same spleen, different location from Fig. 1. Magnification 160x. Note intense expression of IgM in both the germinal center and marginal zone; IgD staining primarily in the mantle zone and faint IgA expression in the germinal center.

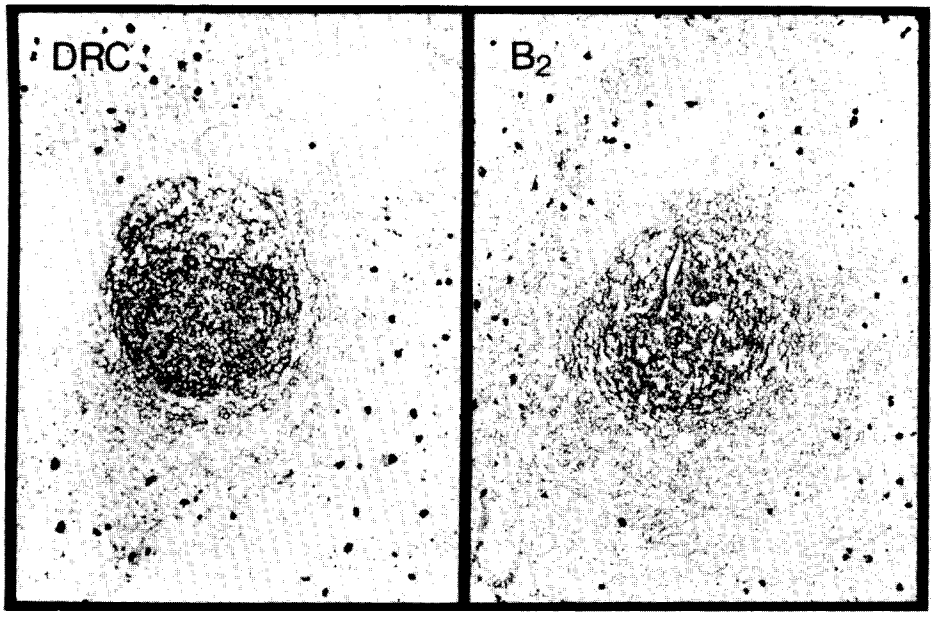

Fig. 3: Localization of dendritic reticulum cells (DRC) and B_2-bearing cells within same white pulp site as Fig. 2. Magnification 160x. Note the prominent staining within in the germinal center and mantle zones.

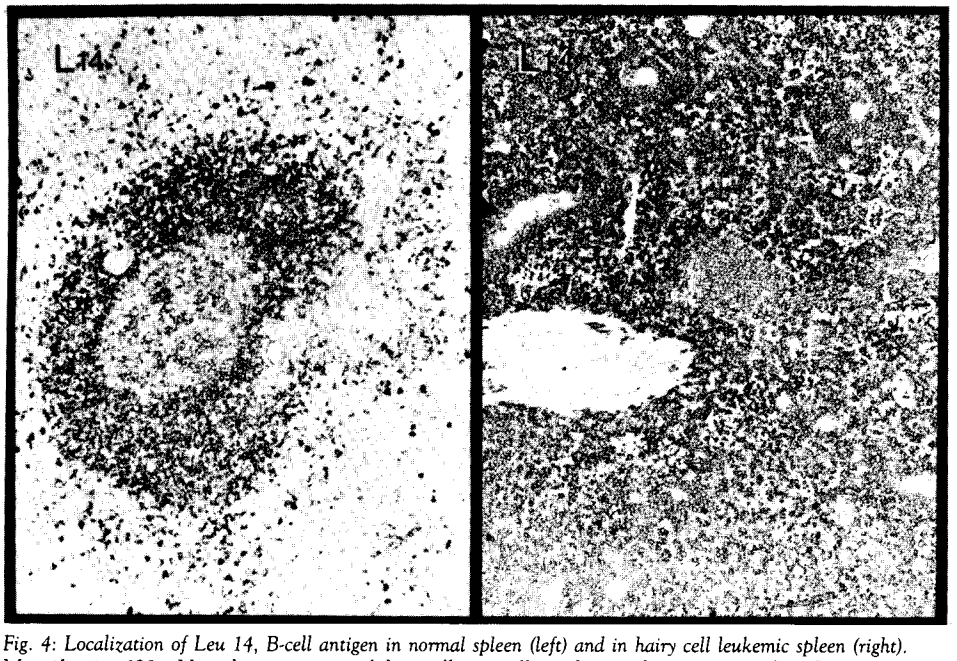

Fig. 4: Localization of Leu 14, B-cell antigen in normal spleen (left) and in hairy cell leukemic spleen (right). Magnification 120x. Note the prominence of these cells normally in the mantle zone surrounding the germinal centers, the likely site of origin of these neoplastic cells in hairy cell leukemia.

B. Localization of heavy chain bearing cells:

Fig. 2 demonstrates in another region of the same spleen, the sharply topographic distribution of heavy chain expression in the splenic B-cell zone. In addition to interstitial IgM and IgG and cellular IgM and IgD expression as in Fig. 1, Fig. 2 demonstrates more conspicuous expression of IgA. Germinal center IgA has primarily an interstitial pattern, while scattered IgA^+ mantle zone cells have a cellular staining pattern. Despite variability of intrasplenic IgA response, (see Fig. 1) IgM, IgD and IgG patterns are constant in each spleen and site examined.

C. Localization of dendritic reticulum cells and B_2 bearing cells:

Fig. 3 indicates the prominent localizaton of dendritic reticulum cells (DRC) within germinal centers and to a lesser intensity within the mantle zone. Although some germinal center and mantle zone B-cells may possess DRC antigen, most of the DRC positive cells morphologically contain discrete dendritic processes.

B_2 staining in the germinal center and mantle zone is both cellular and dendritic, and the latter pattern corresponds closely with DRC localization in germinal centers. This feature suggests some B_2 apparently is localized primarily on B cells in the germinal centers, mantle zone and red pulp.

D. Localization of Leu 14, a B-cell antigen:

Fig. 4 demonstrates conspicuous expression of Leu 14 by splenic B-cells in a normal compared with hairy cell leukemic spleen. Without disease, expression of Leu 14 is most intense in the mantle surrounding the germinal center, less prominent in the germinal center and scant throughout the red pulp. Perhaps, the germinal center pattern represents a mosaic or hodgepodge of Leu 14 positive and negative cells. Note that germinal center Leu 14 pattern of staining is conspicuously cellular, not dendritic/intercellular as with DRC antigen or

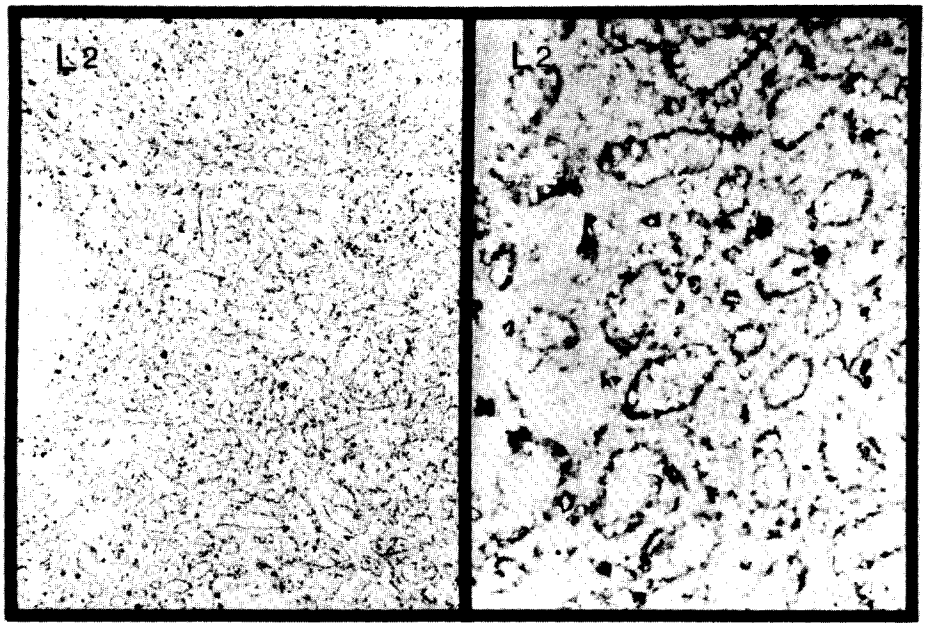

Fig. 5: Localization of Leu 2a (suppressor/cytotoxic T-cell) antigen on splenic T cells and in the sinusoidal wall. Magnification 120x (left), 200x (right).

immunoglobulin. With hairy cell leukemia, leukemic B-cells are visible effacing the entire spleen with strong, constant Leu 14 expression. Especially noteworthy is the large uninvolved trabecular vein and a paler staining, centrally located, intact but rudimentary germinal center.

E. Localization of Leu 2a (suppressor/cytotoxic) antigen:

Fig. 5 demonstrates two patterns of Leu 2a localization. First, Leu 2a positive cells are widely disseminated throughout the red pulp, a finding in distinct contrast with occasional Leu 3a positive cells found at this site (not shown). Second, splenic sinusoids stain with anti-Leu 2a. Although sinusoidal Leu 2a expression may reflect T-suppressor/cytotoxic cells traversing the sinuses, most of the antigen appears to stain lining cells (e.g., either endothelium or sinusoidal macrophages), a phenomenon suggesting non-lymphoid cell Leu 2a expression.

DISCUSSION

These findings illustrate the topography of B- and T-cell antigenic expression in human spleen. Previously, we emphasized the splenic T-cell zones (1); in this study, we detail the complexity of B-cell antigenic expression. The polyclonal light chain expression with kappa predominance we describe conforms to earlier findings in both spleen and reactive lymph nodes (1-5). Coupled with our finding of a polyclonal IgM, IgD, IgA and IgG response it appears that both polyclonal light and heavy chain expression characterizes B-cell zones in the reactive spleen (1,4,5). The topography of heavy chain immunoglobulin expression also corresponds to previous observations in reactive lymph nodes and spleens (3-5). Interestingly, IgG and IgD are mutually exclusive in their distribution: IgG within germinal centers and not mantle zones, and vice versa for IgD. It is well established that heavy chain"switching" (e.g., IgM→IgD→IgM→IgG→IgA) occurs in the process of normal B-cell clonal expansion and ontogeny (6). Each heavy chain switch probably involves a separate additional immunoglobulin gene rearrangement (6). It is intriguing that each gene switching event may relate to a distinct topographic site within the B-cell zone. If so, this arrangement would further signify that heavy chain switching is both a distinct genomic event (e.g. DNA rearrangement) and a microanatomic process (e.g. IgD switching in mantle zones and IgG switching in germinal center), and moreover, that differentiation within the splenic B-cell compartment has a zonal pattern which recapitulates known B-cell maturation (e.g., heavy chain switching). Hence, there now appears an anatomic basis for the known sequence of B-cell heavy chain expression.

Our B_1 findings indicate the substantial utility of a monoclonal antibody which detects a universal or pan-B antigen (4,5). Since this antibody exclusively detects B-cells, and it does so over a broad range of differentiation, it delineates the full extent of the white pulp B-zone, Anti-B_1 allows detection of both immature and mature B-cells so that even immunoglobulin negative B-cells within the germinal center or I_g^- transformed B-cells can be detected in accord with previous findings (4,5).

We demonstrate strong expression of B_2 in germinal centers and mantle zones (7,8). Since B_2 is specifically expressed after antigenic activation of B-cells, yet lost in subsequent transformaton, B_2 is closely associated with antigen activated B-cells (8). Thus, B_2 marks a discrete stage in the maturation process of B-cells and its localization to the germinal center and adjacent mantle zone indicates that antigen activation of B-cells probably occurs within this restricted site. This same pattern of B_2 staining is seen in reactive lymph nodes (5).

In agreement with a previous study (9), we found dendritic reticulum cells substantially localized to white pulp B-cell lymphoid follicles, particularly in secondary follicles. Although a few dendritic reticulum

cells appear in nearby mantle zones most are within germinal centers as previously noted (9). These dendritic reticulum cells have elaborate branching cytoplasmic extensions with a high affinity for immune complexes. They probably function to trap antigen within germinal centers, and the elaborate branching processes enmesh germinal center B-cells facilitating antigen-B cell interaction (9). Within the germinal center, the dendritic reticulum cell with its entrapped, processed and presented antigen probably "drives" B-cell clonal expanson and differentiation. They probably also influence B-cell homing to germinal centers (9). Dendritic reticulum cells with their elaborate meshwork of cytoplasmic tentacles and high affinity for immune complexes is ideally suited and situated to direct B-cell traffic and B-cell clonal expansion and differentiation. Indeed, if B-cell clonal expansion is an antigen directed phenomenon, it is almost certainly a dendritic reticulum cell driven process. Finally, occasional germinal center T-helper cells (Leu $3a^+$) and natural killer cells (Leu 7^+) also probably stimulate and modulate B-cell function at this site (10,11).

Our description of germinal center immunoglobulin expression has emphasized an intercellular or dendritic pattern of staining as opposed to cellular staining. Although a small amount of germinal center staining may relate to germinal center B-cells which are IgM^+, IgG^+ and IgA^+, most of this appears intercellular/dendritic. The letter may represent secreted or shed immunoglobulin from m B-cells (3,4). From the foregoing description of dendritic reticulum cells, it is quite plausible that much of this secreted or shed immunoglobulin is passively absorbed by DRC cells, especially in the absence of evidence that these cells produce immunoglobulin (9). The complexity of immunoglobulin expression, switching, secretion and loss in germinal centers remains controversial, however, and is beyond the scope of this study. Nonetheless, it is reasonable to conclude that I_g expression within the germinal center and mantle zone is the end result of a complex *polyclonal* process of B-cell expansion and differentiation (1,3-5).

Recent studies propose that B_2 antigen (see Figs. 1,3) is a C3d receptor (12), and that these receptors are expressed on both germinal center dendritic reticulum cells (13,14) and B-cells (13). As with immunoglobulin expression, separating germinal center B-cell and dendritic reticulum cell antigenic expression is difficult. Nonetheless, B_2 is probably elaborated by germinal center B-cells, then shed, and perhaps passively absorbed by dendritic reticulum cells (12,13). The exact function of C3d receptor in germinal centers is unknown, but C3 may play a role in immunoregulation (12). Specifically, memory B-cell formation requires entrapped antigen-immunoglobulin-C3 fragments to be held in follicles by complement receptors. Thereafter a complex interplay of dendritic reticulum cells, immunoglobulin, antigen and complement fragments probably sustain B-cell differentiation (12).

Leu 14 B-cell antigen is conspicuously localized to splenic germinal centers and particularly the mantle zone. Strong Leu 14 expression in hairy cell leukemia suggests that these neoplastic B-cells derive from the mantle zone and spread subsequently to the red pulp although derivation from scattered Leu^+ red pulp cells is not excluded.

Localization of Leu $2a^+$ suppressor/cytotoxic T-cells as the dominant red pulp T-cell corresponds with earlier findings (4). Because histiocytes and natural killer cells are also conspicuous in the red pulp, all three cell types may collaborate, perhaps as a triumvirate, to regulate red pulp cytotoxic and phagocytic activity (1). Our finding of Leu $2a^+$ antigen within the sinusoidal walls of the red pulp confirms earlier findings (4,16), and we agree that this finding may signify Leu $2a^+$ antigen on splenic lining endothelial cells (4,16). Since the Leu $2a^+$ sinusoidal lining cells proved $OKM1^-$ previously (16), the staining appears unrelated to macrophage processes in

the sinusoidal wall. Interestingly, the endothelium lining splenic trabecular veins and endothelial cells isolated from umbilical veins do not react with Leu 2a or OKT8 (16). Thus, Leu 2a positivity appears at this time unique to splenic sinusoidal endothelium, a phenomenon suggesting that some non-lymphoid, non-T-cells are Leu $2a^+$ (4). It is uncertain whether the sinusoidal antigen is specifically produced and expressed by these cells or passively absorbed (4). Nonetheless, we agree with Stuart and Warford (16) that sinusoidal Leu $2a^+$ antigens may act as "biochemical signposts which moderate cellular traffic" at a critical point of passage between cord and sinuses in the spleen.

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