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Compartments, domains and migration pathways of lymphoid cells in the splenic pulp

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The spleen is a highly vascularized hemopoietic organ, which can be considered as an encapsulated filter connected to the blood stream. Blood enters the spleen at the hilus through the splenic artery. The splenic artery ramifies into trabecular arteries which become gradually surrounded by lymphatic tissue. This lymphatic tissue forms a compartment known as the white pulp of the spleen. Small terminal arterioles open into the marginal zone, a diffuse area which surrounds the lymphoid white pulp and forms the border between the white pulp and the hemopoietic compartment in the spleen, the splenic red pulp. Alternatively, terminal arterioles extend via capillaries to splenic sinusses and venules. This 'closed' circulation opposes the open circulation where blood cells can freely enter the splenic parenchyma through the marginal zone.

The white pulp constitutes the immunologically active compartment in the spleen. It is a highly organized compartment in which three major domains can be distinguished: a) the peri-arteriolar-lymphoid sheath (PALS), b) lymphoid follicles inserted at the periphery of the PALS and c) a marginal zone which forms the border of the white pulp with the red pulp.

Lymphoid cells in the white pulp are not randomly distributed through the stroma; several types of experiments have shown that the two major subclasses of lymphoid cells, i.e. T lymphocytes and B lymphocytes, localize in distinct domains in the white pulp.

Waksman et al.[24] were the first to notice that in neonatally thymectomized rats the PALS, but not the follicles, were depleted of lymphoid cells. This area was considered as 'thymus dependent'. Parrott et al.[16] confirmed this notion in neonatally thymectomized mice, whereas de Sousa et al.[5] described similar findings in athymic 'nude' mice.

Detection of lymphoid subclasses in frozen sections using specific antibodies and immunocytochemical techniques has confirmed and extended these observations and generally shown that B cells are located in follicles, whereas T cells are localized in the PALS[18]. The marginal zone, being a site of entry of cells into the spleen, comprises both T and B lymphocytes. Recently it has been shown that the marginal zone is not only an important traffic area, it is also a specialized B cell domain. MacLennan et al.[17] showed that this area contains a subpopulation of non-recirculating B cells, i.e., IgM+ve cells, which respond to T independent antigens.

The purpose of this paper is to specify further the localization and migration behavior of various lymphoid subpopulations. The availability of many monoclonal antibodies directed to differentiation antigens on the cell surface of lymphoid cells provides a powerful tool for the study of the precise localization of lymphoid and nonlmyphoid cells in the splenic parenchyma. Furthermore, we will show data on the migration routes of lymphoid cells through the splenic parenchyma towards their specific domains. Finally we will discuss functional aspects of the migration and compartmentalization in the light of the specific immunological potential of the splenic white pulp.

Materials and methods

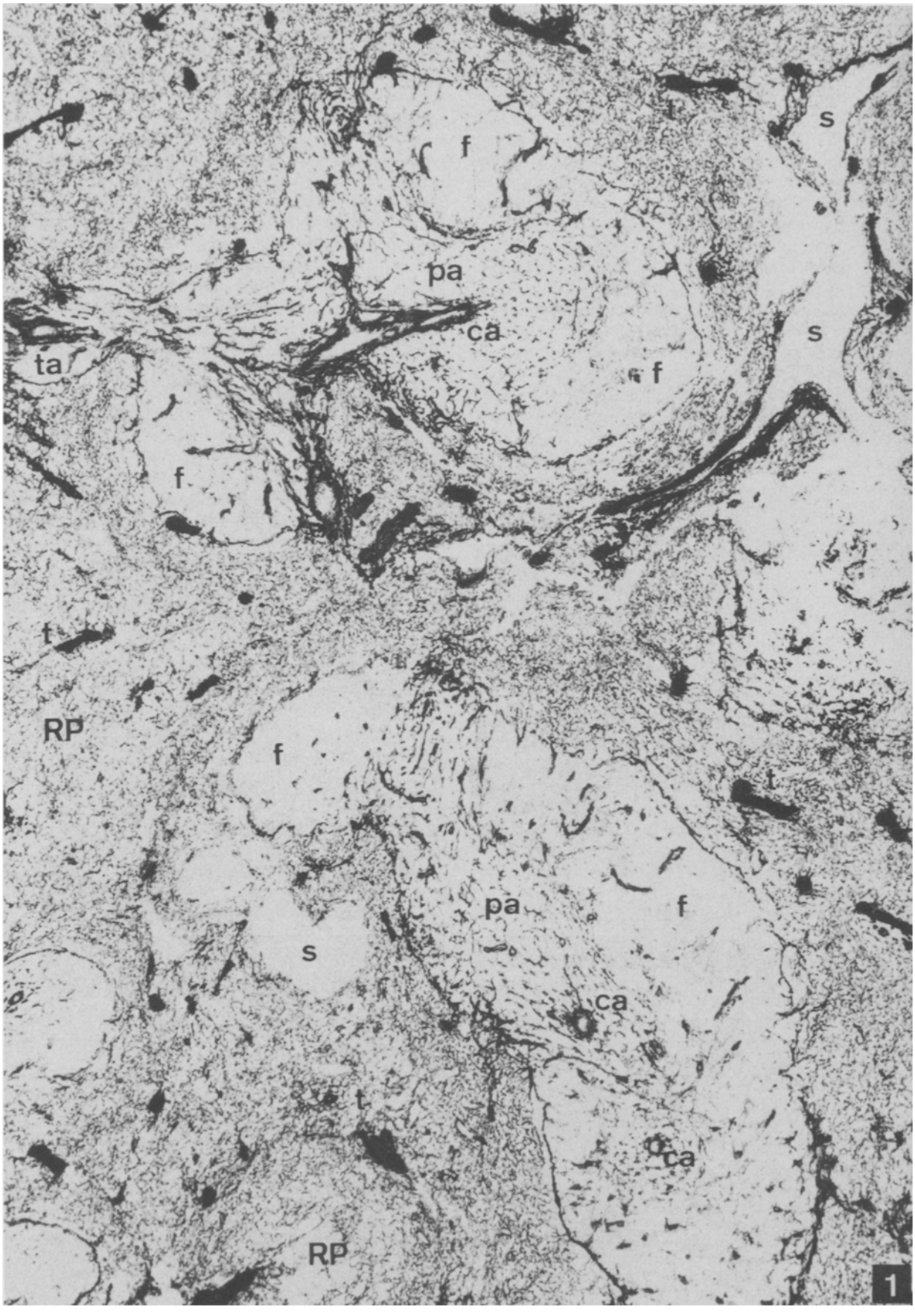
Animals. For the present immunocytochemical studies male or female 10-week-old C3H mice were used. For the autoradiographic studies we used 8–12-week-old highly inbred AO rats. Animals were kept under routine laboratory conditions with free access to food and water.

Antisera. Genetically defined, monospecific antibodies against Thy-1, T-200, Lyt-1 and Lyt-2 determinants were obtained from the tissue culture supernatant of hybrid cell lines. Clone 59AD2.2, secreting anti-Thy-1 antibodies, was the generous gift of Dr J. Haaijman, Department of Genetics, Stanford University, Stanford, CA. Clones 53-7.3.13 and 53-6.72 secreted anti-Lyt-1 antibodies and anti-Lyt-2 antibodies, respectively. These antibodies detect framework determinants and can be used in all strains. Clone 30-G-12 secreted anti-T-200 antibodies. The later clones were obtained from the cell distribution centers of the Salk Institute, San Diego, CA and the Immunobiology Laboratories, Department of Pathology, University of New Mexico. Albuquerque, NM. All clones were originally prepared and characterized by Dr J. A. Ledbetter, Department of Genetics, Stanford University Medical Center[10]. Cells were grown in RPMI tissue culture medium that was supplemented with 10% FCS. Supernatants were harvested, immediately frozen and stored at −70 °C. In addition we used a monoclonal antibody directed to stromal cells in lymphoid organs, clone No. ER-TR7 (Van Vliet et al., in preparation). To demonstrate the binding of these antibodies we used a polyvalent rabbit-antirat antibody conjugated to the enzyme horse-radish peroxidase (RaRa-HRP) (DAKO). For the demonstration of cell surface associated immunoglobulin (sIg) on B lymphocytes we used a polyvalent rabbit-antimouse-Ig peroxidase conjugate (RaM-HRP) (DAKO). All antibodies and antisera were optimally diluted to avoid nonspecific binding.

Tissue preparation for immunocytochemistry. Lymphoid organs were removed from 6–10-week-old mice, collectively embedded in Tissuetek on specimen stubs, frozen on solid carbon dioxide and stored at −35 °C. 6-µm frozen sections were cut on a cryostat (Bright Ltd, Huntingdon, England) and collected on microscope slides precoated with a solution containing 0.1% gelatin and 0.01% chromium potassium sulfate. The tissue was gently fixed by dipping the slide into acetone for 5 sec. Sections were stored at −20 °C for no longer than 1 week before use. Before being incubated with antisera, the sections were soaked in DPBS-FCS-Tw for 30 min to remove the embedding medium. Sodium azide was omitted from the rinsing buffers because this preservative was found to reduce the enzyme activity of horseradish peroxidase. Next, the sections were overlaid with 50 µl of the 1st stage antibody and incubated for 30 min at room temperature. During incubation, the sections were kept in moist chambers to prevent air drying. After rinsing in DPBS-FCS-Tw, the sections were incubated with 50 µl RaRa-Ig-HRP conjugate for 30 min at room temparature. After being rinsed in PBS for 30 min, the conjugate was visualized by incubation of the sections with diaminobenzidene (DAB) (Sigma Chemical, St. Louis, MO) according to Graham and Karnovsky[7]. Next the sections were briefly washed, postfixed in 1% glutaraldehyde in DPBS, dehydrated, and covered with coverslips. Photographs were made with Zeiss plan-apo objectives; the contrast of the image was enhanced with an interference filter at 490 nm.

Collecting cells for migration studies. The procedures for this have been described in detail previously[14]; they are briefly summarized here. Donor rats were cannulated in the abdominal part of the thoracic duct and cells were

Figure 1. Low power magnification of a frozen section of splenic tissue incubated with the monoclonal antibody ER-TR7. This monoclonal antibody reacts with reticular elements in the spleen. The red pulp (RP) contains a fine and dense meshwork of reticular cells. Trabeculae (t) are densely stained, and blood vessels are also manifest. The splenic white pulp is surrounding central arterioles (ca) which branch off in terminal arterioles (ta) and capillaries. The former vessels open in the red pulp or connect to sinuses (s) in the red pulp; the latter open freely into marginal sinuses (ms) which surround the white pulp. The lymphoid compartment in the white pulp is demarcated from the marginal zone by a thin reticular cell layer. Within the white pulp, follicles (f) are present, whereas the periarteriolar lymphoid sheath (pa) is visualized by parallel concentric layers of thin reticular elements. × 100.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collected overnight. Thoracic duct lymphocytes (TDL) consisted of 75% T cells and 25% B cells.

B cells

To study the migration of B-TDL these cells were isolated from normal TDL using a rosetting technique with erythrocytes coated with anti-erythrocyte antibodies and complement (EAC). After rosetting, the cells were spun over a Ficoll-Hypaque gradient. B cells were isolated from the cell pellet by lysing the adhering red cells. Subsequently, isolated B cells were labelled in vitro with ^{3}H-leucine, washed, and injected i.v. into syngeneic recipients.

T cells

No attempt was made to separate T cells from the B cells present among TDL. Instead, we took advantage of the ability of T cells to incorporate ^{3}H uridine about 5–10 times more effectively than B cells. Using standard exposure times (3 weeks) in our autoradiography, this procedure resulted in heavy grain concentration over T cells and light but detectable labeling over B cells. After incubation, cells were washed and injected i.v. into syngenic recipients.

Tissue preparation for autoradiography. Spleen samples were taken from recipient rats at appropriate intervals up to 48 h after injection of labeled cells. Tissues were fixed in 2% glutaraldehyde and processed conventionally. Paraffin sections were cut at 5–7 μm and dipped in Ilford G5 emulsion. After exposure for 3 or 6 weeks at 4 °C the slides were developed, fixed and stained through the emulsion with methyl green pyronin.

Results

In the first part of this section we will describe the various compartments in the splenic parenchyma, and the localization of various subclasses of lymphoid cells within their respective domains. To compare the localization of lymphoid subsets in the white pulp we incubated adjacent frozen sections with monoclonal and polyclonal antibodies. In the second part we will report on the migratory behavior of lymphoid subpopulations towards their specific domains.

A) Immunocytochemical studies

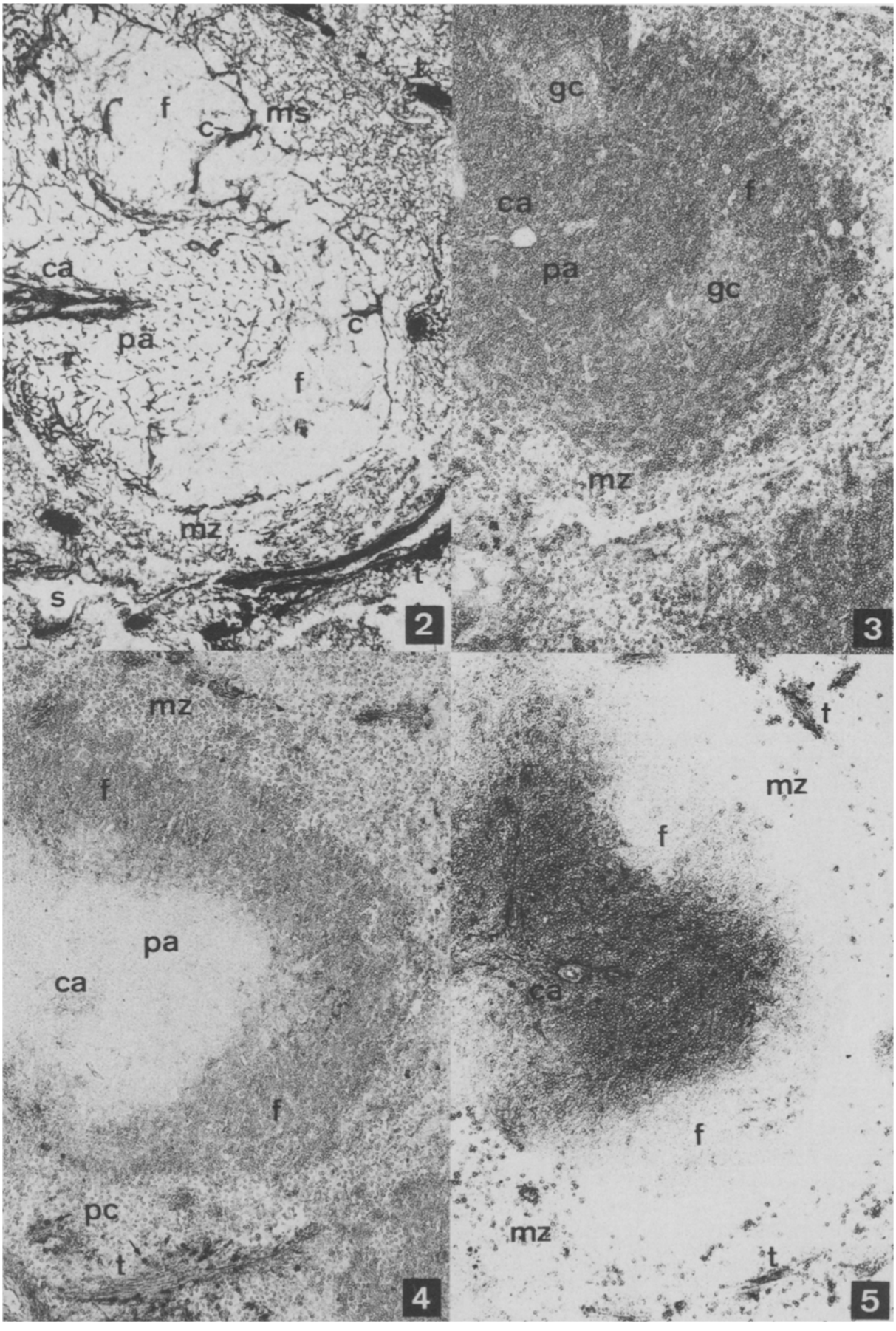
A1) Splenic compartments defined with the monoclonal antibody ER-TR7 (figs 1, 2)

Frozen sections of the spleen incubated with ER-TR7 antibodies show a fine reticular pattern throughout the spleen (fig. 1). This antibody reacts with the reticular framework of lymphoid organs and is most probably directed towards antigens on reticular fibrocytes or their products. Figure 1 shows that the red pulp compartment contains a very fine network of reticular fibrocytes, in between which many trabeculae can be observed. The white pulp compartment shows a clear contrast to the red pulp in its lower incidence of reticular fibrocytes. Sinuses can be noted in the red pulp; frequently they are associated with splenic trabeculae. The lymphoid compartments in the white pulp are clearly demarcated from the red pulp by a dense layer of reticular fibrocytes. These cells are located on the borderline between follicles and marginal zone (fig. 2). Within the white pulp the central arteriole is clearly demarcated and two domains can be recognized based on the reticular staining pattern. First, the staining pattern is relatively dense in the central part of the white pulp, which closely surrounds the branches of the central arterioles (fig. 2). Here, the reticular cells tend to run parallel with branches of the central arteriole and sometimes they appear to be organized in concentric sheaths. The peripheral part of the white pulp is more or less devoid of reticular cells. In this area spheroid accumulations of lymphoid cells can be observed; the lymphoid follicles. These areas are clearly demarcated at the periphery from the adjacent area, the marginal zone, by a dense layer of reticular cells. Small arterioles break away from the central arterioles and traverse the follicles. Most frequently these arterioles extend to capillaries which open freely into the marginal sinus, located at the borderline between follicles and marginal zone (fig. 2). Sometimes these vessels traverse the marginal zone and open directly into sinuses located in the red pulp. Thus, staining of frozen sections of the spleen with these monoclonal antibodies shows that the splenic white pulp in sensu strictu is a highly organized parenchymatous compartment, which is clearly separated from the marginal zone and red pulp.

A2) Localization of lymphoid cells in the splenic white pulp (fig. 3)

Incubation of frozen sections of the spleen with the monoclonal anti T-200 antibody clearly reveals the lymphoid compartments in the spleen (fig. 3). This antibody reacts with a family of cell surface glycoproteins ranging from 180–220 kD which are expressed on T cells as well as B cells. T-200 is expressed at a lower density on the cell surface of germinal center cells. Thus, germinal centers can easily be recognized within the follicles. In addition, this antibody also reacts with a few early hemopoietic cells which are located around trabeculae in the red pulp and beneath the capsule of the spleen (not shown).

Figure 2. Selection of a segment of the white pulp as shown in figure 1. Four areas can be distinguished: a) the periarteriolar lymphoid sheath (pa) containing many fine reticular cells, b) follicles (f), inserted at the periphery of the PALS, c) the marginal sinus (ms) and d) a surrounding marginal zone (mz). Capillaries (c) branch off from the central arteriole (ca) and open freely into the marginal sinus. t = trabeculae, s = red pulp sinus. × 125. Figure 3. Frozen sections of the spleen incubated with monoclonal anti T-200 antibodies (serial section from fig. 2). This antiserum reacts with T cells as well as with B cells and some hemopoietic cells in the red pulp. Germinal center cells (gc) express this antigen at a lower density. ca = central arteriole, pa = periarteriolar lymphoid sheath, f = follicles, gc = germinal centre, mz = marginal zone. × 125. Figure 4. Frozen section of the white pulp incubated with a polyclonal RaM-Ig-PO conjugate. This conjugate reacts with surface Ig molecules on B lymphocytes in follicles (f) and marginal zone (mz) as well as with cytoplasmic Ig in plasmacells (pc). pa = periarteriolar lymphoid sheath, t = trabeculae, mz = marginal zone. This section is serial to the section presented in figure 3. × 125. Figure 5. Serial frozen section incubated with a monoclonal anti-Thy-1 antibody. T cells accumulate in the PALS (p). A few T cells are present in follicles (f) and in the marginal zone (mz). The weak staining of the B cell domains is considered to be background staining of B cells with the RaRa-Ig-PO conjugate. ca = central arteriole, p = periarteriolar lymphoid sheath, f = follicle, mz = marginal zone, t = trabeculae. × 125.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A3) Localization of B lymphocytes in the splenic white pulp (fig. 4)

To localize B cells, frozen sections of the spleen were incubated with a RaM-Ig-PO conjugate. This conjugate reacts with Ig molecules present on the cell surface of B lymphocytes and in the cytoplasm of antibody-forming plasma cells. Figure 2 shows that B lymphocytes localize in the follicles at the peripheral part of the white pulp; they are also located at a lower density in the marginal zone, and a few individual B cells are observed in the red pulp. Comparison with figure 1 shows that B cells mainly locate in areas which are devoid of reticular fibroblasts. Comparison of figure 4 and figure 3 also shows that B cells are the major lymphoid subpopulation in the spleen. From flow-cytometric studies we know that the spleen contains ± 40–50% B cells and 25% T cells.

A4) Localization of T cells and subsets of T cells in the splenic white pulp (figs 5–7)

T cells in the spleen can be identified by the monoclonal antibody 59-AD-22, which detects the Thy-1 molecule, expressed on all T cells. Frozen sections incubated with this antibody show that T cells are localized in the central part of the white pulp (fig. 5). Comparison of figure 5 and figures 1 and 2 reveals that T cells are predominantly located in the white pulp areas which contain a fine meshwork of concentric reticular cells, i.e. the periarteriolar lymphoid sheaths. In addition, a few individual T cells can be observed in B cell domains (compare fig. 5 with fig. 4), as well as in the marginal zone and red pulp. The general weak staining of B cell domains by the anti-Thy-1 antibody is regarded as non-specific. The two major subclasses of T cells, i.e. T helper cells and T suppressor/cytotoxic cells can be distinguished from each other by the allelic Lyt markers[3]. Thus, T helper cells express Lyt-1 antigens at a high level, whereas T suppressor/cytotoxic cells strongly express Lyt-2 antigens. However, the anti Lyt-1 reacts with two subsets of T cells, namely the Lyt-1 'only' cells (T helper lymphocytes) and the Lyt-1,2+ subclass which is considered as a virgin T cell which may differentiate to a Lyt-1+ve or a Lyt-2+ cell[13,17]. However, the latter cell population expresses Lyt-1 antigens at a lower density than the Lyt-1 'only' cells. Lyt-1 cells are distributed throughout the central part of the splenic white pulp, the PALS (fig. 6). Close inspection of figure 6 also reveals Lyt-1 'bright' and Lyt-1 'dull'

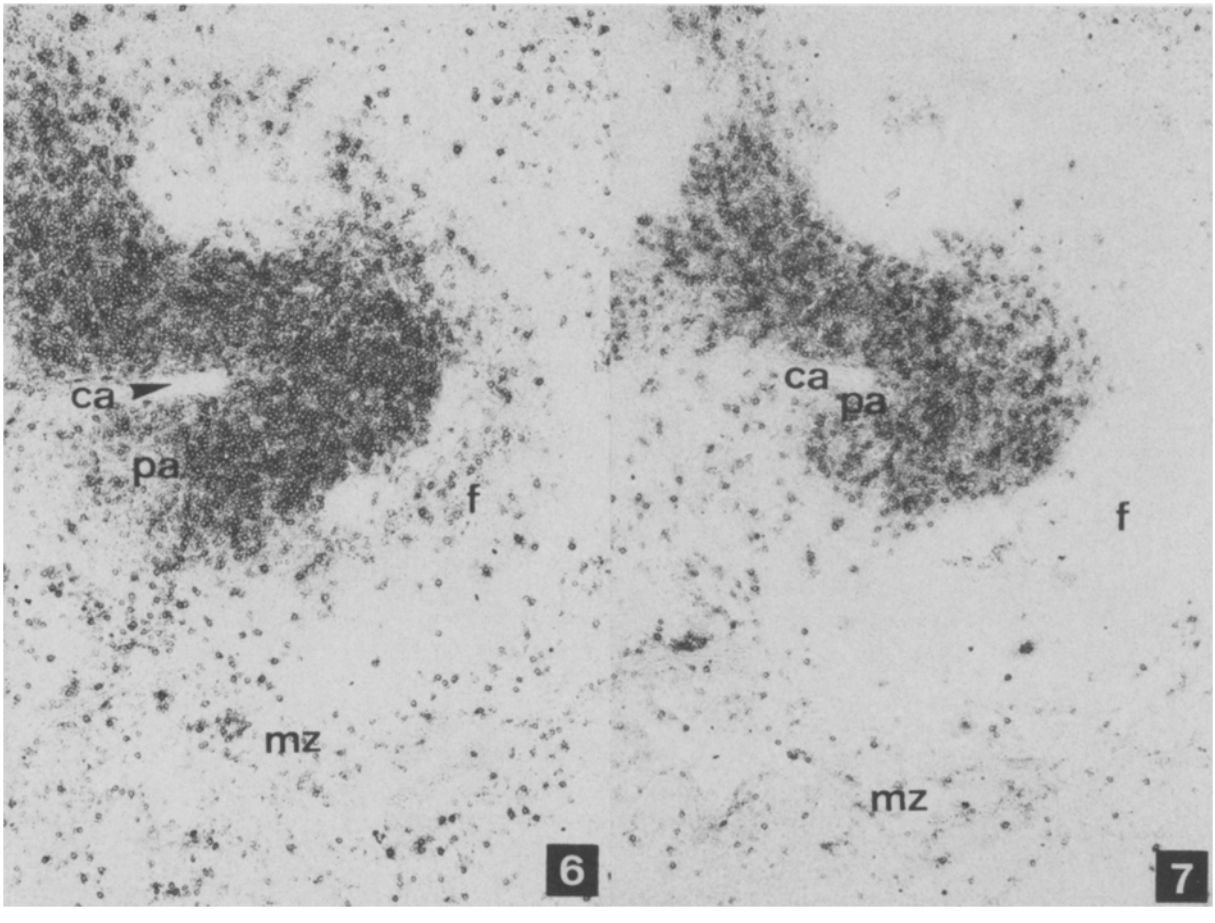

Figure 6. Serial frozen section of the spleen, incubated with monoclonal anti Lyt-1 antibodies. Lyt-1+ve cells accumulate in the periarteriolar lymphoid sheath (pa). Note also their presence in B cell domains. These cells are Lyt-1 'only' cells, since Lyt-2+ve cells are not observed in this area (compare with fig. 7). f = follicle, mz = marginal zone, ca = central arteriole. × 125.

Figure 7. Serial frozen section of the spleen, incubated with monoclonal anti-Lyt2 antibodies. Lyt-2+ve cells exclusively occur in T cell domains. A few Lyt 2+ve cells are seen in the marginal zone. ca = central arteriole, f = follicle, pa = periarteriolar lymphoid sheath, t = trabecula. × 125.

cells, the former population being Lyt-1 'only' cells, the latter probably being Lyt-1,2+ cells[11]. T helper cells are not only located in T cell domains, they are also located in B cell domains (fig. 6), in the marginal zone and in the red pulp.
Lyt-2+ve cells are also located in the PALS (fig. 7). Comparison of figures 6 and 7 shows that Lyt-2+ve cells occur at a lower frequency in the splenic white pulp than Lyt-1+ cells. Furthermore, it may be noted that Lyt-2+ve cells, in contrast to Lyt-1+ve cells, do not occur in B cell domains. They do also occur at a low frequency in the marginal zone and the red pulp.

B) Migration studies

B1) B cell migration pathways (figs 8a, 8b and 9a)
As can be seen from figure 9a, i.v. injected B cells eventually, by 24 h after injection, localize in the lymphocyte corona of the follicular compartments of the white pulp. Only a few labeled cells entered into germinal centres and no labeled cells were found in the marginal zone. The PALS was also devoid of labeled cells. With the exception of the marginal zone this picture correlates nicely with the pattern of B cell localization as shown in figure 4. Figure 9a shows the location of a population of B cells at a certain time after i.v. injection. To obtain information on the migration route of B cells, spleen samples were studied at short intervals after injection of labeled cells. As early as 15 min after injection, large numbers of labeled cells were detected in the marginal zone surrounding the white pulp, where they were especially associated with follicular structures. Much to our surprise, by 1 h after injection, labeled cells had not completed the expected short journey directly into the lymphocyte corona, but were now located at the periphery of the lymphocyte sheaths and had virtually left the marginal zone (data of 15-min and 1-h intervals not shown here). By 3 h labeled cells were concentrated in the outer zone of the PALS with a tendency to accumulate towards the junctional area at a base of the follicles (fig. 8a). By 6 h after injection, most of the labeled cells were at the junctional region between the PALS and the lymphocyte corona. Many had already entered the adjacent part of the corona (fig. 8b). By 18 h most labeled cells were evenly distributed throughouth the lymphocyte corona, and hardly a labeled cell was present any more in the PALS or the marginal zone. A few labeled cells were now also present in germinal centres. This picture persisted for at least 48 h after injection.

B2) T cell migration pathways (fig. 9b)
As for B cells, the localization pattern for T cells 24 h after injection is compatible with the above described presence of T cells in the PALS as shown using monoclonal antibodies (see figs 5–7). There is, however, one exception; labeled T cells could not be detected in follicular areas, in contrast to the demonstration of a T cell subset (T helper cells) in that compartment using the anti-Lyt-1 antibody.
To visualize the kinetics of lymphocyte migration – as for B cells – localization patterns at shorter intervals after injection were studied. Like B cells, T cells were found to enter the spleen via the marginal zone (15 min). Subsequently, this area was cleared rapidly and T cells concentrated around terminal arterioles and at the periphery of the PALS. By 1 h, T cells had already penetrated towards the central region of the larger PALS. Lightly labeled B cells could not be detected among the predominant heavily labeled T cells. At later intervals (3 and 6 h after i.v. injection), T cells were still present in large numbers in the central areas of the PALS, whereas in the junctional area between the PALS and the lymphocyte corona a population of lightly labeled (B) cells was found segregating from the heavily labeled T cells and moving into the corona. By 24 h, lightly labeled B cells were observed evenly distributed over the lymphocyte corona, whereas heavily labeled T cells were still present in the central PALS area, though in smaller numbers than at previous times.

Discussion

The present paper shows that the splenic white pulp is a highly organized lymphoid compartment which is clearly demarcated from the surrounding marginal zone and red pulp. The basis of the structure of the white pulp is most probably formed by a meshwork of reticular cells which surrounds and envelops the splenic white pulp. Within this compartment T and B cells each have their separate domains. Our previous migration studies[14] show that T and B cells migrate towards their domains along different routes. In general it may be concluded that all lymphoid cells enter the spleen by the route through the marginal zone. From here T and B cells enter the white pulp.
T and B domains are not totally exclusive domains for T, respectively B lymphocytes. In the present paper we show that T cells also reside in B cell domains. In a previous paper we have shown that B cells may also occur at the border of T cell domains[19]. It is noteworthy, however, that only a subpopulation of T cells localizes in B cell domains. We showed that only Lyt-1+ve T helper cells are present in follicular areas, whereas Lyt-2 cells are virtually absent from B cell domains. This observation confirms and extends previous observations of our group and others[11,20].

Why do only Lyt-1+ve cells occur in B cell domains, whereas Lyt-2+ve cells are not seen in this area? One likely explanation for this observation could be that Lyt-1+ve cells in B cell domains are involved in triggering B cells during an immune response to proliferate and differentiate towards antibody forming plasma cells or towards B memory cells. Another explanation would be that Lyt-1+ve and Lyt-2+ve cells use different migration pathways towards their specific domains. However, at present there is no firm evidence to support this latter hypothesis.
The present paper combines two techniques. Using one of them, at a particular moment the whereabouts of every T and B cell present in the spleen is established, whether recently arrived and still migrating, or 'at home' in its specific domain. Using the other method we can follow, as it were, a wave of newly arrived T and B cells as part of the total lymphocyte population;

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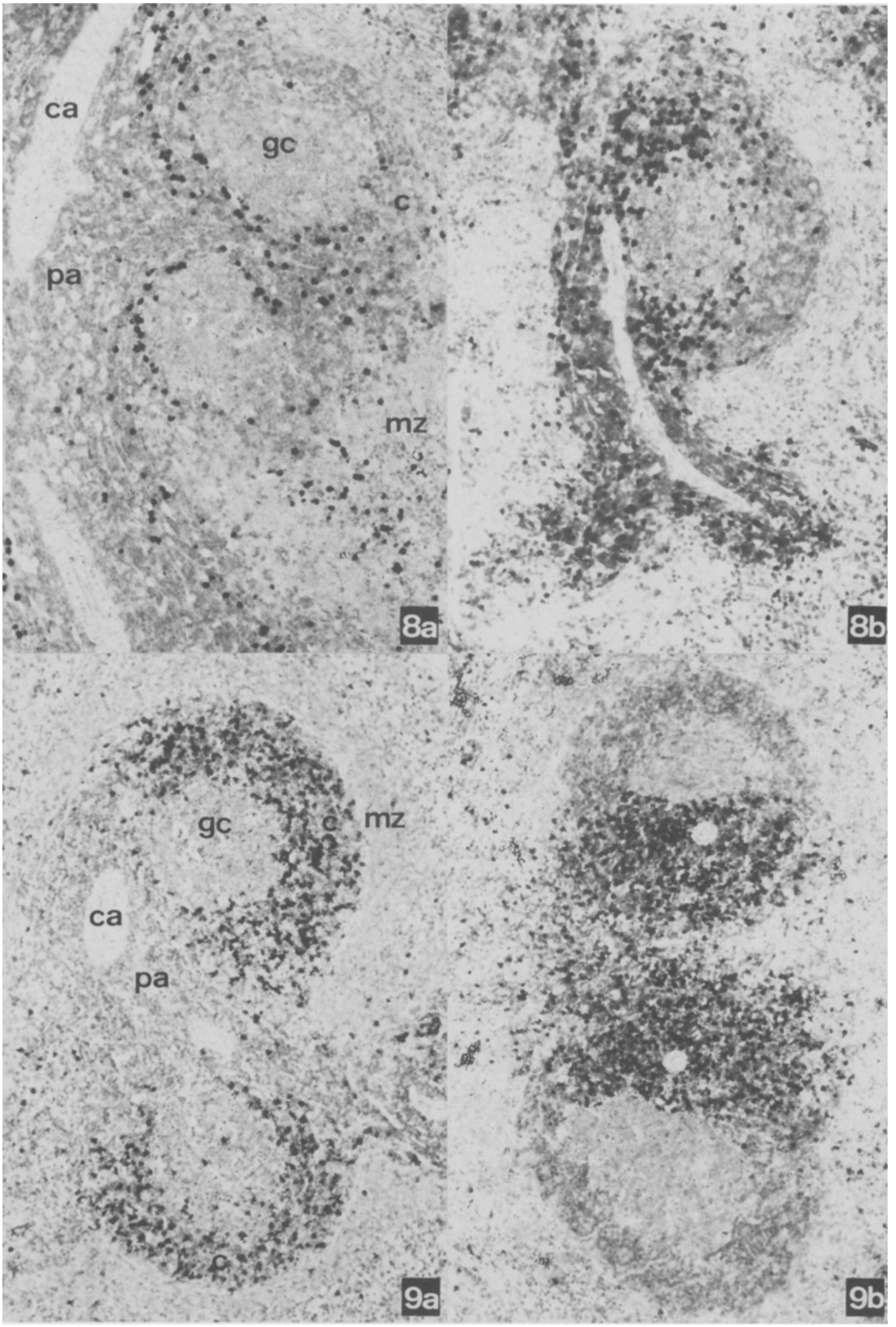

sweeping through the infrastructure of the white pulp, mixing with the other cells around, and eventually reaching their temporary destination, the central PALS or the lymphocyte corona (see also fig. 10). The latter technique, at each particular interval, can only present part of the total picture as obtained with the former technique. However, the addition of these partial pictures should eventually show the same pattern of distribution as is seen at one moment in time using monoclonal antibodies. Two discrepancies, however, merit further discussion: 1) as discussed above, only Lyt-1+ve cells are found in follicular structures in contrast to Lyt-2+ve cells, which are restricted to the PALS. Surprisingly, in our migration studies, T cells were not found to enter follicular structures, at least not over the period observed, i.e. up to 48 h after injection of labeled cells. One possible explanation would be that the inoculum (normal TDL) does not contain this particular subset (T helper cells), but this is not so; another is that the cells detected in follicular structures, although expressing the Lyt-1 antigen, are not T helper cells. Indeed, there is recent evidence that the Lyt-1 marker is not exclusive for T cells[20]. 2) Ig+ve cells were detected all over the nonthymus dependent areas, i.e. germinal centers, lymphocyte corona and marginal zone, but radiolabeled B cells (EAC rosette-forming cells) were not found to enter germinal centers to a significant extent. This might mean that either our population of B cells differs from the B cells as present in germinal centers, or that among TDL only very few germinal center seeking cells are present. In other experiments the presence of germinal center precursor cells among TDL, however, has been demonstrated[15].

T and B cells have been shown to enter the spleen via the same route, i.e. via the marginal zone, and, rather unexpectedly, B cells, at least for some time, were found to follow the same route as T cells, moving through the marginal zone, along the terminal arterioles and at the periphery of the PALS. Only at this point in time do T cells begin to segregate from B cells by penetrating further into the central PALS, whereas B cells, skirting along the T cell area, continue towards the base of the follicle to segregate themselves eventually from the T cells to enter the lymphocyte corona (see fig. 8). As the route for B cells from marginal zone to lymphocyte corona follows this rather devious pattern instead of their directly moving centripetally into the follicular structure, it is tempting to speculate upon this rather enigmatic behavior as being meaningful with regards to the immunological function of T and B cells cooperating in reaction against blood-borne antigens entering the spleen.

What is the series of events during an immune response generated in the splenic white pulp? Earlier studies from our group[18] have shown that i.v. injection of sheep red blood cells (SRBC) in mice leads to trapping of this antigen in the red pulp but also in the marginal zone. 48 h after immunization, SRBC containing macrophages were found within the white pulp especially in the peripheral part of T cell domains. From studies of van Rooyen[22] and from recent studies performed by Kraal et al. (personal communication) we now know that the marginal zone is a major site of antigen trapping. This region contains specialized macrophages, so-called marginal metallophyls, and upon LPS stimulation these cells have been shown to migrate into the white pulp. These cells can be identified with a new monoclonal antibody from our laboratory (clone No. ER-TR9, van Vliet et al., in preparation). The observations mentioned so far all point to the phenomenon that upon i.v. antigenic stimulation, the antigen is picked up from the marginal zone by a subpopulation of mononuclear phagocytes and transported into the white pulp. We assume that these antigen-containing macrophages process and expose antigen to surrounding lymphoid cells. Thus, SRBC, being a thymus-dependent antigen is phagocytosed and transported to the T cell domain. Here, T cells are located around so-called 'interdigitating cells' (IDC). These cells are involved in the generation of antigen-reactive T helper

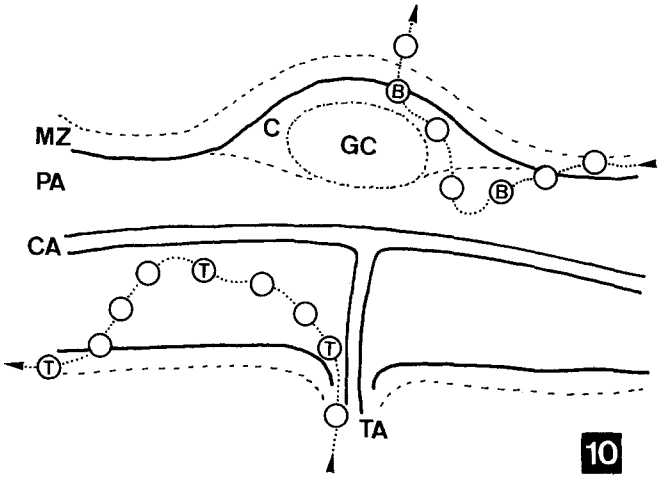

Figure 10. Diagram representing comparative migration of B and T cells in the spleen white pulp. The central arteriole and its sheath are represented in longitudinal section. MZ = marginal zone, PA = peripheral arteriolar lymphoid sheath, CA = central arteriole, TA = terminal arteriole, C = lymphocyte corona, GC = germinal center, T = T lymphocyte, B = B lymphocyte.

Figure 8a. Rat spleen 3 h after the i.v. injection of ^{3}H-leucine labeled B cells. Note labeled cells still mainly at the periphery of PALS and accumulating towards base of follicular structures. ca = central arteriole, pa = PALS, gc = germinal center, c = lymphocyte corona, ma = marginal zone. × 16 obj., × 8 oc. Figure 8b. Rat spleen 6 h after the i.v. injection of ^{3}H-leucine labeled B cells. Note labeled cells entering lymphocyte corona at junctional zone between PALS and follicle. × 10 obj., × 8 oc. Figure 9a. Rat spleen 24 h after the i.v. injection of ^{3}H-leucine labeled B cells. Labeled cells are now evenly distributed over the lymphocyte corona of the follicular structures. ca = central arteriole, pa = PALS, gc = germinal center, c = lymphocyte corona, mz = marginal zone. × 10 obj., × 10 oc. Figure 9b. Rat spleen 24 h after the i.v. injection of ^{3}H-leucine labeled normal TDL. Heavily labeled T cells in PALS. × 10 obj., × 10 oc.

Figure 8 is reproduced from Cell. Immun., *23* (1976) 254–267, and figures 9a and 9b from Malignant Lymphoproliferative Disease (ed. J. G. v.d. Tweel), 1980, p. 3–12, with kind permission of the editors.

cells[21]. We assume that the antigen-containing macrophages together with IDC create a microenvironment which activates T helper cells. Indeed, blast formation in T cell domains is the first morphological sign of responsiveness in the splenic white pulp[15,18]. Activation of T cells is a prerequisite for the activation of antigen-reactive B lymphocytes[1,4].

Where do the first immunoglobulin-containing plasmablasts occur in the white pulp? Already in 1963 Langevoort[9] observed that initial blast formation occurred within the T domains and not in the B domains. Recently we have confirmed this observation with immunofluorescence studies. Thus, the first Ig-containing plasmablasts are observed at the border of B and T cell domains[18]. During a progressive immune response, plasmablasts accumulate in the PALS and leave the white pulp along the thin reticular sheaths surrounding terminal arterioles. Finally, large accumulations of plasmacells may be found in the red pulp in close proximity to trabeculae and sinuses.

What is the explanation for the presence of Ig-containing plasmablasts in T domains? In the normal unstimulated animal, and also in germ-free animals, B cells have been shown to be present at a low frequency in T domains. Autoradiographic studies[14,19] (this paper) point out that these cells are migrating cells. We speculate that these (recirculating?) B cells are antigen-reactive cells which, passing through T cell domains, obtain the stimuli which they need to differentiate into antibody forming cells. Both antigen-containing macrophages and antigen-activated T cells are present to fulfill these criteria.

Which factors govern the ultimate homing of lymphoid subpopulations into their respective domains? At present there is no clue to the answer to this question. There is evidence that the selective migration pathways are indicated by specific cell surface determinants of nonlymphoid cells. Recirculating cells in the lymph nodes, for example, show a receptor for specific determinants on the cell surfaces of the lining endothelial cells in high endothelial venules[2]. It may well be that similar molecules expressed on the cell surfaces of cells which line the white pulp compartment are present, and regulate the migration of lymphoid cells into the white pulp. Further migration into the splenic white pulp and toward T cell domains might be guided by reticular fibroblasts, and chemotactical stimuli might also direct the migration of lymphoid cells. Ultimate homing in the domain might be caused by cell-cell interaction with specific nonlymphoid cells, such as the interdigitating cells in T cell domains[23] and the dendritic reticular cells in B cell domains[8].

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