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THE ROLE OF THE SPLEEN IN LYMPHOCYTE MIGRATION

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Abstract

In all species studied so far it was found that more lymphocytes migrate to the spleen than to all the lymph nodes together. Specific molecules on lymphocytes and endothelial cells regulate their entry into lymph nodes, but none of the known molecules play a role in homing to the spleen. The splenic compartments, comprising the red pulp, marginal zone, periarterial lymphatic sheath (PALS) and follicles, all show different kinetics for migrating lymphocytes. By combining ^{51}Cr lymphocyte labeling with morphometry and two color immunohistochemistry, the migratory route of lymphocyte subsets can be followed through the spleen and absolute numbers of lymphocytes calculated in each compartment. T lymphocytes home preferentially to the PALS and B lymphocytes home not only to follicles but also in large numbers to the marginal zone and red pulp. $CD4^+$ and $CD8^+$ lymphocytes migrate similarly at early time points, but at 24 hours $CD4^+$ lymphocytes are preferentially found in the PALS with $CD8^+$ lymphocytes in the red pulp and marginal zone. The functional significance of the different routes of lymphocytes through the spleen has yet to be defined in relationship to specific immune functions and regulatory factors on splenic lymphocyte homing.

KEY WORDS: lymphocytes, migration, recirculation, spleen, splenic compartments, homing receptors, immunohistology

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Introduction

Lymphocytes are distributed throughout the body and are not only found in the lymphoid organs. Despite this disperse localization there are no "spleen lymphocytes" or "lymph node lymphocytes". Instead there is a continuous traffic of many lymphoid cells from one organ to another, combining to form the lymphoid system. The blood vessels and the lymphatics serve as traffic routes between the different organs. This continuous movement of lymphocytes is an essential part of the immune response to specific antigens all over the body, as the specifically primed lymphocytes can meet their antigen or an antigen-presenting cell in different organs and start an effective immune response. At any given time only about 2% of all lymphocytes are found in the blood compartment, but due to their short mean transit time through the blood this pool is exchanged about 50 times per day (for review see Pabst, 1988a). It has been known for quite a long time that lymphocytes leave the blood in lymph nodes by adhering to and then emigrating via specialized high endothelial venules (HEV) in the paracortex of lymph nodes (Gowans & Knight, 1964). In recent years a lot of effort has been put into characterizing the interaction between lymphocytes and HEV (for review see Jalkanen et al., 1986). The spleen has attracted much less interest, although it too plays an important role. Recently, splenic function in health and disease has been summarized in a monograph (Bowdler, 1990).

The aims of this brief review are 1) to define the role of the spleen in lymphocyte migration in quantitative terms, 2) to compare homing to the spleen with homing to organs with HEV, e.g., lymph nodes, and 3) to describe a combination of techniques for quantitating the localization of lymphocyte subsets on their way through the splenic compartments. In addition, the influence of different techniques on the results will be mentioned as well as future fields of research.

So far most scanning electron microscopy data on the spleen has dealt with red cells in the spleen and the question of whether there is an "open" or "closed" circulation in the red pulp, vascular casting was used in these studies (Barnhart & Lusher, 1976; Fujita et al., 1982; Groom et al., 1991; Suzuki et al., 1977; Weiss, 1974). Scanning electron microscopy might make a

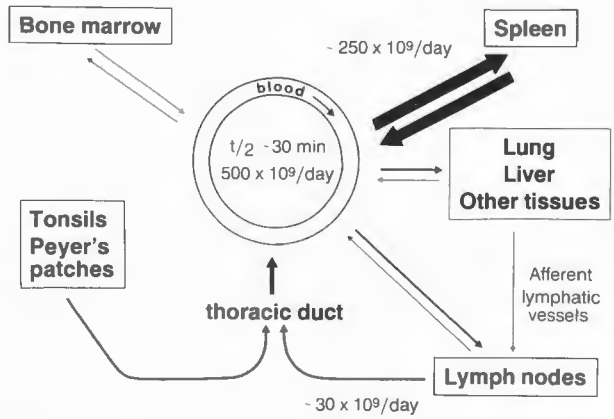

Fig. 1 Schematic drawing of the different routes of migrating lymphocytes between the blood and different organs. The width of the arrows indicates the numbers of lymphocytes involved. The data for young adult man are derived from the literature (modified from Pabst, 1988b).

significant contribution to future experiments on the role of the spleen in lymphocyte migration.

The Spleen Outnumbers All Lymph Nodes With Respect to Numbers of Recirculating Lymphocytes Per Day

Many different approaches and techniques have been used in several species to show that the spleen plays an important role in lymphocyte homing. The published evidence will be summarized briefly.

One traditional way of defining the function of an organ is to remove it and look for differences compared to the normal state. The mean blood residence time of lymphocytes has been compared in normal and splenectomized rats (Whitelaw & Batho, 1972), pigs (Pabst & Trepel, 1975) and man (Christensen et al., 1978). A consistent finding was that the residence time of lymphocytes in the blood was prolonged several times after splenectomy. When the spleen was selectively labeled by a short extracorporeal perfusion, large numbers of labeled lymphocytes were found one day later distributed throughout other lymphoid and non-lymphoid organs (Pabst & Binns, 1981).

Another approach was to destroy lymphocytes migrating through the spleen. If most of the lymphocytes in the spleen were non-migratory, resident cells, this would reduce the lymphocyte content of the spleen but have no effect on other organs. Either ^{32}P-impregnated polythene strips were attached to the rat spleen or a ß-emitting colloid was injected into it. Both these techniques of selective local irradiation resulted in a profound fall in the output of lymphocytes from thoracic duct fistulas as well as severe lymphopenia (Ford, 1968; Roser & Ford, 1972). In several species IV injected labeled lymphocytes were traced to lymphoid and non-lymphoid organs. A consistent finding was a very high number of lymphocytes in the spleen (up to ~40% of the injected dose 1 hour later). This is much more than in all lymph nodes (for reference see Pabst, 1988a).

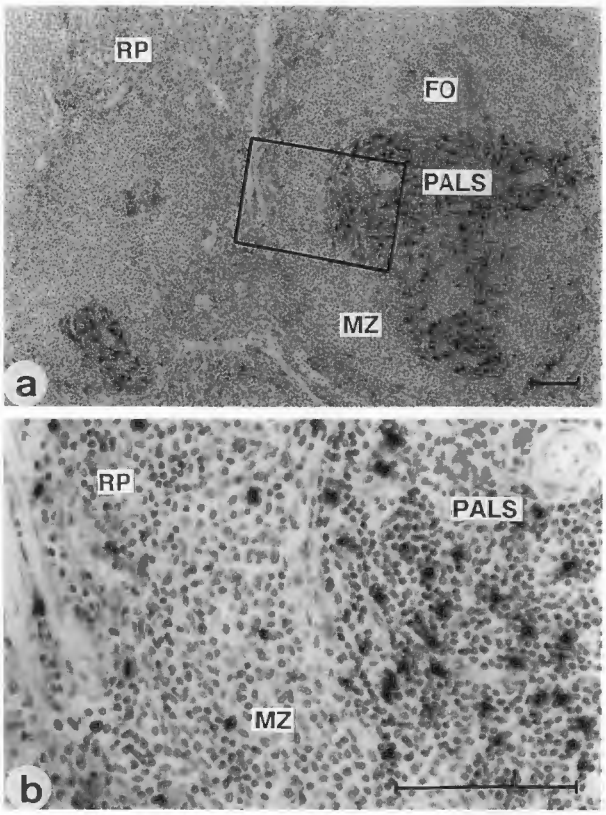

Fig. 2 Immunohistology of a rat spleen 6 hours after the injection of FITC labeled thoracic duct lymphocytes (Willführ et al., 1990). Immigrated lymphocytes were identified by an antibody against FITC and the peroxidase reaction. a. overview (bar = 100 µm), b. higher magnification (bar = 100 µm). RP = red pulp, FO = follicle, PALS = periarteriolar lymphatic sheath, MZ = marginal zone.

Finally, some in vitro experiments should be mentioned: when isolated spleens were perfused in vitro, the number of lymphocytes released in rats was about 80 x 10^6/h or equivalent to twice the thoracic output (Ford, 1969) and in pigs ~10 x 10^9/h or equivalent to more than 25 times the circulating blood pool (Pabst & Trepel, 1975).

It can be concluded from all these different approaches that the spleen plays a predominant role in migration, as it is an organ where lymphocytes leave the blood and to which they return after a certain transit time (Fig. 1).

Different Mechanisms Regulate Lymphocyte Homing to Lymph Nodes and to the Spleen

In recent years many adhesion molecules, often called "homing receptors", have been defined on the lymphocyte surface (for review see Butcher, 1990; Stoolman, 1989; Yednock & Rosen, 1989), although many of these are not "receptors" in its strictest sense. Lymphocyte subsets differ in their expression of homing receptors. This seems to be important in the adhesion to HEV in vivo and also in the HEV adhesion test performed in vitro (Willführ et al., 1990). The in vitro

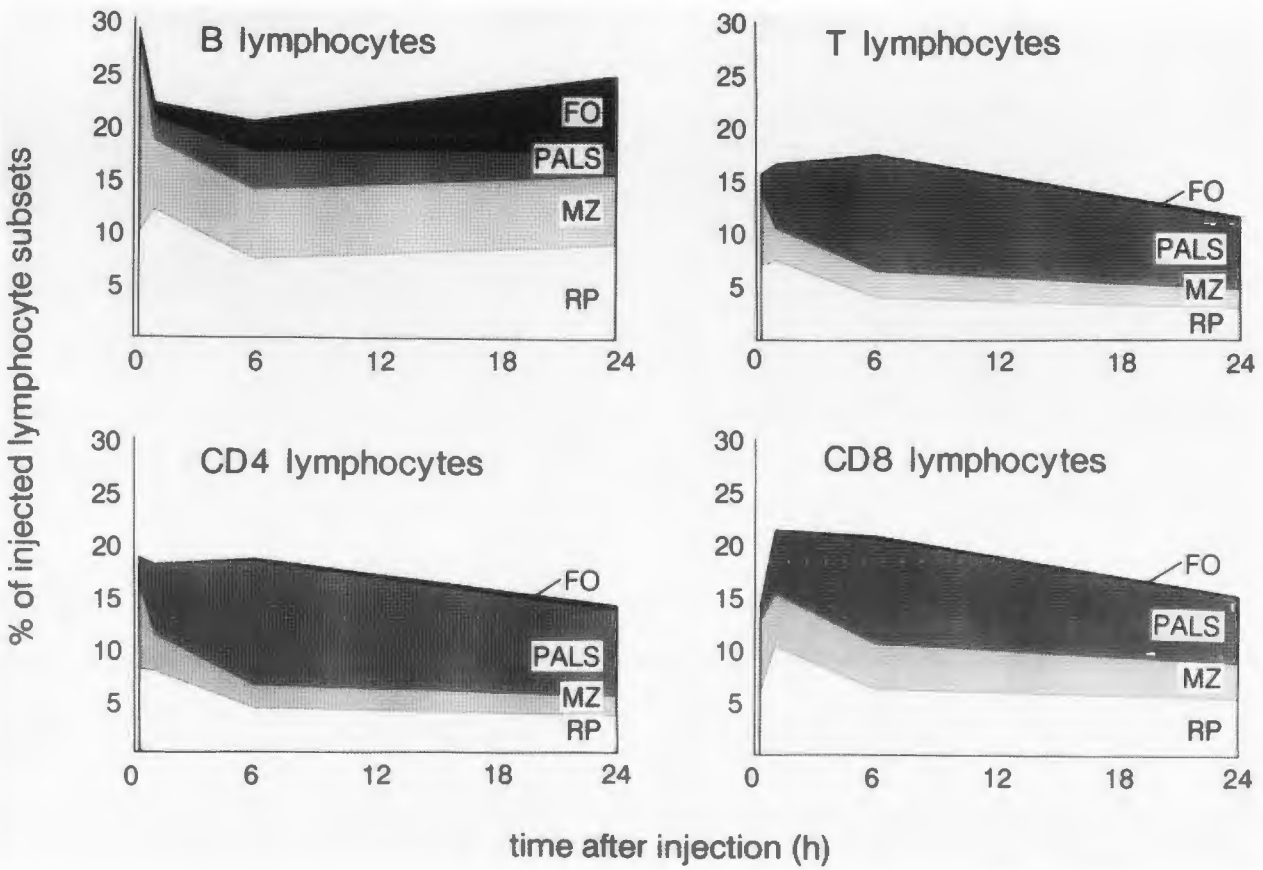

Fig. 3 The number of immigrated lymphocytes in each splenic compartment at different time points, expressed as the percentage of all injected cells of the different subsets respectively (Willführ et al., 1990). Monoclonal antibodies against rat T, B, CD4 and CD8 lymphocytes were used. RP = red pulp, FO = follicle, PALS = periarteriolar lymphatic sheath, MZ = marginal zone.

adhesion test does not work for splenic sections, nor does the blocking of homing receptors have any effect on splenic homing in vivo. The endothelial cells of the HEV also express specific molecules, which play a role in the interaction between lymphocytes and HEV. These "vascular addressins" have been demonstrated on HEV in lymph nodes and Peyer's patches, but are not expressed on any vessels of the spleen. When lymphocytes are incubated with trypsin in vitro and then reinfused, their migration to organs with HEV is dramatically reduced but migration to the spleen remains normal (for review see Pabst & Binns, 1989). When glucocorticoids are injected in rats, reduced numbers of lymphocytes are recovered from lymph nodes and from the blood. Normal numbers of lymphocytes, however, are found in the spleen (Cox & Ford, 1982).

All these experiments do not exclude that there might be "homing receptors" on lymphocytes necessary for entering the spleen or "vascular addressins", but these will definitely be different from the well defined receptors important for lymph node homing. Thus, in this respect the spleen again seems to be unique.

The Splenic Compartments Play Different Roles in the Migration Kinetics of Lymphocyte Subsets

When the migration of lymphocytes and of specific subsets are studied and the data compared to other experiments, a number of technical aspects should be kept in mind to avoid misinterpretations. The source of lymphocytes to be labeled differs between studies: either lymphocytes from the blood, lymph or from cell suspensions have been used. These not only differ in their subset composition, but lymphoid organs also always contain lymphocytes which would not migrate under normal circumstances (e.g., lymphocytes from germinal centers). Often lymphocytes have been enriched by gradients or prepared by enzyme treatment or stored in liquid nitrogen before injection (Bloemena et al., 1990). Even more steps have often been used to study the migration pattern of lymphocyte subsets: the subsets have been enriched by killing other lymphocytes using antibodies and complements, by panning or treatment of donor animals (e.g., thymectomy - irradiation followed by bone marrow reconstitution). These preparation steps might influence the migratory capacity of the lymphocytes. For identification, the injected cells have to be labeled with a radioactive marker, such as ^{51}Cr, ^{111}In, ^{3}H-UdR or a fluorescent dye, e.g. fluorescein isothiocyanate (FITC). The label itself or the labeling procedure can also influence the lymphocyte

migration pattern.

Finally, most studies demonstrated immigrated lymphocytes in certain splenic compartments, but did not try to quantitate the total number or relate the data to the size of the compartment. The outline of the splenic compartments can be precisely defined by using monoclonal antibodies against T or B lymphocytes and macrophage subsets in immunohistochemistry, as demonstrated for the rat spleen at different ages (Westermann et al., 1988). Despite a number of studies using autoradiography to follow the route of lymphocytes through the spleen (Brelinska & Pilgrim, 1982; Carroll & de Sousa, 1984; van Ewijk & Nieuwenhuis, 1985; Mitchell, 1973; Nieuwenhuis & Ford, 1976) and the extremely laborious and detailed studies of Pellas and Weiss (1990a, b), there are still some open questions on the route of lymphocyte subsets through the spleen.

To keep the influence of such techniques as described above to a minimum, the following procedure is recommended: several techniques should be combined, recirculating cells used and cell enrichment procedures for lymphocyte subsets avoided (Willführ et al., 1990). The lymphocytes can be characterized after migrating to the different splenic compartments by double staining immunohistochemistry. The following techniques have been successfully combined: a) using ^{51}Cr labeling the total number of lymphocytes which migrated to the spleen at different time points are determined, b) morphometry of the splenic compartments is performed on splenic sections stained with a monoclonal antibody against B lymphocytes, which nicely outlines the compartments, c) lymphocytes are labeled with FITC and identified on cryostat sections of the spleen by an antibody against FITC, and d) in a second step, monoclonal antibodies against surface molecules of lymphocyte subsets are applied (Fig. 2). The combination of these techniques measured absolute numbers of lymphocyte subsets in the major compartments of the rat spleen at 15 min, 1, 6 and 24 hours. Morphometric analysis of the splenic compartments in SPF rats revealed the following proportions: red pulp 55.4 ± 4.3%, marginal zone 27.6 ± 3.1%, follicles 5.4 ± 0.7% and PALS 11.1 ± 2.8%. The most interesting results of this study (Fig. 3) were that: a) each compartment showed different migration kinetics for all lymphocyte subsets, b) the number of lymphocytes in the red pulp was unexpectedly high at all time points studied and for all lymphocyte subsets, c) not only the typical T cell zone (the periarteriolar lymphatic sheath (PALS) or B cell zone (follicles) were populated by the respective immigrants but other compartments as well, d) $CD4^+$ and $CD8^+$ immigrated lymphocytes hardly differed in their localization initially, but at 24 hours more $CD8^+$ cells were in the red pulp and more $CD4^+$ cells in the PALS, and e) the marginal zone was not the only entry site into the spleen but even at 1 d considerable numbers of labeled lymphocytes were found in this compartment.

In a recent, very detailed study in the mouse Pellas & Weiss (1990a,b) described lymphocytes entering the spleen preferentially at what they called the "distal PALS", which consists of only a few layers of lymphocytes around an arteriole and extends into the red pulp. From this entry site lymphocytes migrated in a longitudinal direction into the classical "proximal PALS". It has yet to be shown whether this is a typical feature of the mouse or also true for other species.

It has to be stressed that the size of the different compartments of lymphoid organs largely depends on age (e.g., until the age of about 2 years there is no marginal zone in the human spleen (Timens et al., 1989)), and on microbial status. The adult rats used in this study were kept under specific pathogen free conditions, so there were very few well developed germinal centers but mostly small follicles (Willführ et al., 1990). Thus, in conventional or antigen-stimulated animals the relative proportion of the splenic compartments and the number of immigrated lymphocytes will be different.

Despite these interesting data, there are still a number of open questions on the role of the spleen and its compartments in lymphocyte migration, which should stimulate further studies, especially on entry, transit and exit: which receptors on the endothelium and lymphocytes are involved in splenic homing? Are cytokines involved in the regulation of splenic homing? Which factors influence transit through the splenic compartments? Are there specific receptors which regulate the release of lymphocytes from the parenchyma to the blood and the efferent lymphatics? Is lymphocyte subset homing or transit altered during different types of immune responses? Finally, how can the route of individual lymphocytes be followed through the spleen? Intravital videomicroscopy (Groom et al., 1991; Schmidt et al., 1990) might be a technique helpful in answering the final question. There is no doubt that the role of the spleen in lymphocyte migration will be a worthy subject of another review at a future Barnhart Memorial Symposium.

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Discussion with Reviewers

A.C.Groom: Your caution that the label itself or the labeling procedure may influence the migration of lymphocytes is well taken. The use of immunochemical staining of lymphocytes in tissue sections, i.e. after migration to the different splenic compartments has occurred, is undoubtedly appropriate. However, in order to provide identification of lymphocyte subset for migrating cells observed under intravital videomicroscopy, it would be necessary to label specific subsets with FITC before injection. Is it your opinion that this would invalidate conclusions that might be drawn from intravital microscopy?

Authors: As there is no specific fluorescent label for one subset of lymphocytes, the subsets have to be separated by different in vitro techniques, to study their migration by intravital videomicroscopy. Very important conclusions can be drawn from such experiments, as long as it is shown in parallel classical migration experiments that the separated cells migrate as well as unseparated lymphocytes.

J.C. Chamberlain: It seems obvious that mechanisms exist which direct T and B lymphocytes to their respective locations in the spleen and that cell recognition and adhesion must occur. Can you speculate what these mechanisms might be? Could physicochemical properties of the migrating cell interact with the anatomy of the vasculature as they seem to do with red cell migration and retention?
Authors: It is likely but unproven so far that T and B lymphocytes express different adhesion molecules which might work as "homing receptors" by interacting with specialized receptors on the endothelial cells. This will undoubtedly be a field of future research, including their upregulation by cytokines, which has been shown for the interaction between lymphocytes and cells of high endothelial venules in lymph nodes.

M. Kashimura: How do the authors distinguish the lymphocytes passing through the red pulp or marginal zone from those homing in these areas? How many lymphocytes labeled with ^{51}Cr or FITC were there in the peripheral blood in your study? If labeled lymphocytes existed in the blood, they were always recirculating through the spleen. Labeled lymphocytes enter the splenic parenchyma from the open end of the arterioles at the marginal zone or the red pulp. A part of them migrate into the white pulp. Others migrate or passively flow into the splenic sinuses and exit the spleen. If the latter is not zero, the data concerning lymphocyte homing in the spleen in this paper may be an overestimate.
Authors: There are labeled lymphocytes in the blood during the period of the experiments. The labeling index, however, shows a different pattern to that of the spleen. As there is no technique available so far to study one cohort of labeled lymphocytes through the splenic compartments, there is the limitation in our studies that we do not know how many lymphocytes stayed for long durations in the individual compartment and how many returned to the spleen after a short transit time. For lymphocytes found in the marginal zone and red pulp the term "homing" might not be adequate, but it has been used in our article for convenience. There are some indications that lymphocytes adhere to certain endothelia without emigration. These lymphocytes form the "marginal pool" which is as big as the circulating lymphocyte pool. It is very likely that adhesion molecules play a role in this margination. The marginal pool of lymphocytes in the spleen, probably mainly in the marginal zone and red pulp, is much larger than could be attributed to the blood volume in this compartment. The kidney and liver for example do not have such large marginal pools and because specific adhesion molecules might be involved in this transient holding back of lymphocytes in the red pulp and marginal zone, one might also use the term homing for these compartments.