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LYMPHOID MICROENVIRONMENTS IN THE THYMUS AND LYMPH NODE

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Abstract

The three-dimensional architecture of the thymus and mesenteric lymph node reveals several different stromal cell types important in the development and function of T cells. In the thymic cortex, T cells proliferate and differentiate in a meshwork of epithelial-reticular cells. They then migrate towards the medulla where they may interact with interdigitating cells. T cells migrate from the thymus through perivascular spaces, surrounding large vessels at the cortico-medullary boundary. In this area also large thymic cystic cavities are found, their function remains at present unclear. Mature "selected" T cells leave the thymus most probably by the venous bloodstream, to enter peripheral lymph nodes.

Upon entering the lymph node they cross the wall of high endothelial venules. On the other hand, lymph enters the node by afferent lymphatics draining into various types of sinuses. Here, macrophages are strategically located to phagocytose and process antigen. These cells then expose antigen to T cells and B cells within the lymph node parenchyma, thus creating a microenvironment for the onset of an immune response.

The various microenvironments important in T cell development and T cell function are shown in this paper using scanning electron microscopy as a dissecting tool. We discuss our morphological findings in the light of recent data on the physiology of T cell differentiation and function.

Keywords: Immunology, microenvironments, thymus, lymph node, T cell diferentiation, stroma, high endothelial venules.

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Introduction

Lymphocytes differentiate in so called "central lymphoid organs", such as the bone marrow and thymus. In young adult mammals, the bone marrow is the major site of B lymphocyte production, whereas the thymus is essential for T lymphocyte production. This paper focusses on various histophysiological aspects of T cell differentiation and T cell function and tries to unravel, at the morphological level, the role of various non-lymphoid, stromal cell types in both processes. Thus, the first part of this paper will deal with microenvironments within the thymus gland, whereas the second part will deal with various microenvironments within the peripheral lymph node.

The process of T cell differentiation within the thymus is a complicated process, involving many differentiation steps (Scollay and Shortman, 1985; Weissman, 1986). Knowledge on T cell differentiation is rapidly accumulating, however, basic questions as (a) why do so few T cell progenitors enter the thymus?, (b) how are T cells selected to leave the thymus?, and (c) why do so many thymocytes apparently die within the thymus?, are still unanswered.

T cell differentiation already starts early in ontogeny within the yolk sac and fetal liver, where pluripotential hemopoietic stem cells are committed to this specific cell lineage (Le Douarin et al., 1984; Owen and Jenkinson, 1984). Postnatally, the bone marrow takes over this role of the yolk sac and fetal liver and is then the major source of committed T cell progenitors (Kadish and Bash, 1976; Boersma et al., 1981; Ezine et al., 1984). Thus, committed progenitors leave these hemopoietic compartments and enter the thymus gland, most probably by traversing the vascular system. Within the thymus these cells start to proliferate and differentiate, resulting in changes in cell size, expression of differentiation antigens, such as CD4 and CD8 (Scollay and Shortman, 1985), expression of receptors for growth factors (Ceredig et al., 1985; Howe et al., 1986) and expression of the Ti antigen receptor (Snodgrass et al., 1985; Pardoll et al., 1987). In addition, thymocytes are selected in the sense that only self tolerant cells, capable of MHC (major histocompatibility complex) restricted antigen recognition may leave the thymus (Zinkernagel and Doherty, 1979; Fink and Bevan, 1979; Kruisbeek and Longo, 1985).

Prothymocytes cannot undergo these differentiation steps outside the thymus. This indicates that thymic microenvironments are essential in T cell differentiation (Van Vliet et al., 1984; Huiskamp and

Van Ewijk, 1985; Huiskamp et al., 1985). The requirement for stromal cells in T cell differentiation has been elucidated in various experimental models including in vitro cultures of thymic fragments. Recently, Kingston et al. (1985) have shown, at the single cell level, that prothymocytes implanted in a cultured fetal, thymocyte-depleted, "stromal" thymus can proliferate and differentiate. These authors provided evidence that (within the stromal compartment of the thymus), a single prothymocyte can give rise to all thymocyte lineages including mature helper T (CD4+ve) and cytotoxic or suppressor T (CD8+ve) cells.

It is very likely that various stromal compartments (**microenvironments**) exert different influences on the T cell differentiation process (Kruisbeek and Longo, 1985). In this context it has recently been proposed that cortical epithelial-reticular cells are involved in "imprinting MHC restriction", whereas medullary bone marrow derived interdigitating cells regulate "tolerance induction" (Lo and Sprent, 1986).

Also in the peripheral organs non-lymphoid cells regulate the immune response. Firstly, endothelial cells determine the homing of lymphocytes in the node (Butcher et al., 1980; van Ewijk et al., 1975; Gowans and Knight, 1964; Streeter et al., 1988); thereafter various types of stromal cells, such as interdigitating reticular cells (IDC) and follicular dendritic cells (FDC) are involved in the T, respectively, B cell microenvironments (van Ewijk et al., 1974, 1977; Veerman and van Ewijk, 1975). Thus, non-lymphoid, stromal cells appear to guide T cell differentiation and T cell function; hence these cells must closely interact with lymphoid cells.

The purpose of this paper is to visualize these types of lymphoid-non-lymphoid cell interactions at various levels of the differentiation of T lymphocytes. In addition, cellular interactions are visualized at the functional level in order to deepen the insight in the complicated architecture of lymphoid organs. Scanning electron microscopy (SEM) is an excellent tool to visualize lymphoid organs and lymphoid microenvironments. We have developed a gentle preparation technique providing "insight" into the complicated microarchitecture of lymphoid organs. Using this technique we will first show the architecture of various microenvironments within the thymus, followed by studies on microenvironments in peripheral lymphoid organs. We will then discuss these morphological findings in the light of recent data on the differentiation of T lymphocytes, and the functional role of these cells in immune responses.

Materials and Methods

Mice

Male CBA and BALB/c mice were kept under routine laboratory conditions. They were used for the present experiment at 6 weeks of age.

Tissue preparation for scanning electron microscopy

Fixation. Mice were anaesthetized by intraperitoneal (ip) injection of 70 mg/ml Nembutal (Abbott). Next, the thorax was opened and a canula was inserted into the left ventricle. The right atrium was incised and total body perfusion was started with phosphate buffered saline (PBS) containing 0.1% procain-HCl, for 30-60 seconds (flow rate 0.5 ml/sec., pressure 40 mm Hg). After this period, perfusion was continued with 0.1% glutaraldehyde (Polysciences) in PBS for an additional period of 15 min. The thymus and the mesenteric node were carefully excised, additional fatty tissue was removed and slices of tissue were prepared using a razor blade. The slices were transferred to vials containing PBS. Next, these vials were vigorously shaken for 5 min to remove unbound lymphoid cells from the tissue. This preparation step is important since it ultimately reveals the various lymphoid microenvironments. The tissue specimens were then postfixed in 1% glutaraldehyde in PBS for a period of at least 60 min.

Specimen preparation. Specimens were transferred to 0.14 M sodium cacodylate buffer, repeatedly rinsed, and incubated in distilled water containing 2% arginine chloride, 2% lysine, 2% guanidine chloride and 2% glycine, for 15 min, in order to block free radicals caused by glutaraldehyde fixation. Specimens were then kept for 2 h in a freshly prepared solution containing 2% tannic acid (Malingrod) in distilled water (adapted from Murakami, 1974). Next, specimens were rinsed in a solution of 1% sodium sulphate in distilled water, for 15 minutes (change 3 times), followed by 6 rinses with distilled water. Postfixation was carried out with a solution of 2% osmium tetroxide in distilled water for 60 min. Specimens were then rinsed in distilled water, dehydrated and critical point dried. Dried specimens were mounted on specimen stubs, gold-palladium sputter-coated and examined in a Philips 505 SEM operating at 30 kV.

Results

Microenvironments in the thymus

The thymus in young adult mice has two lobes, each lobe consists of two major compartments, the cortex and the medulla. It has been previously shown that most immature cells are located in the outer cortex, the more mature cells accumulate in the medulla (Scollay and Shortman, 1985). Close under the capsule, large lymphoblast cells are found: these cells proliferate, differentiate and migrate towards the medulla. Selected T cells leave the thymus through blood vessels, located at the cortico-medullary junction.

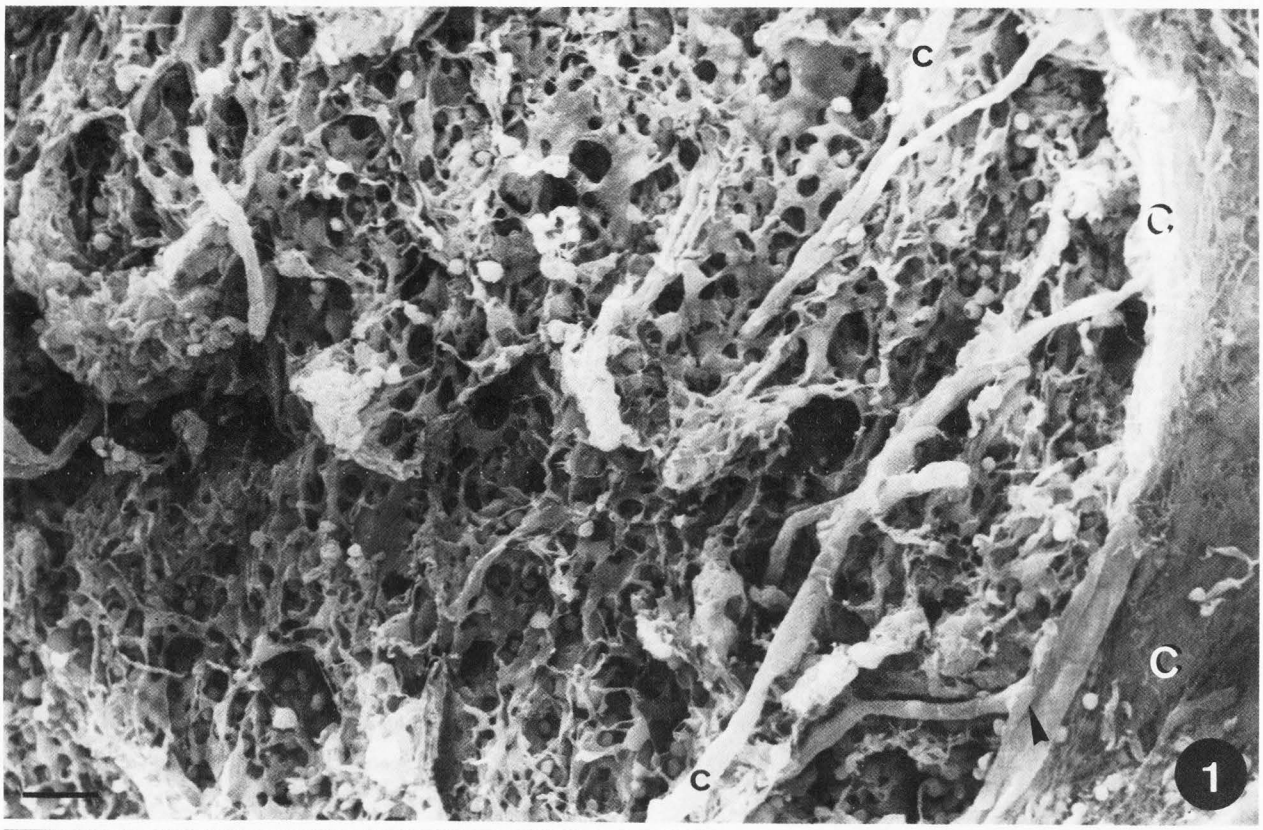
The general microarchitecture of the thymus is shown in Figure 1. Epithelial reticular cells form a meshwork, capillaries run from the cortico-medullary junction towards the capsule (Fig. 1), follow this capsule for a short distance (Fig. 1, arrow) and run back towards the medulla, thus forming capillary loops (so-called "arcade capillaries"; data not shown).

Figure 1. Low magnification view of the thymic cortex. Capillaries (c) run up to the capsule (C) and follow the capsule over short distances (arrow). According to Saint Marie et al. (1986) these capillaries loop back towards the medulla. Cortical epithelial cells form a reticular meshwork.

Figure 2. Thymic cortex, epithelial reticular cells (er) form a reticular meshwork; in between the meshes thymocytes can be observed (arrows). c = capillary.

Figure 3. Close under the thymic capsule, the epithelial reticular cells (er) form thin sheets, or sometimes baskets. Thymocytes contacting epithelial reticular cells project small bulges into the epithelial reticular cells (arrows).

Bars = 30 μm (Fig. 1), and 10 μm (Figs. 2, 3).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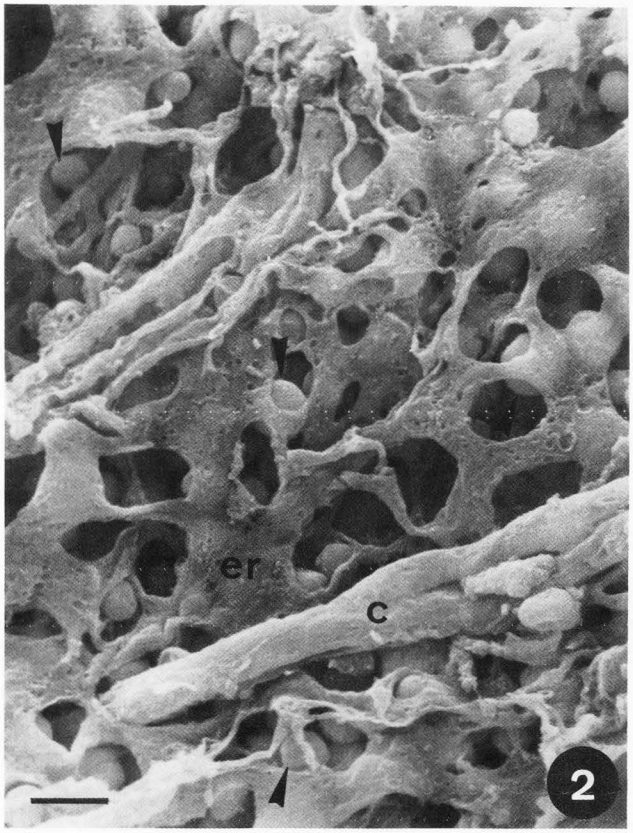
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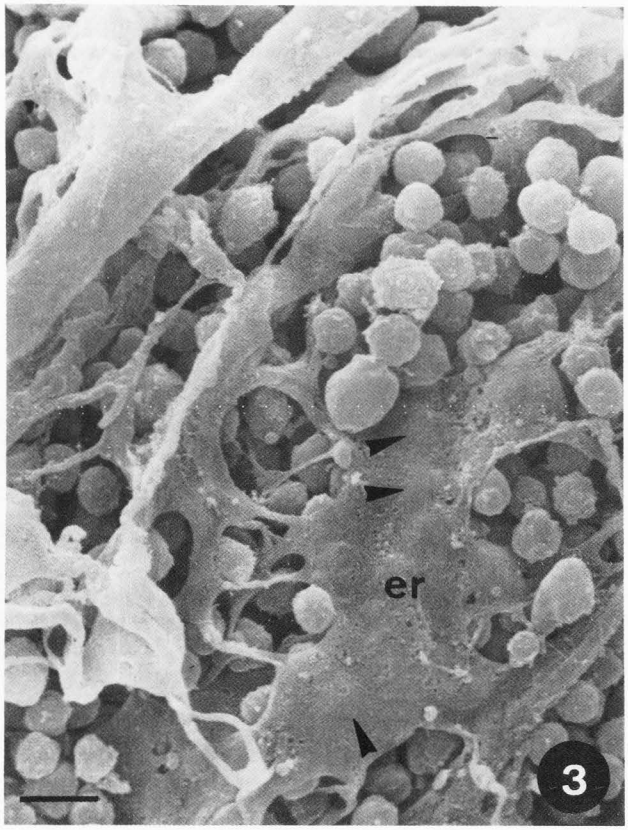
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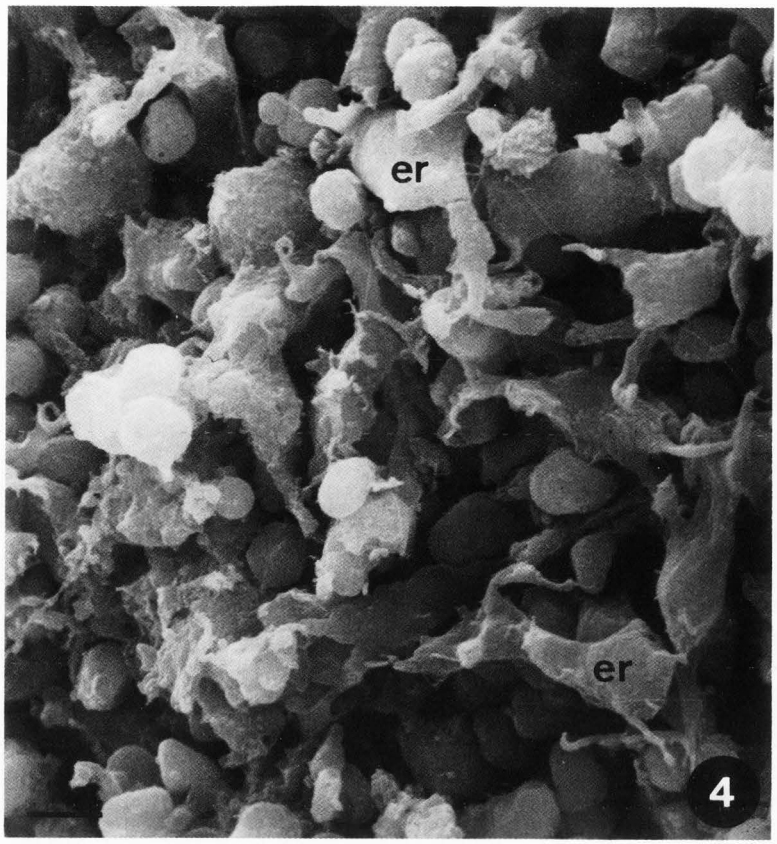


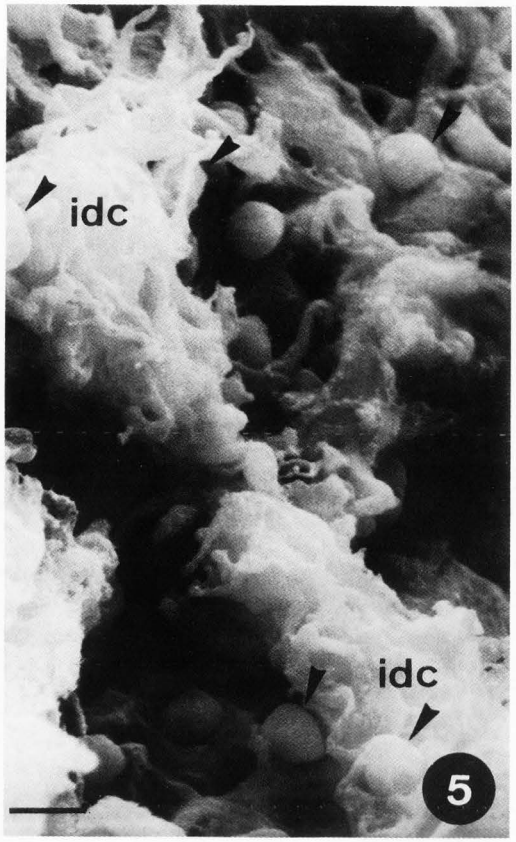


Figure 4 (above). Thymic medulla. Thymocyte/stromal cell to cell interactions are not as obvious as in the cortex. In contrast to cortical epithelial reticular cells, medullary epithelial reticular cells (er) do not show an extended meshwork. Instead they form several blunt processes (arrows). t = thymocyte.
Figure 5 (above). Interdigitating cells (idc) located in the medulla, showing veiled cytoplasmic processes. Medullary thymocytes are sometimes eveloped by these processes (arrows).
Figure 6 (right). Cysts, lined by epithelial cells, are found in the cortico-medullary region (Fig. 6a). Sometimes these cysts contain lymphoid cells (not shown here). The area indicated by an asterisk is shown at higher magnification in Fig. 6b, where border lines between individual epithelial cells are manifest by small grooves. The epithelial cells indicated by an arrow are shown at high magnification in Fig. 6c.

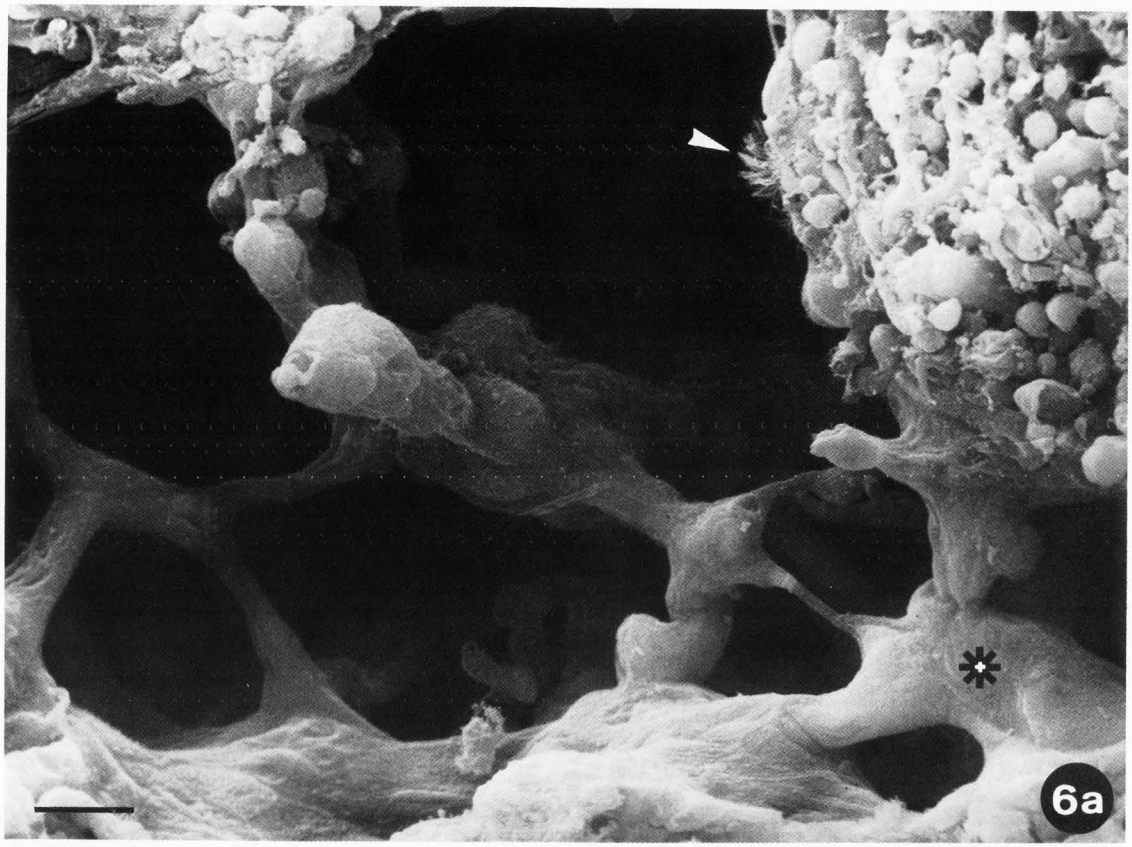
Bars: 10 μm (Figs. 4, 5); 20 μm (Fig. 6a); and 2 μm (Figs. 6b, 6c).

Higher magnification of this cortical compartment shows the typical meshwork of cortical epithelial cells. Lymphoid cells are generally smooth surfaced and are located within the meshes of the epithelial reticulum. They contact the epithelial cells over large surface areas. The epithelial reticulum presents itself in two forms: (a) a typical reticular mesh work is found throughout the cortex (Fig. 2), and (b) more lamillar, flat epithelial cells are found in the subcapsular part of the cortex (Fig. 3). Sometimes these flat cells tend to form baskets, enveloping nests of cortical thymocytes (data not shown).

Medullary epithelial cells (Fig. 4) are clearly different from cortical-epithelial cells (compare with Fig. 3). Here the cells are more spheroid, expressing short blunt processes. Thymocytes in this area are medium-sized expressing short microvilli. Other non-lymphoid cells in this area are bone marrow-derived macrophages and interdigitating cells (Fig. 5). The latter cells are also found in T cell domains in peripheral lymphoid organs.

The medulla also contains typical cysts (Fig. 6a), which are immersed in the epithelial-reticular network, usually occurring at the cortico medullary junction. These structures are lined by several types of epithelial cells, the majority being cells equipped with short, evenly distributed microvilli (Fig. 6b). Other areas in these cysts contain epithelial cells carrying cilia (Fig. 6c). These cysts sometimes contain lymphoid cells.

Lymphoid cells most probably leave the thymus through the vascular lining of the large venules. Our data demonstrate that T cells accumulate in perivascular sheaths which are found around the large venules in the medulla (Fig. 7, arrows). From immunohistochemical studies published elsewhere, it appeared that exclusively phenotypically **mature** cells accumulate here, these cells are either CD4+ or CD8+ and express Mel 14, a marker which represents a homing receptor used by lymphoid cells to migrate through the lymphoid vasculature (See Van Vliet et al., 1986). Cells positive for both markers (CD4+, CD8+) are immature cells. Such cells do not accumulate in these perivascular channels (Van Vliet et al., 1986).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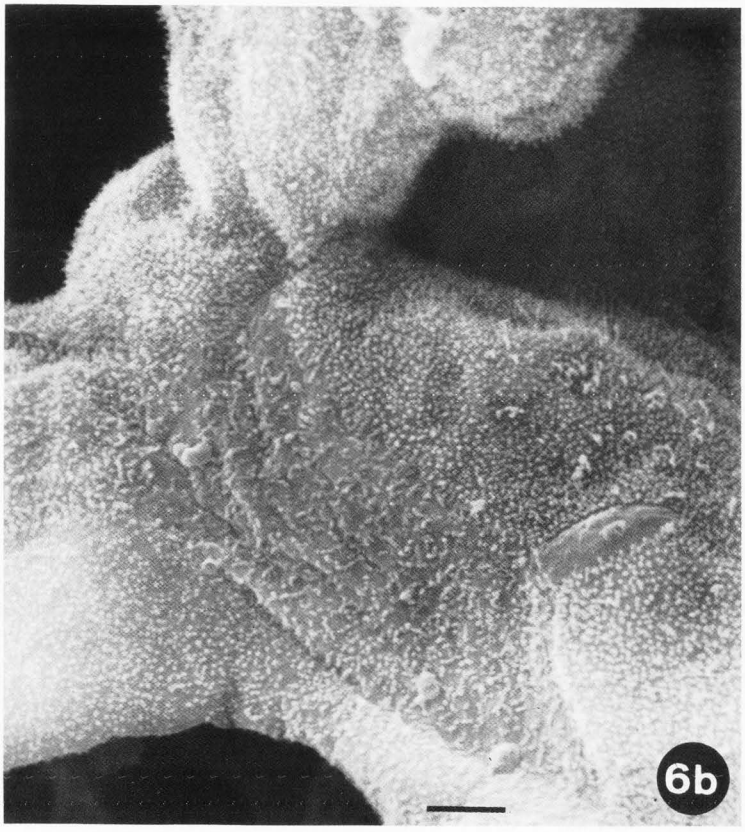
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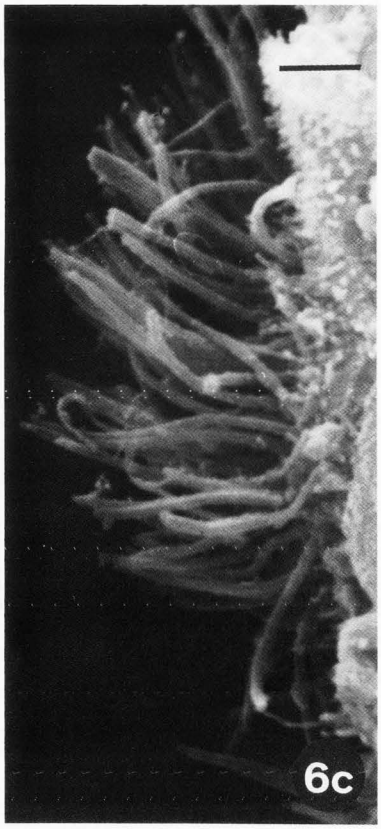
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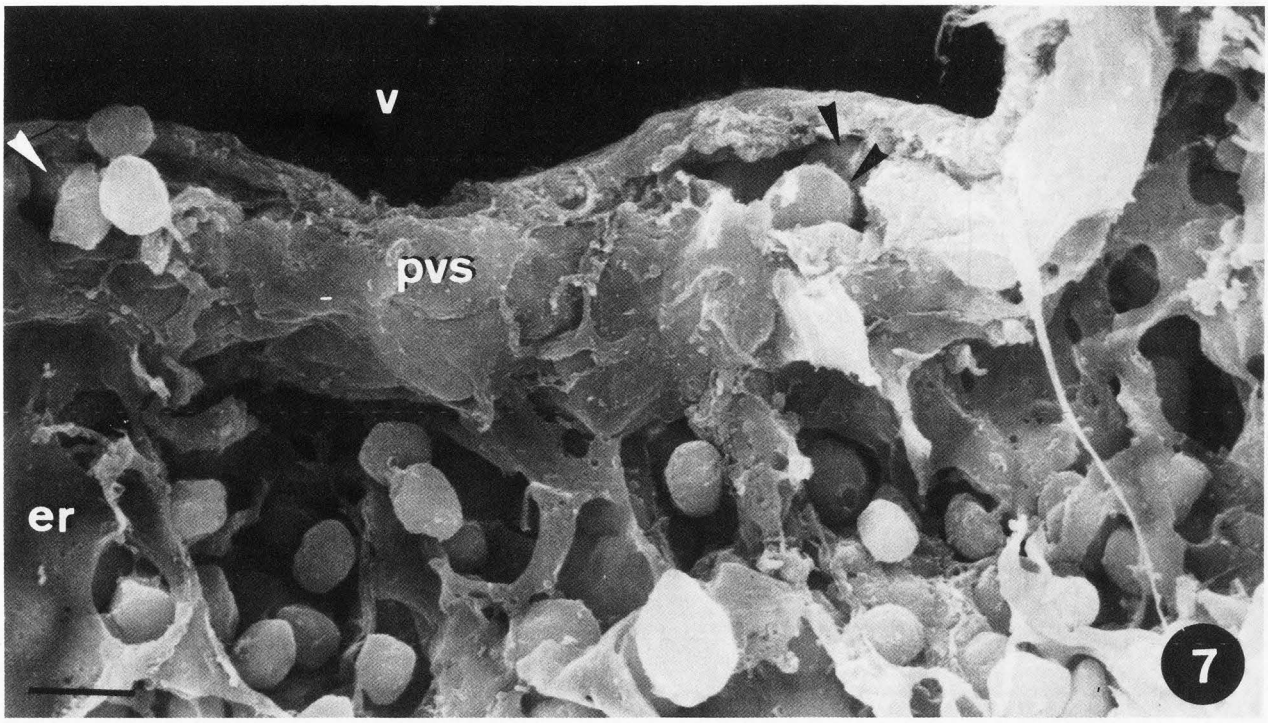


Microenvironments in peripheral lymph nodes

T cells emigrating from the thymus are transported by the peripheral blood to secondary lymphoid organs, such as the lymph nodes. Lymph nodes are situated in a complex network of lymph vessels. Lymph drains into the lymph node through the subcapsular sinus, passes the lymphoid parenchyma through the trabecular sinuses and leaves the node from the medullary sinus and the efferent lymphatic (schematically presented in Fig. 8). Under normal conditions the lymphoid cells do not enter the node through the afferent lymphatics, they enter the node from the blood stream.

Within the node, B and T cells each have their own domains: B cells localize in follicles in the outer cortex, T cells localize in the paracortical area (Van Ewijk et al., 1977). Antibody forming plasma cells are predominantly located in the medulla of the lymph node. Using SEM as a tool, we will first follow the accepted pathway of lymph drainage and subsequently examine the entrance site of lymphoid cells in the node. Finally, microenvironments will be shown where lymphoid and nonlymphoid cells interact during the onset of an immune response.

Lymph fluid, containing antigens, enters the node through the afferent lymphatics, connected to the subcapsular sinus. The inner lining of the subcapsular sinus (Fig. 9a) consists of endothelial cells, but these cells do not form a closed endothelial sheath (Fig. 9b). There are gaps in the endothelial cells and processes of macrophages project into the subcapsular space (Fig. 10). Thus, macrophages are strategically located on the entrance pathways of the lymph fluid in the node. These cells are able to clear the incoming lymph from antigens, a process called "antigen trapping". Macrophages are also located in the trabecular sinus and the medullary sinus (Fig. 11).

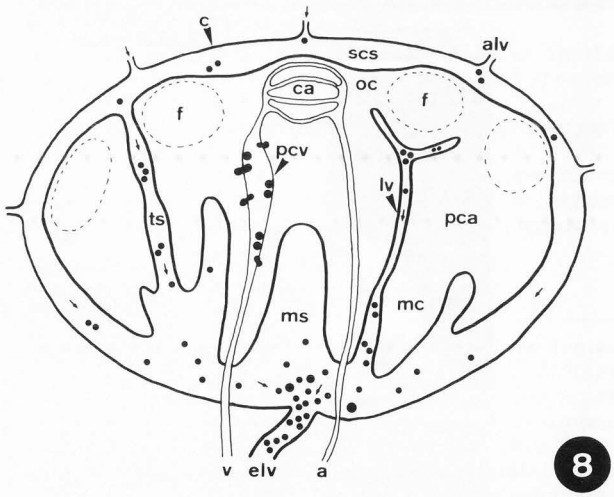


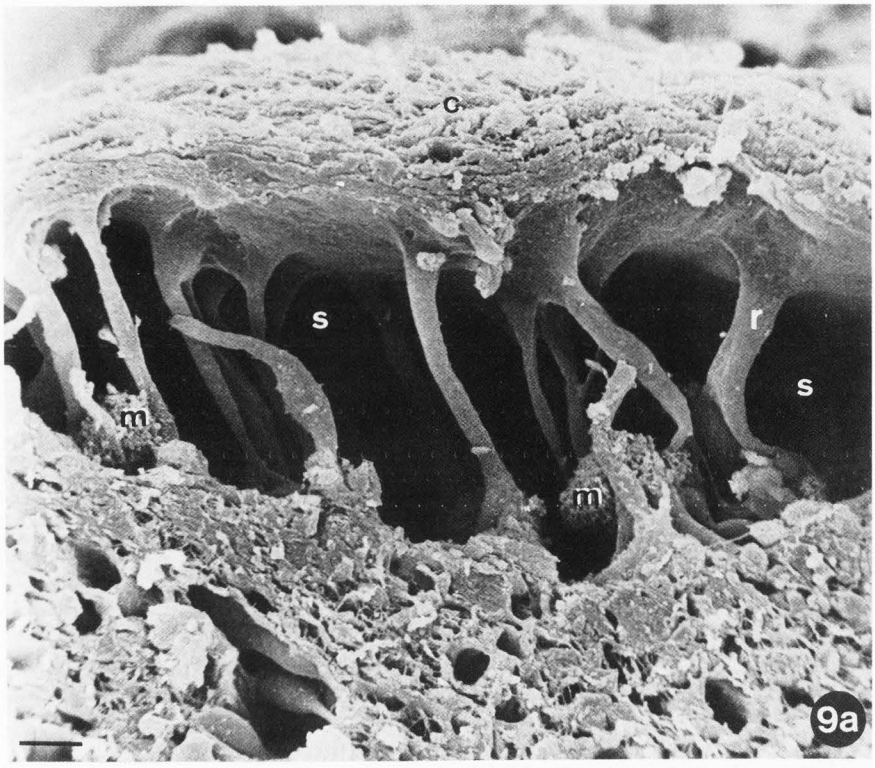
Figure 8. Schematic presentation of a lymph node. Lymph enters the node by afferent lymphatic vessels (alv), follows the subcapsular sinus (scs) to drain into trabecular sinuses (ts) and into the medullary sinus (ms). The lymph exits the node through the efferent lymphatic vessel. Lymphocytes (black dots) enter the node through postcapillary venules (pcv) also called "high endothelial venules". The lymphoid parenchyma contains follicles (f) in the outer cortex (oc). Here B lymphocytes accumulate. T cells home to the paracortex (pca). Lymphocytes leave the parenchyma via short lymphatics (lv) which drain into the medullary sinus. They exit the node through the efferent lymphatic. a=artery, c=capillary, mc=medullary cord.

Figure 7 (facing page top). Venule (v) in the cortico-medullary region, surrounded by a perivascular space (pvs). According to van Vliet et al. (1986) mature thymocytes (arrows) accumulate in these spaces, which may facilitate their exit. Epithelial reticular cells (er) are attached to the perivascular space.

Figure 9a (right). Subcapsular sinus (s) of the mesenteric lymph node. Reticular fibroblasts (r) lined by endothelial cells support the capsule (c) of the lymph node. Macrophages (m) are situated between the reticular cells.

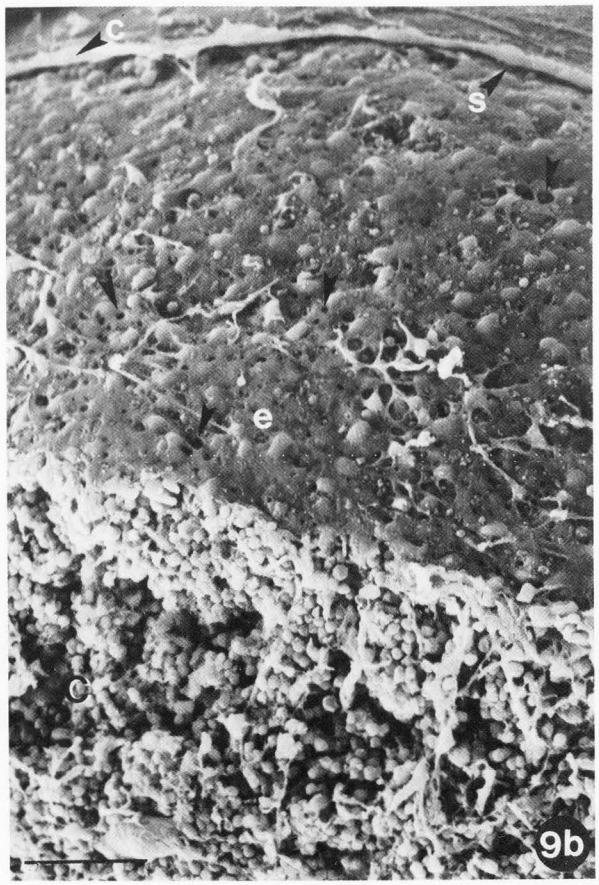
Figure 9b (right, bottom). Mesenteric lymph node, of the cortex (C), at low magnification. The capsule (c) covers the subcapsular sinus (s). The endothelial lining (e), covering the lymphoid parenchyma shows many pores (arrows). Lymph fluid enters the lymph node through afferent lymphatics (not shown) and may penetrate the subcapsular lymphoid tissue through these pores.

Bars = 10 μm (Figs. 7, 9a); and 50 μm (Fig. 9b).



Lymphocytes, both B and T cells, enter the lymph node through the vasculature. Lymphoid cells within the microvasculature are shown in Fig. 12a, where it can be seen that lymphoid cells attach to the endothelial lining of so-called "high endothelial venules", previously called "postcapillary venules". These venules are characterized by their wide lumen and bumpy endothelial lining. Lymphocytes enter these HEV's from the cortical capillaries. Figure 12a suggests that they contact the endothelium by means of microvilli. Attached lymphocytes likely actively migrate over the endothelial lining to clefts between individual endothelial cells. Figure 12b demonstrates lymphocytes in the process of exiting between endothelial cells, where they must pass across the basal lamina and the adventitial lining of the vessel towards the lymphoid parenchyma. During transition, the cell surface morphology of the lymphoid cells changes: microvilli decrease in number, ultimately a smooth surfaced lymphocyte migrates across the endothelial lining (Fig. 12a, 12b, asterisks).

Figure 13 shows lymphocytes, presumably both T and B cells, interacting with macrophages within the lymphoid parenchyma. This type of cellular interaction, found at the boundary of T and B domains, creates an effective microenvironment inductive for the onset of a humoral immune response. Blast transformation, a first morphological sign of lymphocyte activation can be observed in Figure 13. Here, lymphoid cells increase in size up to 12 micrometers (Fig. 13, asterisks). Another feature of lymphoblasts is that they tend to rearrange their microvilli at the cell surface. Ultimately, these cells will leave the macrophage and migrate towards the medullary cords, to differentiate into antibody forming plasma cells.



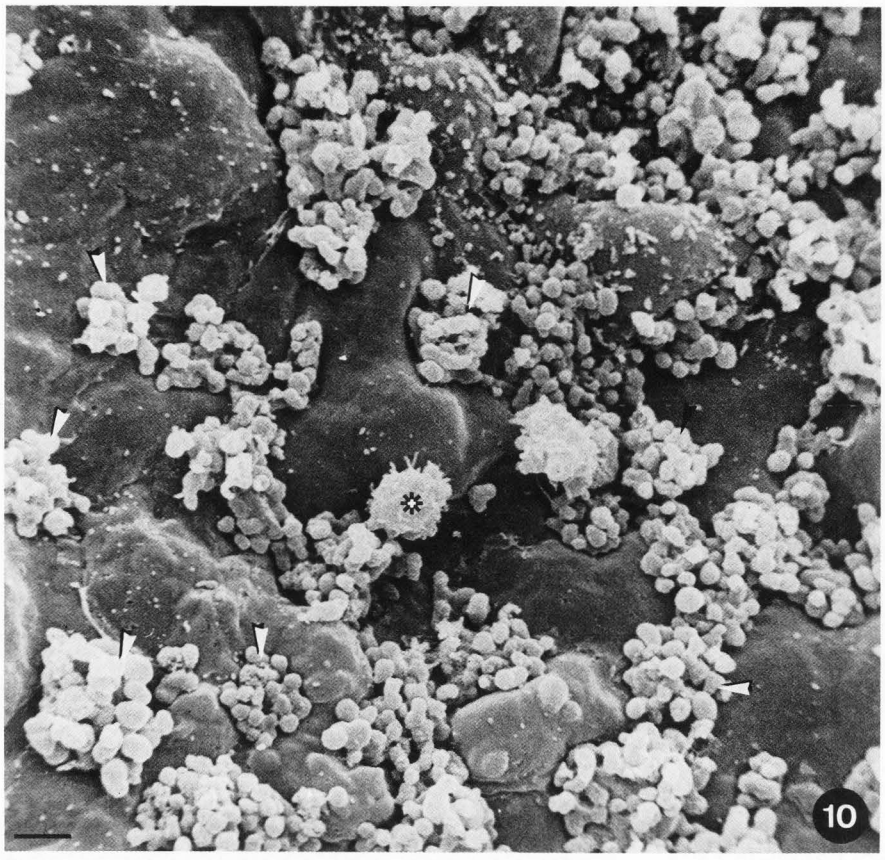


Figure 10 (left). Endothelial lining of the subcapsular sinus. Macrophages project numerous cellular processes through openings in between the endothelial cells (arrows). A single lymphocyte (asterisk) is in contact with the endothelium and macrophage processes.

Figure 11 (left bottom). Trabecular sinus of the mesenteric lymph node. Macrophages (m) showing numerous membrane ruffles attach to reticular fibroblasts (r).

Figure 12a (facing page top). A high endothelial venule (HEV) in the mesenteric lymph node is lined by bulging endothelial cells (e). Lymphocytes are attached to the endothelium by means of short microvilli. Clefts and holes (arrows) are present in between individual endothelial cells. Cells presumed to be in the process of migration from the lumen of the HEV through these clefts and holes towards the lymphoid parenchyma show changes in their morphology from villous to smooth (asterisks).

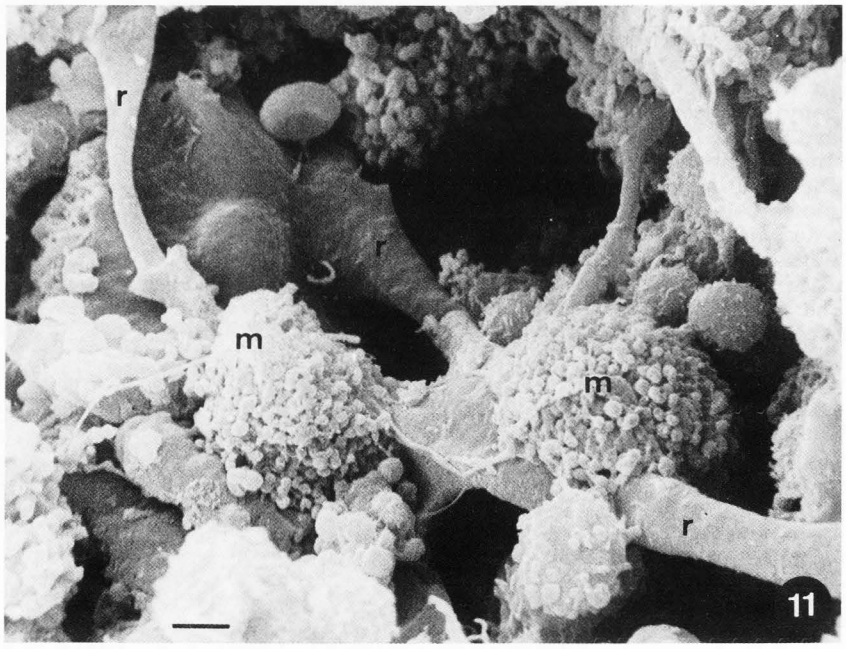
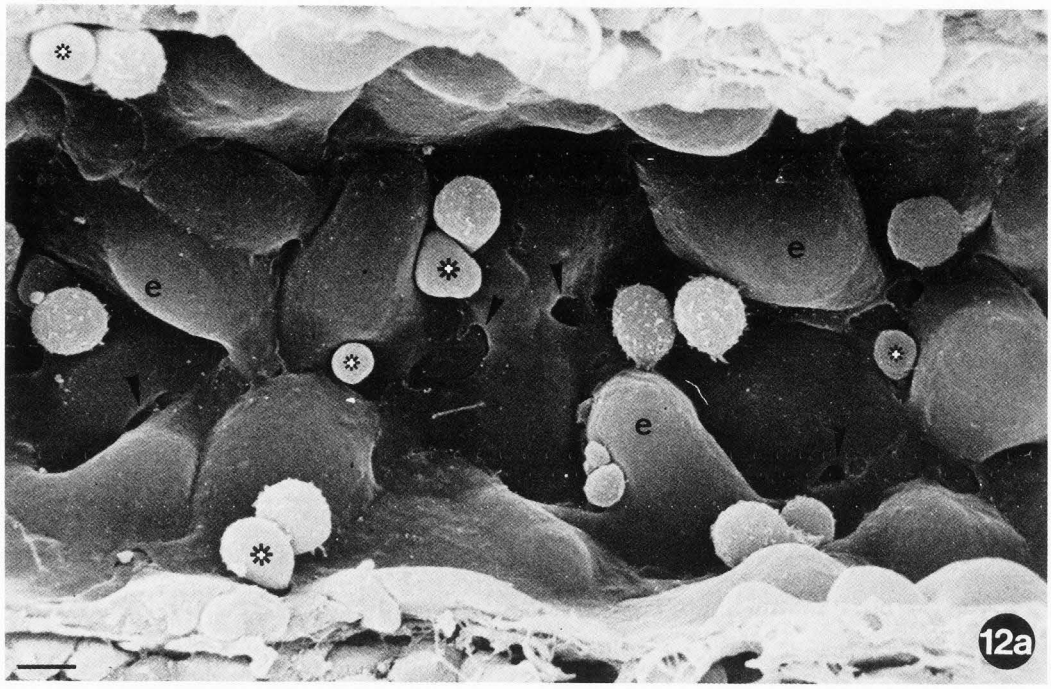


Figure 12b (facing page bottom). Lymphoid cells actively migrate across the endothelial surface and enter clefts between endothelial cells, in order to cross the endothelial lining. Transiting cells lose their microvilli (asterisk).

Bars = 4 μm (Fig. 10, 12a); 3 μm (Fig. 11); and 5 μm (Fig. 12b).

Discussion

The present paper shows morphological evidence that at various levels of the differentiation of T lymphocytes stromal cells are involved in processes as education, migration and activation. T cell differentiation already starts at the level of commitment of the pluripotent stem cells to progenitor T cells. The process of commitment occurs in adult mammals already within the bone marrow (Mulder et al., 1984;



Ezine et al., 1988). Whether specific microenvironments play a role in this initial step in differentiation is still unclear. Recent published evidence suggests, however, that stromal cells isolated from the bone marrow indeed can support the growth of early lymphoid cells (Hurt and Witte, 1987). Progenitor T cells then seed to the thymus (Boersma et al., 1981; Ezine et al., 1984).

The exact entrance pathway of prothymocytes in the thymus still remains obscure, hampered by the fact that progenitor T cells occur at very low frequency. Recently, Ceredig and Schreyer (1984), by using bone marrow chimeras have shown by immunohistochemistry that prothymocytes enter the thymus through the large venules in the cortico-medullary junction. Prothymocytes then migrate up to the subcapsular thymic cortex and start to proliferate and differentiate. Again, the signals which regulate proliferation and differentiation are still not clear, although recently IL1 and IL2 have been shown to influence thymocyte proliferation (Rothenberg and Lugo, 1985; Ceredig et al., 1985; Howe et al., 1986). A next step in the differentiation process is the selection of T cells which are able to recognize MHC antigens in an appropriate way (Kruisbeek and Longo 1985; Lo and Sprent, 1986). This means that T cells in the thymus have to "learn" to recognize MHC antigens in order to interact later on, at the functional level with macrophages exposing antigen. Coordinated recognition of antigen + self MHC antigen is the stimulus to drive the T cell into B cell activation. However, self MHC recognition should be "gentle", T cells should not react **against** the self MHC exposing cells themselves, since this reaction would potentially create an auto-immune response (Jordan et al., 1985). Thus, somewhere during T cell differentiation the developing T cell should learn to be tolerant to self MHC antigens. It has been suggested by various authors that the thymic stroma plays a role in this educational process (Zinkernagel and Doherty, 1979; Fink and Bevan, 1979; Kast et al., 1984; Kruisbeek et al., 1985). In this context, we have previously shown that the epithelial reticular cells express MHC antigens (Rouse et al., 1979; Van Ewijk et al., 1980; Van Ewijk, 1984). Recently published evidence from Lo and Sprent (1986) indicates that the **thymic epithelial reticular cells** are involved in imposing MHC restriction onto developing T cells. According to their hypothesis T cells are positively selected on the basis of a low affinity binding to self MHC antigens on epithelial reticular cells in the

thymic cortex. High affinity binding to self MHC antigens is potentially dangerous, hence these T cells have to be prevented from entering the circulation. Lo and Sprent suggest that this particular process, also called "tolerance induction" takes place in the medulla where bone marrow-derived **interdigitating cells** should be involved in the elimination of autoreactive T cells (for a review see Marrack and Kappler, 1987).

T cells which have undergone this subsequent positive and negative selection are allowed to enter the circulation. There is now evidence that these cells leave the thymus from the cortico-medullary junction, using the perivascular space around large venules as an exit pathway (Van Vliet et al., 1986). However, thymic cysts are also possible candidates for T cell emigration, as suggested by Khosla and Ovalle (1986).

T cells migrate via the bloodstream towards peripheral lymphoid organs such as the mesenteric lymph nodes. Here they enter the node again through the vascular endothelium of high endothelial venules. Initial recognition of high endothelium is mediated by specific "homing" receptors expressed on the cell surface of the T lymphocyte (Gallatin et al., 1983). These receptors interact, most probably with "vascular adressins", determinants exposed on the cell surface of high endothelial cells (Streeter et al., 1988). The exact nature of the passage corridors which lymphocytes use during their migration through the endothelial lining towards the lymphoid parenchyma is still not yet understood. Our own previous studies (Van Ewijk et al., 1975) and our present data indicate migration of cells through extracellular clefts between endothelial cells. Other studies, however, indicate a passage through the cell body of endothelial cells (Cho and De Bruyn, 1979). However, this pathway is most probably not an intracellular pathway since Anderson and Anderson (1976) have shown, using tracers that all lymphoid cells surrounded by endothelial cells are also labeled by tracers, hence they are situated in the extracellular compartment. The present study also fails to demonstrate any transit of lymphocytes through endothelial cell bodies.

Upon arrival in the lymphoid parenchyma, the lymphocytes migrate to their respective domains: B cells migrate to follicles in the outer cortex, whereas T cells localize in the paracortex. Factors governing this specific cell traffic are still unknown. Specific non-lymphoid cells such as interdigitating cells and follicular dendritic cells are candidates for the creation of T domains and B domains, respectively, (Van Ewijk et al., 1977), and it is believed that these cells secrete factors which attract the migrating T and B lymphocytes.

Lymphoid and non-lymphoid cells both function together in the onset of an immune response (Van Ewijk et al., 1977; Stobo, 1982). For example, in the humoral immune response, these events are the following:

(1) macrophages ingest antigens from the lymph and process antigen. (2) processed antigen is exposed at the cell surface of the macrophage. (3) recirculating lymphocytes pass along the antigen presenting macrophages. (4) T helper lymphocytes interact and recognize antigen in the context of self MHC antigens on the cell surface of the macrophage. (5) B cells, migrating through the lymphoid parenchyma, also recognize antigen on the cell surface of the macrophages. (6) T helper cells start the production of interleukins. (7) These factors then trigger antigen reactive B cells to proliferate and differentiate into antibody producing plasma cells.

This complicated set of cell-cell interactions requires optimal microenvironments, where short-range immunomodulating factors can regulate a balanced immune response. It is becoming more and more clear, that various types of non-lymphoid cells play a key role in the constitution of such functional lymphoid microenvironments.

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References

Anderson, AO, Anderson, ND (1976). Lymphocyte emigration from high endothelial venules in rat lymph nodes. Immunology 31, 731-741.

Boersma W, Betel T, Daculsi R, van der Westen G (1981). Post-irradiation thymocyte regeneration after bone marrow transplantation. I.Regeneration and quantification of thymocyte progenitor cells in the bone marrow. Cell Tissue Kinet. 14, 179-196.

Butcher EC, Scollay RG, Weissman IL (1980). Organ specificity of lymphocyte migration: mediation by highly selective interaction with organ specific determinants on high endothelial venules. Eur. J. Immunol. 10, 556-561.

Ceredig R, Lowenthal JW, Nabholz M, McDonald HR (1985). Expression of Interleukin 2 receptors as a differentiation marker on intrathymic stem cells. Nature 314, 98-100.

Ceredig R, Schreyer M (1984). Immunohistological location of host and donor derived cells in the regenerating thymus of radiation bone marrow chimeras. Thymus 6, 15-26.

Cho Y, DeBruyn PPH (1979). The endothelial structure of the postcapillary venules of the lymph node and the passage of lymphocytes across the venule wall. J. Ultrastruct. Res. 69, 13-21.

Ezine S, Jerabek L, Weissman IL (1988). Phenotype of thymocytes derived from a single clonogenic precursor. J. Immunol., in press.

Ezine S, Weissman IL, Rouse RV (1984). Bone marrow cells give rise to distinct clones within the thymus. Nature 309, 629-631.

Fink PJ, Bevan MJ (1979). H2 antigens of the thymus determine lymphocyte specificity. J. Exp. Med. 148, 776-780.

Gallatin WM, Weissman IL, Butcher EC (1983). A cell surface molecule involved in organ specific homing of lymphocytes. Nature 304, 30-31.

Gowans JL, Knight EJ (1964). The route of recirculation of lymphocytes in the rat. Proc. Roy. Soc. Ser. B. (London) 159, 257-265.

Howe RC, Lowenthal JW, McDonald HR (1986). Role of interleukin in early T cell development: Lyt2- L3T4- thymocytes bind and respond in vitro to recombinant IL- 1. J. Immunol. 137, 3195-3200.

Huiskamp R, Van Ewijk W (1985). Repopulation of the mouse thymus after sublethal fission neutron irradiation. I. Sequential appearance of thymocyte

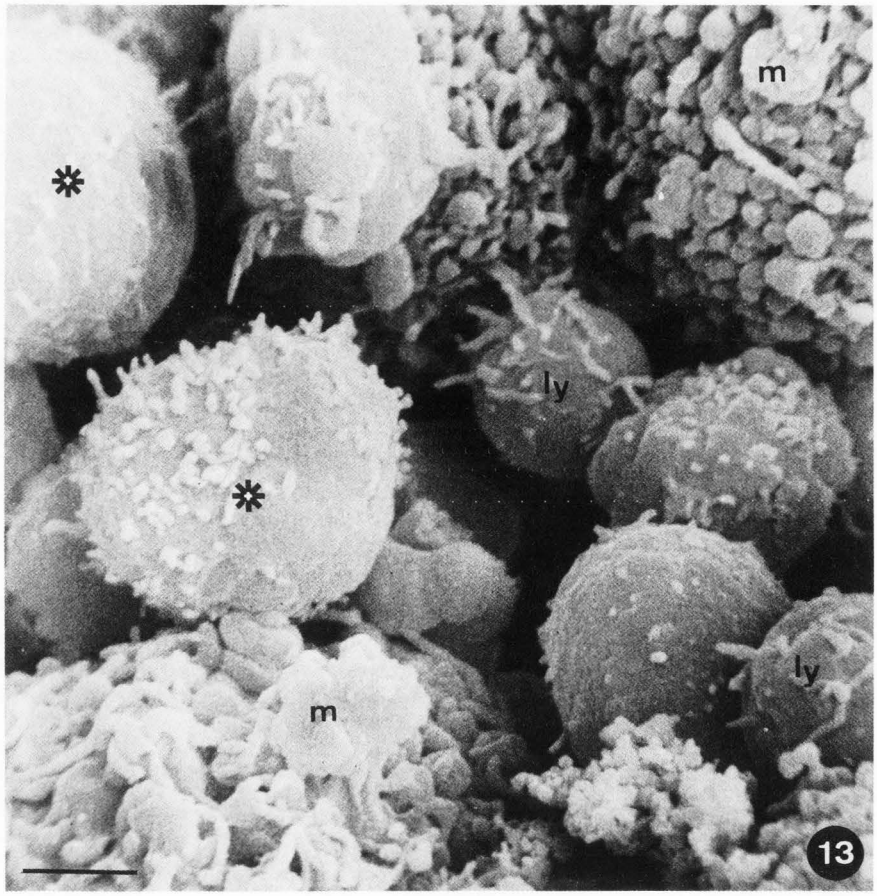

Figure 13 . Cellular interaction between lymphocytes (ly) and macrophages (m) in the paracortex. Small lymphocytes extend prolonged microvilli towards macrophages. Large activated lymphocytes (asterisks) express short microvilli. Cells of this type of microenvironment are necessary for the immune response to proceed.
Bar = 3 micrometers.

subpopulation. J. Immunol. 134, 2161-2169.

Huiskamp R, Van Vliet E, Van Ewijk W (985). Repopulation of the mouse thymus after sublethal fission neutron irradiation. II. Sequential changes in the thymic microenvironment. J. Immunol. 134, 2170-2178.

Hurt P, Witte ON (1987). A single bone marrow derived stromal cell type supports in vitro growth of early lymphoid and myeloid cells. Cell 48, 997-1007.

Jordan RK, Robinson IJ, Hopkinson NA, House KC, Bentley AL (1985). Thymic epithelium and the induction of transplantation tolerance in nude mice. Nature 314, 454-456.

Kadish JL, Bash RS (1976). Hemopoietic thymocyte precursors. I. Assay and kinetics of the appearance of progeny. J. Exp. Med. 143, 1082-1099.

Kast WM, De Waal LP, Melief CJM (1984). Thymus dictates major histocompatibility complex (MHC) specificity and immune response genotype of class II MHC restricted T cells but not of class I MHC restricted T cells. J. Exp. Med. 160, 1752-1765.

Khosla S, Ovalle WK (1986). Morphology and distribution of cystic cavities in the normal murine thymus. Cell Tissue Res. 246, 531-542.

Kingston R, Jenkinson EJ, Owen JJT (1985). A single stem cell can colonize an embryonic thymus, producing phenotypically distinct T cell populations. Nature 317, 811-813.

Kruisbeek AM, Longo DC (1985). Acquisition of MHC restricted specificities: role of thymic stromal cells. Surv. Immunol. Res. 4, 110-119.

Kruisbeek AM, Mond JJ, Fowlkes BJ, Carmen JA, Bridges S, Longo DL (1985). Absence of Lyt2-L3T4+ lineage of T cells in mice treated neonatally with anti-I-A correlates with absence of intrathymic I-A bearing antigen presenting cell function. J. Exp. Med. 161, 1029-1047.

Le Douarin NM, Dieterlen-Lièvre F, Oliver PD (1984). Ontogeny of primary lymphoid organs and lymphoid stem cells. Am. J. Anat. 170, 261-299.

Lo D, Sprent J (1986). Identity of cells that imprint H-2 restricted T cell specificity in the thymus. Nature 319, 672-675.

Marrack P, Kappler. J.W (1987). The T cell receptor. Science 238, 1073-1079.

Mulder AH, Bauman JGJ, Visser JWM, Boersma WJM, Van den Engh GJ (1984). Separation of spleen colony forming units and prothymocytes by use of a monoclonal antibody detecting a H-2K determinant. Cell. Immunol. 88, 401-410.

Murakami T (1974). A revised tannin-osmium method for non-coated scanning electron microscope specimens. Arch. Histol. Jpn. 36, 189-193.

Owen JJT, Jenkinson EJ (1984). Early events in T lymphocyte genesis in the fetal thymus. Am. J. Anat. 170, 301-310.

Pardoll DM, Fowlkes BJ, Bluestone JA, Kruisbeek A, Maloy WL, Coligan JE, Schwartz RH (1987). Differential expression of two distinct T cell receptors during thymocyte development. Nature 326, 79-81.

Rothenberg E, Lugo JD (1985). Differentiation and cell division in the mammalian thymus. Dev. Biol. 112, 1-17.

Rouse RV, Van Ewijk W, Jones PP, Weissman IL (1979). Expression of MHC antigens by mouse thymic dendritic cells. J. Immunol. 122, 2508-2515.

Saint Marie G, Peng FS, Marcoux D (1986). The stroma of the thymus of the rat: morphology and antigen diffusion: a reconsideration. Am. J. Anat. 177, 333-345.

Scollay R, Shortman K (1985). Cell traffic in the adult thymus: cell entry and cell exit, cell birth and cell death. In: Recognition and regulation in cell mediated immunity. Watson JD, Marbrook J, eds. Marcel Dekker Inc., New York, p. 3.

Snodgrass HR, Kisielow P, Kiefer M, Steinmetz M, von Boehmer H (1985). Ontogeny of the T antigen receptor within the thymus. Nature 313, 592-595.

Stobo JD (1982). Cellular interactions in the expression and regulation of immunity. In: Basic and clinical immunology. Stites DP, Stobo JD, Fudenberg HH, Wells JV., eds. pp. 89-96.

Streeter PR, Berg EL, Rouse BTN, Bargatze REF, Butcher EC (1988). A tissue specific endothelial cell molecule involved in lymphocyte homing. Nature 331, 41.

Van Ewijk W (1984). Immunohistology of lymphoid and non-lymphoid cells in the thymus in relation to T lymphocyte differentiation. Am. J. Anat. 170, 311-330.

Van Ewijk W, Brons NHC, Rozing J (1975). Scanning electron microscopy of homing and recirculating lymphocyte populations. Cell Immunol. 19, 245-261.

Van Ewijk W, Rozing J, Brons NHC, Klepper D (1977). Cellular events during the primary immune response in the spleen. A fluorescence, light and electron microscopic study in germfree mice. Cell Tissue Res. 183, 471-484.

Van Ewijk W, Verzijden JHM, Van der Kwast ThH, Luycx-Meijer S (1974). Reconstitution of the thymus dependent area in the spleens of lethally irradiated mice. A light and electron-microscopic study of the T cell microenvironment. Cell Tissue Res. 149, 43-60.

Van Ewijk W, Rouse RV, Weissman IL (1980). Distribution of H-2 microenvironments in the mouse thymus. Immunoelectron microscopic identification of I-A and H-2K bearing cells. J. Histochem. Cytochem. 28, 1089-1099.

Van Vliet E, Melis M, Van Ewijk W (1984). Monoclonal antibodies to stromal cell types of the mouse thymus. Eur. J. Immunol. 14, 524-529.

Van Vliet E, Melis M, Van Ewijk W (1986). The influence of dexamethasone treatment on the lymphoid and stromal composition of the mouse thymus: a flowcytometric and immunohistological study. Cell. Immunol. 103, 229-240.

Veerman AJP, Van Ewijk W (1975). 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. 156, 417-441.

Weissman IL (986). Nursing the thymus. Lab. Invest. 55, 1-4.

Zinkernagel RM, Doherty PC (1979). MHC restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T cell restriction, specificity, functions and responsiveness. Adv. Immunol. 27, 51.

Discussion with Reviewers

Reviewer I: Is the subcapsular space in the thymic cortex a region that is actually separated from the deep cortex, and is there any evidence for the presence of nurse cells in the cortex?
Authors: We feel that there is no absolute separation of the subcapsular cortex from the deep cortex, although within the subcapsular cortex sometimes virtually closed globular epithelial structures are observed. These structures resemble "thymic nurse cells" and these structures could provide a microenvironment separated from the rest of the cortex. The structure of the subcapsular thymic cortex, including thymic nurse cells will be published in more detail elsewhere (Lab. Invest., 1988, in press).

Reviewer I: Were Hassall's corpuscells observed, and if so were they in anyway related to blood vessels?
Authors: We did not observe Hassall's corpuscells. These structures occur at very low frequency in the medulla of the mouse thymus.

Reviewer I: References to epithelial cell types in this paper could use the terminology as described by v.d. Wijngaert et al. (1984, Cell Tissue Res., 237, 227-237), as transmission electron microscopy shows the same morphological types to be present in the thymus of rodents and most other animals.
Authors: We agree with the point that various stromal cell types in the thymus should be properly and uniformly identified. However, we would rather include immunological markers in the definition of stromal cells. A workshop on this topic will be organized at the "Thymic microenvironment" meeting in Seillac, France, September 1988.

A.G. Farr: In collaboration with Drs. deBruyn and Cho, I examined the structure of the sinus wall in the lymph node (Am. J.Anat. 157, 265, 1980). Based on transmission and scanning electron microscopic evidence, we were very convinced that the sinus walls did not have gaps in them. The continuity of the sinus wall was interrupted only by the presence of temporary apertures associated with diapedesing cells. Holes in the sinus wall not associated with migrating cells were not observed.
Authors: In the present study Fig. 9b clearly shows the presence of holes in the sinus wall of the subcapsular sinus in the lymph node. Fig. 10 suggests that macrophages protrude from these holes to clear the lymph fluid in the sinus from antigens. This is particularly manifest in the subcapsular sinus. It may well be that not all sinuses express this phenomenon. Holes in the sinus wall may be more manifest at sites where lymph enters the node, and at places where antigen trapping is known to occur. Another point to add here is that the immunological status of the lymph node (we used for our present studies the mesenteric node, whereas Farr and DeBruyn used the quiescent popliteal node) may influence the presence or absence of apertures in the sinus wall.

A.G. Farr: There is some controversy as to whether the diapedesing lymphocytes traverse transient migration apertures in the endothelial cells or pass between adjacent endothelial cells. Again, work of Farr and DeBruyn (Am. J. Anat. 143, 59), and others provided good scanning and transmission electron microscopic evidence that the migration lymphocytes did not migrate along interendothelial cell junctions.
Authors: We agree about the controversy on the diapedesing lymphocytes in HEV. Certainly, from our work we cannot fully exclude the possibility that lymphoid cells transit through the cell body of endothelial cells, however, we found that the large majority of migrating cells was situated in gaps and ridges in between endothelial cells, as is clearly shown in Figs. 12a and 12b.