Histological, Histochemical, and Fine Structural Observations on the Lymph Node of the Common Seal (Phoca vitulina) and the Grey Seal (Halichoerus grypus)

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ABSTRACT  Background: The recent seal death epizootic prompted interest in their immune system, for which no current morphological data were available.

Methods: Lymph nodes from adult harbor seals (Phoca vitulina) and grey seals (Halichoerus grypus) were investigated by light microscopy, electron microscopy, and lectin histochemistry.

Results: No significant differences in the lymph node morphology were found between the two species, and the overall organization of the nodes comprises of capsule, trabeculae, cortex, paracortex, and medulla. Capsule and trabeculae are composed of tightly packed collagen and elastic fibrils and are rich in fibroblasts, myofibroblasts, and smooth muscle cells. Unmyelinated nerve fibers are common. The cortex contains numerous secondary follicles with well-developed germinal centers and paracortical areas with high endothelial venules. Antigen-presenting cells and phagocytic macrophages were abundantly present. The medullary cords contain numerous plasma cells. Fibroelastic reticulum cells are common throughout the parenchyma and transverse the sinus. Marginal, radial, and medullary sinuses are lined by littoral cells. The visceral lining of the sinuses is marked by macrophages and by numerous mast cells.


Key words: seals; Phoca vitulina; Halichoerus grypus; lymph node; immune system

Seals are amphibious mammals and are exposed to both terrestrial and aquatic environments; they give birth, nurture their young, mate, and molt on land. All their nutritional requirements are met by the resources of the sea, in which they spend usually more time than on land. The grey seal and the harbor seal are members of the family Phocidae (true or earless seals), which together with the Otariidae (sea lions) and Odobenidae (walrus) comprise the pinnipedia, one of the two suborders of the carnivores. All pinnipedia are aquatic mammals but are less modified for aquatic life than the wholly aquatic cetaceans. The seals inhabit coastlines and ice fronts mainly in polar and temperate oceans and adjoining seas.

The pinnipedia are derived from terrestrial arctoid (bear-like) carnivores. Their fossil record dates back to the border oligocene/miocene. Otariids were present in the North Pacific at least 22 million years ago. There is some debate whether all pinnipeds are a mono- or diphyletic group; the majority of anatomical, palaeontological, biochemical and molecular data appears to favor the concept of the monophyly of the group (for a recent discussion see Starck, 1995).

The grey seal is a gregarious coastal species, which does not undertake well-defined long-distance migrations. In many areas harbor seals are present all year-round. Some short migrations may be associated with the seasonal availability of prey and with breeding. Harbor seals are adaptable to a variety of habitats and haul out on, for example, intertidal ledges, rocky islets, reefs, and cobble beaches.
The ultrastructural morphology and histochemistry of lymphoid organs of aquatic mammals is not well elucidated. The published records mainly concern gross anatomy and general histology (Simpson and Gardner, 1972; Banks, 1986) as well as preliminary descriptions of microscopy on the spleen of pinnipeds (Schumacher and Welsch, 1987) and general lymphoid organ histology of sirenians (Cave and Aumonier, 1967) and cetaceans (Romano et al., 1993, 1994). However, detailed morphological investigations on the structure of the lymph nodes of seals are not available. The immune system of the common seal (Phoca vitulina) recently attracted attention because of the seal epizootic of 1988/89 during which more than 17,000 common seals and a smaller number of grey seals died (Dietz et al., 1988/89). The disease has been identified as the causative agent of this epizootic (Romano et al., 1993, 1994). However, since no data on the lymph nodes of the seals were available, the interpretation of lymphocyte depletion was based on observations in other species which can be misleading. Hence, a description of normal seal lymph nodes is desirable to enable the findings of immunodeficiency in seals to be reviewed more critically.

**MATERIALS AND METHODS**

Mesenteric lymph nodes of five healthy adult common seals (Phoca vitulina) and six grey seals (Halichoerus grypus) were available for this study. This lymph node group was chosen since lymph nodes draining the intestine show the greatest degree of immunological stimulation (see Horny and Horst, 1984). The lymph nodes were grouped together at the root of the mesentry and were excised immediately after the death of the animals, which were collected during a hunting expedition at the south coast of Iceland. The animals included in the study did not show any gross signs of disease. Histological examination of the major organs of the hunted seal did not show any pathological changes (data not shown).

**Light Microscopy and Lectin Histochemistry**

The sampling of the organs was performed immediately after death on site. The fixation in 4% neutral buffered formalin was carried out for 48 h before the material was transferred to buffer until further processing for wax histology. The following routine stainings were performed on wax embedded material: haematoxylin and eosin (H&E), silver impregnation according to Gomori, and periodic acid Schiff (PAS) reaction. For lectin histochemistry, deplasted sections were washed in distilled water and trypsinized for 15 min at 37°C in a Shandon Sequenza Immunostaining Centre. After a wash in distilled water, nonspecific endogenous peroxidase was blocked by incubating the sections in 0.3% H$_2$O$_2$ in methanol for 20 min. The sections were incubated with a panel of biotinylated lectins (for the origin of the lectins, their abbreviations, and carbohydrate specificities see Table 1). All lectins except MLs, which were kindly provided by PD Dr. U. Pfüller, University of Witten/Herdecke, were obtained from Sigma (Poole, Dorset, UK) and biotinylated in-house with biotin NHS–long arm spacer (for details see Schumacher et al., 1993). The biotinylated lectins (both 10 and 50 µg/ml were used) diluted in Tris-buffered saline with 1% CaCl$_2$ added (TBS) were applied for 30 min, and after three washes with TBS the biotinilated lectins were detected using a standard avidin-biotin-peroxidase (ABC) complex (Vector, Peterborough, UK). The peroxidase was visualized using diaminobenzidine hydrochloride/H$_2$O$_2$. Controls were performed using 0.3 M of the appropriate inhibitory sugar (see Table 1) except for PHA-L, where 50 or 10 µg of thyroglobulin was used as an inhibitory glycoconjugate, or in the case of SNA, where the sections were incubated with neuraminidase (for details see Schumacher et al., 1994). The sections were counterstained with haematoxylin and photographed using an Olympus photomicroscope with a Kodak Technical Pan black-and-white film.

**Electron Microscopy**

Small cubes of lymph nodes measuring 1–2 mm in length were fixed for 2 h in 4°C phosphate-buffered glutaraldehyde (3.5%, pH 7.5). Afterwards the tissue was rinsed in cold phosphate buffer and stored in 1% glutaraldehyde until further processing. A 2 h postfixation in 2% OsO$_4$ was followed by dehydration in a series of graded ethanol and embedding in Araldite. For orientation, 1 µm thick sections were stained with Toluidine blue. Ultrathin sections were cut on a Reichert ultramicrotome, contrasted for 5 min with uranylacetate and lead citrate, and viewed in a Philips CM 10 electron microscope.

**RESULTS**

No significant differences in lymph node morphology were found between the two seal species studied, and hence the morphology of both species will be described together.

**Capsule**

The connective tissue capsule of the lymph nodes is thick, and a well-developed system of trabeculae penetrates the parenchyma of the lymph node in which a clear subdivision between the cortical and medullary zones can be detected in H&E sections of the bean- or kidney-shaped mesenteric lymph nodes (Fig. 1). Both capsule and trabeculae consist mainly of densely packed bundles of collagen fibrils; the diameter generally is between 25 and 40 nm (Fig. 2a). They form a three-dimensional system of collagen fibrils, which are more tightly packed at the surface of the capsule. Not infrequently elastic fibers are interwoven between the collagen fibrils. Fibroblasts and smooth muscle cells are the principal cellular components of the capsule. The fibroblasts generally have a rather small rim of cytoplasm with only a few cellular organelles. Their small cytoplas-
mic extensions closely approach the surrounding collagen fibrils or the microfibrils of the elastic fibers. Small membrane invaginations and pits indicating endocytosis occur regularly. Typical smooth muscle cells and intermediate cells between fibroblasts and smooth muscle cells, the myofibroblasts, are interspersed in the connective tissue matrix (Fig. 2b). In general, the smooth muscle cells are covered by a basal lamina, which, however, can be absent locally. Unmyelinated nerve fibres are commonly present in the capsule (Fig. 2a).

Valve-containing afferent lymphatic vessels penetrate the capsule occasionally. Larger blood vessels are concentrated in the trabeculae which originate in the hilus of the lymph node, while those trabeculae originating in the capsule lack larger blood vessels and contain only small blood vessels including capillaries.

**Marginal and Intermediary Sinuses**

The connective tissue components of the capsule are continuous with the outer margin of the marginal sinus which is lined by littoral (endothelial) cells (Fig. 3). These 25–50 µm long cells are flat and form a continuous layer resembling closely an endothelium; interdigitations are formed between neighboring cells which are specifically interconnected by zonulae occludentes. The littoral cell has both endocytotic vesicles and secretory granaules. While the outer (parietal) sinus endothelium is continuous and regularly underlain by a basal lamina, the inner (visceral) endothelium is often interrupted by mast cells, macrophages, and lymphocytes; its basal lamina is often not continuous. Opposite the basal lamina, the littoral cells often form small hemidesmosomes. The sinus is packed with lymphocytes; a cell count of 200 cells in the sinus reveals the following cellular distribution: 60% lymphocytes, 13% fibroblastic reticulum cells, 11% macrophages (monocytes), 4% plasma cells, 5% neutrophil granulocytes, and 2% myeloid cells. Mast cells (Fig. 3c) are also rather common in and along the sinus and are often found along the apical membrane of the inner (visceral) littoral cells but can also be found under the littoral cells or within the endothelium built up by the littoral cells.

Fibroblastic reticulum cells cross the marginal sinus and form a loose interconnecting meshwork. They engulf collagen fibrils in their gutter-shaped cytoplasmic invaginations so that collagen fibrils are sheltered from the content of the sinus lumen. Larger connective tissue pillars spanning the lumen of the sinus are ensheathed by the littoral cells and contain collagen, elastic fibers, and even fibroblasts. Intermediate sinuses are found alongside the trabeculae radiating into the medulla and are continuous with the medullary sinuses which have much wider lumina. Strikingly, numerous mast cells are found in association with the visceral lining of the sinuses and within the sinuses. The ultrastructure of the intermediate sinuses (Fig. 4) closely resembles that of the marginal sinuses.

**Cortex**

In the cortex of the lymph node, two regions can be clearly distinguished: the areas where the follicles are located and the paracortical zone between the follicles which is continuous with the medullary region. The vast majority of the follicles are secondary, with clearly delineated germinal centers surrounded by a darker stained mantle zone containing tightly packed monomorphic intensely stained small lymphocytes (Fig. 3a). The mantle zone forms a thick cap on the side where the follicles face the sinus. The relation of the feret diameter of the mantle zone to the feret diameter of the germinal center is generally between one-third and two-thirds; however, in larger follicles it can be one-fifth to four-fifths.

The germinal center can be divided into two parts. First is a lighter stained zone consisting predominantly of centrocytes, which can be recognized by their oval cytoplasm and their cleaved nucleus (Figs. 5, 6). This zone is oriented towards the marginal and radial sinus. The second zone is stained darker and consists of centroblasts which can be recognized by their basophilic cytoplasm and their large nucleus containing several marginally located nucleoli. Occasionally mitotic figures can be seen in this area. Macrophages with heteromorph lysosomes and multinucleated giant cells with smooth rimmed euchromatin-rich nuclei are occasionally found (Fig. 5c). Circular reticulum fibers seem to embrace the whole germinal center, though only sparsely distributed and without a particular orientation within the germinal center.

Dendritic reticulum cells are characterized by a deaved nucleus with a fine granular, electron-dense heterochromatin and a centrally located nucleolus (Fig. 6b). The cytoplasm is pale and on many sections is relatively poor in organelles; however, not infrequently these cells can contain considerable accumulations of mitochondria. Irregular cytoplasmic fingerlike protrusions extend into the surrounding tissue. Numerous small lymphocytes can be found adjacent to these cells.

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**TABLE 1. The lectins used in this study, their abbreviations, and their carbohydrate specificities.**

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Abbreviation</th>
<th>Nominal sugar specificity</th>
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<tbody>
<tr>
<td>Griffonia simplicifolia-I</td>
<td>GSA-I</td>
<td>N-acetylgalactosamine</td>
</tr>
<tr>
<td>Helix pomatia</td>
<td>HPA</td>
<td>N-acetylgalactosamine</td>
</tr>
<tr>
<td>Soy bean</td>
<td>SBA</td>
<td>N-acetylgalactosamine</td>
</tr>
<tr>
<td>Sophora japonica</td>
<td>SJ A</td>
<td>N-acetylgalactosamine</td>
</tr>
<tr>
<td>Vicia villosa</td>
<td>VVA</td>
<td>N-acetylgalactosamine</td>
</tr>
<tr>
<td>Wisteria floribunda</td>
<td>WFA</td>
<td>N-acetylgalactosamine</td>
</tr>
<tr>
<td>Mistletoe III</td>
<td>ML-III</td>
<td>N-acetylgalactosamine</td>
</tr>
<tr>
<td>Mistletoe II</td>
<td>ML-II</td>
<td>N-acetylgalactosamine</td>
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<tr>
<td>Mistletoe I</td>
<td>ML-I</td>
<td>Galactose</td>
</tr>
<tr>
<td>Griffonia simplicifolia-B4</td>
<td>GSA-B4</td>
<td>Galactose</td>
</tr>
<tr>
<td>Peanut</td>
<td>PNA</td>
<td>Galactose</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>WGA</td>
<td>N-acetylgalactosamine</td>
</tr>
<tr>
<td>Canavalia ensiformis</td>
<td>ConA</td>
<td>Mannose, glucose</td>
</tr>
<tr>
<td>Galanthus nivalis</td>
<td>GNA</td>
<td>Mannose</td>
</tr>
<tr>
<td>Lens culinaris</td>
<td>LCA</td>
<td>Mannose</td>
</tr>
<tr>
<td>Lotus tetragonolobus</td>
<td>LTA</td>
<td>Fucose</td>
</tr>
<tr>
<td>Ulex europaeus-I</td>
<td>UE-A-I</td>
<td>Fucose</td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td>PHA-L</td>
<td>Complex carbohydrates</td>
</tr>
<tr>
<td>Sambucus nigra</td>
<td>SNA-I</td>
<td>Sialic acid</td>
</tr>
</tbody>
</table>
Fig. 1. Phoca. **a:** Low power magnification of the lymph node. Note the well-developed capsule (C), the cortical region (CR) below the capsule, and the well-developed system of trabeculae (T) radiating into the medulla (M). H&E. Scale bar = 200 µm. **b:** Capsule (C) and cortical region of the lymph node. Note the presence of argyrophilic fibers (arrowheads) in the capsule and around the secondary follicle (F). Note the almost complete absence of argyrophilic fibers in the germinal center. Gomori. Scale bar = 40 µm.
Fig. 2. Capsule of the lymph node in Phoca. a: Note the presence of nerve fibers (N) between the bundles of collagen fibrils (CF). Elastic (E) fibers are also present. Scale bar = 0.2 µm. b: A myofibroblast (M) is surrounded by collagen fibrils. Scale bar = 0.2 µm.
Fig. 3.
In addition, larger lymphocytes, centroblasts, with a pale nucleus and large nucleolus, large rim of cytoplasm with mitochondria, ribosomes, and cisternae of rough endoplasmic reticulum can be found. These cells are tightly packed in groups with close apposition of the neighboring cell membranes. The region containing the centrocytes is located next to the centroblast-containing region. They are characterized by their medium size, a cytoplasm poor in cellular organelles, and a deeply cleaved nucleus (Fig. 6a). The nuclei contain relatively little heterochromatin and only very occasionally more than two nucleoli. Fibroblastic reticulum cells are regularly found in germinal centers. Their nuclei are rich in heterochromatin and contain one nucleolus, and cytoplasmic extensions of these cells branch out and usually surround collagen fibrils. They form desmosomes with adjacent fibroblastic reticulum cells.

The whole cortical region—mainly the paracortical region—is rich in blood vessels, particularly veins. The endothelium rests on a well-developed basal lamina which is covered by collagen fibrils.

**Paracortex**

This area stretches from the region between the follicles into the medullary part of the lymph node and is characterized by small-sized monomorphic lymphocytes tightly packed into small groups which are often oriented in radial strands towards the medullary sinus. The lymphocytes are morphologically rather uniform with a moderate density of the heterochromatin and often containing several nucleoli. Due to the uniform morphology of the lymphocytes, no further morphological subdivision of the lymphocyte population is possible. Interdigitating reticulum cells are rarely found. These pale cells are characterized by their elongated, irregular nucleus and their small cytoplasmic granules and tubular cisternae. Multiple cytoplasmic extensions are in close contact with surrounding lymphocytes (Fig. 7). Birbeck granules as found in interdigitating cells of humans have not been detected in these seal cells. In addition to the interdigitating reticulum cells, fibroblastic reticulum cells are present in the paracortical region. In general, they are not different from those in the follicles. Typical, and indicative for the paracortical region, is the presence of numerous high endothelial venules (HEVs) (Fig. 8). The large nuclei (in comparison to those in other endothelial cells) are rich in euchromatin and contain a prominent nucleolus. The endothelial cells are linked by complex contact zones, and the apical membrane can form long and branched microfolds (Fig. 8a). Both smooth and clathrin-coated pits and vesicles can be seen at the apical surface of these cells (Fig. 8b).

Intermediate filaments are well developed in these cells (Fig. 8c). Single lymphocytes or groups of two lymphocytes often penetrate the cytoplasm of these cells in the regions where no cell-to-cell junctions are found (Fig. 8d).

**Medulla**

Medullary cords separated by medullary sinuses, which have broad lumina and are built up by a loose meshwork of reticulum fibers filled with lymphocytes and plasma cells, are the distinguishing features of the medullary zone of the lymph node (Fig. 9). The extracellular matrix is made up of collagen fibrils which are either oriented in parallel fibrils or in a feltwork-like fashion. In Halichoerus, frequently a filamentous matrix with a relatively indistinct cross-striation was found, which is interpreted to represent collagen type VI. Plasma cells (Fig. 9) are common, often grouped together, and are characterized by their round to oval, eccentrically located nucleus with the typical cartwheel pattern of heterochromatin distribution. The large cytoplasm is filled with partly dilated rough endoplasmic reticulum. In the whole medullary region fibroblastic reticulum cells are found on rare occasions. mast cells are also common in the medullary region and are often grouped together or in conjunction with plasma cells (Fig. 9). The diameter of the mast cells is up to 20 µm, and the large round to oval nucleus contains between one and four nucleoli. The cytoplasm of each mast cell is characterized by the presence of numerous round granules containing either coarse particles or meandering filamentous structures. Neutrophil granulocytes are another frequent cell population. Their cytoplasm contains electron-dense round or dumble-shaped granules, sometimes containing crystalline material. Eosinophils are often clustered together and tightly packed. They are characterized by their lentil-shaped, electron-dense granules with a mean diameter of about 1 µm. The periphery of the granules is often lighter stained than the center which frequently is built up by a crystalloid inclusion body. Groups of eosinophils are often surrounded by a wall of plasma cells. Regularly macrophages have been found stuffed with lipid droplets; such lipid droplets also occur freely in the sinus lumen.

The medullary cords are separated from the broad lumina of the medullary or central sinus by an endothelium consisting of flat littoral cells, underlain by a narrow discontinuous basal lamina. The endothelium is similar to that of the other sinuses described above. Again, as in the other sinuses, fibroblastic reticulum cells and bundles of collagen fibrils cross the lumen. The collagen is again ensheathed by fibroblastic reticulum cells.

**Lectin Histochemical Findings**

From the N-acetylgalactosamine- and galactose-specific lectins, HPA, ML-I,-II,-III, SBA, SJA, and VVA did not react with the lymph nodes. The other lectins generally showed a stronger reactivity at 50 µg/ml lectin used, and the description will be based on this concentration. GSA-I showed a strong reactivity with
the collagenous capsule and with reticulum cells and macrophages in the medullary sinuses (Fig. 10a). GSA-I/IB4 showed essentially the same binding pattern as GSA-I but had an additional reactivity with mononuclear cells in the germinal center and the paracortical region. WFA strongly bound to the collagenous capsule of the lymph node, to the endothelium of blood vessels including the endothelium of the sinuses, and to erythrocytes, lymphocytes, and macrophages particularly in the medullary sinusoids. In HEV it only bound to the apical surface of the cells and not consistently to all HEVs. Macrophages in the germinal centers were also labeled.

Con A also reacted with the collagen capsule, with the cytoplasm of macrophages in the germinal center, with the multinucleated giant cells, and with the cell membrane of lymphocytes in the region around the germinal centers. GNA reacted with macrophages in the germinal centers and with macrophages in the paracortical region (Fig. 10b). LCA, LTA, and UEA-I did not react with the tissue, while PHA-L showed the same binding pattern as GSA-I.

SNA-I bound to cell membranes of lymphocytes in the germinal centers and even more intensively to macrophages and dendritic cells (Fig. 10c). Lymphocytes in the cap region were also intensively stained, as was a lymphocyte population in the deeper paracortical zones.

WGA showed the broadest binding pattern: macrophages/reticulum cells in the germinal centers were labeled, as were lymphocytes in the paracortical zone. The capsule was very intensively stained, as were endothelia in the larger blood vessels. Sinus-lining cells, erythrocytes, and lymphocytes in the central medullary sinuses were also stained.

The reactions, especially with the N-acetylgalactosamine-specific lectins, were not uniform; some seal nuclei centered on those specific lectins at all.

In control sections lectin reactivity was abolished except for the reaction of the loose connective tissue around the collagenous capsule and, in the case of the erythrocytes, where the intensity of the lectin reaction only decreased.

DISCUSSION

The present study was undertaken to assess the functional morphology of the structure of normal lymph nodes from two seal species of the North Sea, the grey seal (Halichoerus grypus) and the common seal (Phoca vitulina). Besides the intrinsic scientific interest in the normal structure and function of the immune system of aquatic mammals, a practical need for the knowledge of these structures arose during the seal death epidemic in 1988/89. From an evolutionary point of view, no specific structural adaptations have been found in respect of the lymph nodes of the two pinniped species studied. The entire lymph node structure falls entirely into the general mammalian pattern. The numerous fully developed large secondary follicles and the broad T-cell regions indicate that also the coastal marine habitats are endowed with abundant antigens and that the seals are normally able to respond to these antigens powerfully.

In respect to the practical need of the present study, histological examination of the lymphatic organs from the deceased animals revealed what appeared to be a lymphocyte depletion in the lymphatic organs, including lymph nodes, when compared to other mammals (Schumacher et al., 1990); however, the structure of normal lymph nodes from P. vitulina was not known at that time, and hence the conclusions drawn in that report were based on limited knowledge. This report has now ascertained that the lymph nodes of seals which died during the epidemic were indeed lymphocyte-depleted, not only in comparison with other mammals but also when compared to normal P. vitulina lymph nodes. This observation confirms the assumption, already implied, that the lymph node structure of seals is similar to that of other mammals, in particular to that described of other carnivores such as dog (Fournel et al., 1995) and cat (Banks, 1986). This relative similarity of the lymph node structure between seals and other mammals is also reflected in the fact that lymphocytes from P. vitulina can be stimulated in vitro by the same mitogens as used for studies in humans (de Swart et al., 1993).

Despite the general structural similarity of seal lymph nodes with those of other mammals, morphological peculiarities can be found. Capsule and trabecular systems are, compared to human standards, relatively broad and contain numerous smooth muscle cells embedded in a dense network of collagenous and elastic fibers, which have also been described in the lymph node of rats (Miyata and Takaya, 1981). This contractile apparatus apparently forms a functional unit with collagen and elastic fibers facilitating the flow of lymphatic fluid. Judging from the density of innervation within the capsule and in the trabeculae, this flow seems to be controlled and influenced by the autonomous nervous system. To what extent this innervation is particular to the mesenteric lymph nodes, however, has to be elucidated further by analysis of lymph nodes from different regions.

The ultrastructure of the seal mast cell granules resembles that of several other mammals but is different from those of primates (Weiss, 1988), which are characterized by a fingerprint pattern produced by membrane and tubular formation absent in the seals. All sinuses are lined by flat cells (littoral cells, sinus endothelial cells) characterized as endothelium due to the presence of apical intercellular contacts and a basal lamina. Their similarity with vascular endothelium is further emphasized by the fact that WFA stains endothelium in the blood vessels as well. The labeling of both endothelium and sinus-lining cells by the same specific marker is of interest since some authors argue that these cells, at least in humans, are flattened fibroblastic reticulum cells (Mori and Lennert, 1969; Weiss, 1988, Leonhardt, 1990), while Compton and Ravìola (1985) see the histogenetic origin of these cells in the endothel-
Fig. 5.
lum, an interpretation more in line with the findings reported here. A similar difference in interpretation exists for the histogenetic origin of the cells which cross the lumen. As with the sinus-lining endothelia, the first group of authors (Mori and Lennert, 1969; Weiss, 1988; Leonhardt, 1985) see the fibroblastic reticulum cell as the histogenetic origin of these cells, while Compton and Ravria (1985) argue that these cells are of endothelial origin. The question of the histogenetic origin of these cells is complicated by the observation that in some species both fibroblastic cells and endothelial cells can line the lumina of vertebrate blood vessels (Hughes and Welsch, 1972). The situation in the seals is complicated by the fact that both single cells and broader tissue strands bridge the lumen. The larger strands are covered at the outside by littoral cells, while the single cells crossing the lumen appear to be, as in humans, fibroblastic reticulum cells. The simple cell contacts found between the cytoplasmic extensions covering the collagen fibrils do not as such qualify these cells as endothelial since similar contact zones can be found between nonepithelial cells such as smooth and heart muscle cells. Since these cell contacts are also seen in the fibroblastic reticulum cells, where their fibroblastic character is in no doubt, and the sinus spanning cells surround collagen fibrils presumably secreted by them, it seems justified to assume that these cells are of fibroblast origin.

The principal subdivision of the cortex into follicles and the paracortical region as seen in other mammals can also be made in *P. vitulina* and *H. grypus*. Most of the follicles appear as germinal centers which are indicative of an active humoral immune response since the priming and maturation of B-cells takes place in this location (Stein et al., 1982). Indeed, quantitative studies of the immune response in sterile-reared rats revealed that germinal centers were absent in non-treated control groups, while they formed after antigen administration (Novotny et al., 1994). Four months after immunostimulation, germinal centers could be found only in a few lymph nodes (Novotny et al., 1994). The presence of an active immune response in the mesenteric lymph nodes of *P. vitulina* and *H. grypus*, which directly drain the paracolic lymph nodes, is in accordance with quantitative immunohistochemical data from human lymph nodes, indicating that paracolic lymph nodes are immunologically more active than axillary lymph nodes (Horny and Horst, 1984).

As typical for lymph follicles in other species including man (Stein et al., 1982), the lymphocyte wall forms a cap and is thicker on the side directed towards the sinuses. The internal division of the germinal centers into the areas which contain predominantly centrocytes and those containing predominantly centroblasts, both containing dendritic reticulum cells as found in other mammals (Horton and Ratcliffe, 1993), is also observed in lymph nodes of *P. vitulina* and *H. grypus*. It is unusual that several of the cells interpreted to be dendritic cells contain numerous mitochondria. Normally multinucleated giant cells are not found in germinal centers. Since they contain relatively lightly stained nuclei resembling those of dendritic reticulum cells and also contain only a modest amount of lysosomes, one would assume the histogenetic origin of these cells in the monocyte/macrophage/dendritic cell lineage.

The similarity of the fine structure of the lymph node cortex of seals with that of other mammals also extends to the organization of the paracortex which is dominated by small monomorphic lymphocytes and the presence of high endothelial venules which could be labeled by GalNac-specific lectins (see below) and through which lymphocytes penetrate. Interdigitating reticulum cells are similar in morphology to those described in humans (Kaiserling and Wollburg, 1988). The last two features, namely the high endothelial venules and the interdigitating reticulum cells, are features of the T-region of lymph nodes, and hence in correlation with the other findings it can be assumed that the paracortical region in *P. vitulina* and *H. grypus* serves as the T-cell region does in other mammals.

The last distinguishable region is the medullary region characterized by medullary cords separated by medullary sinuses, some of which have a very broad lumen in *H. grypus*. The interstitium of the medullary rays contains interstitial cross-striated bundles which presumably represent a particular collagen. Due to the morphological similarity between the collagen type VI described in chick embryos and in human fibroblast cell culture (Bruns et al., 1986; van der Rest and Garrone, 1991), it is assumed that these formations are built up of collagen VI. Collagen VI fibrils are found all over the medullary region and are described here for the first time in this particular location.

The medullary region as well as the sinusaloidal walls in general are characterized by the abundant presence of mast cells, often found in small clusters. The presence of mast cells has been regarded as a sign of immunological activation in the lymph nodes of man (Lennert and Illert, 1959), and this interpretation would be in accordance with the high number of germinal centers observed in the lymph node cortex observed in this study, which is also interpreted as a sign of immunological stimulation (see above). In addition to the immunological function of mast cells, it has to be borne in mind that mast cells contain heparin ("clearing factor" for lipids) in their granules, and hence an accessory function in the processing of the ingested lipids passing through the mesenteric lymph nodes, possibly in conjunction with the fat phagocytosing and storing cells, can be assumed.

Plasma cells are another very common feature of this region, and in experimental studies their number in the medullary region of lymph nodes increases after antigenic stimulation (Novotny et al., 1994), again arguing for the immunologically active nature of these lymph nodes. A similarly high content of plasma cells was described in the spleen of Weddell seals in which it was interpreted as a high antigen exposure (Schumacher and Welsch, 1987), a conclusion which seems also justified in this study.

Fig. 5. Germinal centers in *Phoca*. a: Low power magnification showing the lymphocyte cap (LC) around the germinal center (GC). The lymphocyte cap seems to be thicker nearer the capsule (C). Scale bar = 20 µm. b: Transition between the lymphocyte cap (LC) and the germinal center (GC). Note that the centrocytes (arrows) which make up the majority of cells in the light zone of the germinal center are oriented towards the capsule, while the centroblasts (arrowheads) are oriented at the opposite side. Note the presence of germinal center macrophages (star). Scale bar = 10 µm. c: Multinucleated giant cells (arrows) in the germinal center. Scale bar = 10 µm. Semithin sections, Toluidine blue.
Fig. 6. Germinal center in Phoca. a: Centrocytes (CC) with convoluted nuclei. Scale bar = 1 µm. b: Dendritic cell with several sections through the nucleus (N) and numerous mitochondria (arrows). Scale bar = 1 µm.
Monoclonal antibodies to subtype the lymphocyte population in the lymphatic organs are not available; hence, functional tests have been used to assess the immune status (Kendall et al., 1992). However, several cell populations in the lymph node could be labeled by the use of lectins, carbohydrate-binding proteins which have been widely used across the species barrier in histochemical studies (Spicer and Schulte, 1992). In

Fig. 7. Paracortical region in Phoca. The interdigitating cell (IDC) branches out into the surrounding tissue with many interdigitations. Note the lymphocytes (L) which are in close contact to the interdigitating cell. Scale bar = 1 µm.
particular the cytoplasm of macrophages and/or dendritic cells could be labeled by the mannose/glucose-specific lectins ConA and LCA. The labeling of the giant cells with Con A argues for the histogenetic origin of these cells from the monocyte/macrophage lineage. This intracytoplasmic labeling pattern is in accordance with findings in humans, where Con A labeled cells of the monocyte/macrophage lineage in lymph nodes (Strauchen, 1984; Horst et al., 1992), and findings in the spleen of antarctic seals, where Con A also labeled monocytes/macrophages (Schumacher and Welsch, 1987). In addition to the reaction with the cytoplasm of macrophages, Con A also particularly reacted with the cell membranes of lymphocytes outside the germinal center, where particularly T-cells are found. This labeling of T-cells by Con A is in accordance with functional studies of peripheral blood mononuclear cells in P. vitulina, where it has been shown that Con A acts predominantly as a T-cell mitogen (deSwart et al., 1993).

Macrophages in the medullary sinuses reacted strongly with GSA-I; this lectin also stained the same macrophage population in mesenteric lymph nodes in Wistar rats (Sminia and van der Ende, 1991). While this marker was a constant one in Wistar rats, it was
not the case in seal lymph nodes. The marked differences, especially in GalNac-specific lectin binding between the individuals in this study, may indicate that such differences are due to blood group substances or related antigens. In humans GalNac-specific lectins such as Helix pomatia agglutinin are blood group specific (Hammarström and Kabat, 1969), and the differences in the lectin binding pattern in this study may therefore also relate to blood groups in seals. These blood group-specific differences would of course not be apparent in inbred strains such as the Wistar rats, and hence a more uniform lectin binding pattern as reported by Sminia and van der Ende (1991) can be observed.

Using the limited set of lectins, no overall marker of vascular endothelium of all types of blood vessels, including arteries, capillaries, and veins and their
respective subdivision in the lymph node of seals, could be detected. However, WGA showed binding to endothelia in larger blood vessels in accordance with the findings of Alroy et al. (1987), who reported that WGA labeled endothelia of several mammalian species including man, while GSA-I was a good marker for endothelia of all mammals investigated excluding man. However, in their study no lymph node were investigated and, since carbohydrate residues play a role in lymphocyte traffic, the distribution of carbohydrate residues may be more complex than in other organs where no such traffic takes place. Carbohydrate heterogeneity of the endothelium as detected by lectin histochemistry is not unique but has also been described in the mouse kidney using the GalNac-specific Dolichos biflorus agglutinin (Spicer and Schulte, 1992). Lymphocyte interchange occurs in particular in the high endothelial venules, and WFA bound to the apical membrane of high endothelial venules but not to all apical surfaces of high endothelial venules. Since not all high endothelial venules displayed the same WFA binding capacity, it is attractive to speculate that the presence of GalNac residues recognized by WFA can act as a partner in the lymphocyte/high endothelial venule interaction. However, further experiments are needed to support such a proposition. This interaction could be considered species-specific since glucosamine- and mannose-specific lectins are the main binding partners for high endothelial venules in other species such as pigs (Whyte et al., 1993).

The much more detailed morphological investigation of the lymph nodes of P. vitulina presented in this study might be of future use if a seal death epizootic should recur or if the effects of other environmental toxic substances have to be evaluated. In the previous studies, no attempt was been made to investigate the distribution of plasma cells or mast cells in the lymph nodes of the diseased seals. This future, more detailed analysis of the lymph nodes might be particularly appropriate since it has been suggested that the environmental pollutants polychlorinated biphenyls (PCBs) led to immunocompromised seals, which made them more susceptible to the phocine distemper infection (Schumacher et al., 1990). Since both phocine distemper (Baker, 1992) and PCBs lead to an immunodepression, a more detailed morphological analysis of the immune system of seals might help to dissect out which part of the immunodepression is caused by the virus and which by the environmental pollution. Since the seals investigated in this study originate from a population of seals relatively uncompromised in terms of environmental pollution when compared with North Sea seals (Schumacher et al., 1995), it serves particularly well as a control group.

LITERATURE CITED


