Lacrimal Drainage–Associated Lymphoid Tissue (LDALT): A Part of the Human Mucosal Immune System

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PURPOSE. Mucosa-associated lymphoid tissue (MALT) specifically protects mucosal surfaces. In a previous study of the human conjunctiva, evidence was also found for the presence of MALT in the lacrimal sac. The present study, therefore, aims to investigate its morphology and topographical distribution in the human lacrimal drainage system.

METHODS. Lacrimal drainage systems ($n = 51$) obtained from human cadavers were investigated by clearing flat whole-mounts or by serial sections of tissue embedded in paraffin, OCT compound, or epoxy resin. These were further analyzed by histology, immunohistochemistry, and electron microscopy.

RESULTS. All specimens showed the presence of lymphocytes and plasma cells as a diffuse lymphoid tissue in the lamina propria, together with intraepithelial lymphocytes and occasional high endothelial venules (HEV). It formed a narrow layer along the canaliculi that became thicker in the cavernous parts. The majority of lymphocytes were T cells, whereas B cells were interspersed individually or formed follicular centers. T cells were positive for CD8 and the human mucosa lymphocyte antigen (HML-1). Most plasma cells were positive for IgA and the overlying epithelium expressed its transporter molecule secretory component (SC). Basal mucous glands were present in the lacrimal canaliculi and in the other parts accompanied by alveolar and acinar glands, all producing IgA-rich secretions. Primary and secondary lymphoid follicles possessing HEV were present in about half of the specimens.

CONCLUSIONS. The term lacrimal drainage–associated lymphoid tissue (LDALT) is proposed here to describe the lymphoid tissue that is regularly present and belongs to the common mucosal immune system and to the secretory immune system. It is suggested that it may form a functional unit together with the lacrimal gland and conjunctiva, connected by tear flow, lymphocyte recirculation, and probably the neural reflex arc, and play a major role in preserving ocular surface integrity. (*Invest Ophthalmol Vis Sci.* 2001;42:566–574)

The mucosa-associated lymphoid tissue (MALT) represents an outpost of the immune system located at mucosal surfaces of the body.[1,2] It is responsible for antigen detection and immune responses[3,4] by the cellular system of T cells[3,5] and the so-called secretory immune system.[6,7] The latter consists of immunoglobulin-producing plasma cells in the subepithelial connective tissue and a transepithelial transport of immunoglobulins to the mucosal surface, where they act as a protective shield against pathologic invasion.[8,9]

In contrast to other organs, relatively little is known about this tissue at the ocular surface and within the lacrimal drainage system, especially in the human.[10,11] This is surprising because the mucosal immune system has been shown to be important for the preservation of mucosal integrity.[3,4,12] There is also growing evidence that lymphoid cells and their immune modulators (cytokines) are involved in alterations of the ocular surface. This is especially true for inflammatory conditions[13,14] that are associated with a variety of ocular surface disorders, including dry eye.[15,16] It may be hypothesized that the nasal mucosa and probably also that of the lacrimal drainage system contribute to the integrity of the ocular surface by the reflex stimulation of aqueous tears[17,18] and through the mechanism of lymphocyte recirculation.[19–21]

During a systematic study of lymphoid tissue in the human conjunctiva, which provided evidence for the regular presence of a conjunctiva-associated lymphoid tissue (CALT),[22] we noticed similar tissue also within the lacrimal drainage system.[23–25] This represents an appropriate location for MALT because the tear flow conceivably carries foreign materials and antigens from the ocular surface into the lacrimal drainage system. Contact time with the mucosa of the lacrimal sac and the nasolacrimal duct may be prolonged here because of the decreased velocity of tear flow resulting from a widening of the lumen. This situation favors increased contact between the immune system and transported antigens capable of promoting a cellular and humoral immune response.[3,4]

In 1910, Merkel and Kallius[26] stated that the lacrimal drainage system was the most frequently investigated part of the eye and ocular appendage. Hence, the presence of lymphoid cells was known early and has also been reported later, but the results are still fragmentary; their functional significance has not yet been correctly interpreted or they have been implicated as pathologic.[27–30]

To date, clarification is still required as to the composition of the lymphoid tissue in the lacrimal drainage system, its frequency, and the types of lymphoid cells and their distribution in the mucosa. The presence of immunoglobulin A (IgA) was reported,[31] but its source and distribution are unclear. Therefore, the aim of the present study was to perform a thorough investigation of the lacrimal drainage system, focusing on the morphology of the mucosa and the associated lymphoid tissue, its components, distribution, and probable function.

MATERIALS AND METHODS

Tissue

The lacrimal drainage system ($n = 51$) was obtained complete, with ($n = 22$) or without the lacrimal canaliculi ($n = 29$), from human cadavers ($n = 31$) from 1994 to 2000 at the Department of Anatomy, Medical School Hannover. The average age of the donors was 79.5 years (± 13.8 years; mean $\pm$ SD), and the sex distribution was 19:12 (female:male). The average postmortem time before fixation was 1.8 ± 0.8 days (mean $\pm$ SD). Specimens were only used if the respective conjunctival tissue appeared normal upon macroscopic inspection. They were taken from donors who had given previous informed

consent to donate their bodies for education and science. This study complies with the Declaration of Helsinki.

Preparation

The complete lacrimal drainage system ($n = 22$) was excised together with the conjunctiva as described previously[22] (Fig. 1A). Identification of lacrimal tissue was performed starting from the lacrimal punctum and passing along the lacrimal canaliculi together with their encircling tissue toward the nasal canthus, until the nasal canthal tendon was reached. This tendon was held in position to allow dissection of the lacrimal sac from its osseous bed in the lacrimal and maxillary bone, to prevent destruction of the more delicate tissue of the sac itself. Starting superiorly and proceeding dorsally and nasally, the lacrimal sac and nasolacrimal duct could be followed downward to the point of its connection with the nasal cavity, resulting in a specimen of total length of approximately 20 mm (Fig. 1B).

The complete lacrimal drainage system was removed together with the conjunctiva, placed on a plastic board, and mounted in the anatomic position (Fig. 1A). The lacrimal sac and canaliculi were then separated from the conjunctiva by dissecting the lid margin laterally from the lacrimal punctum and along the lacrimal canaliculi toward the nasal canthus. The isolated lacrimal drainage system was mounted
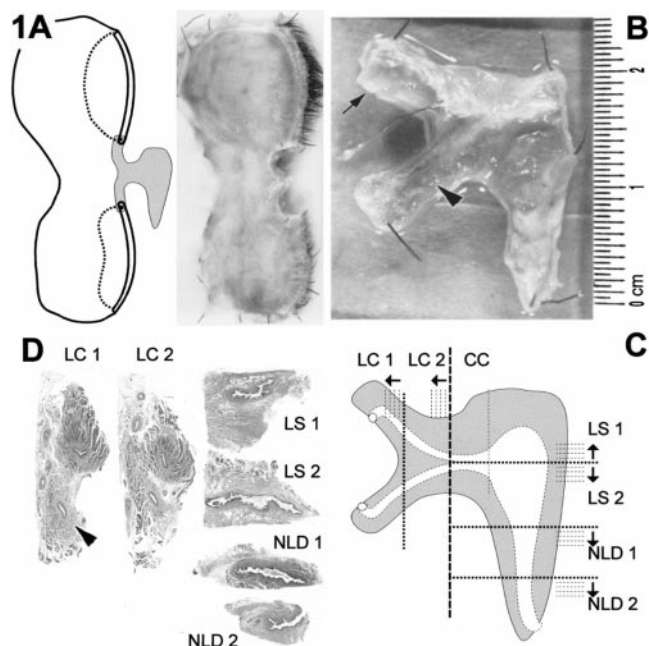

FIGURE 1. The anatomy of the lacrimal drainage system. (**A**) Position of the lacrimal drainage system in relation to a flat wholemount of the conjunctiva. It is *shaded* in a schematic drawing (*left*) and already dissected in a flat tissue preparation (*right*), resulting in the defects on the nasal side of the conjunctiva. The respective specimen of the complete lacrimal drainage system, as obtained by the described preparation technique, is separately mounted on a plastic mat (**B**). The lacrimal canaliculi are surrounded by dense connective tissue and a skeletal muscle layer (*arrowheads* in **B** and **D**). An *arrow* (in **B**) points to the upper lacrimal punctum. The upper lacrimal canaliculus is still covered in part by the epidermis, whereas in the lower one, the encircling layer of skeletal muscle fibers is visible and can be followed onto the lacrimal sac. (**C**) Scheme indicates the drainage system to be composed of the lacrimal canaliculi (divided into tissue blocks LC1 and LC2), common canaliculus (CC) and lacrimal sac (both divided into tissue blocks LS1 and LS2), and the nasolacrimal duct (divided into tissue blocks NLD1 and NLD2). All tissue blocks were embedded together en bloc into one paraffin mold and serially sectioned; the planes of section are indicated in (**C**). Using this technique, it was possible to show all the different parts of the lacrimal drainage system in a single section (**D**).

TABLE 1. Data of the Immunochemicals Used

Immunochemicals	Company	Type	Dilution
CD3	Sigma C7930	Rabbit, polyclonal	×800
CD8	Dako M0707	Mouse, monoclonal	×100
HML-1	Dako M0847	Mouse, monoclonal	×100
CD20	Dako M0755	Mouse, monoclonal	×200
MHC II	Dako M0775	Mouse, monoclonal	×200
IgA	Dako M0728	Mouse, monoclonal	×160
IgM	Dako M0702	Mouse, monoclonal	×80
Secretory component	Dako A0187	Rabbit, polyclonal	×1000
IgA-protein	Sigma I1010	Human colostrum	

separately with the lacrimal canaliculi, forming an angle in the anatomic position (Fig. 1B).

Division of the Lacrimal Drainage System into Tissue Blocks

The lacrimal canaliculi (LC) were dissected, just up to the point of entry into the sac (Fig. 1C) and divided into two portions (initial segment, LC1, and terminal segment, LC2) at a point about halfway between the sac and the punctum. The lacrimal sac (LS) and the nasolacrimal duct (NLD) were cut into four parts along the long axis: first by dividing the region where the canaliculi converge into the sac into an upper piece (LS 1 in Fig. 1C, representing the fundus) and a lower piece. The latter was further divided into three tissue blocks (LS2 and NLD 1 + 2, Fig. 1C). All tissue blocks of one specimen were embedded together and serially sectioned in the direction indicated by arrows in Figure 1C. Additional lacrimal sacs ($n = 29$), removed without the lacrimal canaliculi, were halved before embedding and were sectioned later.

Histology

Specimens ($n = 26$) were immediately fixed by immersion in 4% formaldehyde in 0.1 M cacodylate buffer, pH 7.4. The tissue was dehydrated and immersed in paraffin (Histo-Comp; Vogel, Giessen, Germany). Before embedding, the specimens were divided as described above, and all tissue blocks of one specimen were embedded together into a single paraffin mold. Using this technique it was possible to show all the different parts of one lacrimal drainage system in a single section (Fig. 1D). Continuous serial sections (5 μm in thickness) were performed on all blocks over a distance of 500 μm, on average. At intervals of 50 μm, sections were stained with Mayer's hematoxylin and eosin or Masson-Goldner for investigation of morphology. At locations of interest, intermediate sections were used for immunohistochemistry. Specimens for cryosections ($n = 8$) were obtained from unfixed tissue blocks divided as above and frozen embedded in OCT compound (Tissue Tek; Ted Pella Inc., Irvine, CA), using liquid nitrogen. Sections of 10-μm thickness were performed and stained as described.

Immunohistochemistry

Primary antibodies (Table 1) were applied according to the indirect avidin-biotin-complex (ABC) method as described previously.[22] For negative controls, primary antibodies were replaced by normal serum and anti-IgA antiserum was additionally preadsorbed with the respective protein (Sigma, Munich, Germany) to confirm the identity of staining. Accessory lacrimal gland tissue was used as a positive control.

Electron Microscopy

For transmission electron microscopy (TEM), specimens ($n = 9$) were fixed by immersion in a mixture of 2.5% glutaraldehyde and 2% formaldehyde diluted in cacodylate buffer. The tissue was dehydrated, divided as described above, and embedded in Epoxy resin (Epon). Semithin sections (1-μm thick) were stained with toluidine blue, thin

FIGURE 2. Aspects of lymphoid tissue associated with the lacrimal canaliculi. (**A**) A lacrimal canaliculus at about half of its length (overview in *inset*) is surrounded by skeletal muscle fibers (*arrowheads* in **A** and *inset*) and dense connective tissue. It shows a narrow subepithelial layer of lymphocytes (*arrow*) in the lamina propria. At a location where a vessel approaches the epithelium (*open arrow* in **B** and **D**), numerous lymphocytes are seen inside and around vessels with a flat or high endothelial lining (*asterisks* in **B** and **D**). Intraepithelial lymphocytes (*arrowheads* in **B** and **D**) and lamina propria lymphocytes are both mostly positive for CD3 (**D**). In the epithelium are occasional scattered cells (**B**, *black arrow*) that may correspond to those with a dendritic shape in MHC class II staining (**C**, *arrow*). The superficial cell layers of the stratified squamous epithelium show a positive stain for secretory component (**E**; **B**, **D**, and **E** are consecutive sections). In a terminal lacrimal canaliculus close to the common canaliculus, the lymphoid layer hosts an increased amount of cells (**F**). In the basal epithelium are multicellular mucous glands (m) that may show a duct-like opening (*black arrow*). Intraluminal secretions (*open arrow*) are continuous with the gland opening. Staining for secretory component (**G**) is observed in these glands (m), in the superficial epithelial layers, and inside the canalicular lumen (*open arrow*). (**H**) IgA is expressed inside the roundish subepithelial plasma cells and seen in glandicular cells (m) and inside the lumen (*open arrow*). Single CD20-positive B lymphocytes are interspersed and can accumulate in the natural folds of the canaliculi (**I**); (**F**) through (**I**) are consecutive sections. In a section where the two lacrimal canaliculi (**J**, lc at *open arrows*) are seen to merge separately into the lacrimal sac (ls), the increasing occurrence can be seen of lymphoid cells (*black arrow*) and basal mucous glands (*arrowhead*). Along one of these canaliculi is a lymphoid follicle (f) with a flattened overlying epithelium containing groups of intraepithelial lymphocytes (**K**, *arrowheads*) and high endothelial venules in the periphery (*asterisks*). Immunohistochemistry on consecutive sections shows that this follicle is composed of peripheral T and central B cells (flat follicle-associated epithelium is indicated by *arrowheads* in **L** and **M**). Bars, 100 μm; staining is indicated in the figures.

sections (70-nm thick) were stained with uranyl acetate and lead citrate, and then observed in a Zeiss EM 10 electron microscope.

Clearing Procedure

Lacrimal sacs (*n* = 8) were prepared as flat wholemounts stained en bloc in undiluted Mayer's hematoxylin (Merck, Darmstadt, Germany) for 8 minutes and consecutively cleared by embedding in anise oil or 2-hydroxy-methacrylate resin (Kulzer, Hanau, Germany) as described previously.[22]

RESULTS

Cleared and stained specimens of the lacrimal sac and nasolacrimal duct revealed an inhomogeneous layer of lymphoid tissue with embedded roundish spots corresponding to lymphoid follicles similar to those in the conjunctiva[22] (not shown). Because of the high distortion of lymphoid morphology due to the multitude of large vessels in the saccular wall and the difficulty in applying the flat preparation technique to the whole lacrimal drainage system, an approach

FIGURE 3. Lymphoid tissue of a common lacrimal canaliculus. Two terminal canaliculi (*arrowheads* in *inset* of **A**) merge into a common canaliculus. Its stratified squamous canalicular epithelium (**A**) shows basal mucous glands that can be intraepithelial (m) or extraepithelial (me). Alveolar (a) glands are also seen. The lamina propria contains a zone of small dark lymphocytes and plasma cells (l and p in **B**) with frequent small vessels that occasionally have a high endothelium and adjacent lymphocytes (*asterisk*). Sometimes a single granulocyte is observed (*arrow* in **B**). In the epithelium are frequent intraepithelial lymphocytes (*arrowheads* in **A** through **C**), also associated (*double arrowheads* in **A** through **C**) with the mucous glands (m). Most of the lymphocytes are CD3-positive T cells (**C**); few CD20-positive B cells are interspersed (**D**). IgA immunostaining (**E**) characterizes plasma cells in the lamina propria (*arrowhead*), as well as mucous glands and the lumen of an alveolar gland. SC (**F**) shows little apical staining in the squamous epithelium but is strongly expressed in the mucous (m) and alveolar (a) glands and, after the transition into the pseudostratified saccular epithelial type, also in the epithelium (**F**, *black arrows*). Luminal secretions are strongly positive for IgA and SC (*open arrows* in **E**, **F**). IgM-positive plasma cells (*arrowhead* in **G**) are relatively rare. Follicular lymphoid accumulations have a complementary arrangement of T and B lymphocytes, as shown in serial sections of the same follicle (**A**, **H**, **I**): T cells form loose arrangements in the periphery (**H**), whereas B cells are densely aggregated in the center (**I**). In the T-cell area is a rich network of high endothelial venules (**J**, *asterisks*) originating from approaching vessels with a flat lining (**J**, *open arrows*). Bars, 100 μm; staining is indicated in the figures.
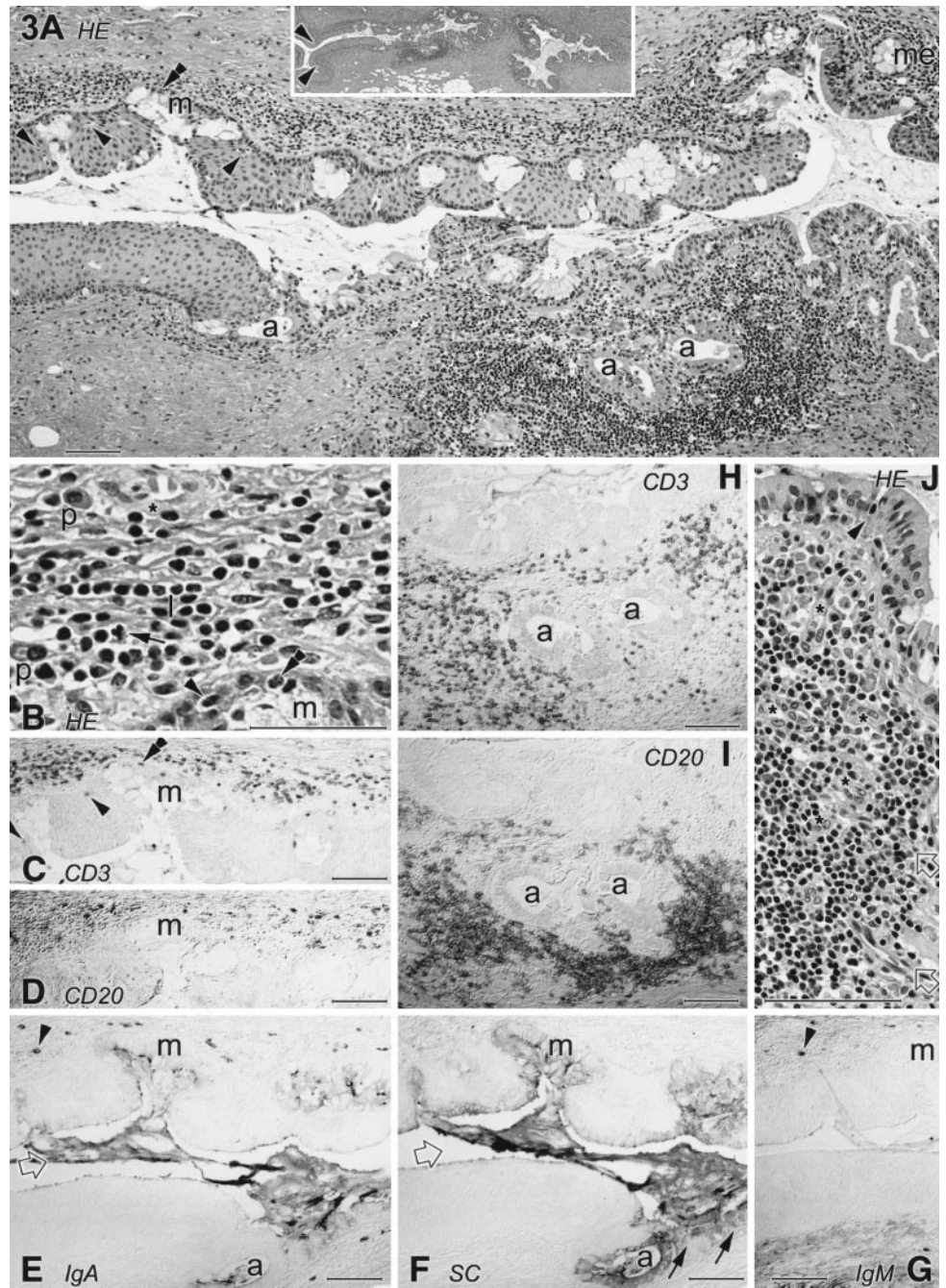
involving serial sectioning of embedded tissue was preferred in this study.

Lacrimal Canaliculi

The mucosa of the lacrimal canaliculi was surrounded by a dense connective tissue encircled by skeletal muscle fibers and covered by the skin (Figs. 1B, 1D, 2A, inset). It was lined by a stratified squamous, nonkeratinized epithelium on a loose lamina propria, which contained a narrow but distinct layer of lymphoid cells (Figs. 2A, 2B). Inside the epithelium were MHC class II–positive cells of dendritic morphology (Fig. 2C). Intraepithelial lymphocytes were preferably located in the basal layers of the epithelium (Figs. 2B, 2D). At locations where vessels approached the epithelium, lymphocytes were more numerous (Fig. 2B). Among vessels with the usual flat endothelium, high endothelial venules (HEV) were occasionally

observed. The lymphocytes consisted mainly of CD3-positive T cells (Fig. 2D), whereas CD20-positive B cells and plasma cells were rare (both not shown). Staining for the transepithelial immunoglobulin transporter molecule secretory component (SC) was observed in the superficial layers of the epithelium (Fig. 2E).

Close to the termination of the lacrimal canaliculi, the amount of lymphoid cells was seen to increase (Fig. 2F). From a region of approximately 2 to 3 mm distance between the merging canaliculi, there was an additional transformation of the epithelium, with the occurrence of multicellular mucous glands in the basal layers of the epithelium showing occasional duct-like openings (Fig. 2F). The glands were positive for SC and IgA (Figs. 2G, 2H). Plasma cells (Fig. 2H) and B lymphocytes (Fig. 2I) were more frequent in the lamina propria of the terminal canaliculi. T lymphocytes together with some B lymphocytes and HEV were seen to
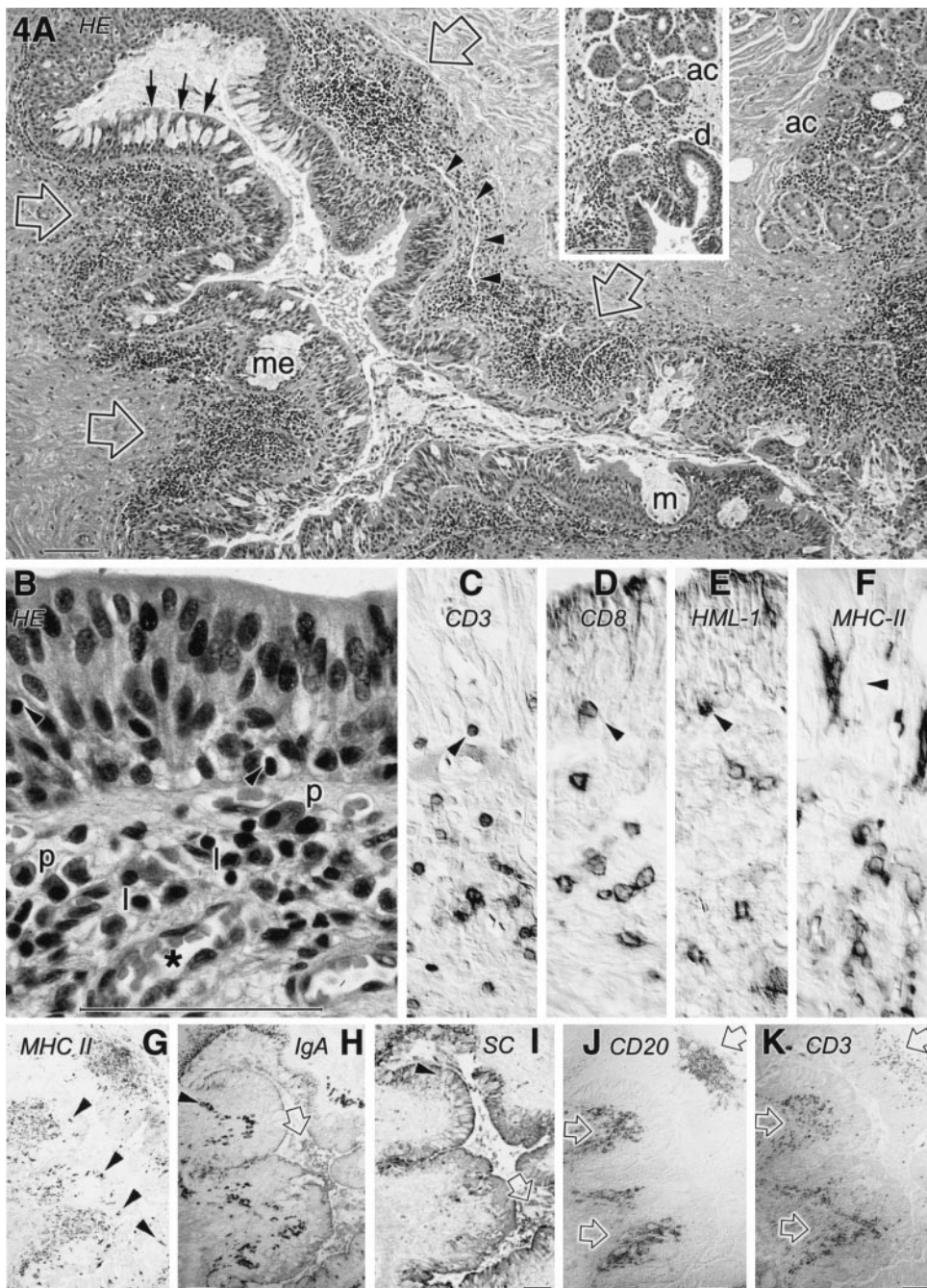
FIGURE 4. Aspects of a lacrimal sac with several lymphoid accumulations. The mucosa of the lacrimal sac (**A**) has an undulated outline with protrusions filled with accumulations of lymphoid tissue (*open arrows*). These are often connected by narrow lymph vessels (*arrowheads*). The mucosa contains goblet cells (*black arrows*) and intraepithelial (m) and extraepithelial (me) mucous glands. The wall of the lacrimal sac contains serous acinar glands (ac in **A** and *inset*) of considerable size that open with their secretory ducts into the lumen of the sac (d in *inset*). The saccular epithelium is pseudostratified with two to three (occasionally up to five) nuclear layers and intraepithelial lymphocytes (*arrowheads* in **B** through **E**). The lamina propria contains lymphocytes and plasma cells (l and p in **B**) and vessels of different sizes (*asterisk*). Immunostaining characterizes CD3-positive T cells (**C**) in the lymphoid layer and epithelium that are frequently CD8-positive (**D**) and carry the HML-1 antigen (**E**). B cells and macrophages in the lamina propria and dendritic cells (*arrowheads* in **F** and **G**) in the epithelium stain positive for MHC class II. IgA-positive plasma cells build up a broad zone (**H**, *arrowhead*). The epithelium stains positive for IgA and strongly for SC (except in goblet cells, *arrowhead* in **I**), and both accumulate at the mucosal surface and inside the luminal secretions (*open arrows* in **H** and **I**). Lymphoid accumulations (*open arrows* in **A**, **J**, and **K**) consist of clusters of B cells (**J**) embedded in loose arrangements of T cells (**K**); one cell type predominates in a particular location, but overlap is often present (**G** through **K** are serial sections of *left* part of **A**). Bars, 100 μm (**B** through **F** are of the same enlargement and share one bar in **B**); staining is indicated in the figures.

accumulate in the natural folds of the canaliculi (as in the top left corner of Fig. 2F).

Distinct lymphoid follicles were also found, flanking a terminal lacrimal canaliculus, as observed in Figure 2J, where both canaliculi opened separately into the lacrimal sac. The amount of lymphoid cells is seen here to increase along the canaliculi in the direction of the lacrimal sac. Follicles were formed by an accumulation of lymphocytes (Fig. 2K). These consisted of loose peripheral T cells (Fig. 2L) with HEV that surrounded a dense central B-cell area (Fig. 2M). They were covered by a flattened epithelium containing groups of intraepithelial lymphocytes.

Common Lacrimal Canaliculus

Frequently, before entering the sac, the two lacrimal canaliculi joined into a common canaliculus (Fig. 3A, inset).

Increasing numbers of mucous glands were embedded in the stratified squamous epithelium until its transformation into the pseudostratified columnar epithelium of the lacrimal sac. Their size increased toward the sac, and they sometimes formed extraepithelial extensions. The transformation of the epithelium occurred either gradually or was abrupt (compare upper and lower wall of the common canaliculus in Fig. 3A). The lymphoid layer consisted of numerous lymphocytes and interspersed plasma cells (Fig. 3B) accompanied by a subepithelial network of vessels including occasional HEV. Single mast cells, granulocytes, and macrophages were observed in the layer, and occasionally a granulocyte was present in the epithelium. T cells (Fig. 3C) dominated in the lamina propria and epithelium, but few B cells (Fig. 3D) were regularly present in the lamina propria.
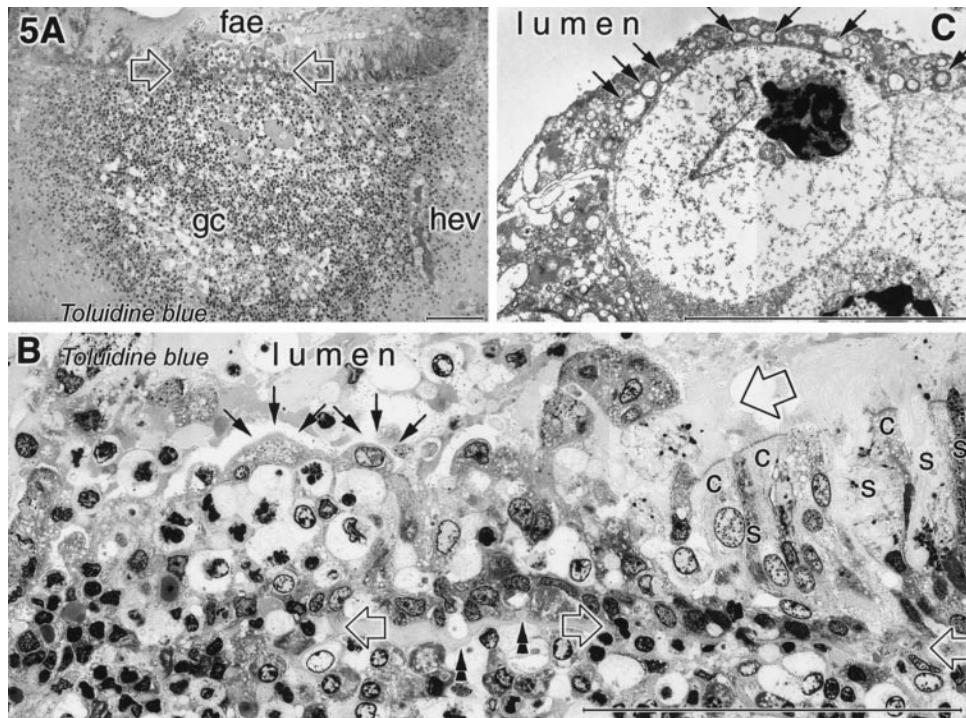
FIGURE 5. Aspects of secondary follicles. A secondary follicle in the lacrimal sac (**A**) shows a typical roundish morphology and a bright germinal center (gc). It is covered on the apex (*open arrows*) by a follicle-associated epithelium (fae, label is inside the saccular lumen) containing groups of intraepithelial lymphocytes and is connected to high endothelial venules (hev). High magnification (**B**) shows that the usual pseudostratified epithelium of the lacrimal sac, containing bright and dark secretory (s) cells interspersed between ciliated (c) epithelial cells, becomes flat toward the apex of the follicle (*large open arrow*). It transforms into a delicate cellular meshwork of hollow spaces filled with lymphoid cells and covered by a thin epithelial lining (*black arrows* in **B**). Inside the lumen are some cells present because of destruction of the epithelium on the left side of the follicle. Some of the immigrated cells inside the epithelium appear apoptotic. The basement membrane becomes thin and discontinuous (**B**, *double arrowheads*) and is, in some places, disrupted by migrating cells (**B**, *small open arrows*). The thin cytoplasm (**C**) of the covering cells toward the lumen contains numerous vesicles as in M cells (*arrows*). Bar, (**A**, **B**) 100 μm; (**C**) 10 μm; staining is indicated in the figures.

Plasma cells, most of which were positive for IgA (Fig. 3E) were regularly found, and different glands secreted IgA-positive material into the lumen of the common lacrimal canaliculus. Besides the mucous glands there were others with alveoli, formed of a monolayer of cuboidal or sometimes columnar cells resembling modified sweat glands (Fig. 3A). Occasional larger glands had serous acini and resembled accessory lacrimal glands (shown later). All these glands, which were also encountered distally in the lacrimal drainage system, showed a strong expression of SC (Fig. 3F) and to varying extents of IgA. IgM-positive plasma cells were rare in the lamina propria and also the epithelial or luminal staining for it (Fig. 3G). In the squamous region, the epithelium showed weak staining for SC and occasional faint staining for IgA; however, more distinct staining was revealed as soon as the saccular pseudostratified type of epithelium appeared. T and B cells in the lamina propria formed accumulations with a complementary composition of peripheral T cells and aggregated central B cells (Figs. 3H and I); the peripheral areas always contained HEV (Fig. 3J). These follicular lymphoid accumulations were found underneath the epithelium or around glandular tissue (as seen in the Figs. 3A, 3H, 3I).

Lacrimal Sac

The epithelium of the lacrimal sac was composed of two to three nuclear layers on average, occasionally of up to five layers, and contained goblet cells besides the mucous glands (Fig. 4A). The mucosa was usually markedly undulated forming protrusions alternating with recesses. The lymphoid cells often built up a broad zone in the lamina propria (Figs. 4A, 4B). CD3-positive T cells (Fig. 4C), which were frequently CD8-positive suppressor/cytotoxic T cells (Fig. 4D), were present in the lamina propria and in the basal layers of the epithelium. HML-1–positive lymphocytes were also found (Fig. 4E). MHC class II–positive cells with a dendritic morphology could be demonstrated (Fig. 4F, 4G) inside the

epithelium, as well as B cells and macrophages in the lamina propria. IgA-secreting plasma cells (Fig. 4H) formed a broad band in the lymphoid layer with a strong concomitant staining for SC in the epithelium, excluding the goblet cells (Fig. 4I).

Inside the connective tissue of the mucosal protrusions, lymphoid cells formed follicular accumulations (Figs. 4A, 4J, 4K). These consisted of B-cell clusters that were highly compact or diffuse (4J), embedded into a broadened zone of T cells (4K) with HEV, and often interconnected by small lymph vessels (Fig. 4A). They did not always show a follicle-associated epithelium devoid of goblet cells (Fig. 4A). Besides these, distinct secondary follicles with a bright germinal center, dense corona, and parafollicular HEV were observed (Fig. 5A). Over the apex, the regular pseudostratified epithelium transformed into a follicle-associated epithelium (Fig. 5B). This was characterized by a flattening of cell shape, loss of the integrated secretory cells, and the occurrence of a loose epithelial meshwork with spaces occupied by lymphoid cells. The subjacent basement membrane became thin and was sometimes disrupted by lymphoid cells, probably resulting in holes of the basement membrane sheet. The thin cytoplasm of the flat covering epithelial cells contained numerous small vesicles (Fig. 5C), as reported for the M cells of intestinal Peyer's patches.

Acinar serous glands reached a considerable size in the wall of the lacrimal sac (Fig. 4A) and nasolacrimal duct. In the loose connective tissue between the acini (Fig. 6A), few T and B cells (Figs. 6B, 6C) and numerous plasma cells were detected. Most of the latter were strongly positive for IgA (Fig. 6D). Only a few IgM-positive cells were present (not shown). Although the glandular epithelium exhibited only a moderate amount of IgA staining, it was strongly positive for SC (Fig. 6E), and both accumulated apically in the cells and in the intraluminal secretions.
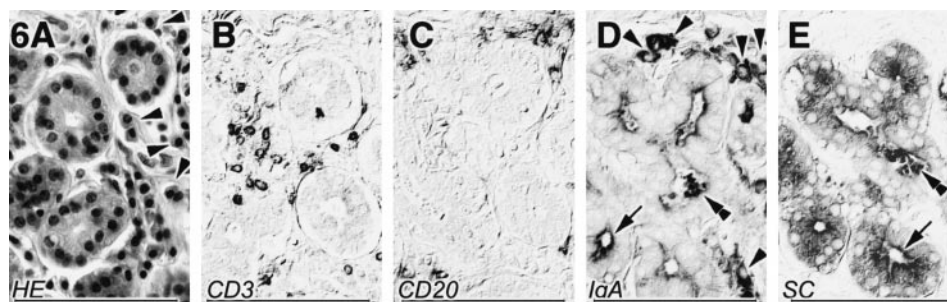
FIGURE 6. Aspects of an intramural acinar gland. Large intramural acinar serous glands (**A**) contain some T and B lymphocytes (**B** and **C**) as well as numerous IgA-positive plasma cells (*arrowheads* in **A** and **D**) in the lamina propria. The acinar epithelium expresses moderate amounts of IgA and high amounts of secretory component. Both accumulate apically in the cells (*arrows* in **D** and **E**) and intraluminally (*double arrowheads* in **D** and **E**). Bars, 100 μm; staining is indicated in the figures.

Nasolacrimal Duct

In the nasolacrimal duct, the mucosal undulations observed in the sac were reduced, and the mucosal outline was smoother (Fig. 7). The epithelium was narrower than that observed in the sac and assumed a regular, pseudostratified morphology of two (or sometimes three) nuclear layers thickness, not unlike that of the respiratory epithelium of the nasal cavity. The number of lymphoid cells and the thickness of the lymphoid layer was reduced in general, but follicular accumulations of lymphocytes also occurred here. As elsewhere, plasma cells were abundant in the lamina propria, and the expression of IgA and SC in the epithelium was substantial.

Lymphoid Follicles

Follicles were observed in 19 (44%) of the 43 specimens sectioned, representing 13 (52%) of 25 individuals. They had a diameter ranging from 0.1 to 0.8 mm, with an average of approximately 0.5 mm. In most of these specimens (28%), primary follicles occurred, whereas secondary follicles, with a distinct germinal center were seen less frequently (16%). Investigation of the right/left symmetry of follicles in 18 donors where specimens of both sides were sectioned showed 14 donors (i.e., 78%) with bilaterally equal expression (6 with follicles and 8 without) and 4 donors with unilateral follicles.

DISCUSSION

Using the described technique of serial sections with histologic, immunohistochemical, and electron microscopical investigation of the total lacrimal drainage system, we were able to characterize the regular presence of a lacrimal drainage–associated lymphoid tissue, which thus constitutes a part of the mucosal immune system and for which we propose the term LDALT. This can be interpreted as a continuation of the lymphoid tissue that we observed in the conjunctiva (CALT)[22] and found to accumulate there toward the lacrimal punctum. It may also be related to the nasal lymphoid tissue (NALT),[32] because the lacrimal drainage system is interposed between the CALT and the NALT.

Topographical Distribution

The LDALT is more extensive in the wider, cavernous parts of the lacrimal drainage system, that is, the lacrimal sac and nasolacrimal duct, where a low flow rate can be assumed because of the widening of the lumen and the limited amount of tear fluid, but it is also prominent in the common canaliculus. In the lacrimal canaliculi, which have narrow lumina and conceivably a rapid flow, because of the proposed pumping mechanism,[29] there is only a thin layer of lymphoid cells. Hence, this topographical distribution corresponds to the velocity of tear flow in the system. This would, in turn, reflect the relative contact time of the mucosa with the tear fluid and its exposure to antigens with the resulting ability for antigen probing but also with the necessity for protective immune responses such as IgA secretion.

Diffuse Type of LDALT and the Secretory Immune System

A so-called "diffuse" lymphoid tissue,[3] represented by a zone of lymphocytes and plasma cells in the lamina propria, together with mostly basal intraepithelial lymphocytes is found in all investigated specimens in a varying density. The predominance
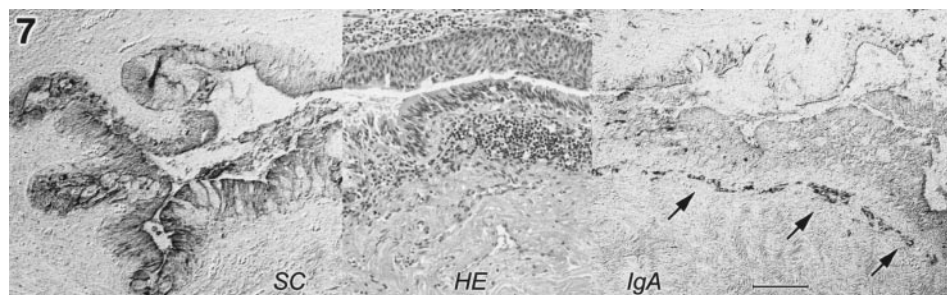

FIGURE 7. Aspects of a nasolacrimal duct. A composite image shows the secretory immune system of a nasolacrimal duct with a lymphoid layer (HE, *middle*) containing a regular lining of IgA-positive plasma cells (*arrows*) as well as IgA deposition in the epithelial cells (*right*), and a strong expression of the IgA transporter SC in the epithelium (*left*). Staining for both factors is more pronounced in the middle and apical parts of the epithelium. Bar, 100 μm for all three segments; staining is indicated in the segments.

of T cells in this lymphoid layer (whereas B cells are relatively confined to lymphoid accumulations) is similar to other lymphoid organs of the MALT system such as the conjunctiva[22,33–36] or the intestine.[37] CD8-positive T cells are thought to play a role in the generation of tolerance, suppression of inflammatory reactions, and preservation of tissue integrity in the conjunctiva[35] and elsewhere.[5] Intraepithelial and lamina propria lymphocytes are positive for the human mucosa lymphocyte antigen (HML-1).[34,38–40] This $\alpha E\beta 7$ integrin is an adhesion molecule that characterizes lymphocytes specific for mucosal tissues and thus indicates that the lymphoid tissue of the lacrimal drainage system is a part of the MALT system.[1,2] HEV, which allow an effective homing of lymphocytes into mucosal tissues were also found in the lacrimal drainage system and hence provide regulated access of the trafficking lymphocytes[19–21] to the lacrimal drainage system.

Immunoglobulin-positive plasma cells in the lamina propria, their transporter molecule SC[41] in the epithelium, and intraluminal secretions positive for both of these characterize LDALT as a part of the secretory immune system.[6,7,42] These immunoglobulins are spread over mucosal surfaces and provide a protective shield that can bind, block, and neutralize pathogens at the surface and prevent them from entering the tissue or, alternatively, mark and opsonize them inside the tissue.[4,6-9] The clear predominance of IgA-positive plasma cells over IgM indicates the absence of an acute immune reaction in the investigated tissues and thus supports the normal character of the lymphoid tissue in the lacrimal drainage system. The multitude of associated glands that release immunoglobulin-rich products onto the mucosal surface contribute to the secretory immunity. The intraepithelial mucous glands observed in the lacrimal canaliculi seem to represent as yet undescribed structures.

Follicular LDALT

Most of the follicular lymphoid accumulations that are present in LDALT have characteristics of primary lymphoid follicles,[43] although secondary follicles are also present. This could indicate that the immune capacity to detect antigens and to present them to lymphoid cells, resulting in lymphocyte activation and proliferation, is low in LDALT. However, the proliferation and differentiation of specific mucosal IgA-secreting plasma cells that allow effective protection may not solely depend on the presence of a distinct follicular architecture as found in the intestine.[44] Additionally, the follicles observed here show cells that resemble follicular, antigen-transporting M cells.[45] MHC class II–positive cells observed throughout the nonfollicular epithelium may also assist in antigen detection and presentation. The restriction of follicles to about half of the investigated specimens is most likely influenced by the relatively old age of the tissue donors, because follicles are known to be reduced with increasing age.[46] This is supported by the finding that one young donor had four secondary follicles in his drainage system without any signs of inflammation. The noninflammatory character of LDALT is also supported by the relative right/left symmetry observed in about three quarters of individuals. This suggests that the occurrence of follicles is independent of pathologic unilateral affections (e.g., infections) and reflects a normal and physiologic tissue component that may be modulated because of age or environmental conditions that equally affect both sides. The lower amount of follicles compared with the proximal conjunctiva[22] may be due to the different methodological approach in the two studies. Although extensive serial sections were performed here, only a part of the tissue blocks could be investigated, so that the amount of follicles in the lacrimal drainage system may be higher than that observed in the present study.
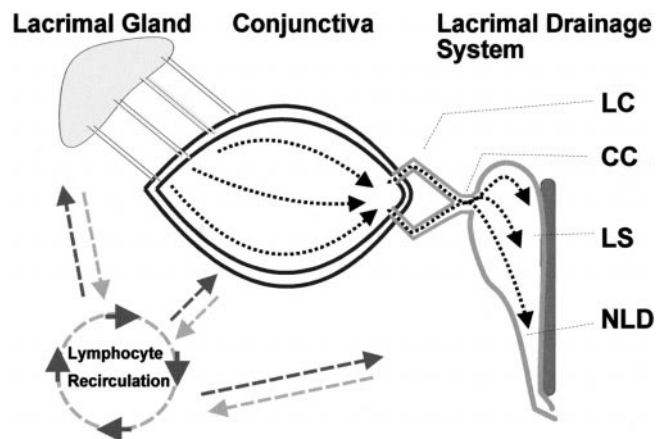
GRAPH 1. Functional unit for ocular surface immune protection.

CONCLUSION

In conclusion, our study shows that the normal human lacrimal drainage system usually contains all components of a mucosa-associated lymphoid tissue. We hypothesize (as illustrated in Graph 1), that the specific immune protection of the ocular surface, as performed by the main and accessory lacrimal glands,[10,42,47] the conjunctiva,[11,22,33–36,48,49] and the lacrimal drainage system, as described in this article, acts as an integrated system. This can be assumed, because its parts are connected with each other by the flow of tears that allows them to share protective factors, cytokines, and also similar antigens, which are finally washed away into the lacrimal drainage system. Furthermore, all three tissues belong to the MALT system, are connected by lymphocyte recirculation via HEV[50] to the homing and exchange of protective lymphocytes,[19–21] and should hence together be addressed as "Eye-Associated Lymphoid Tissue." Finally, the lacrimal drainage system may also be connected to the ocular surface and lacrimal gland via the neural reflex arc that is shown to influence ocular surface integrity and possibly dry eye development.[51] The presence of another lymphoid tissue (LDALT) closely downstream to the conjunctiva has to be taken into account if local immunity of the conjunctiva is investigated or considered.

The extent to which lymphocyte recirculation actually takes place within this system is still unknown, nor is it known whether these tissues have a differential immunologic importance. The elucidation of these important questions for the regulation and preservation of ocular surface integrity requires further physiological studies.

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