HUMAN LACRIMAL DRAINAGE-ASSOCIATED LYMPHOID TISSUE (LDALT) BELONGS TO THE COMMON MUCOSAL IMMUNE SYSTEM

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1. INTRODUCTION

The lacrimal drainage system constitutes the part of the ocular adnexa where the tear flow drains after production in the lacrimal gland and subsequent to bathing the ocular surface. It is lined by a mucosa that continues that of the conjunctiva. The lacrimal drainage system begins at the two lacrimal puncta of the lid margin that lead via the lacrimal canaliculi into the cavernous lacrimal sac and via the nasolacrimal duct into the nose (Fig. 1).

The tear flow enables the tissue of the lacrimal drainage system to share soluble factors with the upstream ocular surface and also with the lacrimal gland. These factors can be of a protective nature (e.g. IgA, antimicrobial peptides and proteins, growth factors etc.) but can also be potentially harmful as inflammatory cytokines or as the antigenic materials and microbes that reach the ocular surface via the open palpebral fissure.

Therefore, the lacrimal drainage system also shows evidence of a specific

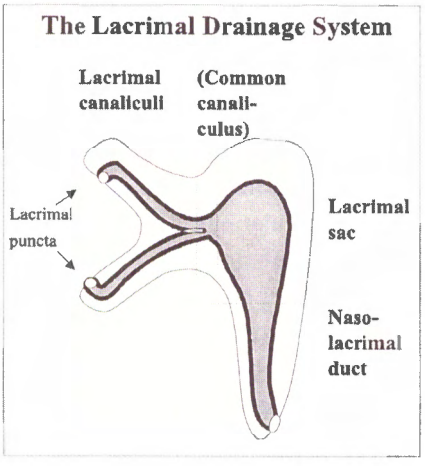

Figure 1. Anatomy of the lacrimal drainage system showing the different regions.

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immune protection by a mucosa-associated lymphoid tissue (MALT)[1–4] as reported by us previously.[5–8] It was identified in studies on the upstream conjunctiva-associated lymphoid tissue (CALT)[8] that it continues and was accordingly later termed lacrimal drainage-associated lymphoid tissue (LDALT).[9]

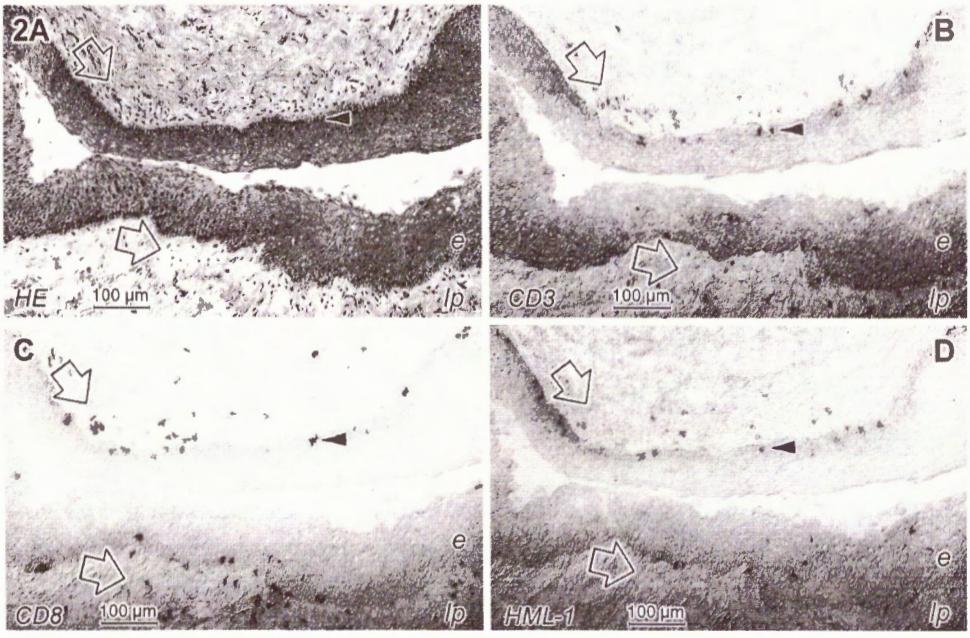

Figure 2. Lymphoid cells (**A**) in the lamina propria (open arrows, lp) and basal epithelial layers (arrowheads, e) consist mainly of CD3 positive T-cells (**B**), CD8-positive cells are frequent (**C**), HML-1-positive cells (**D**) always occur. Plasma cells are rare in this initial part of the lacrimal canaliculi but increase towards the sac.

Although the presence of lymphoid cells in the lacrimal drainage system had been reported for a long time,[9] no detailed description and no conclusive analysis of their functional significance had been given. The lymphoid cells and, in particular, their follicular accumulations which had been occasionally described,[10–12] were usually considered as pathological. Meanwhile our results on the presence of LDALT are supported by other studies. Some, focusing on follicles, consider it in a pathological context,[13] others as a *local* immune system.[14] However, due to the technical difficulty to obtain complete preparations of the total lacrimal drainage system, most investigations are restricted to parts of the system such as sac or nasolacrimal duct and hence also lose the continuity with CALT. In order to clarify these points, we aimed here to investigate the presence, distribution, histology and immunohistochemical characterization of lymphoid tissue only in complete lacrimal drainage systems.

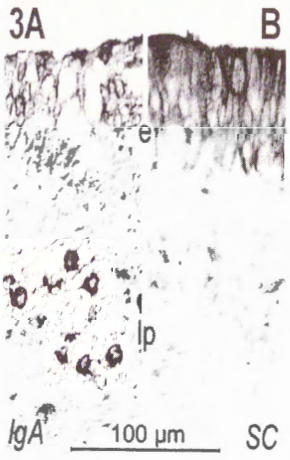

Figure 3. IgA-positive plasma cells are seen in the lamina propria (lp) and IgA (**A**) and SC (**B**) are in the epithelium (e) of the lacrimal drainage system.

2. Material and Methods

Complete normal human lacrimal drainage systems (n = 18) were obtained from cadavers of body donors (average age 81.7 ±16.1 years) at the Department of Anatomy, Hannover Medical School. For histological and immunohistological analysis, the tissue was either fixed in 4% formaldehyde solution and embedded in paraffin or fresh frozen in OCT. Sections of all regions (Fig. 1) were stained with hematoxylin and eosin (HE) or immunohistochemistry. For the latter, sections were rehydrated, incubated with primary antibodies for components of the secretory immune system (IgA, IgM, SC), for lymphocyte subtypes (CD3, CD8, HML-1) or for macrophages (CD68). Primary antibodies were marked with secondary biotinylated ones and visualized with streptavidin-peroxidase and DAB.

3. RESULTS

A narrow layer of diffusely arranged lymphoid cells was observed along the lacrimal canaliculi (Fig. 2), consisting mainly of CD3-positive T-lymphocytes in the lamina propria and in the basal epithelial layers. CD8 positive lymphocytes were frequent and lymphocytes carrying the human mucosa lymphocyte antigen (HML-1) were always seen. Small vessels were located in the lamina propria including occasional specialized high endothelial venules (HEV). Plasma cells were rare in the initial part of the lacrimal canaliculi but increased in their terminal part towards the lacrimal sac. In the common canaliculus, in the lacrimal sac (Fig. 3) and in the nasolacrimal duct a dense zone of diffuse

lymphoid tissue was observed, including frequent plasma cells among the lymphocytes. Most plasma cells were positive for IgA (Fig. 3), few for IgM. IgA was also seen in the epithelium, diffusely or as deposits. The IgA transporter molecule SC was strongly positive in the pseudostratified columnar epithelium of all parts of the drainage system; however, it was restricted to patches in the outermost layers of the stratified squamous epithelium in the lacrimal canaliculi. Staining for both IgA and SC was also observed in associated glands and in the drainage lumen.

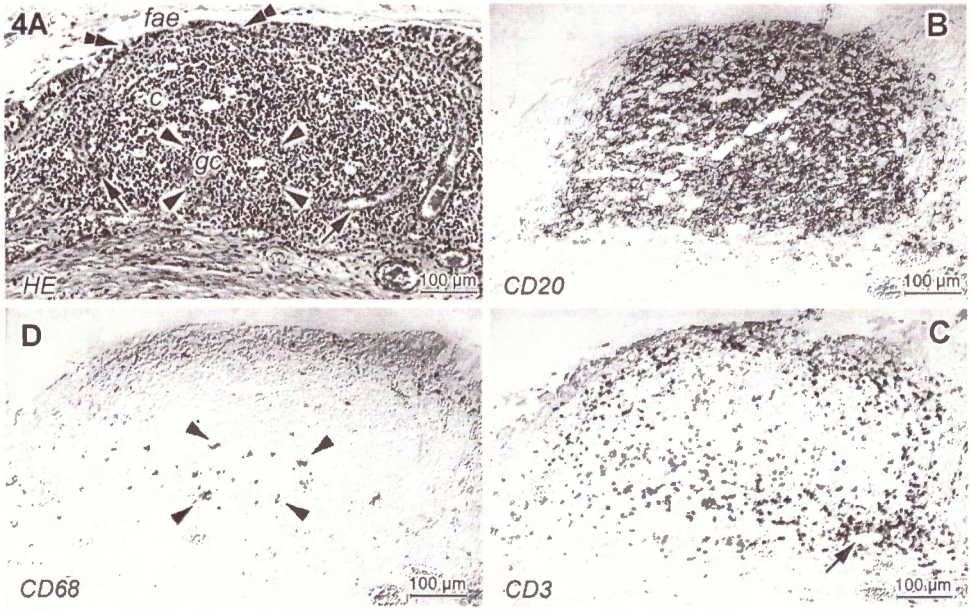

Figure 4. A secondary follicle in the human lacrimal sac with a germinal center (gc, arrowheads) and a denser lymphocyte corona (c) has a flat overlying follicle-associated epithelium (fae, double arrowheads) and is surrounded by numerous parafollicular vessels (arrows) including high endothelial venules **(A)**. It is composed of B-cells **(B)** and has parafollicular T-cells, clustering here around one of the vessels **(C, arrow)**. In the germinal center, not very obvious in HE-staining, CD68-positive macrophages (arrowheads) are detected **(D)**.

Lymphoid follicles (Fig. 4) were observed in 56% of these complete drainage systems and occurred in all regions, rarely also in the terminal canaliculi. They had a roundish shape, an average diameter around 0.5 mm, and were usually composed of accumulations of B-cells (Fig. 4B) and accompanied by parafollicular T-cells. In the parafollicular T-cell areas, HEV were regularly observed, sometimes with lymphocytes clustering around them (Fig. 4C). Secondary follicles with a germinal center were seen. The follicle-associated epithelium had a flat cell shape and frequent intraepithelial lymphocytes. In the germinal center, CD68 positive macrophages were characterized (Fig. 4D) which may indicate phagocytosis of newly formed lymphocytes with unsuitable antigen specificity. Most

follicles were primary; sometimes lymphoid accumulations were seen without a flat overlying epithelium.

4. DISCUSSION AND CONCLUSION

LDALT of the diffuse and follicular type regularly occurs in the normal human lacrimal drainage system similar to the conjunctiva[8] and other organs,[1-4] and contains mucosa specific lymphocytes, further supporting its integration into the common mucosal immune system.

Follicles were found in the majority (56%) of specimens in this study which contained only complete lacrimal drainage systems that were analyzed in all regions. This supports the previous assumption[9] that their amount may easily be underestimated if incomplete tissue is included or a section approach used, and further underlines that follicles represent a normal tissue component here. IgA-positive plasma cells in the lamina propria and secretory component in the epithelium characterize LDALT as a part of the secretory immune system.[2] This represents the efferent limb of immunity and is able to exclude antigens from the mucosa mainly by the production of secretory IgA. Follicles with a germinal center indicate that antigens had been presented here to lymphoid cells resulting in their activation,[3,4] and give evidence that LDALT can also perform an afferent immune function. This may be one of its main functions because antigens that are washed away from the ocular surface have a higher probability of contact with the mucosal immune system due to the slower flow of tears in the drainage system and can hence prime immune responses here that are also relevant for the ocular surface. Antigen transporting M-cells[15] were observed in ultrastructure.[9]

LDALT is physically continuous with CALT and is connected with it by tear flow and by the regulated recirculation of lymphocytes[16] as suggested by the presence of high endothelial venules and discussed in detail in another contribution to this book[17]. We propose that the lacrimal drainage system, conjunctiva, and lacrimal gland form a functional unit for ocular surface immune defense, to be termed "Eye-Associated Lymphoid Tissue" (EALT).

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