Eye Associated Lymphoid Tissue (EALT) and the Ocular Surface

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Summary

Although immune based inflammation is increasingly recognized as an important pathogenetic factor in several forms of ocular sur-face di-se-ase, information about the physiologic ocular mucosa-associated lym-pho-id tissue (MALT) was sparse and controversial. In complete nor-mal hu-man tissues components of MALT were consistently found to ex-tend from the lacrimal gland along the conjunctiva (as CALT) into the lacri-mal drain-age system (as LDALT). Together this consti-tutes an eye-asso-ciated lym-phoid tissue (EALT). Lymphoid cells at the healthy hu-man ocular surface contribute to the normal homeostasis. However, ana-logous to other mu-co-sae, a dysregulation of the mu-cosal immune system resulting from al-tera-tions of the ocular surface can con-tribute to inflammatory processes and may therefore deserve increasing interest for future therapeutic stra-tegies.

Introduction

Immune based inflammation is increasingly recognized as a primary or secondary pathogenetic factor in several forms of ocular surface disease, even in those that did not necessarily appear to be associated with inflammation, as for example in non Sjögren dry eye disease[16,17].

Although lymphoid cells were identified as an important modulator of clinical and subclinical ocular inflammation, the information about MALT at the ocular surface was sparse and controversial[10]. Better knowledge can be advantageous for understanding and modulating ocular surface immunology in diseases.

MALT is an outpost of the immune system located at almost all mucosal surfaces of the body. It is composed of diffusely interspersed lymphoid cells, mainly lymphocytes and plasma cells, and of accessory cells such as dendritic cells, macrophages and mast cells. They are located in the loose connective tissue of the subepithelial lamina propria and lymphocytes also occur inside the epithelium as intraepithelial lymphocytes. Organized lymphoid follicles are embedded into the mucosae and constitute a second form of MALT. They have a different but complemen-tary function because they are the sites for uptake of environmental antigens and generation of antigen specific effec-tor cells which are later distributed via regulated migratory processes (lymphocyte recirculation)[9] within the mucosal organs and the central immune system in order to act against their cognate antigens. At the ocular surface however, lymphoid cells have frequently been regarded as inflammatory cells (for review see[8]).

MALT has certain differences compared to the central immune system that have only recently achieved a broader recognition and may in the future allow a more differentiated view on mucosal immunity also in the eye. A lot, if not the majority, of antigens at mucosal surfaces are non-patho-genic and derive, in case of the ocular surface, from environmental antigens such as dust and pollen or from the non-pathogenic bacterial flora but also from own tissue constituents of intact or disintegrated cells following mucosal injury. Therefore, in order to prevent con-stant unnecessary adverse inflammatory immune reactions that en-danger the integrity of the mucosal tissues, one of the main functions of MALT is the generation of immune tolerance. This is maintained by an anti-inflammatory environment characterized by respective cytokines and a highly regulated antigen presentation. Furthermore, immunoglobulin production by local mucosal plasma cells preferably consists of the anti-inflammatory isotype immunoglobulin A (IgA)[2]. The balance between immunity and tolerance must be tightly regulated by the mucosal immune system. If it is dysregulated by external influences this may result in mucosal disease.

There is a historical misconception of lymphoid cells at the ocular surface

Lymphocytes and plasma cells are known at the ocular surface from histological investigations dating back to the nineteenth century but their significance has remained largely unclear until recently. In contrast to the lacrimal gland where plasma cells were accepted as the source of specific immune protection, the same cells were regarded as an inflammatory infiltration in the conjunctiva and lacrimal drainage system[8]. This reflects a historical misconception of lymphoid cell function. Early immuno-histochemical studies revealed contradictory results

about the number and types of lymphoid cells. Only in the last decade of the twentieth century authors could agree on the presence of lymphoid cells and related them to the increasing knowledge about the mucosal immune system. Since these studies were usually based on small clinical biopsies, some disagreement remained on the actual types of lymphoid cells, their topographical distribution and on the occurrence of organised lymphoid follicles.

Material and Methods

Therefore a thorough investigation of whole-mounts from normal tissues of the lacrimal gland, conjunctiva and lacrimal drainage system of human body donors admitted to the department of anatomy was performed by histology, immuno-histo-chemistry, scanning and transmission electron microscopy and molecular biology.

Results

We found that all tissues of the normal human conjunctiva and lacrimal drainage system contained a diffuse lymphoid tissue composed mainly of lymphocytes and plasma cells. These were found in the subepithelial lamina propria but lymphocytes regularly occurred also in the epithelium. The lamina propria lymphocytes and plasma cells were arranged in a thin zone (Figure 1). This had an inhomogeneous density but showed

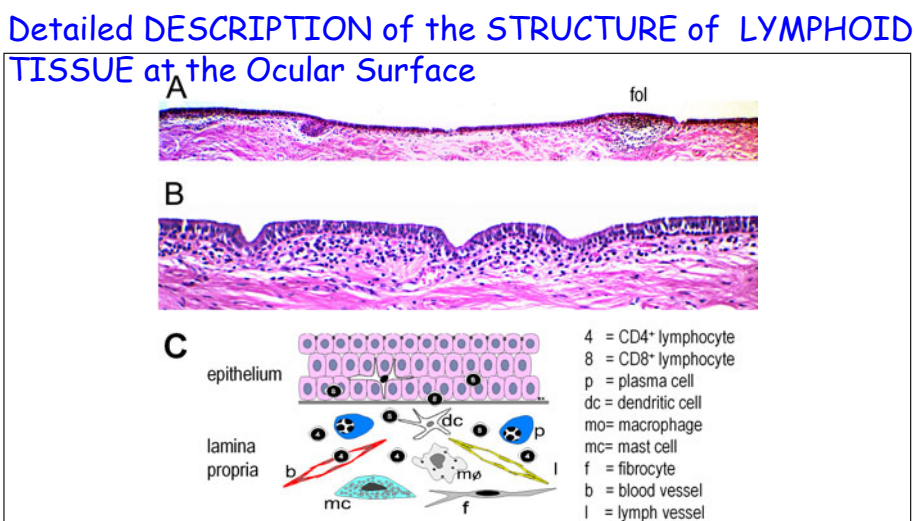

Figure 1. Diffuse lymphoid tissue and an organized lymphoid follicle (fol) in the normal human conjunctiva. (A). Higher magnification clearly shows the lymphoid cells (B) that are schematically indicated with their accessory cells in (C).

an overall trend of highest cell density in the tarso-orbital conjunctiva and fewer cells in the bulbar region. The plasma cells were strongly positive for IgA and the overlying epithelium expressed its transporter molecule secretory component which is necessary for transepithelial transport of the protective immunoglobulins onto the ocular surface. This was observed in the conjunctiva and lacrimal drainage system and in fact it directly continued from the known tissue inside the lacrimal gland. Numerous small vessels including high endothelial venules for the regulated migration of lymphoid cells also occurred.

A majority of tissues (about 60%) from the conjunctiva and lacrimal drainage system contained organised lymphoid follicles with a high bilateral symmetry of more than 75% in fellow eyes. These follicles were usually relatively flat in the conjunctiva but showed typical histologic and immunohistologic characteristics of lymphoid follicles as a thin apical epithelium that contained groups of lymphocytes together with absence of the immunoglobulin transporter.

Conclusions

Lymphoid cells are a physiological component of the normal human ocular surface

These results show that a resident lymphoid population is present at the normal human ocular surface and lymphocytes do not need to immigrate prior to any kind of physiological or pathophysiological action. Their continuity from the lacrimal gland throughout the conjunctiva into the lacrimal drainage system and their typical characteristics allow to address them as an Eye-Associated Lymphoid Tissue (EALT) at the ocular surface that stays in line with the other organs of the MALT system of the body. Since the conjunctiva-associated lymphoid tissue predominates in the tarso-orbital area which covers the cornea during eye closure, it can be assumed that it has an important function for protection of the cornea which is itself devoid of lymphoid cells.

Local inhomogeneities of lymphoid cells may be one reason why they have previously either been regarded as an inflammatory infiltration or were simply missed. Another reason for a limited view on lymphocytes in general and plasma cells in particular is that they were generally regarded and addressed as “inflammatory cells” even when observed in every normal tissue. More recent evidence shows that lymphoid cells as such are not inflammatory but instead their function is tightly regulated and there are several subtypes with specific and partly opposing functions. The balance between immunity and tolerance and the maintenance of the latter is the basis for a healthy ocular surface (Figure 2). It depends, apart from the primary usage of the anti-inflammatory IgA, on the tight regulation of antigen presentation.

Detailed DESCRIPTION of IMMUNE REGULATION at the Ocular Surface

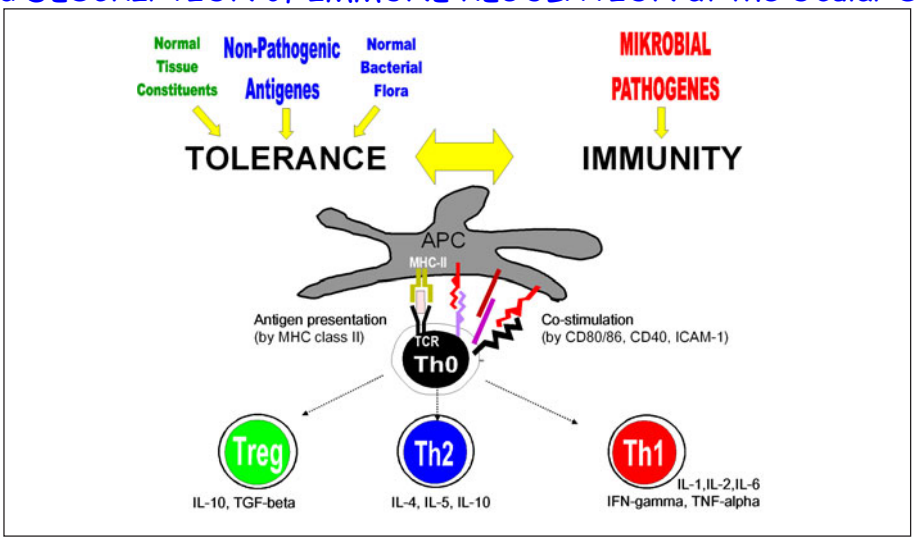

Figure 2. The main function of the mucosal immune system is the balance between immunity and tolerance. This is regulated by the mode of antigen presentation by APC which results in different subpopulations of T-cells.

Lymphocyte function is tightly regulated by antigen presentation

Antigen presentation is normally performed by specialized antigen presenting cells (APC), mainly dendritic cells. They regulate, depending on their functional status the generation of different populations of T-helper cells which in turn determine the direction of the resulting immune response by the specific pattern of their secreted cytokines[1].

The interaction of the antigen specific T-cell receptor with a peptide antigen presented on the major-histocompatibility complex class II (MHC-class II) of an APC alone is not sufficient to activate the lymphocyte to a destructive action against the antigen. It rather leads to generation of tolerance by anergy or deletion of the respective lymphocyte, or, under certain conditions, to T-helper cells type 2 (Th2) cells that normally support production of anti-inflammatory IgA at mucosal surfaces[1]. Only if non-physiological stimuli ("danger signals")[5] are provided by microbial infection, cell destruction or surgical procedures, the physiological anti-inflammatory mode of MALT is skewed towards inflammation. Dendritic cells mature by up-regulation of co-stimulatory molecules (such as CD80/86, CD40, ICAM-1) and generate inflammatory Th1 lymphocytes that produce pro-in-flam-matory cytokines (IL-1, IL-2, IL-6, IFN-gamma, TNF-alpha) and a T-cell mediated mucosal inflamma-

tion may result, as reported for example in inflammatory bowel disease[11].

Dysregulation of the physiological mucosal immune system can contribute to inflammatory ocular surface disease

Also at the ocular surface and appendage it has been shown that several diseases include an up-regulation of inflammatory markers.

Inflammatory cytokines are observed in the lacrimal and salivary glands in Sjögren´s syndrome[4,14] and in the conjunctiva in Sjogren´s syndrome[7] and in non-Sjogren dry eye[16]. Epithelial cells of the conjunctiva[3,7] and lacrimal gland[4] appear to produce them in addition to the inflammatory lymphoid cell infiltrates that are characteristic for lacrimal gland destruction in Sjogren´s syndrome[15] but also occur in the conjunctiva[17].

The primary alteration in inflammatory ocular surface disease conceivably occurs by activation of the epithelial cells either by viral infection as proposed in Sjogren´s syndrome[4] or by infection, mechanical irritation and eventual disruption and leakage of the epithelium[16] (Figure 3). Such alterations, as supported by findings in inflammatory mucosal disease of the intestine, explain the observed up-regulation of inflam-

Detailed DESCRIPTION of INFLAMMATORY PATHWAYS in DRY EYE

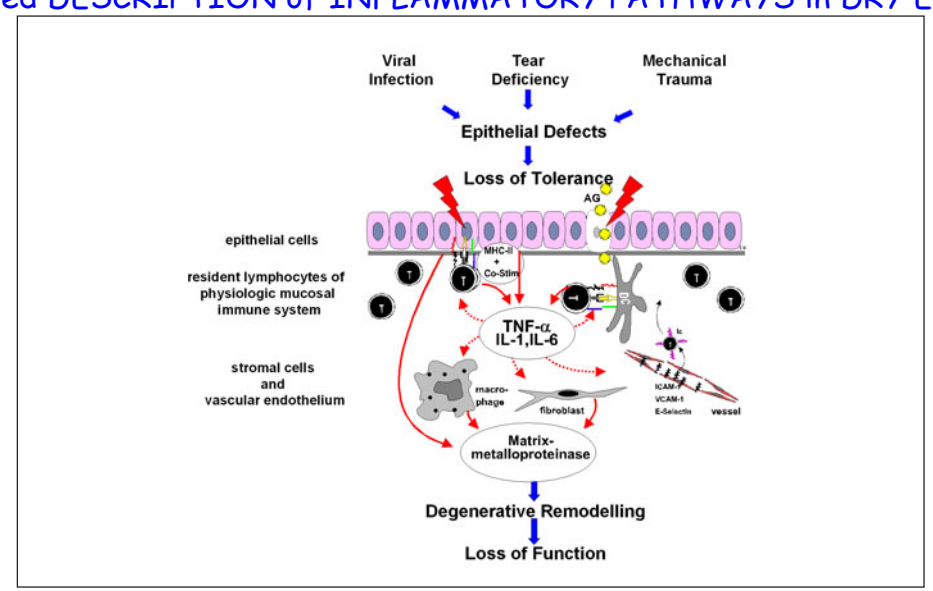

Figure 3. A dysregulation of the physiologically protective mucosal immune system re-sults in a loss of mucosal tolerance with upregulation of in-flam-matory events that finally lead to tissue destruction and loss of function.

matory epithelial markers[3] and the presentation of auto-antigens observed in the lacrimal gland[13]. These events can lead to a breakdown of the physiological mucosal immune tolerance and are sufficient to induce an inappropriate activation of the normally protective lymphocytes of the resident MALT which results in the dysregulation of mucosal homeostasis and the observed release of inflammatory cytokines. Downstream effects of these signalling molecules include an activation of vascular endothelial cells leading to an influx of additional lymphoid cells. Matrix metal-lo-proteinases produced by activated stromal macrophages and fibrocytes[12] and by epithelial cells[6] finally lead to a degenerative re-model-ling of the tissue in the conjunctiva and lacrimal gland with a conse-quent loss of function that causes the symptoms presented to the ophthalmologist.

Newly discovered biological therapeutics that were characterised by several speakers in this congress can interact with all steps during cellular activation, antigen presentation and tissue remodelling in order to provide future effective strategies against inflammatory ocular surface disease.

References

1. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998;392:245-252.
2. Brandtzaeg P. History of oral tolerance and mucosal immunity. *Ann.N.Y.Acad.Sci.* 1996;778:1-27.
3. Brignole F, Pisella PJ, Goldschild M, De Saint JM, Goguel A, Baudouin C. Flow cytometric analysis of inflammatory markers in conjunctival epithelial cells of patients with dry eyes. *Invest Ophthalmol.Vis.Sci.* 2000;41:1356-1363.
4. Fox RI, Kang HI, Ando D, Abrams J, Pisa E. Cytokine mRNA expression in salivary gland biopsies of Sjogren's syndrome. *J Immunol.* 1994;152:5532-5539.
5. Gallucci S, Matzinger P. Danger signals: SOS to the immune system. *Curr.Opin.Immunol.* 2001;13:114-119.
6. Garrana RM, Zieske JD, Assouline M, Gipson IK. Matrix metalloproteinases in epithelia from human recurrent corneal erosion. *Invest Ophthalmol Vis Sci* 1999;40:1266-1270.
7. Jones DT, Monroy D, Ji Z, Atherton SS, Pflugfelder SC. Sjogren's syndrome: cytokine and Epstein-Barr viral gene expression within the conjunctival epithelium. *Invest.Ophthalmol.Vis.Sci.* 1994;35:3493-3504.
8. Knop E, Knop N. A functional unit for ocular surface immune defense formed by the lacrimal gland, conjunctiva and lacrimal drainage system. *Adv.Exp.Med.Biol.* 2002;506:835-844.
9. Knop E, Knop N. Lymphocyte Homing in the Mucosal Immune System to the Eye-Associated Lymphoid Tissue (EALT). In: Zierhut M, Sullivan DA, Stern ME, eds. Immunology of the Ocular Surface and Tearfilm. Amsterdam, Swets & Zeitlinger (in Press), 2004

10. Knop N, Knop E. Conjunctiva-associated lymphoid tissue in the human eye. *Invest Ophthalmol Vis Sci* 2000;41:1270-1279.
11. MacDonald TT, Bajaj-Elliott M, Pender SL. T cells orchestrate intestinal mucosal shape and integrity. *Immunol Today* 1999;20:505-510.
12. Meller D, Li DQ, Tseng SC. Regulation of collagenase, stromelysin, and gelatinase B in human conjunctival and conjunctivochalasis fibroblasts by interleukin-1beta and tumor necrosis factor-alpha. *Invest Ophthalmol Vis Sci* 2000;41:2922-2929.
13. Mircheff AK, Xie J, Qian L, Hamm-Alvarez SF. Diverse perturbations may alter the lacrimal acinar cell autoantigenic spectra. *DNA Cell Biol.* 2002;21:435-442.
14. Ohyama Y, Nakamura S, Matsuzaki G, Shinohara M, Hiroki A, Fujimura T, Yamada A, Itoh K, Nomoto K. Cytokine messenger RNA expression in the labial salivary glands of patients with Sjogren's syndrome. *Arthritis Rheum.* 1996;39:1376-1384.
15. Pepose JS, Akata RF, Pflugfelder SC, Voigt W. Mononuclear cell phenotypes and immunoglobulin gene rearrangements in lacrimal gland biopsies from patients with Sjogren's syndrome. *Ophthalmology* 1990;97:1599-1605.
16. Pflugfelder SC, Solomon A, Stern ME. The diagnosis and management of dry eye: a twenty-five-year review. *Cornea* 2000;19:644-649.
17. Stern ME, Gao J, Schwalb TA, et al. Conjunctival T-cell subpopulations in Sjogren's and non-Sjogren's patients with dry eye. *Invest Ophthalmol.Vis.Sci.* 2002;43:2609-2614.