Virchows Arch (2000) 437:185–189

ORIGINAL ARTICLE

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Mucosa-associated lymphoid tissue in human efferent tear ducts

Received: 1 March 2000 / Accepted: 17 April 2000 /

Abstract The recent description of primary marginal zone lymphomas in human efferent tear ducts with typical features of lymphomas arising in mucosa-associated lymphoid tissue (MALT) infers the presence of MALT in the human efferent tear ducts. To date, studies have not established clearly whether organised MALT occurs in normal human efferent tear ducts. To elucidate this problem, efferent lacrimal pathways from unselected body donors with unknown prior history of efferent tear duct, ocular, or nasal disease were examined for the presence of organised MALT. Organised lymphoid tissue was found with the cytomorphological and immunophenotypic features of MALT in 41% of the cases examined. These findings suggest that MALT is a feature that, although it need not be present in normal efferent tear ducts, is acquired during life in a proportion of apparently asymptomatic individuals.

Key words Extranodular tissue · Immune defences · Lymphoma · Ocular adnexa

Introduction

The evidence of primary extranodal marginal zone B-cell lymphoma [low-grade B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT) type] of the efferent tear ducts [13, 24, 25] infers the presence of pre-existent organised lymphoid tissue at that site. The mucosa of the lacrimal sac and the nasolacrimal duct consists of a double-layered epithelium with scattered lymphocytes or groups of lymphocytes. Although it has been shown that the mucosa of the efferent tear ducts plays an important role in defence against foreign antigens and varying types of microbial pathogens, it is not clear whether organised MALT is a normal component of the human efferent lacrimal system [19]. In the present study, the lacrimal sac and nasolacrimal duct from each eye of 41 unselected adult body donors were examined for the presence of lymphoid tissue with the structure and immunophenotype of organised MALT.

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Materials and methods

Both efferent lacrimal systems, each consisting of both canaliculi, the lacrimal sac and the nasolacrimal duct, were excised in their entirety from the heads of 41 unselected adult (23 female and 18 male; age range 25–93 years) body donors obtained at the Department of Anatomy, Christian Albrecht University of Kiel, Germany, during the period from 1997 to 1999. In all cases, the efferent tear duct systems, dissected and freed from the surrounding bone, appeared macroscopically normal with no pre-mortem history of efferent lacrimal system, ocular, or nasal disease. The efferent lacrimal pathways measured between 35 mm and 42 mm. They were routinely fixed in formalin and embedded in paraffin. Sections 7-µm thick were stained with toluidine blue, hemalaun, according to Goldner, and resorcin–fuchsin–thiazine picric acid [21]. Immunohistochemical staining was performed using antibodies against CD20 (L26; concentrated), CD3 (1:100), immunoglobulin (Ig) M (1:100), IgA (1:20), IgG (1:50), follicular dendritic cells (KiM4; concentrated), CD45RA (KiB3; concentrated) and CD68 (KiM1; concentrated). With the exception of Ki, all antibodies were from Dako (Glostrup, Denmark); the Ki antibodies were provided by the Department of Pathology, Christian Albrecht University of Kiel, Germany. They were applied using a standard peroxidase-labelled streptavidin-biotin technique [1], either with microwave heating pretreatment [5] or using conventional methods with trypsinisation where appropriate. Two negative control sections were used in each case. One was incubated only with the second antibody, the other only with the primary antibody. As a positive control, sections of human spleen (all other antibodies) were used. All slides were examined using a microscope (Zeiss-Axiophot).

Results

Organised lymphoid tissue was identified in 17 of the 41 adult specimens. No significant differences were found between females and males. The distribution by age group and by percentage is shown in Fig. 1 and Table 1, respectively. A 27-year-old was the youngest person in whom MALT was identified.

In the 17 cases in which organised MALT was identified, it was distributed at intervals along the lacrimal sac and/or the nasolacrimal duct and was not necessarily present to the same extent in both efferent lacrimal systems (nine times in both systems in the same person, five times in the left system only, three times in the right system only), or in the lacrimal sac and the nasolacrimal duct of the same eye. Similarly to other mucosal sites, MALT in the efferent tear ducts, was characterised by the presence of reactive germinal centres (Fig. 2) containing tingible-body macrophages (Fig. 2a), a network of KiM4-positive follicular dendritic cells (Fig. 2c) and CD3-positive T cells (Fig. 2e). The germinal centres were surrounded by mantle zones and marginal zone cells (MZCs; Fig. 2b). The mantle zones consisted of small CD20-positive lymphocytes (Fig. 2d) expressing CD45RA (Fig. 2b) and IgM. These lymphocytes merged into a population of small- to medium-sized B cells with moderately abundant cytoplasm and irregular nucleus outlines, features typical of MZCs. The MZC exhibited the following immunophenotype: CD20+, CD45RA–, CD3–, IgM+ and IgA+. Some of the cells also expressed IgG, but these cells were rare. The MZC extended into the overlying epithelium to form a characteristic lymphoepithelium (Fig. 2f). In the parafollicular area, T lymphocytes, B lymphocytes and high endothelial venules were present. In no case were features suggestive of lymphoma observed.

In the 24 cases in which no organised lymphoid tissue was found, there was a diffuse infiltrate of variable intensity within the lamina propria of the lacrimal sac and the nasolacrimal duct consisting predominantly of CD3-positive T lymphocytes with scattered CD20- and CD45RA-positive B cells and plasma cells. Solitary CD3-positive T cells and CD45RA-positive B cells were detected inside the epithelium, although the overall extent of infiltration was quite low.

Discussion

An understanding of the structure and function of MALT is central when considering extranodal lymphomas arising at mucosal sites [10, 11]. The term MALT is most often associated with the well-defined lymphoid structures in the gut wall, the Peyer's patches. According to Isaacson [12], MALT is typified by Peyer's patches in the terminal ileum and comprises four lymphoid compartments

Table 1 Distribution by age and percentage of cases with organised mucosa-associated lymphoid tissue

Age (years)	20–30	31–40	41–50	51–60	61–70	71–80	81–90	>90
Cases	1	1	2	3	4	3	2	1
Percentage	2.4	2.4	4.9	7.3	9.8	7.3	4.9	2.4

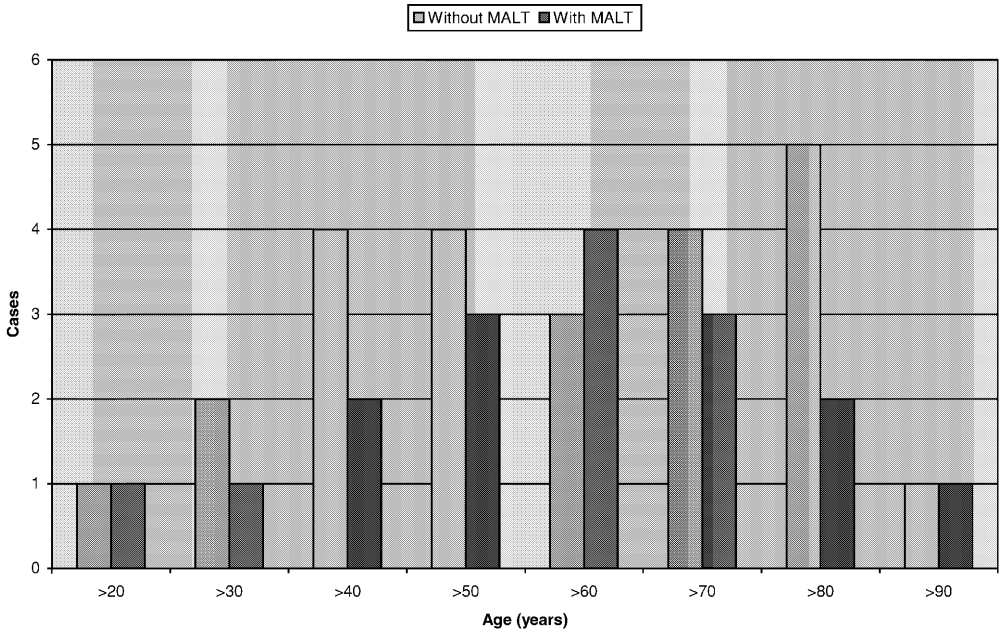

Fig. 1 Distribution by age of cases with and without organised mucosa-associated lymphoid tissue

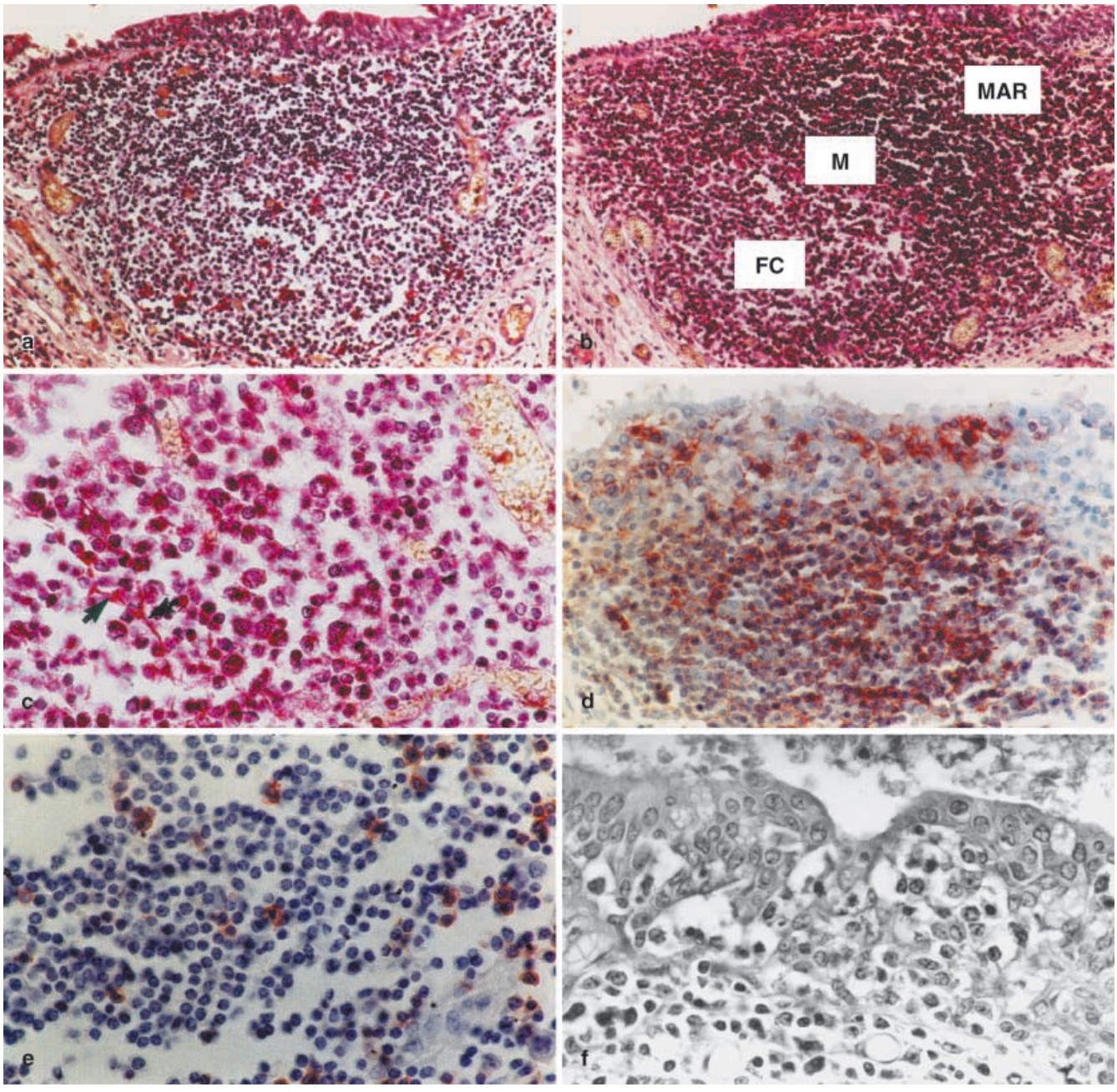

Fig. 2 It must be stated that the preparations are from body donors and are not taken until 48 h after death. **a** Acquired mucosa-associated lymphoid tissue (MALT) of the efferent tear ducts with a well-developed follicle centre and surrounding mantle zone. Macrophages are stained *red* by KiM1p; ×115. **b** B-cell component of efferent tear duct MALT showing follicle centre (*FC*), mantle zone (*M*) and marginal zone (*MAR*). Immunostaining with KiB3; ×115. **c** Anti-KiM4 immunostaining shows follicular dentritic cells (*arrows*) in a germinal centre within a lymphoid follicle of the nasolacrimal duct; ×363. **d** Anti-CD20 immunostaining confirms the cells within the lymphoepithelium as B cells; ×231. **e** Anti-CD3 immunostaining shows T cells within a lymphoid follicle of a nasolacrimal duct; ×363. **f** High magnification of lymphoepithelium in the area of the lacrimal sac showing clusters of intraepithelial B cells with irregular nuclei and pale-staining cytoplasm. Goldner staining; ×363

as follows: (1) organised mucosal lymphoid tissue, (2) a lamina propria, (3) intra-epithelial lymphocytes and (4) the mesenteric lymph nodes. The organised lymphoid tissue comprises a B- and T-cell component. The B-cell component consists of a follicle with a centre and mantle zone and a more or less well-developed marginal zone. There is also an interfollicular area of T cells with high endothelial venules. The lamina propria is diffusely infiltrated by plasma cells, most of which are synthesising IgA, and lymphocytes, most of which are $CD4^+$ T cells with some memory B cells, macrophages and other accessory cells [12].

The human paired palatine tonsils of Waldeyer's ring are believed to form an interface between MALT and nodal lymphatic tissue, with features of gut-associated

lymphoid tissue and to function as a peripheral lymphoid organ with connections to other peripheral lymphoid organs via efferent lymphatics [2]. Primary low-grade B-cell lymphomas of the MALT type arising in Waldeyer's ring have been described [16, 18]. Organised lymphoid tissue from outside the gut is supposed to form MALT in response to antigenic stimulation, which may be part of an autoimmune condition such as Sjögren's syndrome in the salivary gland [9]. In the conjunctiva [27], nose [14], larynx [22] and lung [7, 8, 17], MALT is absent in neonates and is acquired in early childhood in response to antigenic stimulation. Wotherspoon et al. [26] demonstrated that gastric MALT is acquired specifically in response to local infection by *Helicobacter pylori*.

In a small proportion of cases of developed MALT, subsequent and as yet unclarified oncogenic events occur, leading to development of lymphomas with histological and immunotypical features of MALT. Most of these lymphomas are of the low-grade B-cell type.

In humans, the lacrimal sac and the nasolacrimal duct contain a double-layered epithelium resting on a broad basement membrane. Perra et al. [20] demonstrated large amounts of IgA inside and on the surface of the lining epithelium of the lacrimal ducts which could interact with T and B lymphocytes and macrophages present inside the epithelium and the underlying substantia propria. Duke-Elder [6] described a layer of adenoid tissue beneath the epithelium, sometimes aggregated into follicles. The adenoid tissue has been shown to consist of immunocompetent cells differentiated as T and B lymphocytes and macrophages showing a special distribution inside the lamina propria [19]. According to Markovitch [15] and Tsuda [23], the occurrence of intraepithelial lymphocytes in the nasolacrimal ducts is frequent.

In the present study, we were able to demonstrate subepithelial visibility of aggregated follicles in some of the efferent tear ducts investigated. These aggregations fulfilled the criteria for designation as MALT. They consist of organised mucosal lymphoid tissue characterised by the presence of reactive germinal centres and mantle zones. Around the mantle zone, one can identify an additional zone of somewhat larger cells corresponding to MZCs. These cells extend into the overlying epithelium, forming a lymphoepithelium.

Specific secretory immunity depends on sophisticated co-operation between the mucosal B-cell system and an epithelial glycoprotein called the secretory component [3]. Initial stimulation of Ig-producing B cells is believed to take place mainly in organised MALT [4]. It has become evident that considerable regionalisation or compartmentalisation exists in MALT, perhaps being determined by different cellular expression profiles of adhesion molecules and/or the local antigenic repertoire. Antigenic stimulation of B cells results in the generation of predominantly IgA-synthesising blasts that leave the mucosae via efferent lymphatics, pass through the associated lymph nodes into the thoracic duct and enter the circulation. The cells then return selectively to the lamina propria as plasma cells or memory B cells [12] by means of homing mechanisms.

The lamina propria within the efferent lacrimal pathways is normally devoid of organised lymphoid structures, suggesting that its immunological effector functions depend on the generation of immune responses elsewhere. In this context, experimental work has focused on the paired lymphocytic cell aggregates present at the entrance to the nasopharyngeal duct in rodents [14]. This organised lymphatic tissue has many similarities to gut-type MALT, and its inductive function may be crucial to the immune response of mucosal surfaces in the head and perhaps beyond this region. Wotherspoon et al. [27] advise caution when extrapolating animal experimental data to humans, since the distribution and structures of MALT vary among different species.

In the present study, organised MALT was found in 41% of the efferent tear ducts from unselected body donors with unknown previous history of disease regarding the eye, efferent lacrimal pathway or nose. This suggests that MALT is not a ubiquitous finding in the efferent lacrimal system and is rather acquired in response to antigenic stimulation. It may develop in response to bacterial or viral infections or as a result of allergic reactions. The development or acquisition of MALT in individual human efferent tear ducts remains unclarified, but, once present, it can provide the basis from which primary low-grade B-cell lymphoma of the MALT type may arise.

It can be concluded that this is the first description of organised MALT in the human efferent tear ducts. It is not present in the lacrimal sac and nasolacrimal duct of all humans, and it is suggested that this lymphoid tissue may be acquired either in reaction to immunological changes in lymphatic tissue inside the body or in reaction to specific infections.

Acknowlegements The authors would like to thank Karin Stengel for her excellent technical assistance. We gratefully acknowledge gifts of monoclonal KiM4, KiB3 and KiM1 antibodies from Dr. H.C.R. Parwaresch (Department of Pathology, Christian Albrecht University of Kiel, Germany). The study was supported by DFG grant Pa 738/1-1 – a programme of the German Research Foundation.

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