The Presence of a Local Immune System in the Upper Blind and Lower Part of the Human Nasolacrimal Duct

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Summary. The nasolacrimal duct is exposed to exogenous agents, including potentially harmful microorganisms, coming from the eye surface by the lacrimal sac, and from the nasal cavity by the inferior meatus of the nose. The upper blind and lower part of the human nasolacrimal duct were examined immunohistochemically to ascertain the presence and localization of immunoglobulin-producing cells and the epithelial expression of IgA, IgM, and IgG in order to verify the possible antimicrobial properties of this duct. IgA-, IgM-, and IgG-positive immunocompetent cells were recognizable in the lamina propria of the upper blind and lower part of the human nasolacrimal duct, while an evident immunoreactivity for sIgA, IgM, and IgG was demonstrated in the cytoplasm of the apical epithelial cells. The results suggest that all the effector components of the mucosal immune system are present in that area of the human nasal mucosa next to the opening of the nasolacrimal duct as well as in the human lacrimal sac.

The ocular surface, one of the external structures of the body, provides a potential substrate for the adhesion of a wide variety of bacteria and other pathogens. Under normal circumstances a combination of non-specific and specific factors prevent allergic, inflammatory, and infective diseases of the external eyes (ALLANSMITH, 1979; GILLETTE et al, 1981; CHANDLER and GILLETTE, 1983; SMOLIN, 1985). A critical role in this defense is played by the lacrimal apparatus composed of two parts: the lacrimal gland, which secretes a complex fluid (tears), and the excretory ducts which convey the fluid from the surface of the eyes into the nasal cavity (GRAY, 1973; DUKE-ELDER, 1974; KUWABARA, 1983; HURWITZ, 1996). This communication suggests not only that some ocular infections could arise from the nose, but also that the nasolacrimal duct is exposed to exogenous agents,

including potentially harmful microorganisms, coming from the eye surface by the lacrimal sac, and from the nasal cavity by the inferior meatus of the nose.

Although investigations have demonstrated that the excretory ducts of the human lacrimal apparatus are not passive structures but act as a barrier against pathogenic bacteria (GROELL et al., 1997; PAULSEN et al., 1997, 1998; THALE et al., 1998), the existence of a mucosal secretory immune system has not been completely explained (GREGORY and ALLANSMITH, 1987; MCCLELLAN, 1997). Our previous study could demonstrate that the human lacrimal sac, which represents the upper blind of the nasolacrimal duct, is protected from pathogen insult by a local IgA-mediated immune response (PERRA et al., 1995). However, human tears allow one to detect not only levels of IgA but also levels of IgG and IgM (LITTLE et al., 1969; MCCLELLAN et al., 1973; SEN et al., 1978). The detection of IgG and IgM originating from local synthesis and secretions of the lacrimal gland (SMOLIN, 1987; FRANKLIN, 1989; KIVELA, 1992; SULLIVAN, 1994) have led us to study, by immunohistochemical methods, the presence and localization of the cells necessary to activate an Ig-mediated immune response and the epithelial expression of IgM and IgG, in order to obtain information about a local immune system in the mucosal surface of the human lacrimal sac.

Because the lower portion of the nasolacrimal duct is closely related to the nasal mucosa which represents the first site of contact with inhaled antigens to induce an immunoglobulin-mediated response (BERNSTEIN, 1992; BRANDTZAEG, 1995; BRANDTZAEG et al., 1997), we employed the same methods to attempt a study of the local immune system in the human nasal mucosa close to the opening of the nasolacrimal duct, in comparison with that of the lacrimal sac mucosa.

MATERIALS AND METHODS

Human biopsy specimens of the lacrimal sac were obtained by surgery from 12 patients (five males and seven females) ranging in age from 30 to 70 years at the time of surgery for dacryocystitis; two samples were obtained from patients undergoing repair for midfacial trauma (age 24, 38).

Human biopsy specimens of the nasal mucosa next to the inferior meatus were obtained from nine patients (four males and five females) ranging in age from 12 to 43 undergoing surgery for symptomatic nasal obstruction caused by polypoid rhinosynusitis. All patients had normal immunological responses.

Tissue segments were fixed by immersion in cold 10% paraformaldehyde in 0.2 M phosphate buffer, pH 7.3, for 4–6 h, and processed for paraffin embedding. Microtome sections (6–7 μ m) were treated for the immunohistochemical demonstration of sIgA, IgM, and IgG using the ABC method: they were rehydrated in PBS, pre-treated with 0.1% trypsin (Sigma, St Louis, MO) in PBS at 37°C for 20 min to enhance the intensity of the specific staining, and then immersed for 30 min in a solution of 100% methyl alcohol and 30% hydrogen peroxidase to inactivate endogenous peroxidase. The sections were treated for 15 min with 10% non immune rabbit serum. Rabbit polyclonal antibody to human sIgA (Cappel, Durham, NC; working dilution 1:4000), and mouse monoclonal antibodies to human IgM and IgG (Dako, Glostrup, Denmark; working dilution 1:100) were used as primary antisera overnight at 4°C. Both anti-human IgA and anti-human IgG were F(ab')₂ fragments. Biotinylated anti-rabbit and anti-mouse IgG were used as secondary antisera (Chemicon International, Temecula, CA; 1:200) for 30 min at room temperature. The sections were further incubated in an avidin-biotin-peroxidase complex (Biomedica, Milano, Italy; 1:250) for 30 min at room temperature, reacted with 3,3-diaminobenzidine (Sigma), and then counterstained with hematoxylin. The sections were thoroughly rinsed in PBS between each step.

Further adjacent sections were incubated using a rabbit anti-human IgA specific for alpha-chains, F(ab')₂ fragment, (Dako; working dilution 1:4000) as the primary antiserum for the concurrent demonstration of IgA class immunoglobulins.

Other microtome sections were treated for the immunohistochemical demonstration of CD3, CD20, CD68, HLA-DR and Protein S100 using the ABC method. Rabbit polyclonal antibodies to human T-cells CD3 (Dako; prediluted), mouse monoclonal antibody to human B-cells CD20 (Dako; working dilution

1:500), mouse monoclonal antibody to human macrophages CD68 (Dako; working dilution 1:100), mouse monoclonal LM3/HLA-DR to human HLA-DR antigen (Clonab®, Biotest, Milan, Italy; working dilution 1:4), and rabbit polyclonal antibody to cow protein S100 (Dako; working dilution 1:100) were used as primary antisera and biotinylated anti-rabbit and anti-mouse IgG were used as secondary antisera.

In the control sections, the specificity of the antisera was tested by replacing the primary antibodies with normal serum.

RESULTS

In the nasal mucosa of human biopsy specimens, marked immunoreactivities for sIgA, IgM and IgG were detected. The immunoreactivities for sIgA, IgM and IgG were localized in the apical cytoplasm of the superficial epithelial cells. Along the surface such an immunoreactivity as for and IgM IgG was not continuous; in fact, areas of strong, weak, and completely absent immunoreactivity were observed. IgA-immunocompetent cells were localized in the subepithelial layers of the stroma, while scattered IgM- and IgG-immunoreactive cells were detected in the lamina propria (Fig. 1A–C).

A similar distribution pattern of sIgA-, IgM- and IgG-immunoreactivity in the mucosa of the lacrimal sac was detected, with an IgA- and IgG-immunoreaction localized in the apical cells and an immunoreactivity for IgM localized in the apical cytoplasm of the most superficial cells. Scattered IgA-, IgM-, and IgG-immunocompetent cells in the lamina propria was observed (Fig. 2A–C).

The immunoreactions for CD20, CD3, CD68, HLA-DR and S100 protein showed a different pattern of distribution between the nasal and lacrimal sac mucosa. In fact, we could not find any lymphoid follicles in the examined specimens of the nasal mucosa as we could in the lacrimal sac. In the nasal mucosa, B- and T-lymphocytes respectively stained with anti-human CD20 and anti-human CD3 showed a similar distribution, being scattered in the epithelial layers and in the lamina propria (Fig. 3A, B). Macrophages, stained with anti-human CD68, appeared scattered in both the subepithelial layers of the lamina propria and the epithelium (Fig. 3C). These cells showed a morphology varying from that of small cells with regular outlines to cells with typical dendrite-like processes; this heterogeneity seemed to be linked to their enzymatic store of tryptase and/or chymase and histamine. HLA-DR expression was found in the lamina propria around the basolateral

Fig. 1. Light micrographs of the human nasal mucosa reacting to anti-human secretory IgA (A), anti-human IgM (B) and anti-human IgG (C). An intense immunoreactivity for sIgA is observed in the apical cytoplasm of the epithelial superficial cells. The distribution of immunoreactivity for IgM and IgG appears inhomogeneous along the surface. Scattered immunoreactive cells are detected in the lamina propria (arrows). Counterstained with hematoxylin. $\times 400$

Fig. 2. Light micrographs of the human lacrimal sac reacting to anti-human secretory IgA (A), anti-human IgM (B), and anti-human IgG (C). An intense immunoreactivity for sIgA, IgM, and IgG is localized in the cytoplasm of the epithelial superficial cells. IgA-, IgM- and IgG-immunocompetent cells in the lamina propria are observed (arrows). Counterstained with hematoxylin. $\times 400$

side of the glands; HLA-DR-positive cells scattered in the stroma were also present. Numerous HLA-DR-immunoreactive cells with dendritic-like processes were recognizable in the epithelium (Fig. 5A).

Immunoreactivity for S100 protein was localized in the acinar and ductal cells of the human nasal mucosa glands in which it also showed nuclear localization (Fig. 5B), as observed in other tissues such as human vascular and intestinal smooth muscle (MANDINOVA et al., 1998) and within Schwann cells (MATA et al., 1990).

In the lacrimal sac mucosa, B- and T-lymphocytes and macrophages showed a similar distribution of localization in both the lamina propria and the epithelium.

In the stroma, B and T lymphocytes were organized in typical secondary follicles surrounded by parafollicular areas, where high endothelial venules were present (Fig. 4A, B). CD68-positive cells were distributed throughout the lamina propria and directly subepithelially; some CD68-immunoreactive cells in a lymphoid follicle were recognizable (Fig. 4C).

In the lacrimal sac, the HLA-DR antigen expression was found in the epithelium and in the lamina propria, in which some immunoreactive cells with dendritic-like processes were also recognized. Numerous HLA-DR-positive cells were detected in the lymphoid follicles as well as in the parafollicular areas (Fig. 6A).

The staining for protein S100 revealed the presence of immunoreactive cells in the epithelium and in the lamina propria, mainly in the parafollicular areas, where cells with dendritic-like processes were noticed (Fig. 6B).

In all control sections the immunoreactivity was completely abolished.

DISCUSSION

It has been considered that the principal tissues involved in the immunological protection of the ocular surface are the lacrimal gland and the conjunctiva (ALLANSMITH et al., 1973; SACKS et al., 1986; SETO et

Fig. 3. Light micrographs of the human nasal mucosa reacting to anti-human CD20 (A), anti-human CD3 (B), and anti-human CD68 (C). B and T lymphocytes have a similar distribution, being scattered in the lamina propria; some T-cells in the epithelium are noted (*arrow*). Macrophages are present in both the epithelium and the lamina propria (*arrows*). Counterstained with hematoxylin. $\times 400$

al., 1987; FRANKLIN, 1989; KIVELA, 1992; SULLIVAN, 1994).

The excretory ducts of the human lacrimal apparatus have long been considered to be merely passive conduits. However, recent morphological and histochemical studies have indicated that different tracts of the lacrimal apparatus not only play an active role

in the tear outflow mechanism (THALE et al., 1998) but they also can function in the local defence against ascending infections (PERRA et al., 1995; PAULSEN et al., 1998). Among the specific defences, the local immune system acts as main barrier against pathogens; moreover, it appears to be able to potentiate the effect of non-specific antibacterial factors adding

Fig. 4. Light micrographs of the human lacrimal sac reacting to anti-human CD20 (A), anti-human CD3 (B), and anti-human CD68 (C). The lamina propria shows a rich infiltration of lymphocytes, either scattered in the lamina propria or aggregated into typical lymphoid follicles. CD68-positive cells are distributed throughout the lamina propria and the epithelium (arrows). Counterstained with hematoxylin. A: $\times 200$, B: $\times 100$, C: $\times 400$

a specific element (McCLELLAN, 1997).

A previous study in the human lacrimal ducts demonstrated the presence of a variety of cell types including macrophages, T cells, and B cells in the submucosa partly penetrating the epithelium (PAULSEN et al., 1998). Nevertheless, the presence of immunoreactive cells in the inspected organs may not

be enough to demonstrate the existence of a mucosal secretory immune system. The secretory IgA, detectable in the external secretions, is a product of two cell types: B lymphocytes that produce polymeric IgA, and epithelial cells that produce secretory component (SC) (MAZANEC et al., 1993). Secretory IgA is particularly stable among the immunoglobulins pres-

Fig. 5. Light micrographs of the human nasal mucosa reacting to anti-human HLA-DR (A), and anti-cow protein S100 (B). HLA-DR positive cells are recognizable in the epithelium and in the stroma. S100 protein is localized in the acinar and ductal cells of the glands in which it has also nuclear localization (*arrows*). Counterstained with hematoxylin. $\times 400$

Fig. 6. Light micrographs of the human lacrimal sac reacting to anti-human HLA-DR (A), and anti-cow protein S100 (B). Numerous HLA-DR positive cells are detected in the epithelium and in the lamina propria, either in the lymphoid follicles or in the parafollicular areas. S100-immunoreactive cells are present in the epithelium and in the lamina propria (*arrows*). Counterstained with hematoxylin. $\times 400$

ent in mucosal surfaces as a result of active secretion or of transudation. This stability is largely ascribed to the secretory component that has been recognized to be part of the poly Ig receptor (pIgR) that transports IgA from mucosal tissue to its secretion.

IgM in the external secretions is also associated with SC, which results from its transport to secretions by pIgR. However, IgM as well as pIgA may not be transported because of the molecular weight restriction, which is SC-dependent transport. Consequently, the concentration of secretory IgM is substantially lower than that of sIgA. On the other hand, a compensatory increase of IgM has been observed in mucosal tissues and secretions of IgA-deficient individuals (BRANDTZAEG et al., 1986). Our observations indicate that the secretory component is a specialized transport protein necessary for the transcytosis process of IgA and IgM antibodies. One reason for the undemonstrated secretory component in the recent study by PAULSEN et al. (1998) could be that the reactivity for this important component, synthesized by epithelial cells, was altered in the autoptic samples used.

The presence of IgG at the epithelial level and that of clusters of IgG-positive immunocompetent cells in the subepithelial layers of both organs inspected in this study are not easy to explain, considering that most of the IgG present in mucosal secretions is derived from two sources: local antibody production and transudation from the circulation. The present state of our knowledge and the amount of immunoglobulins of the IgG class present in human tears may suggest that the organs we studied could contribute to their production presumably not only by a transudation process, but also by a selective synthesis. In such a case, IgG is thought to enter the mucosal secretions non-specifically via paracellular transport or fluid phase endocytosis (UNDERDOWN and MES-TECKY, 1994).

The present observations indicate that the immunoreactivities for IgM and IgG do not appear uniformly distributed in the whole epithelium. Although we cannot prove it, this characteristic distribution pattern seems likely due to the different functional moments of the organs from which such immunoglobulins derive.

Our findings suggest that the upper blind and the lower part of the nasolacrimal duct are protected from pathogen insults by an antibody-mediated local immune response, because all the effector components of an active local immune system are present. In fact, the first and the second lines of defence in both mucosae are represented. The first line, mediated by secretory IgA and lower amounts of secretory

IgM and of IgG together with various non specific protective factors, binds pathogens and their toxic products in the extracellular spaces of the body. The second line, mediated by T cells and, most importantly, APC cells, recognizes antigens that may derive from pathogens, such as viruses or intracellular bacteria, that replicate within cells, or from pathogens or their products that cells internalize by endocytosis from the extracellular fluid.

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