A Histochemical and Immunohistochemical Study of Certain Defense Mechanisms in the Human Lacrimal Sac Epithelium*

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Summary. The mucosal surface of the human lacrimal sac represents an area exposed to exogenous agents including potentially harmful microorganisms. The human lacrimal sac was examined histochemically to identify glycoproteins, and immunohistochemically to identify secretory IgA. Neutral and acid glycoconjugates were detected mainly in the cytoplasm of the surface cells of the columnar stratified epithelial lining. The same reactions were recognized in occasional clusters of secretory cells forming intraepithelial glands in the lining of the lacrimal sac. The presence of secretory IgA in the cytoplasm of the apical epithelial cells was demonstrated. The results indicate that the lacrimal sac mucosa possesses certain active defense mechanisms against ascending infections.

The human lacrimal apparatus consists of two parts: the lacrimal glands, which secrete a complex fluid (tears); and the excretory ducts, which convey the fluid to the surface of the eyes and into the nasal cavity (GRAY, 1973). The lacrimal sac is the upper blind end of the nasolacrimal duct; its only ascertained function is a pumping action that causes tears to flow (FERNÁNDEZ-VALENCE and GÓMEZ PELLICO, 1990).

The lacrimal sac, like all other excretory ducts of the lacrimal apparatus, has been considered a passive structure which neither acts as a barrier against pathogenic bacteria nor modifies the luminal content. However, morphological observations and ultrastructural features of the lacrimal sac epithelium suggest that it could play some role in local defense mechanisms (ADENIS et al., 1980; RIVAS et al., 1991). The mucosal tissues typically possess an almost impervious array of specific and nonspecific defenses erected against initial encounters between a bacterial pathogen and the mucosal surfaces. Among the nonspecific defenses are the cleansing mechanisms of the lacrimal flow, the barrier functions of the luminal mucus, and the desquamation of mucosal cells. Of the specific immune defenses, secretory IgA (sIgA) in mucosal secretions is predominant (MCNAAB and TOMASI, 1981; ABRAHAM and BEACHEY, 1985; TOMASI and PLAUT, 1985).

The purpose of this study has been to evaluate the histochemical and immunohistochemical characteristics of the human lacrimal sac mucosa in order to ascertain some of its defense mechanisms. As a nonspecific defense mechanism we have studied the histochemical properties of luminal mucus, and as an immunospecific defense mechanism, the occurrence of sIgA in the luminal secretions was investigated.

MATERIALS AND METHODS

Human biopsy specimens, apparently normal at histological examination, were obtained by surgery from four male and six female patients ranging in age from 40 to 60 years at surgery for dacryocystitis. Two lacrimal sacs were obtained from patients undergoing repair for midfacial trauma (ages 24, 38) and one lacrimal sac from a patient undergoing removal for epiteloma of the skin adjacent to the excretory duct of the lacrimal apparatus. All of the patients were immunologically tested with normal results.

Some tissue segments were fixed and processed for paraffin embedding. Microtome sections (6–7 μm) were

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treated for the localization and identification of glycoproteins according to methods described by Pearse (1985).

Other samples were rapidly frozen and cryostat sections (10 μm) were cut after a few hours. The sections were collected on gelatin coated glass slides, fixed 10 min in 100% acetone, and air dried. The sections were then processed for immunohistochemical demonstration of sIgA using the ABC method: they were rehydrated in PBS, immersed for 30 min in a solution of 0.1% phenylidrazine in PBS to inactivate endogenous peroxidase, then treated for 15 min with 10% non-immune rabbit serum. Rabbit polyclonal antibody to human sIgA (Cappel, Durham, NC) was used as the primary antiserum (working dilution 1:1000) for 1 h at room temperature, and biotinylated anti rabbit IgG was used as secondary antiserum (Chemicon International, Temecula, CA; 1:200) for 30 min at room temperature. The sections were further incubated in avidin-biotin-peroxidase complex (Biomed, Milan, Italy; 1:250) for 30 min at room temperature, reacted with 3,3’-diaminobenzidine (Sigma Chemical Company, St Louis, MO), then counterstained with hematoxylin. The sections were thoroughly rinsed in PBS between each step.

Furthermore, adjacent sections were incubated using rabbit anti-human IgA specific for alpha-chains, F(ab’)_2 fragment (Dako, Glostrup, Denmark; 1:1000),
as primary antiserum for the concurrent demonstration of IgA class immunoglobulins. In the control incubations, the specificity of the sIgA and IgA specific for alpha-chains antisera was tested in adjacent sections, replacing the primary antibodies with non-immune rabbit serum at the same dilution.

RESULTS

The lining of the lacrimal sac mucosa consists of a columnar stratified epithelium. No differences were observed between the specimens examined, which appeared normal at histological examination. Scattered within the epithelium are numerous groups of intraepithelial glands (Fig. 1). The lamina propria is rich in lymphocytes sometimes aggregated into follicles, and contains many elastic fibres. It is also supplied by a rich venous plexus which transforms it into erectile tissue continuous with that underlying the nasal mucosa, confirming what was described by Duke-Elder (1961).

The cells nearest the lumen appeared strongly PAS-positive (periodic acid-Schiff reaction) (Fig. 2), weakly alcianophilic at pH 1 and strongly alcianophilic at pH 2.5 (Fig. 3), whereas the other epithelial cells were unreactive. PAS reactivity was not aboli-

Fig. 3. Alcian blue (pH 2.5). A marked reactivity is observed in the cytoplasm of the cells nearest the lumen and in the intraepithelial glands. ×250

Fig. 4. Toluidine blue. The lining epithelium and the interspersed glands appear orthochromatic. ×400
shed after treatment with diastase. After treatment with toluidine blue, the epithelium appeared orthochromatic (Fig. 4). After high iron-diamine or low iron-diamine methods, the cytoplasm of the epithelial cells was unreactive, but if alcian blue staining was added to these procedures, only the superficial layer of cells was reactive. The same pattern of reactivity was observed in the intraepithelial glands (Figs. 2-5).

An evident immunoreactivity for sIgA was observed both in the apical cells of the lacrimal sac epithelium and in the immunocompetent cells of the lamina propria, in the form of small granular deposits (Fig. 6). Immunostaining in the control sections was completely abolished (Fig. 7).

We did not observe any differences in the distribution pattern of the reactions.

**DISCUSSION**

The results obtained after the histochemical treatment to identify glycoproteins indicate that the material detected consists of sialoproteins and acid mucopolisaccharides containing carboxyl groups, but not sulphated mucosubstances. This glycoproteic material seems concentrated in the apical cytoplasm.
of the epithelial cells nearest the lumen and in the secretory cells interspersed within the lacrimal sac epithelial lining.

Among the nonspecific defenses in the lacrimal sac mucosa are the cleansing brought about by the tear flow, the antibacterial effect of the lysozyme highly concentrated in human tears, produced by the acinar cells of the lacrimal glands (KLOCKARS and REITAMO, 1975; SAND et al., 1986), and the barrier to invasion resident in the luminal mucus detected in this study. The mucus forms a protective, waterproof coating over the mucosal surface. In addition to its lubricating and transporting functions, the mucus coats the glycoprotein and glycolipid receptors for several bacterial adhesins and toxins, and thereby prevents adhesion of the respective bacteria (ABRAHAM and BEACHEY, 1985). However, several pathogens possess enzymes that degrade the mucus; such mucous coat degeneration enables bacteria to reach the mucosal epithelium, but the products of mucus degradation, at the same time, provide nutrients that further facilitate bacterial colonization (GIBBONS, 1982; KILIAN et al., 1988). Thus, although the mucus can provide a natural barrier, it alone is not sufficient to prevent bacterial adhesion.

The sIgA detected in this study adds a specific element to the mucus protective activity by being incorporated in the mucus layer on the mucosal surface. The presence of sIgA in the secretions is, in fact, the predominant specific immune defense of the mucosa (TOMASI and PlAUT, 1985). Moreover, IgA in various forms appears to be able to potentiate the effect of some of the nonspecific antibacterial factors in esocrine secretions, such as lactoferrin, lactoperoxidase, and lysozyme (KILIAN et al., 1988).

Mucosal sIgA is generally viewed as an immune barrier to prevent the adherence and absorption of antigens, such as viruses, and bacterial adhesins and toxins. IgA antibodies in the mucosa have three locations: in the lumen, inside the epithelial cells, and in the lamina propria. In this way they might have the opportunity to inhibit the surface spreading of infection along the mucosa, and, in the instance of a virus, to limit the infection to the mucosa via intra-epithelial neutralization and interception in the lamina propria (MAZANEC et al., 1993). The immunoreactivity detected in the lamina propria of the lacrimal sac suggests that IgA could be of local origin.

Although the lacrimal sac possesses both specific and nonspecific defense mechanisms, it is frequently the site of infections spreading from neighboring structures, like the nose and sinuses. Infections lead to onset of inflammation, particularly in those cases where anatomical factors predispose the luminal content to stasis.

It is known that inflammatory changes usually start and are more marked in the lower reaches of the lacrimal passages, and it is probable that in a large number of cases their incidence is determined by the direct spread of infection from the nose. On the other hand, the healthy lacrimal passages, when functioning normally, are more than resistant to ascending infections (DUKE-EELDER, 1974; KILIAN et al., 1988).
Although there are many factors which tend to initiate and influence the inflammatory process, the breakdown of the defensive mechanisms could play a primary role in the aetiology of some pathological diseases such as dacryocystitis.

REFERENCES


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