Immunohistochemical Localization of Secretory Immunoglobulins in the Main Excretory Duct of the Human Submandibular Gland*

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Summary. The localization of IgA, IgG, and IgM was investigated immunohistochemically in the mucosal surface of the main excretory duct of the human submandibular gland in order to verify the possible antimicrobial properties of this duct. Only secretory IgA-immunoreactivity was recognized in the epithelial cells of the duct. An intense immunoreactivity was observed in the cytoplasm of some cells and at the luminal surface of most of the cells. Clusters of IgA-positive immunocompetent cells were also recognizable in the subepithelial layers. No reactivity for IgG and IgM was noticed. The results suggest that the ductal epithelium may actively be involved in the release of secretory IgA, which could play a prominent role in the local defense mechanism of the duct.

The oral mucosa is protected by non-specific (MANDEL and ELLISON, 1985; MANDEL, 1987; NIEUW AMERONGEN et al., 1995) and specific defense mechanisms which normally prevent the penetration of harmful microorganisms and macromolecules (BRANDTZAEG, 1975; IACONO, 1980; LEHNER, 1981; MCGHEE and MICHALEK, 1981; GIBBONS, 1984; MESTECKY, 1993). Antimicrobial proteins, such as lactoferrin, lysozyme, and lactoperoxidase are involved in the protection of the oral cavity, either through their individual antimicrobial activities or in concert with immunoglobulins (KRAUS and MESTECKY 1971; ARNOLD et al., 1977, 1980; MANDEL, 1979; SKURK et al., 1979; LEHNER, 1982; MORO et al., 1984; RUDNEY, 1989; CHALLACOMBE and SHIRLAV, 1995). The salivary glands are known as the principal source of antimicrobial substances (TOMASI et al., 1965; TOURVILLE et al., 1969; BRANDTZAEG, 1974, 1976, 1977; KORSRUD and BRANDTZAEG, 1982) and have long been recognized to play a part in the common mucosal immune system (BIENENSTOCK and BEFUS, 1980).

Immunoglobulins (Ig) were identified in human saliva more than 30 years ago by ELLISON et al. (1960), and the prevalence of IgA was demonstrated by TOMASI and ZIEGELBAUM in 1963. IgG and IgM are also present in saliva, though in markedly lower amounts (CHALLACOMBE et al., 1978, 1995). It has been well established that salivary IgA is represented by the typical secretory IgA molecule (CRAWFORD et al., 1975; TOMASI and PLAUT, 1985), that salivary glands are infiltrated by IgA-producing plasma cells, and that ductal and acinar cells express receptors specific for polymeric IgA molecules (CRAWFORD et al., 1975; BRANDTZAEG, 1981, 1989; MESTECKY and RUSSELL, 1986; MESTECKY, 1987, 1993; MORRIER and BARSOTTI, 1990). In addition to locally-produced and plasma-derived immunoglobulins, the purely local component of this immunity has been attributed to the duct-associated lymphoid tissue (DALT) (NAIR and SCHROEDER, 1986, 1987). A typical DALT unit consists of a local collection of organized lymphoid tissue in juxtaposition to a secretory-duct (KLEIN et al., 1979). MATSUDA et al. (1997) demonstrated that the epithelial cells of the DALT in the monkey parotid gland take up antigens from the duct lumen and transport them to adjacent immune cells, and that the DALT may serve as part of the inductive sites in the common mucosal immune system.

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The main excretory duct (MED) of the submandibular gland had long been considered to be merely a passive conduit. However, recent histochemical and ultrastructural studies have indicated that the MED not only modifies the composition of saliva (Testa-Riva et al., 1981; Perra et al., 1988), but also plays some important roles in local defense mechanisms. It has been suggested that probably most of the secretary IgA in saliva passes through the duct epithelium, with IgA itself being synthesized by interstitial immunocytes and secretory components by the duct cells.

The findings of IgA in human saliva led us to this study by immunohistochemical methods of the localization of secretary IgA in the lining epithelium of the human submandibular MED, in order to verify the possible antimicrobial properties of this epithelium. In addition to IgA, IgG and IgM immunoglobulins were also immunohistochemically investigated.

**MATERIALS AND METHODS**

Human biopsy specimens, apparently normal at histological examination, were obtained from 10 patients ranging in age from 30 to 65 years at surgery for the removal of tumors from the neck. All of the patients were immunologically tested with normal results.

Tissue pieces were fixed and processed for paraffin embedding. Microtome sections, 6–7 μm thick, 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 (Difco Laboratories, Detroit, MI, USA) in PBS at 37°C for 20 min to enhance the intensity of specific staining, and then immersed for 30 min in a solution of 30% hydrogen peroxide in methyl alcohol to inactivate endogenous peroxidase. The sections were treated for 15 min with 10% non-immune horse serum. Rabbit polyclonal antibody to human sIgA (Cappel, Durham, NC, USA; 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 antimouse IgG were used as secondary antisera (Chemicon International, Temecula, CA, USA; 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, treated with 3,3’-diaminobenzidine (Sigma, St. Louis, MO, USA), and 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; 1:4000), as the primary antisera for the concurrent demonstration of IgA class immunoglobulins.

In the control incubations, the specificity of sIgA, IgM and IgG antisera was tested in adjacent sections, replacing the primary antibodies with normal serum at the same dilution.

**RESULTS**

The epithelium lining the MED of human submandibular gland is pseudostratified, consisting of tall principal cells and small basal cells with few goblet cells. No differences in this fundamental structure were observed between the specimens examined, which appeared normal at histological examination.

Immunohistochemical staining for sIgA appeared as small granular cytoplasmic deposits. Only sIgA-immunoreactivity at the duct epithelial layers level was present (Fig. 1A–C). An intense immunoreactivity for sIgA and IgA specific for alpha-chains was observed in the entire cytoplasm of a small part of the epithelial cells. Their luminal surface was intensely immunostained in the major population of the cells. The immunoreaction showed in some areas to be discontinuous, being interrupted by less dense or completely unstained portions along the luminal surface (Fig. 1B,C).

Clusters of ovoid and irregular-shaped cells immunoreactive for sIgA were occasionally found in the subepithelial layer (Fig. 1D). We did not note any differences in the distribution pattern of the immunoreactivity when using both antibodies against sIgA and IgA specific for alpha-chain.

No reactivity for IgG and IgM in the epithelial layers was recognizable, and no IgM and IgG scattered immunoreactive cells were noticed in the subepithelial layer (Fig. 2A,B).

Immunostaining in the control sections treated with normal serum was completely abolished (Fig. 2C).

**DISCUSSION**

Our previous histochemical study of the MED of the submandibular gland revealed the presence of glyco-proteic material concentrated in the apical zone of the epithelium (Perra et al., 1988). The glycoproteins, which represent the glicocalix covering the ductal
Fig. 1. Light micrographs of the human submandibular MED reacting to anti-human secretory IgA (A, C, D), and anti-human IgA specific for alpha-chains (B). An intense immunoreactivity is observed in the apical cytoplasm of the epithelial superficial cells (A). A marked immunoreactivity is localized in the entire cytoplasm of some cells and/or at the luminal surface of the apical cells (B, C). In D clusters of immunocompetent cells can be observed in the subepithelial layers (arrow). Sections A and D are counterstained with hematoxylin. Scale bars: 50 μm.
epithelium (TESTA-RIVA et al., 1981), were suggested to provide a protective barrier against bacteria in addition to their lubricating and transporting function, since receptors for several kinds of bacterial adhesins and toxins have been demonstrated in the luminal coat (ABRAHAM and BEACKEY, 1985). The ductal epithelium also exhibited activity for peroxidase (PERRA et al., 1988) which inhibits the growth and metabolism of many species of microorganisms (ABRAHAM and BEACKEY, 1985; PRUITT et al., 1994; UNDERDOWN and MESTECKY, 1994). The above knowledge indicates that the human MED contains remarkable barriers acting in different ways against the attachment of invading bacterial pathogens.

The sIgA detected in this study adds another specific and powerful element to the mucus protective activity in this duct organ. The presence of sIgA in the secretions of different organs is of predominant significance in the specific immune defense of the mucosa (TOMASI and PLAUT, 1985). Moreover, IgA in various forms has been suggested to potentiate the effect of nonspecific antibacterial factors in exocrine secretions, including lactoferrin, lactoperoxidase, and lysozyme (KILIAN et al., 1988).

The present observations indicate that the immunoreactivity does not appear uniformly distributed in the whole epithelium. Such a characteristic distribution pattern is likely due to a particular functional stage of the IgA secretion. However, the

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**Fig. 2.** Light micrographs of the human submandibular MED reacted with anti-human IgG (A), anti-human IgM (B), and normal horse serum (C). No reactivity for IgG and IgM in the epithelial layers is observed, and no IgM and IgG scattered immunoreactive cells are noticed in the subepithelial layers (A,B). In the control section, the immunoreactivity for sIgA has been completely abolished (C). Counterstained with hematoxylin. Scale bars: 50 μm.
absence or the reduction of immunostaining in some cells of the epithelium could indicate that they have limited secretory activity and probably play only a minor role in the composition of saliva.

Clusters of IgA positive cells in the submucosal layers are very likely to be source of IgA that eventually are transported into epithelial cells. Submucosal plasma cells have been known to synthesize predominantly polymeric, J chain containing IgA that, after being secreted, selectively binds to secretory component (SC) localized on basolateral plasma membrane, and is internalized into epithelial cells, followed by its release from the apical membrane after the proteolytic cleavage of SC (BECKENKAMP, 1985; MESTECKY, 1993).

In this immunohistochemical study, neither IgG nor IgM was detected in the human salivary main duct system. CHALLACOMBE et al. (1995) have indicated the presence of both IgG and IgM in human saliva, though the levels were markedly low. It is very likely that the concentrations of IgG and IgM in ductal tissue were too low to be detected by means of the immunohistochemistry applied in the present study. Detection of IgM and IgG in saliva may indicate a compensatory protection of the mucosa against infections. In fact, in patients with selective IgA deficiency, IgM occurred in a pattern similar to that normally seen for IgA (KORSRUD and BRANDTZAEG, 1980; SMITH, 1992).

In conclusion, our detection of sIgA in the entire cytoplasm and at the luminal plasma membrane of the epithelial cells in human salivary MED may be significant in the immune defences of the oral cavity. The evidence supports the present knowledge that the organ plays an important role in not only passive but also active protective function.

REFERENCES


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