Identification of Secretory Immunoglobulin A in Human Sweat and Sweat Glands

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Secretory immunoglobulin A (sIgA) plays an important role in local immune defense mechanisms. Although skin is always exposed to external antigens, the role of local immune defenses involving sIgA in the skin has not been adequately studied. In order to evaluate the presence of sIgA in sweat, we have measured the concentration of sIgA in human sweat by enzyme immunoassay and have localized the components of sIgA in the sweat glands of human axillary skin. The concentration of sIgA in sweat was found to be 10 times higher in men than in women (13.0 ± 0.9 μg/ml versus 1.6 ± 0.9 μg/ml). Secretory component (SC) was localized immunohistochemically in protein synthetic organelles, such as the perinuclear spaces and Golgi complex, in cytoplasmic vesicles, and along the external surface membranes of mucous cells on the terminal segment of eccrine sweat glands. IgA and J chain were present in plasma cells in the protein synthetic organelles. The luminal aspects of eccrine sweat ducts also strongly express SC, as well as IgA and J chain. Neither SC, IgA, or J chain were identified in epithelial cells of apocrine sweat glands. These findings are consistent with the theory that J chain complexed with dimeric IgA is synthesized in plasma cells and is transported by SC-mediated endocytosis transfer across mucous cells of eccrine sweat glands and thus into sweat. *J Invest Dermatol 90:648–651, 1988*

Secretory immunoglobulin A (sIgA) is a major immunoglobulin in various human mucosal secretions and plays an important role in local immune defense mechanisms against microbial antigens on mucosal surfaces [1,2]. SIgA consists of 2 molecules of IgA linked by a peptide, J chain, and a glycoprotein, secretory component (SC) [3,4]. These constituents of sIgA are derived from two different types of cells: plasma cells and epithelial cells. In the intestine, IgA and J chain molecules are synthesized in plasma cells in the lamina propria, whereas SC is synthesized in epithelial cells and expressed on the basolateral surfaces of the cells [5].

SIgA inhibits bacterial adherence and has a role in controlling the uptake of antigen-like macromolecules from the small intestine [6–8]. SIgA complexed with lactoferrin inhibits the growth of *Escherichia coli in vitro* [9]. SIgA antibodies to *Streptococcus mutans* have also been studied as a means of possible control of dental caries [10,11].

Although the skin is always exposed to external antigens, a possible role for the local immune system involving sIgA in the skin has not been studied sufficiently. Sweat has been reported to contain only small amounts of IgA [12,13], present as a result of transudation rather than secretion [13]. However, Ishiguro et al [14] reported that immunoglobulins were secreted into sweat and that sIgA, but not free IgA, was present. There has not been careful confirmation of sIgA in sweat or any careful immunochemical documentation of the components of sIgA associated with sweat glands or ducts.

The purpose of the present study was to identify the presence of sIgA in human sweat and sweat glands. We have measured the concentration of sIgA in sweat by enzyme immunoassay and have immunohistochemically demonstrated the location of SC, IgA, and J chain in the sweat glands and ducts of human axillary skin.

MATERIALS AND METHODS

Sweat Samples and Tissue Sweat samples were collected from 10 men and 8 women (25 to 34 years old) after jogging. Tissue samples from axillary skin were obtained from adults (3 women) at opportune surgical operations. Skin specimens were fixed with periodate-lysine-paraformaldehyde [15] and embedded in OCT compounds. The sections were sliced at 8 μm thick in a cryostat and dried in room air.

Enzyme Immunoassay of sIgA Assay of sIgA was performed by the enzyme immunoassay system of secretory immunoglobulin A for saliva (Amano Pharmaceutical Co. Aichi, Japan). This is a solid phase sandwich enzyme immunoassay system that employs immobilized anti-IgA antibody to bind the sIgA and anti-SC Fab′ labeled with β-D-galactosidase to identify the bound sIgA [16]. The concentration of sIgA can be determined with a minimum detectable sensitivity of 3 ng/assay tube without interference from free IgA and SC in the same samples.

Antibodies As the primary antibodies, rabbit anti-human IgA and anti-human SC were purchased from Dakopatts (Copenhagen F, Denmark) and rabbit anti-human J chain antibodies were produced by the method of Kobayashi et al [17] in Tokai University. As the second antibody, horseradish peroxidase (HRP)-labeled goat IgG Fab fragments against rabbit IgG were purchased from Medical and Biological Laboratories Co., Nagoya, Japan.

Immunohistochemical Identification of IgA, SC, and J Chain for Light Microscopy IgA, SC, and J chain were local-

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Abbreviations:
DAB: 3,3′-diaminobenzidine.4HCl
HRP: horseradish peroxidase
SC: secretory component
sIgA: secretory immunoglobulin A

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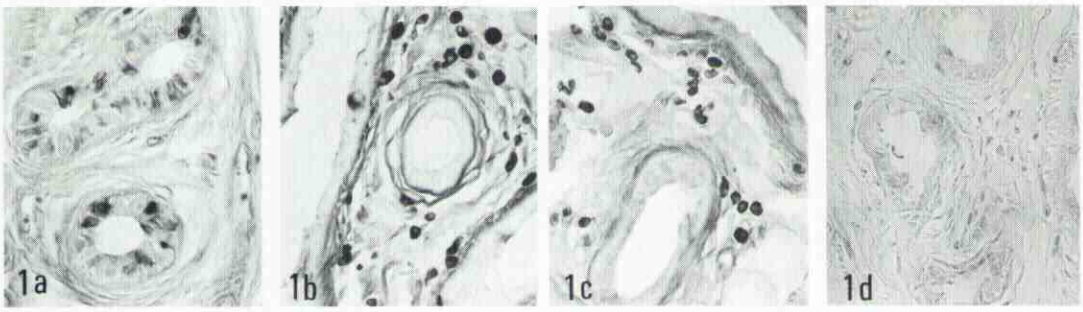

Figure 1. Light micrographs of eccrine sweat glands reacted with *(a)* anti-human SC; *(b)* anti-human IgA; *(c)* anti-human J chain; and *(d)* normal rabbit serum. *a,* Reaction products of HRP indicating SC sites in some of secretory cells. *b,* IgA is present in numerous plasma cells. *c,* J chain is present in numerous plasma cells (×150).

ized in the axillary skin tissues by the indirect immunoperoxidase technique [18]. Endogenous peroxidase in the tissue sections was inactivated by incubation with 0.3% hydrogen peroxide in methanol, and sections were incubated with primary antibodies overnight at 4°C. As a control, the sections were reacted with 10% non-immune rabbit serum. After incubation with the primary antibodies, sections were washed and then incubated with the HRP-labeled Fab fragments for 6 hours. Binding was visualized after incubation with 0.25% diaminobenzidine (DAB) solution containing 10 mM hydrogen peroxide and 10 mM sodium azide.

Immunoelectron Microscopy For immunoelectron microscopic identification of the components of sIgA, sections were incubated with the first and second antibodies, as described above, and were then postfixed in 0.5% glutaraldehyde in phosphate-buffered saline. Sections were then incubated sequentially in 0.25% DAB solution for 30 min and in 0.25% DAB solution containing 10 mM hydrogen peroxide and 10 mM sodium azide. The sections were then washed, fixed in 2% osmium tetraoxide, dehydrated in graded ethanol, and embedded in Quetol 812. Ultrathin sections stained with lead citrate were viewed with a Hitachi H-600 electron microscope.

RESULTS

SIgA Content in Sweat SIgA content in sweat was shown in Table I. The mean value for sIgA in men was 13.0 μg/ml, which was about 10 times greater than that in women, i.e., 1.6 μg/ml. This difference was significant ($p < 0.005$) by Student's t test.

Table I. sIgA Content in Sweat

Sex	sIgA Content (μg/ml)	Range
Male	13.0 ± 9.0 (10)	2.6–32.0
Female	1.6 ± 0.9 (8)	0.7–3.5

Values are mean ± SD.
Numbers in parentheses denote number of samples.

Light Microscopy SC was localized in some of secretory cells of eccrine sweat glands and was not found in apocrine sweat glands (Figs 1*a* and 2*a*).

IgA positive cells, appearing like typical plasma cells, surrounded the terminal secretory segment of eccrine sweat glands. A few of them surrounded the terminal secretory segment of apocrine sweat glands. IgA immunoreactivity was also found within some of the secretory cells, along the basement membrane, and on the luminal surface of eccrine sweat glands (Figs 1*b* and 2*b*).

The distribution of J chain immunoreactivity corresponded to that of IgA (Figs 1*c* and 2*c*).

HRP-reaction products of SC, IgA, and J chain were also found in the intraepidermal and/or intradermal ducts of eccrine sweat glands (Fig 3*a–c*).

Control sections were uniformly negative (Figs 1*d*, 2*d*, and 3*d*).

Electron Microscopy Immunoelectron micrographs corroborated the light-microscopic findings. In epithelial cells of eccrine sweat glands, SC was localized in protein synthetic organelles, such as the perinuclear spaces and Golgi complex, in cytoplasmic vesicles, and along the external surface membranes of mucous cells on the terminal segment of eccrine sweat glands. Non-specific HRP-reaction products, representing endogeneous peroxidase, were present in lysosomes (Fig 4). The control was negative for the staining identified as representing SC.

IgA and J chain were present at the sites of protein synthesis (i.e., perinuclear spaces and rough endoplasmic reticulum) in typical plasma cells (Fig 5). IgA was also identified by immunoelectron microscopy on cell membranes on the luminal surface and in the cytoplasm of some secretory cells of eccrine glands (data not shown).

SC, IgA, and J chain were not found in the secretory cells of apocrine glands.

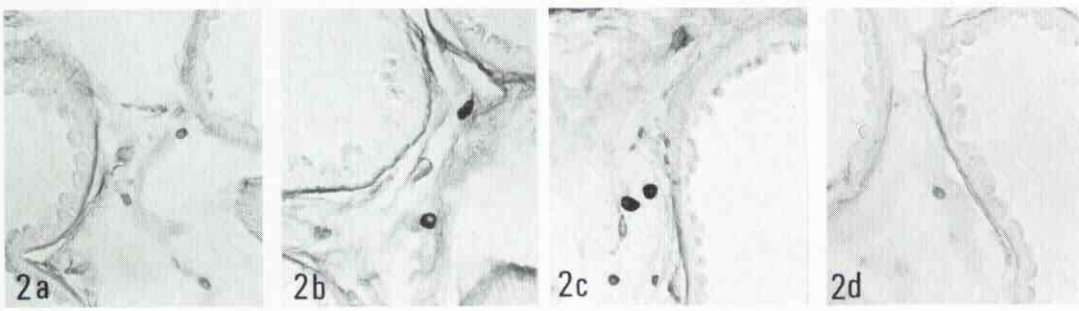

Figure 2. Light micrographs of apocrine sweat glands reacted with *(a)* anti-human SC; *(b)* anti-human IgA; *(c)* anti-human J chain; and *(d)* normal rabbit serum. *a,* Reaction products of HRP: SC sites are not present. *b,c,* IgA and J chain are present in only a few plasma cells (×150).

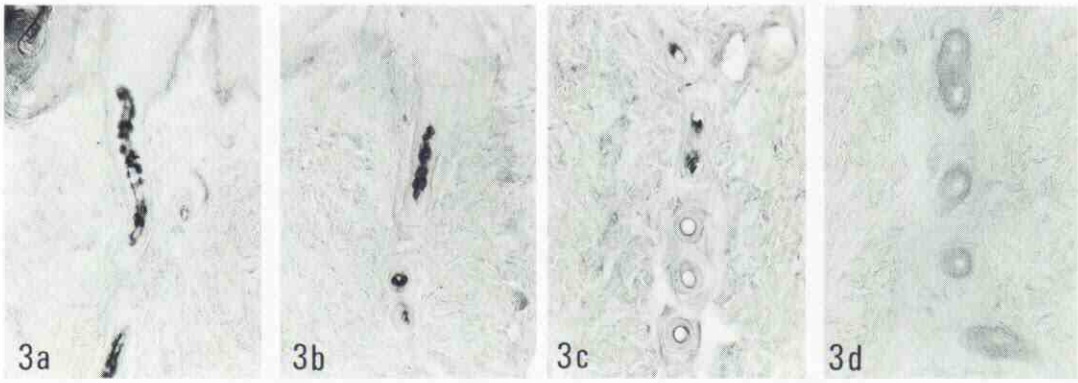

Figure 3. Light micrographs of ductal segment in eccrine sweat gland reacted with *(a)* anti-human SC; *(b)* anti-human IgA; *(c)* anti-human J chain; and *(d)* normal rabbit serum. *a–c,* HRP-reaction products of SC, IgA, and J chain are found into the intraepidermal and/or intradermal ducts of eccrine sweat glands (×150).

DISCUSSION

We have measured the concentration of sIgA in sweat by enzyme immunoassay and have immunohistochemically demonstrated the location of SC, IgA, and J chain in sweat glands of human axillary skin.

By enzyme immunoassay, the concentration of sIgA in male sweat was significantly higher than that in female sweat. Although the secretion of sIgA into human sweat has been previously reported [14], the difference in results between men and women was not noted. Higher concentrations in men than in women have been previously reported for both sIgA in saliva [19] and IgA in plasma [20]. However, there is no clear-cut explanation for these differences. Concentrations of sIgA in both male and female sweat were less than those measured in serum and saliva. It is unclear whether sIgA secreted into sweat plays any role in the local immune system in the skin at such low concentrations. However, skin is exposed to air, and the water contained in sweat evaporates. Therefore, the effective concentration of sIgA on the skin surface may be much higher than that in sweat.

SC was located in the protein synthetic organelles, such as perinuclear spaces and Golgi complex, in cytoplasmic vesicles, and along the external surface membrane of mucous cells on the terminal segment of eccrine sweat glands. IgA and J chain were present in the protein synthetic organelles; that is, perinuclear spaces and rough endoplasmic reticulum in the typical plasma cells. These plasma cells positive for IgA and J chain were identified surrounding the terminal secretory segments of eccrine sweat glands. A small number were also present near apocrine glands.

It has been reported that the low concentrations of IgA and/or sIgA present in sweat are the result of transudation rather than secretion [13]. The immunohistochemical findings in the present study, however, are consistent with the hypothesis that dimeric IgA synthesized in plasma cells is actively transported across mucous cells of eccrine sweat glands by SC-mediated endocytic transfer to produce sIgA in sweat. Such transportation of sIgA into external secretions is consistent with that previously reported in the intestine and in salivary glands [21–23].

In previous studies, SC and sIgA have been reported in human skin. Tourville et al [24] demonstrated the presence of SC in eccrine sweat glands, and Kaneko et al [25] detected components of sIgA at the lumen and inner cell membranes of eccrine sweat glands and in the upper epidermis. Harris and South [26], in contrast, reported that SC was absent from the epidermis. Our observation in this study was that the components of sIgA were also detected by weak HRP-reaction products in the epidermis. The explanation for this finding is not clear. One explanation might be the permeation of sIgA from sweat into the epidermis.

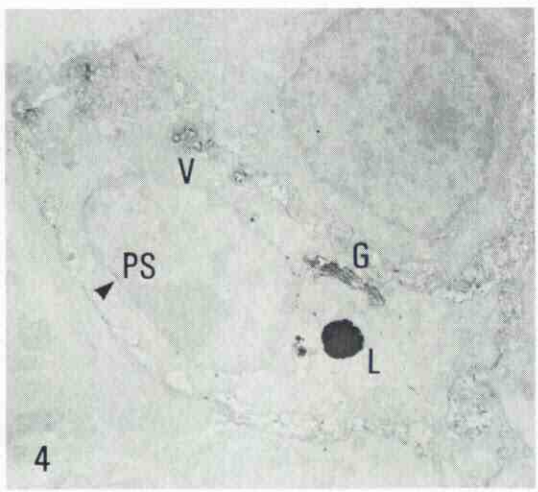

Figure 4. Electron micrograph of mucous cell of eccrine sweat gland reacted with anti-human SC. SC is found along external surface membrane and in perinuclear spaces (PS, *arrow head*), Golgi complex (G), and in cytoplasmic vesicles (V). Lysosome (L) is stained by endogenous peroxidase. The control was negative for the staining identified as representing SC (×5,700).

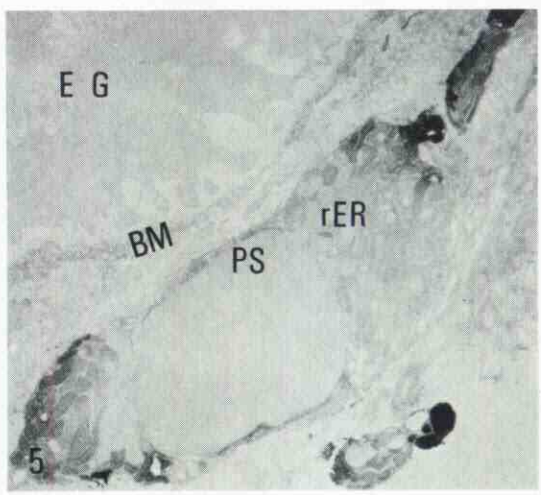

Figure 5. Electron micrograph of plasma cell reacted with anti-human J chain. IgA is present in protein synthesis sites (i.e., perinuclear spaces (PS) and rough endoplasmic reticulum [rER]) of plasma cell basement membrane (BM), eccrine sweat glands (EG) (×5,900).

The previous reports have not identified differences in sIgA secretion between eccrine and apocrine sweat glands. From our identification of SC and IgA in cells of eccrine sweat glands, but not in cells of apocrine sweat glands, we suggest that sIgA secretion into sweat is predominantly the function of the eccrine glands.

SIgA is present in various human mucosal secretions and is known to have bacteriostatic effects on some micro-organisms [1,27,28] and to be protective against external antigens [1,2,6–8]. Although the function of sIgA in the skin is not yet known, we speculate that the sIgA secreted into sweat has some role in the skin's defense mechanisms.

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