Epithelial Polymeric Immunoglobulin Receptors

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The secretory immune system, which leads to secretion of polymeric immunoglobulins along mucosal surfaces, has not been shown to have any definite role in cutaneous immunology, although the polymeric immunoglobulin receptor, secretory component (SC), has been found in sweat glands and possibly in the epidermis. The purpose of this study is to examine normal human skin and cultured human keratinocytes for the presence of SC.

Positive staining for SC was found in sections of normal human skin along the basement membrane zone with use of a polyclonal antibody to SC and focally on the surfaces of epidermal cells with use of a monoclonal antibody to SC. Granular cell-surface fluorescence of an intensity far less than that of the positive control HT 29 cells was seen when cultured human keratinocytes were stained for SC by indirect immunofluorescence (IF). Study of lysates of both HT 29 cells and HK by immunoblotting have been negative, perhaps due to destruction of the protein or loss of antigenicity during the extraction process.

If human keratinocytes are capable of expression of SC, and the receptor can interact with IgA and IgM, this might be a mechanism for protection of the skin from microbial agents or foreign antigens and might be relevant to the deposition of IgA seen in certain skin diseases. *J Invest Dermatol 94:74S–78S, 1990*

Investigations over the last 30 years have led to recognition of a unique segment of the immune system, one involved in protection of mucosal surfaces from microbial agents and foreign antigens [1–4]. The immunoglobulins involved are polymeric immunoglobulin, including dimeric IgA and pentameric IgM, which are thought to be produced largely by plasma cells beneath the mucosal surfaces. These immunoglobulins are specifically secreted along mucosal surfaces and by glands to produce concentrations in secretions out of proportion to their concentrations in the blood. This important segment of the immune system has been termed the "secretory immune system."

A special protein with a molecular weight of about 80 kD, which has been named secretory piece or secretory component (SC), is added by the epithelial cell as part of the secretory immunoglobulins [5]. The current concept of SC is that it is an epithelial receptor for polymeric immunoglobulins and that binding of polymeric immunoglobulins to this receptor leads to specific transport of the immunoglobulins across an epithelial surface and to secretion of the immunoglobulin along with a portion of the receptor molecule [6–9]. In addition to its role as a receptor and transport molecule, SC may serve the further purpose of preventing degradation of the secreted immunoglobulin [2].

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Abbreviations:
BMZ: basement membrane zone
FCS: fetal calf serum
FITC: fluorescein isothiocyanate
HK: human keratinocytes
HRP: horse radish peroxidase
IF: immunofluorescence
NCP: nitrocellulose paper
PAGE: polyacrylamide gel electrophoresis
PBS: phosphate-buffered saline
PPD: para-phenylenediamine
SC: secretory component
TBS: Tris-buffered saline

Most studies of human SC synthesis have been in the human adenocarcinoma cell line, HT-29. Human SC is synthesized as a peptide of about 95 kD, which contains multiple immunoglobulin-like domains [10]. After post-translational glycosylation, producing a glycoprotein of about 100 kD, this protein is expressed predominantly along the basolateral plasma membranes of the epithelial cells [7,8]. Polymeric immunoglobulin binds covalently to this receptor molecule, and this combination of SC and polymeric immunoglobulin is actively transported across the cell, where it is released at the apical surface. As part of the secretory process, the extracellular receptor portion of the SC molecule is clipped from the transmembrane and cytoplasmic portions, producing a SC moiety of about 80 kD bound to the immunoglobulin [7,8].

Epithelial cells at a number of sites have been shown to express SC, primarily the columnar cells lining the gastrointestinal tract and the respiratory tract, and exocrine glands, such as salivary glands, lacrimal glands, and mammary glands. The endometrial, biliary, and renal tubular epithelia also may express SC.

Synthesis and expression of SC by epithelial cells are often quite variable and appear to be modulated by local cytokines and hormonal agents. Gamma interferon [11], tumor necrosis factor [12], sex hormones [13,14], and pituitary hormones [13] have all been shown to modulate SC expression by epithelial cells.

Direct evidence of a relationship between the secretory immune system and the skin is scanty. In sweat, there appears to be an enrichment for IgA, and the epithelium of sweat glands and ducts has been described as expressing SC and IgA [2,15–17]. In a recent study, Kaneko et al reported positive immunofluorescence staining of the epidermis for SC and for IgA in 20% of skin biopsies [18].

If a specific, receptor-mediated interaction of polymeric immunoglobulins with the epidermis were to occur, such a mechanism might play a role in cutaneous immunity and might prove relevant to the cutaneous IgA deposits that are involved in the pathogenesis of certain blistering diseases. The mechanism of IgA deposition in such diseases as dermatitis herpetiformis (DH) and intraepidermal IgA dermatosis remains unknown [19,20].

The ultimate purposes of these studies are to examine whether a receptor-mediated interaction of IgA with human epidermis might

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occur, to learn what factors may modulate such interaction, and to study the relevance of such mechanisms to certain skin diseases, such as the IgA dermatoses. The initial studies that we have undertaken are directed toward the examination of human epidermis for expression of the polymeric immunoglobulin receptor protein, also known as SC.

MATERIALS AND METHODS

Immunofluorescence (IF) Study of Skin Biopsies Normal skin biopsies from 28 individuals which had been snap-frozen in liquid nitrogen and submitted to the Skin Immunofluorescence Laboratory at the University of Colorado and which had no deposition of immunoreactants on routine direct IF examination, were stained for SC by direct IF with use of rabbit anti-human SC labeled with FITC (Dakopatts, Denmark). The antibody, diluted 1:50 in PBS, was used to stain 4-μm cryostat sections of normal skin biopsies, as previously described [21], and a coverslip mounting medium containing para-phenylenediamine (PPD) (Sigma, St. Louis, MO) was used as a means of preventing fading of fluorescence [22]. Sections were examined under an Olympus epi-fluorescence microscope with a filter-reflector system for FITC and a HBO-100 mercury light source.

Unlabeled rabbit anti-human SC (Dakopatts) was used to stain sections of five normal skin biopsies. The antibody, diluted 1:50 in PBS with 5% normal goat serum and 0.1% sodium azide, was incubated with skin sections for 2 h at room temperature. After a wash in PBS, sections were incubated with FITC-labeled goat anti-rabbit $F(ab')_2$ fragments (Tago Immunologicals, Burlingame, CA), diluted 1:50. After a final wash, coverslips were applied over PPD containing mounting medium.

Sections from two normal skin biopsies from two individuals were stained by indirect IF technique with a murine monoclonal antibody to human SC, available from Australian Monoclonal Development (AMD, Artarmon, NSW). Sections were incubated at 4°C for 12 h with the monoclonal antibody diluted 1:20 in PBS containing 5% normal goat serum and 0.1% sodium azide. After washing, sections were incubated for 2 h at room temperature with FITC-labeled goat anti-mouse IgG $F(ab')_2$ fragments (Tago), diluted 1:50 in PBS. A monoclonal antibody to human IgA1 (Becton Dickinson, Sunnyvale, CA) was used in a similar manner as a control antibody.

Cell Culture HT-29 cells, a colon adenocarcinoma cell line obtained from the American Type Culture Corporation (ATCC, Rockville, MD), were grown in two-chamber Lab Tek slides (Nunc Inc., Naperville, IL) to 90% confluence and then used for indirect IF staining for SC. HT-29 cells were grown in RPMI-1640 (GIBCO, Gaithersburg, MD) with 10% fetal calf serum (FCS) (Irvine Scientific, Santa Ana, CA).

Second-passage human keratinocytes (HK), isolated from neonatal foreskins, were grown to 90% confluence on Lab Tek slides in serum-free keratinocyte growth medium (Clonetics, Mountain View, CA), as described by Boyce and Ham [23]. These cells were examined for SC by indirect IF.

Fibroblasts, isolated from neonatal foreskins, were grown in Lab Tek chambers as negative control cells for SC expression. Fibroblasts were grown in M199 (GIBCO) with 10% FCS.

Immunofluorescence (IF) Studies of Cultured Cells Cultured cells on Lab Tek slides were stained for SC by indirect IF technique. The rabbit antibody to SC (Dakopatts), diluted 1:20 in tissue-culture medium, was incubated over the monolayer at 37°C for 2 h. Cells were washed thoroughly in tissue-culture medium, fixed for 1 min in cold acetone, and air dried. Incubation in the second antibody, FITC-labeled goat anti-rabbit $F(ab')_2$ fragments (Atlantic Antibodies, Scarborough, ME), diluted 1:50 in PBS for 2 h at room temperature, was followed by three washes in PBS and application of coverslips in PPD mounting medium. As a control antibody for this technique, a rabbit antibody to herpes simplex virus, type 1 (Dakopatts) was used at the same dilution in the second chamber on the slide.

Indirect IF staining of cultured cells with the monoclonal antibody was accomplished by a similar technique with the monoclonal antibody diluted 1:20 and with the FITC-labeled goat anti-mouse IgG $F(ab')_2$ fragments diluted 1:50. The monoclonal antibody to IgA1 was used at the same dilution in the second chamber on each slide as a control antibody.

Isolation of Secretory Component Secretory component was isolated for antibody absorption experiments with human saliva as the source. Briefly, saliva collected from volunteers was maintained on ice and clarified by centrifugation for 30 min. Saliva samples were combined to make a pooled volume of 500 ml and passed through a 0.22-μm filter. Saliva was then concentrated 100 times over an XM 50 membrane (Amicon, Danvers, MA), and 2 ml was fractionated over a Sephacryl S200 HR column (Pharmacia, Upsala, Sweden). The fractions containing proteins with molecular weights approximately 100 kD were concentrated to 2 times the application volume and used for absorption. The material used for absorption was shown to contain SC by immunoblot analysis.

Polyacrylamide Gel Electrophoresis (PAGE) and Immunoblotting Flasks (T 75), containing either confluent HT-29 cells or HK, were lysed as described by Labib et al [24]. Cell lysates were diluted 3:1 with sample buffer (Tris buffer, pH 6.8, with 5% 2-mercaptoethanol, 1.5% sodium dedecyl sulfate, glycerol, and bromophenol blue), and 100 μl was applied to the wells of a 7.5% polyacrylamide gel, with a 5% stacking gel. Secretory IgA from colostrum (Cappel, Westchester, PA) and human saliva were used as positive controls for SC for these studies.

Polyacrylamide gel electrophoresis (PAGE) was performed according to the methods of Laemmli [25] with use of prestained molecular weight standards (Bio Rad, Richmond, CA). Electrophoretic transfer of proteins separated by PAGE to nitrocellulose paper (Life Sciences, Denver, CO) was achieved by the method of Towbin et al [26]. Nitrocellulose papers (NCP) were stained for SC by first blocking protein binding on the NCP by 1 h incubation with 3% milk powder (Carnation) in Tris-buffered saline (TBS), pH 7.5, then incubating the NCP overnight at 4°C with rabbit anti-human SC (Dakopatts) 1:5 in TBS with 3% milk powder, and finally incubating the NCP for 2 h at room temperature with protein A, labeled with horseradish peroxidase (HRP) (Bio Rad) diluted 1:500 in TBS with 3% milk powder. Color development of the sites of HRP binding was achieved with a 4-chloro-1-naphthol color reagent (Bio Rad), in the presence of hydrogen peroxide. Between each incubation, strips were washed 3 times, 5 min per wash, in TBS with 0.05% Tween 20. A final wash in TBS before the color development prevented inhibition of the reaction by Tween 20.

RESULTS

Direct IF staining of normal human skin sections for SC produced a faint linear band of fluorescence along the basement membrane zone (BMZ) in 18 of 28 specimens (Fig 1). Faint fluorescence was also present on the surface of epidermal cells. Indirect IF staining of skin sections with the rabbit anti-SC yielded a higher background, but with a similar pattern of staining in all biopsies examined (Fig 2). With indirect IF staining with the monoclonal antibody to SC, the pattern of staining was somewhat different, with focal granular staining on the surface of epidermal cells (Fig 3). Repeat of the IF staining on a specimen positive for SC with antibodies absorbed with purified SC produced negative or markedly diminished fluorescence.

Indirect IF staining of cultured HT-29 cells with the polyclonal rabbit antibody to SC was strongly positive, with a granular pattern on cell surfaces (Fig 4), while the control antibody produced negative staining. Cultured human fibroblasts were negative for SC expression. HK cultures, when stained with the polyclonal antibody to SC, were positive for granular fluorescence cell surfaces at an intensity far less than that of the HT-29 cells (Fig 5). The control antibody staining was negative.

Indirect IF staining of HT-29 cells with the monoclonal antibody

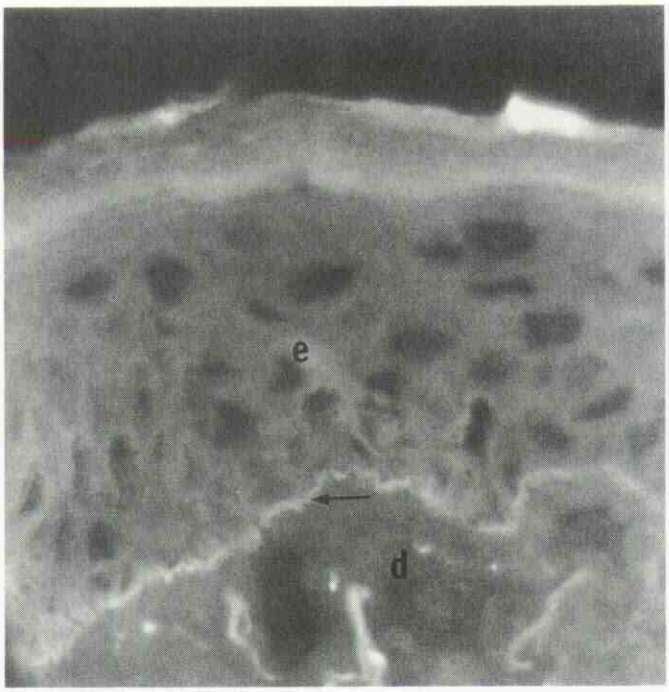

Figure 1. Direct immunofluorescent staining of a section of normal human skin for SC with use of FITC-labeled rabbit polyclonal antibody to SC. A faint band of staining along the BMZ *(arrow)* was observed. (e, epidermis; d, dermis) (magnification ×400).

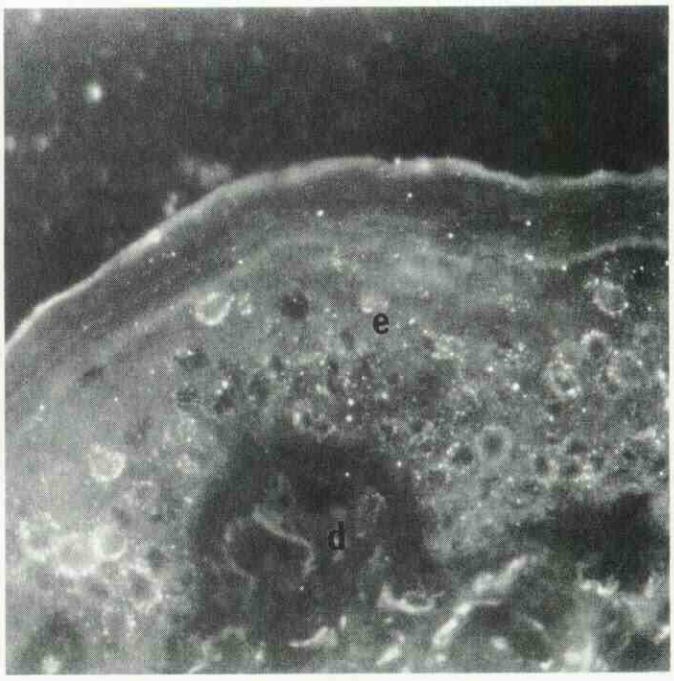

Figure 3. Indirect immunofluorescence staining of a section of normal human skin for SC with use of the monoclonal antibody to SC. Granular fluorescence on the surface of epidermal cells, primarily in the mid epidermis, was the predominant finding. (e, epidermis; d, dermis) (magnification ×400).

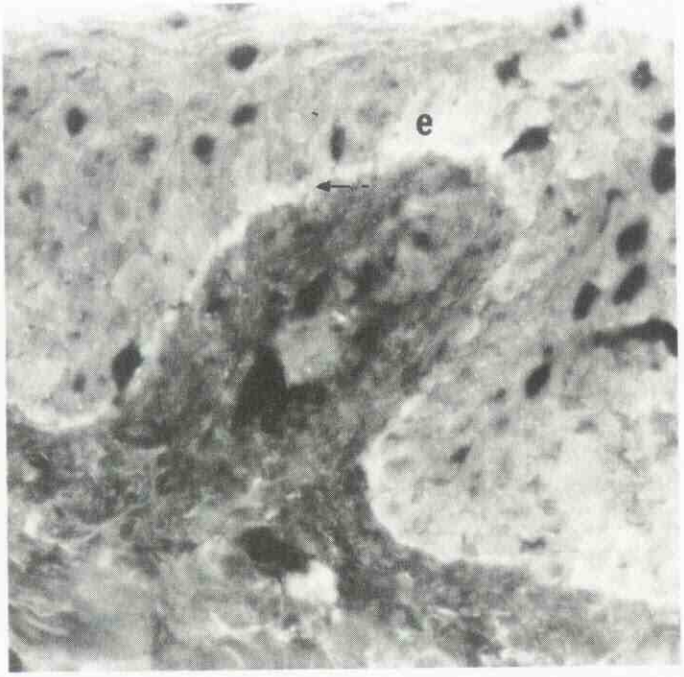

Figure 2. Indirect immunofluorescent staining of a section of normal human skin for SC with use of the rabbit antibody to SC. Staining along the BMZ *(arrow)* was again observed. (e, epidermis; d, dermis) (magnification ×400).

for SC was strongly positive, while that with the control antibody was negative. Human fibroblasts were negative by this indirect IF technique. HK cultures, when examined by indirect IF with the monoclonal antibody, were faintly positive, with a granular fluorescence on cell surfaces (Fig 6). Staining with the control monoclonal antibody on HK cultures was negative.

Indirect IF staining of HK with both the polyclonal antibody and the monoclonal antibody SC absorbed with SC produced marked diminution of fluorescence of cultured HK.

By immunoblotting, we have been able to identify strong SC staining at a molecular weight of approximately 80–90 kD from purified secretory IgA and from saliva. Saliva reproducibly yields two separate bands of SC staining, one of which may represent free SC. However, immunoblotting, thus far, has not identified SC from HT-29 cells or from HK (Fig 7).

DISCUSSION

The secretory immune system plays an important role in local immunity on mucosal surfaces but currently is not recognized to be of significance in the skin. However, recent reports have documented that IgA is present in sweat and that the receptor for polymeric immunoglobulins is present in sweat glands and ducts [15–17]. Immunofluorescent staining for SC has also been reported in the epidermis in a small percentage of samples [18]. One clue that there may be a specific interaction of polymeric immunoglobulins in the skin is the occurrence of certain skin diseases with unusual deposits of IgA in the skin. In diseases such as DH and intraepidermal IgA dermatosis, the IgA is deposited in the skin but not in other tissues. Perhaps derangements in some normal interaction of IgA in the skin leads to these deposits and thus to these diseases. The hypothesis for this study is that some specific interaction of immunoglobulins occurs in the skin. Because the polymeric immunoglobulin receptor, also known as secretory component, is responsible for specific interactions of other epithelia with IgM and IgA, an examination of human skin and human epidermal cells for the presence of SC was undertaken.

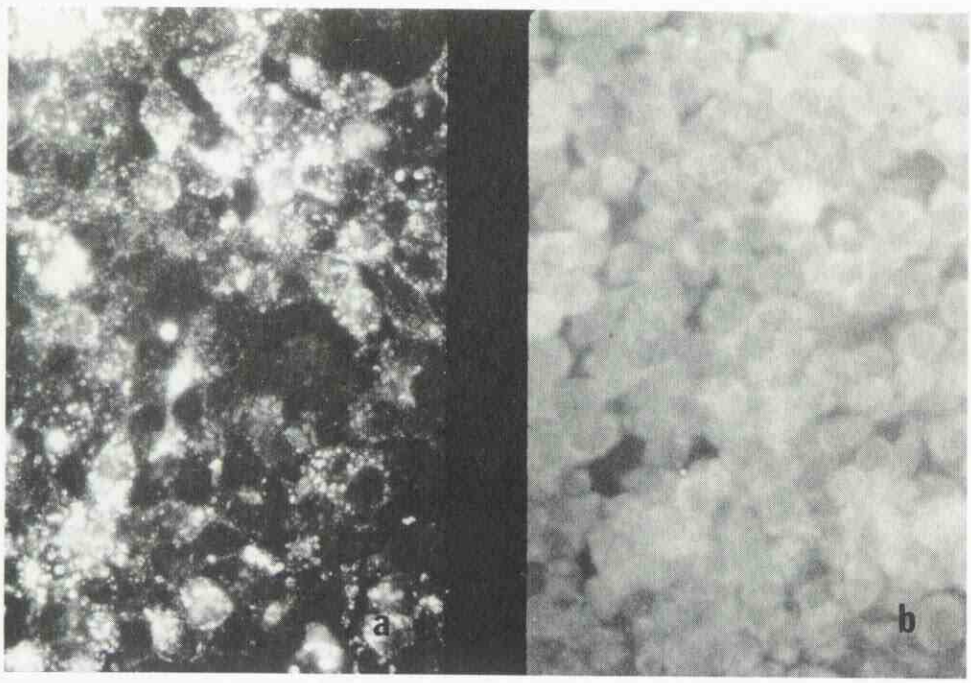

Figure 4. Indirect immunofluorescence staining of cultured HT-29 cells for SC with use of the rabbit antibody to SC. Strong granular staining, primarily on cell surfaces, was seen *(a)*. HT-29 cells stained with the control antibody were negative *(b)* (magnification ×630).

Staining for SC that appears to be specific was found in the epidermis in sections of human skin. The pattern of staining differed, depending on the antibody to SC used. Differing pattern of expression could be related to variable expression of epitopes at different levels of the epidermis. Possibly more convincing was the cell-surface staining for SC detected on cultured human keratinocytes. Although the intensity of SC was far less than that of cultured HT-29 cells, this observation points to the fact that HK may be capable of SC expression. If SC expression is modulated in HK by factors such as cytokines and hormones, expression might be further enhanced under stimulated conditions.

By immunoblotting, we have not been able to detect the SC protein from cultured HK, but we have also not been able to detect it in cultured HT-29 cells. We believe that in the technique for lysis of cells the receptor protein is either being destroyed or is being denatured. Improvements in this technique should allow detection of cell-associated SC and identification of SC synthesized by HT-29 cells and by HK.

Based on these observations, additional studies of the expression of SC by the human epidermis are being undertaken. Studies to learn what factors that might enhance SC expression by HK, to evaluate the binding of polymeric IgA to cultured HK, and to examine the presence of the RNA message for SC in HK are now underway.

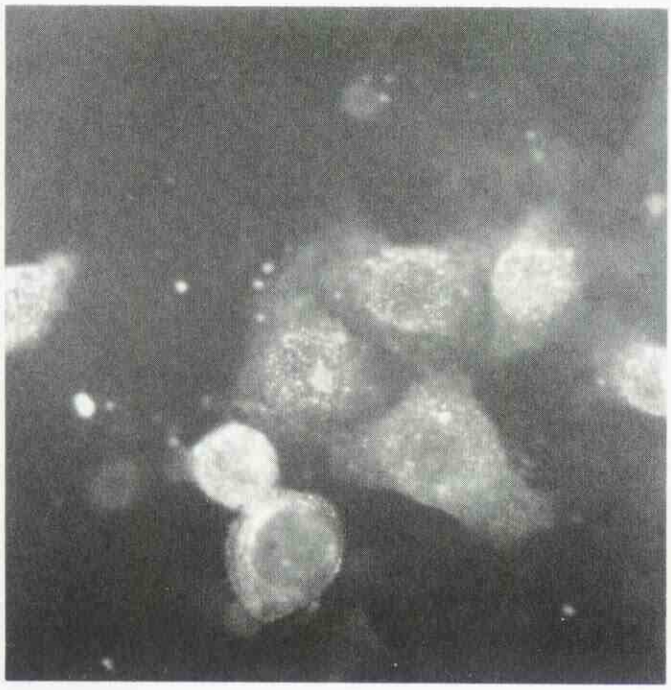

Figure 5. Indirect IF staining of cultured HK for SC with use of the rabbit antibody to SC. Faint granular fluorescence on cell surfaces was seen (magnification ×630).

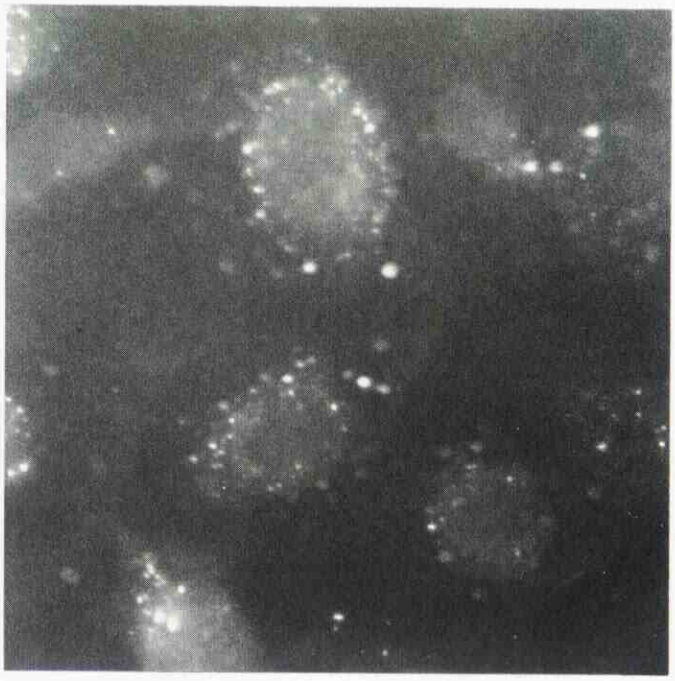

Figure 6. Indirect immunofluorescence staining of cultured human HK for SC with use of the monoclonal antibody to SC. Granular fluorescence on cell surfaces was seen (magnification ×630).

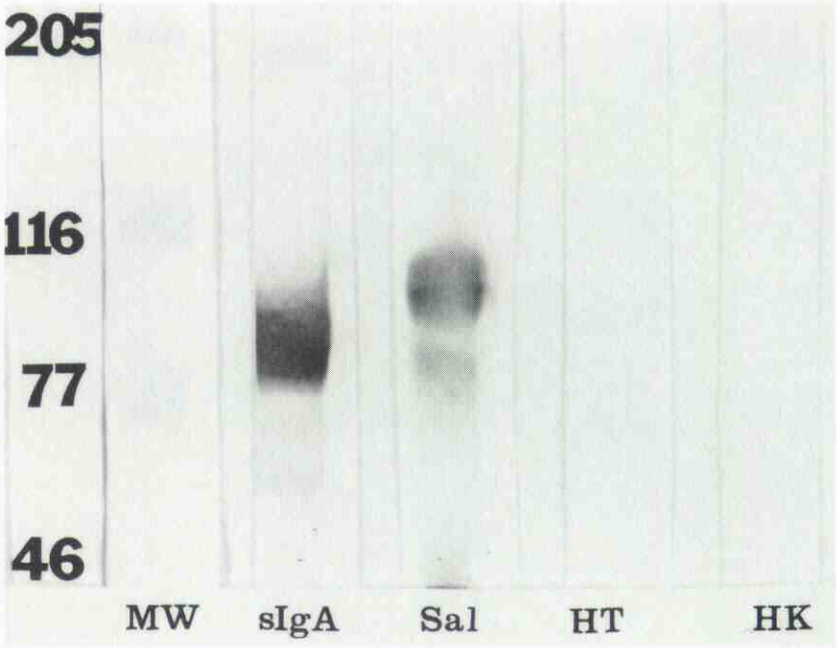

Figure 7. Immunoblot of proteins from secretory IgA (sIgA), saliva (Sal), HT-29 cells (HT) and HK, stained for SC. Molecular weight standards (MW) are on the left. One strong band of SC staining is seen in secretory IgA, while two bands are seen in saliva. The strong higher molecular weight band in saliva may represent free SC. Lysates from HT-29 cells and from HK do not show SC, presumably due to destruction or denaturation of the protein during the extraction process.

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