Brief Definitive Report

SECRETORY COMPONENT ON EPITHELIAL CELLS IS A SURFACE RECEPTOR FOR POLYMERIC IMMUNOGLOBULINS*

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The transport of immunoglobulins across mucous membranes is a selective process that involves different classes of immunoglobulins in different species (1). In human external secretions, the principal immunoglobulin is secretory IgA (s-IgA). This molecule is the product of plasma cells that secrete polymeric IgA with J chain, and of epithelial cells that synthesize secretory component (SC). The SC becomes linked to the IgA during transport through the epithelial cells (2). In some cases of IgA deficiency, secretory IgM (s-IgM) rather than s-IgA appears in external secretions (2, 3). Several models of active transport proposed to explain the selective appearance of IgA and IgM in secretions suggest that initially these immunoglobulins are selectively bound to a receptor on the surface of exocrine epithelial cells (2, 4). The possibility that SC may serve as an immunoglobulin receptor on epithelial cells is implied by the following findings: J chain-containing polymeric IgA and IgM molecules exhibit an in vitro affinity for SC (2); the infusion of an animal with anti-SC serum lowers the ratio of intestinal s-IgA to serum IgA (5); and immunofluorescence studies on tissue sections of the intestine reveal the coincidental appearance of SC and IgA on the basal and lateral surfaces of epithelial cells (2).

The present studies were undertaken to determine whether SC is indeed a receptor for immunoglobulins of various classes, subclasses, and molecular configurations. The human epithelial cell line HT-29, which displays SC on the cell surface (6), and sections of human fetal intestines were examined for their ability to bind various immunoglobulins on the cell membrane or in the cytoplasm.

Materials and Methods

Preparation of Immunoglobulins and SC. Secretory IgA from colostrum, and polymeric or monomeric IgA from sera of patients with IgA myelomatosis, and IgG from normal human serum were purified by ammonium sulfate precipitation, followed by molecular sieve chromatography on Sephadex G-200 and Sepharose 6B, and DEAE-cellulose chromatography as described in detail (7). IgM proteins were purified from sera of patients with Waldenström's macroglobulinemia by euglobulin precipitation with a 15-fold excess of cold distilled water and subsequent Sephadex G-200 gel filtration (8). SC was purified by the method of Brandtzaeg (9) from colostrum and milk of normal mothers and a patient with panhypogammaglobulinemia. The purity of these preparations was determined by immunoelectrophoresis and double immunodiffusion with polyvalent

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antisera to human serum and colostrum (Behring Diagnostics, American Hoechst Corp., Sommerville, N.J.).

Reagents. Monovalent antisera (specific for the heavy (H) chains of IgA, IgM, or IgG) were prepared in this laboratory as previously described (7), or were obtained from a commercial source (Behring Diagnostics). Anti-SC reagents were prepared by adsorption of anti-s-IgA antisera with purified serum IgA (10) or by immunization of rabbits and goats with SC that was purified as described above; a commercially available rabbit anti-human SC was also used (Behring Diagnostics). Preparations of anti-J chain and anti-lactoferrin reagents have been described (7, 11). Procedures used for the preparation and fluorochrome labeling of γ-globulin fractions and $F(ab')_2$ fragments of anti-SC and anti-H chain reagents have been described (12).

Tissue and Cell Processing. Tissue from the small intestine was obtained from human fetuses (age determined by crown to rump measurements). Tissues were either immediately frozen in liquid nitrogen or were fixed in cold 95% ethanol and embedded in paraffin by the method of Sainte-Marie (13); frozen sections (4 μm) were fixed in 95 ml of 95% ethanol + 5 ml of glacial acetic acid. The presence of SC was determined by incubation with tetramethylrhodamine-isothiocyanate (TRITC) labeled anti-SC. To determine the binding of immunoglobulins, tissue sections were incubated with TRITC-labeled monomeric or polymeric myeloma IgA, colostral IgA, macroglobulin IgM, or IgG.

A line of human epithelial cells, HT-29 (developed at the Sloan-Kettering Institute for Cancer Research, New York) was propagated in tissue culture and allowed to grow in monolayers, according to the technique of Huang et al. (6). In some experiments, cells were recovered from monolayers by a 5-min treatment at 37°C with 0.25% trypsin and 10^{-2} M EDTA, washed, and then allowed to adhere to sterile glass cover slips for 48–72 h before examination. In other experiments, cells were scraped off the flask wall, washed, and resuspended in phosphate-buffered saline (PBS) + 1% bovine serum albumin + 0.3% sodium azide for immediate examination. Cultures were maintained in McCoy's Modified 5A medium (Grand Island Biological Co., Grand Island, N. Y.) that contained 15% fetal bovine serum (Reheis Chemical Co., Kankakee, Ill.), penicillin and streptomycin, and L-glutamine. All cultures were incubated at 37°C in 5% CO_2.

The presence of SC on the cell membrane was determined with the fluorochrome-labeled $F(ab')_2$ fragment of anti-SC reagents. Binding affinities were determined by incubation of cells with various immunoglobulin preparations (50 μl of a solution containing 4 mg/ml) for 45 min at room temperature. Cells were washed in PBS and then incubated with fluorochrome-labeled anti-H chain reagents or their $F(ab')_2$ fragments.

Cell suspensions were prepared as described by Winchester (12), and all specimens were examined with a Leitz Orthoplan (Leitz, Wetzlar, Germany) fluorescence microscope equipped with epi-illumination.

Results

Immunoglobulin Binding by Intestinal Tissues. Sections of human small intestine obtained from fetuses at 8, 10, 11, 12, 13½, 14, and 16 wk of gestation were examined by direct immunofluorescence for the presence of SC and endogenous immunoglobulins. Intestinal tissue sections incubated with labeled anti-H chain reagents alone exhibited no cellular fluorescence, which indicated that endogenous immunoglobulins were absent. SC was not detected in fetal tissues until the 16th-wk of gestation. These findings match those of Ogra et al. (14) as to the time of appearance of SC in human fetal development. At this stage, the epithelial cells fluoresced on staining with fluorochrome-labeled polymeric IgA (Fig. 1a), whereas epithelial cells of tissue sections from younger fetuses (which displayed no SC) did not exhibit immunoglobulin binding. Specificity of the immunoglobulin binding (at 16 wk) was confirmed by the absence of fluorescence when the cells were pre-incubated with unlabeled polymeric IgA. TRITC-labeled monomeric IgA and serum IgG did not bind to the cytoplasm of epithelial cells from fetuses of any age. The fluorescence observed after incubation with fluorochrome-labeled IgM was weaker than with poly-

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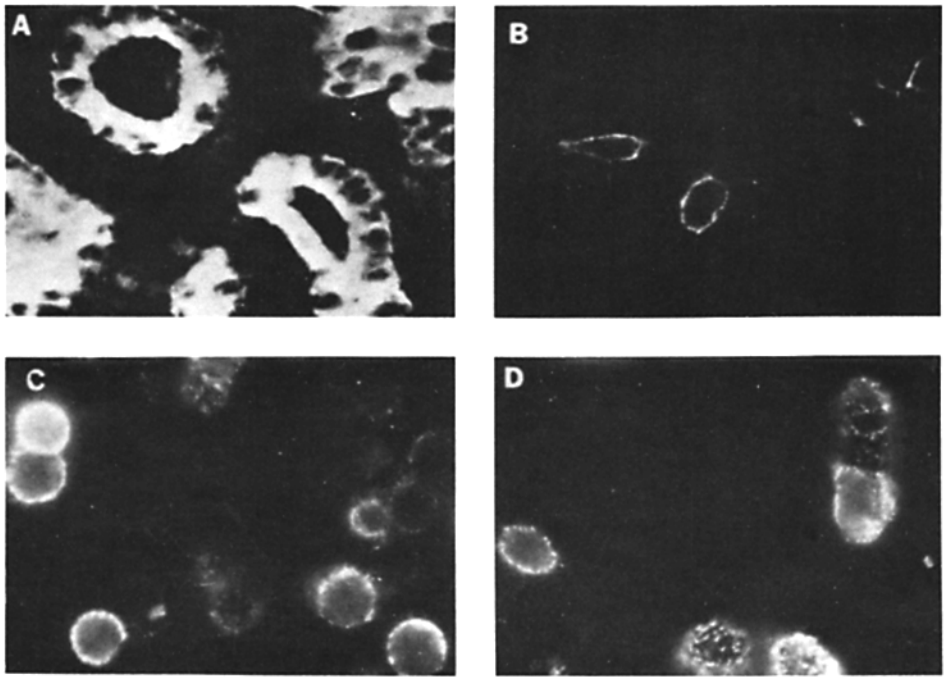

FIG. 1a. Binding of RITC-labeled polymeric IgA to the cytoplasm of fetal intestinal epithelium at 16 wk of gestation; b. Expression of SC on the surface of HT-29 colon carcinoma cells as disclosed by RITC-labeled anti-SC reagent; c. Binding of polymeric IgA to the surface of HT-29 cells; d. Binding of IgM to the surface of HT-29 cells.

meric IgA; fluorescence of marginal intensity was observed with TRITC-labeled s-IgA.

Binding of Immunoglobulins to the Surface of Colonic Carcinoma Cells. HT-29 cells attached to a cover slip or in single cell suspensions were examined for the presence of SC on the membrane and for their ability to bind immunoglobulins of various classes, subclasses, and molecular configurations. SC was expressed most prominently after 72 h of cultivation; at that time 35% of the cells fluoresced with labeled anti-SC reagents (Fig. 1b). Incubation of these cells with polymeric IgA myeloma proteins and subsequent addition of fluorescein isothiocyanate-labeled alpha chain specific antisera revealed immunoglobulin binding to the cell surface. Both IgA1 and IgA2 subclasses with kappa and lambda chains were included in these IgA myeloma proteins. Characteristics of these immunoglobulins and their capacities for binding to HT-29 cells are given in Table I and Fig. 1c. Binding of immunoglobulins to epithelial cells was not affected by IgA subclass or light (L) chain type, but was dependent on molecular configuration, as shown by the failure of monomeric IgA and s-IgA to bind to HT-29 cells. Three of four IgM proteins also bound to the surface of HT-29 cells. The properties of the immunoglobulins are indicated in Table I and Fig. 1d. IgG or labeled anti-H chain reagents did not react with the cells.

The binding of polymeric IgA and IgM to the surface of epithelial cells could be blocked by incubating the cells with anti-SC before adding immunoglobulins;

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TABLE I
Binding of Immunoglobulins by HT-29 Epithelial Cells

Class	Configuration*	J Chain‡	L Chain	Binding
IgA2	Polymeric	+	λ	+
IgA1	Polymeric	+	κ	+
IgA1	Monomeric	–	κ	–
IgA1	Monomeric	–	κ	–
s-IgA	Polymeric	+	κ and λ	–
IgM	Polymeric	+	κ	+
IgM	Polymeric	+	λ	+
IgM	Polymeric	+	κ	–
IgM	Polymeric	+	κ	+
IgG	Monomeric	–	κ and λ	–

* Configuration determined by polyacylamide gel electrophoresis and ultracentrifugation (26).
‡ Presence of J chain determined by polyacylamide gel electrophoresis and immunoelectrophoresis (26).

anti-lactoferrin or anti-J chain reagents had no blocking effect.

HT-29 cells treated with trypsin lost their membrane-associated SC as evidenced by their failure to react with fluorochrome-labeled anti-SC; these SC-negative cells did not bind monomeric or polymeric immunoblobulins of any class. However, when these treated HT-29 cells were cultivated for at least 24 h, surface SC reappeared and the binding of polymeric immunoglobulins was restored.

Discussion

SC is a glycoprotein constituting an integral part of the s-IgA molecule. A number of conjectures have been put forth to account for its function. Within the s-IgA molecule, SC affords protection against attack by proteolytic enzymes (15) and contributes to the stabilization of the quaternary structure of s-IgA (4, 16, 17). South et al. (18) suggested that SC might be instrumental in the transport of IgA from serum into external secretions. However, the parenteral infusion of large amounts of serum IgA did not increase the levels of IgA in secretions because serum-derived 7S IgA is not transported into external secretions (4). Subsequent investigations revealed that only polymeric, J chain-containing IgA and IgM were transported into exocrine fluids (2, 4).

It has also been postulated that SC might be involved in the homing of IgA precursor cells to secretory tissues. This possibility was prompted by the absence of IgA-containing cells from the submucosal lymphoid tissues of an SC deficient patient (19). However, the failure to reveal SC receptors on the surface of normal or stimulated peripheral blood lymphocytes (20, 21) did not support this contention.

Earlier investigations strongly implied that SC mediates the transport of polymeric immunoglobulins (2, 4, 5), and proposed that SC acts as a specific receptor (2). However, evidence has not been offered for the binding of polymeric immunoglobulins to membrane-associated SC on living epithelial cells. Results of the present study validate the concept that SC does indeed function as an immunoglobulin receptor. We have demonstrated that the binding of polymeric immunoglobulins to the surface or cytoplasm of epithelial cells requires the presence of SC and is inhibited by anti-SC. We have also shown that only polymeric immunoglobulins devoid of SC will bind to intestinal epithelial cells

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and will do so irrespectively of IgA subclass and L-chain type. The participation of J chain in the process of SC binding of polymeric immunoglobulins remains unresolved (2, 22).

This study concerns only the initial event in the transport of immunoglobulins through the epithelial cells. Reports of IgA associated with SC in cytoplasmic vesicles within epithelial cells (23–25) suggest that membrane-bound IgA is then internalized and transported to the secretory surface of the exocrine cell.

Summary

Epithelial cells of human fetal intestines and of a colonic carcinoma cell line (HT-29) exhibited intracellular and surface binding of polymeric immunoglobulins of IgA and IgM classes; monomeric IgA and IgG did not bind to these cells. Secretory component was identified as the receptor involved in the immunoglobulin binding. This conclusion was confirmed by the following experiments: trypsin abrogated the surface binding of polymeric immunoglobulin, reappearance of surface secretory component (SC) restored immunoglobulin binding; the appearance of SC in developing fetal tissues coincided with their potential to bind polymeric immunoglobulin; anti-SC reagents inhibited the binding of immunoglobulins to epithelial cells; and SC-containing secretory IgA did not bind to the surface of HT-29 cells.

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