Selective Transport of γA[1] (34240)

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Immunofluorescence studies (1) of various normal exocrine gland tissues have revealed γA and secretory "piece" (SP) staining in the intercellular spaces between glandular acinar and mucous membrane epithelial cells. These results are consistent with the studies of South *et al.* (2) showing that only γA was present in the saliva of agammaglobulinemic patients following plasma infusion experiments even though the level of γG in the serum was much greater than that of γA. Based on these studies (1) and a previous report by Tomasi *et al.* (3), a revised model was proposed involving in part, the intercellular as well as intracellular transport of γA. However, the exact function of SP in the transport of γA, if any, remains obscure.

One of the questions which arises in this regard is whether or not the selective transport of γA could be demonstrated in highly inflamed tissues into which exudation of serum proteins occurs and which contained large amounts of other immunoglobulins. Brandtzaeg *et al.* (4) showed that nasal mucosal tissue from allergic subjects contained relatively large amounts of γG compared to normal nasal tissue, the amounts of γG present depending on the degree of inflammation. The present studies were undertaken to determine by the immunofluorescent technique, whether or not there is selective transport of γA in inflamed allergic tissues in the presence of high concentrations of other immunoglobulins. In addition, the localization of SP and lactoferrin (LF) in these tissues was studied.

Material and Methods. Tissue specimens. Immunofluorescent studies were carried out on nasal mucosae from five patients with nasal polyps who were diagnosed clinically as being allergic. Two nasal mucosal specimens from apparently normal subjects were obtained at autopsy.

Preparation of tissue. Both formalin fixed and fresh frozen tissues were prepared for cryostat sectioning according to previously described methods (1).

Fluoresceinated antisera. The antisera employed in this study were prepared as described in an earlier report (1). In brief, rabbit antisera directed against human γG, γA, and γM proteins were made specific for their respective heavy chains by appropriate absorptions to remove L chain antibodies as previously described (1). Antisera to secretory γA was prepared by immunizing goats with purified colostral γA. This antiserum was made specific for SP by absorption with lyophilized normal human serum and with purified LF (1). Antiserum specific for LF was prepared in rabbits. The specificity of each antiserum was ascertained by immunoelectrophoresis and Ouchterlony analyses. All conjugated antisera were absorbed with rabbit liver powder and human ABO red blood cells before use.

Immunohistological studies. Consecutive sections of the various tissue specimens were stained with the five antisera. Control experiments included incubation of sections of each tissue with fluoresceinated normal rabbit globulin and fluorescence blocking of γA, γG, and γM by prior incubation of the tissue with nonconjugated specific antisera. Blocking was also accomplished by absorption of the appropriate antiserum with purified preparations of γG, γM, and γA. Controls for SP staining included absorption of this antiserum with purified preparations of SP, secretory γA, and LF. Staining with LF an-

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tisera was abolished by absorption with purified LF obtained from human colostrum. In addition, it was shown that SP and LF antisera failed to stain human spleen and lymph node tissues.

Results. Nasal mucosae. The γA staining of all five nasal polyp specimens showed considerable variation depending upon the degree of inflammation. The extent of eosinophil cell infiltration also varied depending on the severity of inflammation. The γA staining was pronounced along the basement membrane of both glandular and surface epithelial cells. In addition, large numbers of γA producing plasma cells located in the interstitium fluoresced brilliantly in areas near the glands and in close proximity to the surface epithelial basement membrane. It is important to note that γA staining was localized in the intercellular spaces and apical portion of both glandular and surface epithelial cells. This type of staining was most striking in the glandular epithelial cells, however, considerable variation was observed between specimens and in different areas of sections obtained from the same tissue. In tissues which were more inflamed there appeared to be considerable diffuse γA and γG staining in the interstitium not localized in cells. This was particularly evident with formalin-fixed tissue. Photographs of representative sections are shown in Fig. 1.

The γG staining was also striking in intensity and in some cases was equal or even greater than γA staining. This was particularly evident in more acutely inflamed tissue. The γG staining was localized along the basement membrane of the glandular and surface epithelium, diffusely in the interstitium and in numerous plasma cells in the interstitium. However, γG staining was rarely observed in the intercellular spaces between epithelial cells while γA was consistently observed in this location. No intercellular or apical epithelial cell γG staining was seen even in areas where the intensity of γG staining along the basement membrane was as great or greater than that of γA (see Fig. 1).

The γM staining was localized in blood vessels and in plasma cells that were sparsely dispersed throughout the interstitium. Only

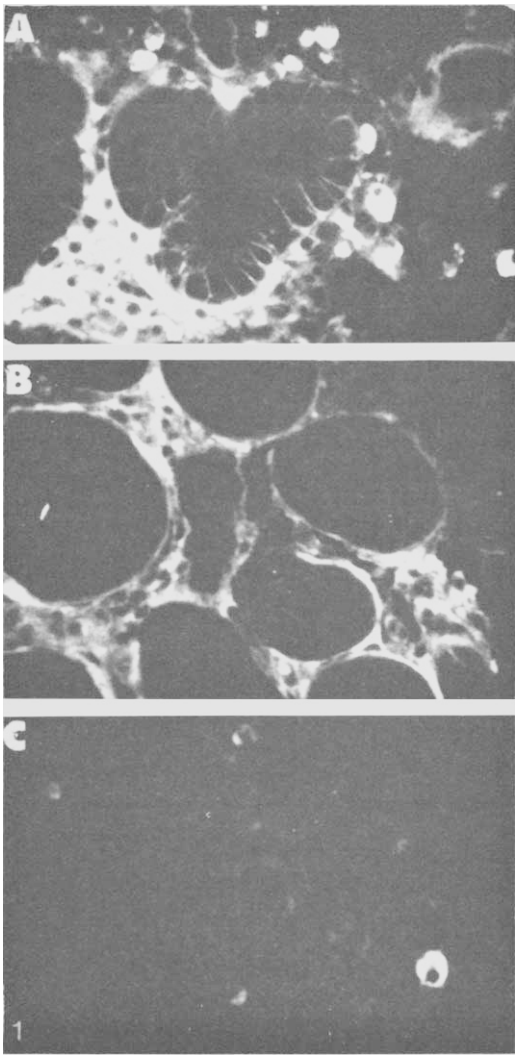

FIG. 1A. Section of nasal mucosa stained with 7SγA antiserum: The γA staining is intense in numerous plasma cells and along the basement membrane of the glands. Note the γA staining in the intercellular spaces between the glandular epithelial cells; original magnification 250×. (B). Consecutive section obtained from same tissue as in (A) stained with γG antiserum. Note the intense γG staining along the basement membrane of the glands and the absence of staining in the intercellular spaces between the glandular epithelium. Several plasma cells are present near the glands; original magnification 250×. (C). Section of nasal mucosa stained with γM antiserum showing γM staining in sparsely dispersed plasma cells; original magnification 250×.

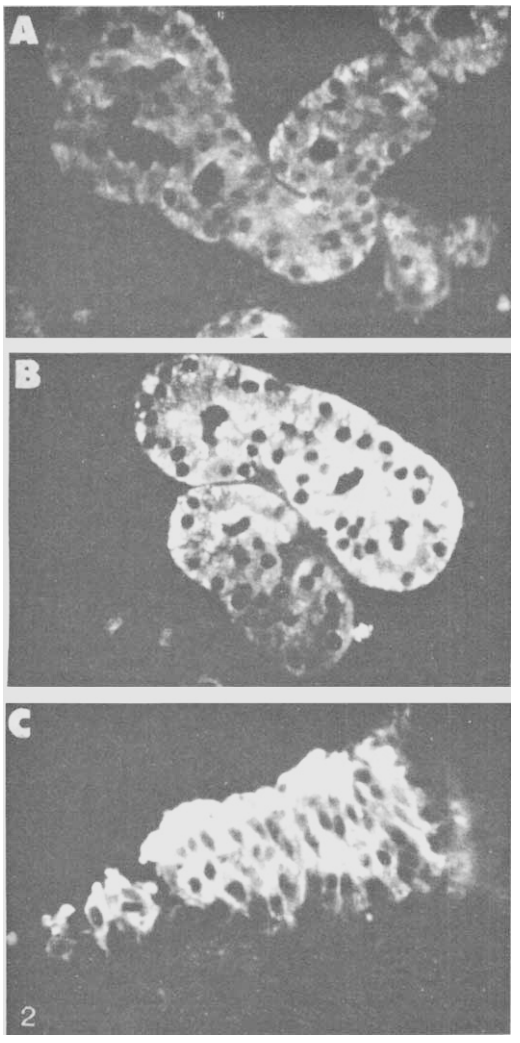

FIG. 2A. Nasal mucosal glands stained with SP specific antiserum. Note the absence of plasma cell staining in the interstitium; original magnification 250×. (B). Consecutive section of same tissue as in (A) stained with LF antiserum; original magnification 250×. (C). Section of nasal mucosal tissue stained with SP antiserum. Note the SP staining of the columnar epithelium. LF antiserum does not stain these cells; original magnification 250×.

slight basement membrane staining was observed in some sections regardless of the degree of inflammation. Figure 1 shows typical γM staining of nasal mucosal tissue.

The SP staining was particularly striking in the submucosal glands and in the surface epithelial cells. In addition, intense SP staining was observed along the mucous layer covering the surface epithelial cells. No SP staining was observed in the lamina propria except for occasional nonspecific granulocyte staining. Both serous and mucous type glandular epithelial cells stained. Typical epithelial cell staining is shown in Fig. 2.

The LF was localized only in the glands present in the submucosa. This staining was very intense in the glandular epithelial cells and was found in consecutive sections to be present in the same glands that stained for SP. It is of particular interest that LF staining was restricted to the glandular epithelium and was not detected in the surface epithelium while SP staining was present in both types of epithelial cells. Typical LF and SP staining of nasal mucosa is shown in Fig. 2.

Normal nasal mucosal tissue appeared to stain very similarly to the tissue obtained from allergic subjects. Noninflamed mucosa showed less diffuse immunoglobulin staining in the interstitium and the number of plasma cells were less and the granulocyte staining was nearly absent in most sections. There was no discernible difference in the staining patterns for SP and LF in normal tissue as compared to nasal mucosal specimens obtained from allergic subjects.

Discussion. The distribution of immunoglobulins in nasal mucosae obtained from individuals with nasal polyps, was in general agreement with the studies reported by Brandtzaeg *et al.* (4). γA producing plasma cells were predominant in the interstitium although large numbers of γG staining cells were also present. γM staining was observed in occasional plasma cells and blood vessels which is in accord with earlier reports (4, 5). One of the most striking observations was that in all cases only γA and not γG staining occurred in the intercellular spaces and apical portion of the glandular epithelial cells even though the intensity of γG staining along the basement membrane of the same glands was approximately as intense as that of γA. (see Fig. 1). This was a consistent observation in this study and supports our earlier observations (1) for a number of different exocrine gland tissue. This intercellular distribution of γA was also present in the

surface epithelium although the intensity of staining appeared to be less.

These findings suggest that selective transport of γA occurs even in tissues where severe inflammatory reactions are present and large amounts of γG are found. This evidence not only adds support to the occurrence of selective transport but also to the recently proposed model (1) for the transport of γA across mucosal epithelial cell surfaces. However, it is not yet clear what function, if any, SP has in the transport process. In this regard Brandtzaeg *et al.* (6) have demonstrated that in patients with hereditary ataxia telangiectasia immunofluorescence staining due to γM was observed in the intercellular spaces between epithelial cells. In addition, these investigators showed that SP was not associated with γM. Brandtzaeg (7) in a recent report, suggested that the selective transport of γA and γM may be due to some unique characteristic of the heavy chains of these two immunoglobulin classes and that SP may not be involved in the secretion (transport) of immunoglobulins across the glandular epithelium.

Heremans and Crabbe' (8) detected apical and intercellular γM staining in tissues from a patient with γA deficient sprue. Thus it appears that under certain conditions other immunoglobulins may be selectively transported across the mucosal epithelial cell surfaces of exocrine glands.

The localization of γG, γM, and γA in the two apparently normal autopsy specimens was in general the same as was observed in the tissue obtained from allergic subjects with nasal polyps. The most obvious differences were fewer numbers of γG and γA staining plasma cells and the large amorphous deposit of these immunoglobulins which may be directly related to the inflammatory state of the allergic mucosa as opposed to the nonallergic tissue. These results are in accord with the observations of Brandtzaeg *et al.* (4) in their studies of both normal and allergic nasal mucosae.

It is quite clear from the data that both SP and LF may be produced within the same glandular epithelial cells (see Fig. 2) as determined by staining consecutive sections with SP and LF specific antisera. The localization of LF in glandular but not surface epithelium is an interesting observation in view of the finding that SP was localized in both types of epithelial cells. The absence of LF staining in surface epithelial cells is consistent with previous findings in studies involving bronchial biopsy tissue (1, 9) and various other exocrine gland tissues (1). This raises the question as to why such differential epithelial cell function should exist with respect to these two secretion specific components.

In this regard, Masson *et al.* (10) and more recent observations made in this laboratory involving specimens of human endometrium have shown that depending upon the phase (proliferative vs. secretory), one may or may not detect LF in the glandular epithelium. We have also made similar observations for SP in human endometrium tissue.

Summary. The direct immunofluorescence technique was employed to localize γA, secretory "piece" (SP) and lactoferrin (LF) in human nasal mucosal tissue obtained from five allergic and two nonallergic individuals. The γA staining was localized in the apical portion of the mucosal epithelium, intercellular spaces, basement membrane, and in plasma cells dispersed throughout the interstitium of the nasal mucosal tissue. The γG was present in approximately the same amounts in the interstitium and basement membrane area but unlike the γA did not gain access to the intercellular space or epithelial cell cytoplasm. The SP staining was localized in both glandular and surface epithelial cells. In addition SP staining was observed in the intercellular spaces between epithelial cells. The LF staining was localized only in the glandular epithelium of both allergic and nonallergic nasal mucosae and not in the surface epithelium.

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