Immunochemical and Immunohistochemical Studies on Chronic Paranasal Sinus Infection

Noritake WATANABE M.D.
Department of Otolaryngology, Yamaguchi University School of Medicine Ube, Japan.
(Chief: Prof. Toru Sekitani)
(Received February 2, 1977)

SUMMARY

Mucosal immunity as well as enzymatic and mucociliary defense mechanism acts in the mucous membrane of nasal and paranasal sinus cavities, preventing viral, bacterial and other antigenic invasions. Chronic nasal and paranasal sinus infection is very common disease, but the etiology and pathogenesis of this entity is still a subject of controversy. To obtain an additional information about the pathogenesis of chronic paranasal sinusitis, we investigated secretory component (SC), lactoferrin and immunoglobulins in nasal secretions and nasal and paranasal sinus mucosae.

Forty-seven specimens of nasal secretion were obtained by adsorption onto filter papers from patients with chronic rhinosinusitis, 74 specimens were from patients with allergic rhinitis and 40 from normal subjects.

Tissue specimens of the inferior turbinate, nasal polyp, and maxillary and ethmoidal sinus mucosa were obtained from 32 patients who underwent paranasal sinus operation. These tissue specimens were subjected to the immunofluorescent observation and immunohistochemical SC affinity test of IgA immunocytes.

Concentrations of IgA and IgG in both nasal secretions and sera were determined by single radial diffusion technique, while radioimmunoassay was employed to measure concentrations of SC and lactoferrin in nasal secretions.

Mean concentrations of total protein, IgG and IgA in nasal secretions for patients with chronic rhinosinusitis exceeded significantly those of normal subjects. However, mean concentrations of SC and lactoferrin in nasal secretions from the group of chronic rhinosinusitis were less than those of normal subjects. Lactoferrin was chiefly synthesized in serous secretory cells of nasal and paranasal sinus mucosae. However, the production of this protein was reduced in chronic rhinosinusitis, even

though glands were apparently abundant. Although IgA content in nasal secretions was elevated in chronic rhinosinusitis, the number of IgA immunocytes capable of binding SC was smaller than that of IgA immunocytes not binding SC. Findings of this investigation suggest that the function of local mucosal immunity is limited in the nasal and paranasnasat sinus cavities of patients with chronic rhinosinusitis, and that antibodies originating from the plasma contribute significantly to the constitution of immunoglobulins in nasal secretions.

INTRODUCTION

Chronic paranasal sinusitis is a common disease. Although infection, allergy and dysfunction of vasomotor nerve have all been regarded as major causative factors, the definite etiology and pathogenesis of this disease are still unsettled.

Secretions bathing the mucous membrane of nasal and paranasal sinus cavities serve to mucosal biologic defense systems, such as mucociliary transportation, enzymatic activity and local mucosal immunity. The concept of local immunity was suggested by Besredka[1] almost 50 years ago. However, the intensive attention to the local immunity has been paid since Tomasi et al.[2)3)] demonstrated that the immunoglobulin content of certain non-vascular fluid is quite different from that of serum, that IgA being a relatively minor immunoglobulin in serum is the predominant immunoglobulin class in external secretions, and that IgA in external secretions has a structure significantly different from IgA in serum. The characteristic IgA in external secretions possesses an extra antigenic determinant, called secretory component (SC) and IgA associated with SC is now called secretory IgA. SC is a nonimmunoglobulin glycoprotein, existing as bound SC (associated with IgA) and/or free SC (unassociated with IgA). It has been suggested that SC facilitates the transport of IgA to the epithelial surface and that secretory IgA is more resistant to proteolysis than serum IgA lacking SC[4)5)6)]. Recently, Strober et al.[7)] proposed another function of SC that is to provide a homing signal for IgA cells or to stimulate differentiation or proliferation of IgA precursor cells that move into mucosal areas.

On the other hand, since Mach[8)] and Brandtzaeg[9)] showed that free SC combines *in vitro* with 10S serum IgA having J chain (but not 7S monomer IgA) producing 11S secretory IgA, it has been suggested that there are two types of IgA immunocytes, monomer IgA-forming cells and dimer IgA-forming cells. Brandtzaeg[10)] first demonstrated the presence of these two types of IgA forming cells in salivary glands and colon.

Mogi[11] reported the existence of dimer IgA-forming cells in tonsils.

Secretory IgA and SC are probably the key factors in mucosal immunity. In the present study, we investigated SC, lactoferrin and immunoglobulins in nasal secretions, and nasal and paranasal sinus mucosae of patients with chronic sinusitis to clarify whether the local immune system is suppressed or activated in the chronically diseased mucosa, with the intention of obtaining an additional information about the pathogenesis of chronic paranasal sinus infection. Lactoferrin is an iron binding protein, being synthesized locally in the submucosal glands. This protein was found by recent studies[12][13][14] to have an inhibitory activity in certain bacterial growth.

MATERIALS AND METHODS

Forty-seven specimens of nasal secretion were obtained from patients diagnosed as having chronic rhinosinusitis, 74 specimens were from patients with allergic rhinitis, and 40 specimens were from normal subjects. Patients with chronic rhinosinusitis consisted of 31 male and 16 female and the ages were between 9 and 72 years (median, 29). Diagnosis of chronic rhinosinusitis was based on findings of patient's history, rhinoscopic findings, x-ray examination, and cytologic examination of the nasal smear. Patients with allergic rhinitis consisted of 37 male and 37 female and the ages ranged 4 to 66 years (median, 30). Allergic patients were subjected to allergological investigation. Forty-five of 74 patients (61%) were found to have house-dust or mites allergy and the remaining 29 patients (39%) were to have pollen allergy (Japanese ceder). Forty normal subjects (28 male and 12 female) were selected from healthy persons of 12 to 68 years of age (median, 25), who were free from symptoms of nasal and other respiratory disease and judged to have "normal nasal mucosae" by rhinoscopic examination and cytological examination of the nasal smear.

Collection of Nasal Secretions: Nasal secretions were collected from all patients and control subjects by adsorption onto strips of filter paper according to the method described by Lorin et al.[15]. In this investigation, nasal secretions were absorbed by 10 strips (5 mm×50 mm) of filter paper*, eluted by 2 ml of 0.2M phosphate buffer, pH 6.5, containing 2 mg NaN_3, and centrifuged at 1500 g for 30 minutes to remove any insolube material. The supernatant fluid was frozen at −20°C until use. A blood sample for serum was taken from each subject at the same

*Toyo No. 4, Toyo Roshi Co., Ltd., Tokyo, Japan.

time that the nasal secretion was colledted.

Determination of Protein Concentration in Original Nasal Secretion:

The concentration of a protein content in the original nasal secretion was calculated by the formula:

$$\begin{pmatrix}\text{concentration}\\ \text{in secretion}\end{pmatrix} = \begin{pmatrix}\text{concentration}\\ \text{in eluate}\end{pmatrix}\left(\frac{\text{volume eluent} - \text{weight secretion}}{\text{weight secretion}}\right)$$

according to the method described by Lorin et al.[15] In this method, a negligible error is introduced by assumption that the weight of the secretion in grams equals its volume in milliliters.

Collection of Tissue Specimens: Tissue specimens of the inferior turbinate, nasal polyp, and maxillary and ethmoidal sinus mucosa were obtained during surgery from 32 patients with chronic rhinosinusitis, who supplied the nasal secretions and underwent paranasal sinus operation. Three specimens of the maxillary sinus mucosa were obtained from patients without nasal and paranasal sinus infection. The maxillary antrum of these three patients was exposed for another reason.

Isolation of Secretory IgA, Free SC and Lactoferrin: The fractionation of secretory IgA, free SC and lactoferrin from colostrum was done by methods of Kobayashi[16] and Mogi[17]. Figure 1 illustrates the outline of the isolation. The pooled colostrum was centrifuged at 16100 g for one hour to remove fat and then acidified to pH 4.2 with 2% acetic acid to precipitate casein. After the centrifugation to remove casein, the supernatant was immediately readjusted to pH 7.0 with 1N NaOH solution. The colostral whey was treated with saturated ammonium sulfate at 50% final saturation. The precipitate was used as the source of secretory IgA and free SC, and the supernatant was used for lactoferrin. The precipitate was redissolved and dialyzed against 0.01M phosphate buffer pH 7.6, and applied to diethylamino-ethyl (DEAE)-cellulose* colum chromatography. The DEAE-cellulose chromatography was performed by a stepwise gradient procedure. The elution was done first by 0.01M phosphate buffer at pH 7.6 and then succeeded by 0.02M buffer at pH 7.6 and 0.1M buffer at pH 6.4. Eluates of 0.01M and 0.02M buffers at pH 7.6 were collected and subjected to carboxy-methyl(CM)-cellulose* chromatography for fractionation of free SC, using linear gradient between 0.01M and 0.5M sodium acetate buffers at pH 5.0, while eluates of 0.1M buffer at pH 6.4 was chromatographed on CM-cellulose for isolation of secretory IgA. The fraction containing IgA, checked by immunodiffusion analysis using rabbit

*Pharmacia Fine Chemicals AB, Uppsala, Sweden.

antihuman α-chain antiserum, was concentrated and passed through Sepharose 6B* gel filtration using borate buffer saline (BBS) at pH 7.8. The second peak was further fractionated by G-200 Sephadex gel filtration with BBS. The first excluded fraction contained pure secretory IgA. For isolation of free SC, eluates of 0.01M and 0.02M buffers at pH 7.6 were concentrated and applied for CM-cellulose chromatography. Three peaks appeared, and the third was rich in free SC. The fraction was

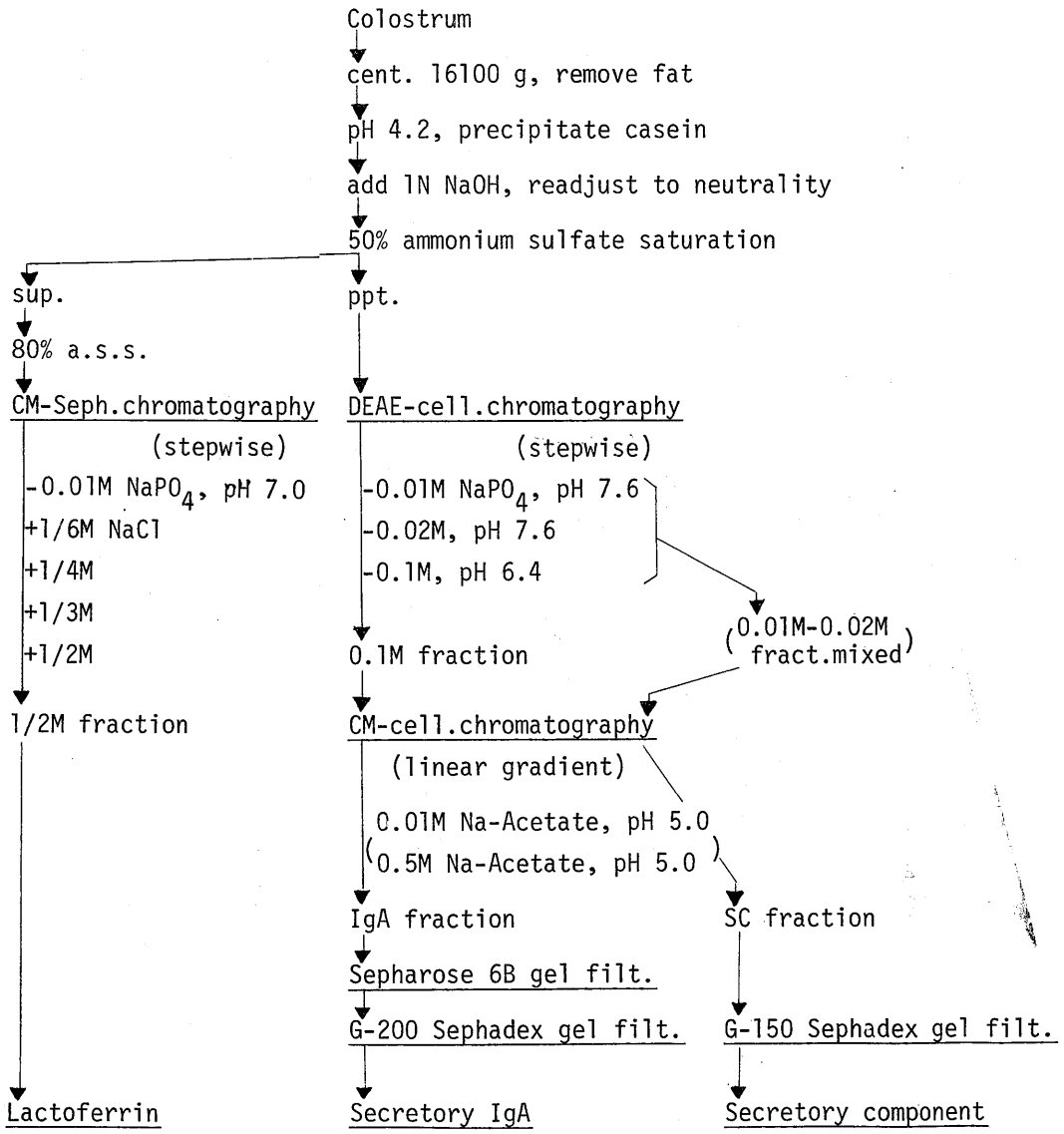

Figure 1 Isolation of secretory IgA, free SC and lactoferrin from human colostrum.

*Pharmacia Fine Chemicals AB, Uppsa,a, Sweden.

further fractionated by G-150 Sephadex gel filtration. For isolation of lactoferrin, the supernatant of colostral whey by 50% saturated ammonium sulfate was dialyzed against distilled water, concentrated and treated with saturated ammonium sulfate at 80% final saturation. The precipitate was redissolved and dialyzed against 0.01M phosphate buffer at pH 7.0. The solution was then applied to CM-Sephadex* column chromatography by a stepwise gradient procedure with 1/6M, 1/4M, 1/3M and 1/2M NaCl in 0.01M phosphate buffer at pH 7.0. Eluates of the buffer with highest concentration of NaCl contained pure lactoferrin.

Antisera: Antisera to human secretory IgA, SC and lactoferrin were made by immunizing rabbits with the purified secretory IgA, free SC and lactoferrin, respectively. The monospecificity of antisen to SC and lactoferrin was tested by immunodiffusion and immunoelectrophoresis (Fig. 2). Antisera to IgA (anti-α-chain) and IgG (anti-γ-chain) were purchased from a commercial source**.

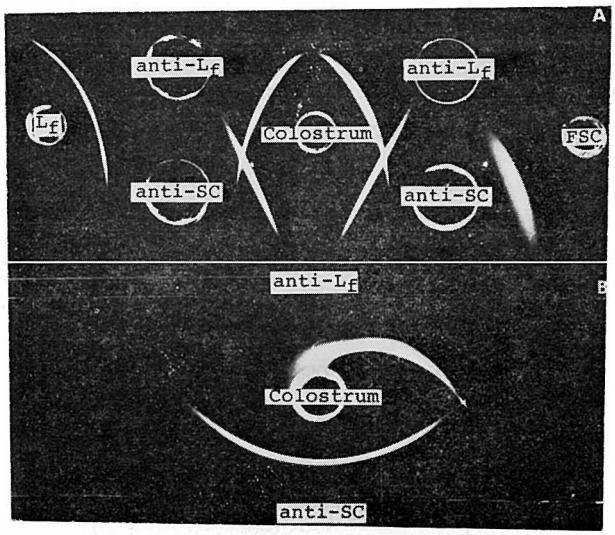

Figure 2. Immunodiffusion and immunoelectrophoretic analysis of the isolated free secretory component (SC) and lactoferrin (Lf).
A) Immunodiffussion. Precipitation lines of the colostrum against anti-SC and anti-lactoferrin antisera cross over each other.
B) Immunoelectrophoresis. Colostrum develops a single precipitation line with each of anti-SC and anti-lactoferrin, and the lines cross over each other.

*Pharmacia Fine Chemicals AB, Uppsala, Sweden.
**Behringwerke, Marburg, Lahn, Germany.

Measurement of Total Protein Concentration: The total protein concentration in nasal secretions and sera was determined by the method of Lowry et al.[18].

Quantitative Analysis of IgG and IgA: Concentrations of IgG and IgA in nasal secretions and sera were determined by a single radial immunodiffusion according to Mancini et al.[19]. Secretory IgA was used as standard to estimate IgA concentration in nasal secretions, while serum IgA (7S IgA) was used as standard to measure IgA in sera.

Coupling of Antibodies to Gels: The IgG fraction purified from the rabbit anti-SC antiserum or anti-lactoferrin antiserum was adjusted to concentration at 5 mg/ml in phosphate buffer saline at pH 8.0. One ml of this protein solution was coupled to one gram of DOTITE Chromagel A-2* activated with cyanogen bromide (CNBr). The activation of gels and coupling of antibodies were performed by the method of Wofsy and Burr[20].

Labeling of Free SC and Lactoferrin with ^{125}I: Ten mg of the purified free SC or lactoferrin Were labeled with one mCi of ^{125}I using sodium iodide and chloramine T[21]. Free iodine was removed by G-25 Sephadex** gel filtration. The ^{125}I-labeled free SC or lactoferrin was mixed with non-labeled free SC or lactoferrin solution.

Radioimmunoassay for the Quantitation of SC and Lactoferrin:

Quantitative determination of SC and lactoferrin in nasal secretions and sera was carried out by a radioimmunoassay using immunosorbents originally described by Wide[22]. The principle of this assay is a competitive inhibition test. Briefly, the assay system used was as follows: 100 μl of elutes of an unknown sample were mixed with 100 μl of the radioactive tracer antigen (^{125}I-SC or ^{125}I-lactoferrin) solution consisting of equal amount of hot antigen and cold antigen (0.0025% in an assay buffer solution). After mixing, one ml of immunosorbent solution, consisting of one gram of the coupled Chromagel A-2 in 2,500 ml of the assay buffey solution (NaCl, 0.9%; phosphate buffer 0.05M; bovine serum solution, 0.3%; sodium azide, 0.05%; final pH 7.4), was added to the mixture. After incubation with shaking overnight at room temparature, the particles of the coupled Chromagel A-2 were centrifuged at 2000 g for 5 minutes, washed with BBS 3 times, and the radioactivity bound to the gels was measured by a gamma-counter***. The radioactive

*Wako Pure Chemical Industries Ltd., Osaka, Japan.
**Pharmacia Fine Chemicals AB, Uppsala, Sweden.
***Packard Model 3330, Tri-Carb Scintillation Spectrometer, Packard Instrument Company, Inc., Downers Grove, Ill.

uptake on the coupled Chromagel A-2 varied inversely with quantity of antigen present in the original incubation mixture. The count-rate for each standard and mean count-rate of the "zeros" (containing no antigen) were determined, and the count-rate of each standard was expressed as a percentage of the mean count-rate of the "zeros". The persentage values of standards were plotted semi-logarithmically against the concentration to provide the dose response curve, as shown in Figure 3. The concentration value for each of the unknown samples was obtained by reading off from the standard curve. The mean count-rate of "zeros" was kept to range 10 to 25% of the mean count-rate of total activity.

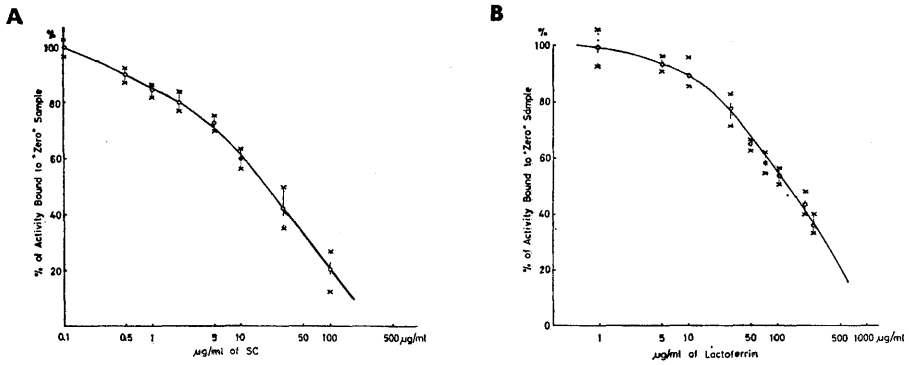

Figure 3. Dose response curves of secretory component (SC) and lactoferrin by a radioimmunoassay.
A) Secretory component (SC); B) Lactoferrin.

Fluoresceinated Antisera: Antibodies to SC or lactoferrin were conjugated with fluorescein isothiocyanate (FITC) by the method of Hamashima and Kyogoku[23]. Fluorescein conjugated rabbit anti-human IgG, IgA and IgM antisera were purchased from a commercial source*. These five antisera were absorbed with human liver powder in order to remove nonspecific staining for connective tissue, as previously described by Mogi[17].

Preparation of Tissues and Immunofluorescent Studies: Specimens of the fresh tissues were fixed in cold ethanol (95%) for paraffin sectioning according to the method described by Hamashima and Kyogoku[23], and tissue specimens were preparated for immunofluorescent studies, as previously described by Mogi[17]. Consecutive sections approximately 4 μ

*Behringwerke, Marburg, Lahn, Germany.

thick were obtained from each tissue, and the sections were stained with each FITC-conjugates. Blocking tests were also performed by supplying the appropriate unconjugate antibodies to the consecutive tissue sections prior to staining with FITC-labeled antibodies. The specimens were examined with a Leitz incident light fluorescence microscope (Dialux)* using KP 500 and BG 38 excitation filters and a dichroic beam splitting mirror (TK 510). Paraffin sections from each block were stained with H.E. and PAS-AB for light microscopy. After the observation by the fluorescence microscopy, sections treated with anti-SC or anti-lactoferrin conjugate were further stained with H.E. or PAS-AB for light microscopic observation.

SC Affinity Test: In order to identify 10S IgA producing cells, tissue sections of the nasal specimens were assayed by SC affinity test as reported by Mogi[11]. Briefly, tissue sections were incubated with a purified free SC solution (20 μg/ml in a 0.3% solution of rabbit IgG), washed with a phosphate buffer at pH 7.4, incubated with FITC-labeled antibodies to SC, washed again, and then subjected to fluorescence microscopic observation.

RESULTS

Total Protein Concentrations of Nasal Secretions and Sera: Results are shown in Table I. The mean concentration of total protein in nasal secretions obtained from patients with chronic rhinosinusitis was higher than that in secretions from groups of allergic rhinitis and normal subjects. This difference was statistically significant ($p<0.001$). The mean concentration of nasal sceretions in the group of allergic rhinitis exceeded significantly that in the group of normal subjects ($p<0.02$). There was no significant difference of the mean value in total protein concentrations of sera between these three groups.

Table I Total Protein concentration of nasalsecretions and sera

	Normal Subjects	Chronic Rhinosinusitis	Allergic Rhinitis
Number of Case	A 40	B 47	C 74
Nasal Secretions mg/100ml	733 (668-804)*	1334 (1167-1524)	879 (780-990)
Sera mg/100ml	7480 (7350-7610)	7430 (7080-7780)	7260 (7140-7380)

*±2SE of Mean.
A<B ($p<0.001$), B>C ($p<0.001$), A<C ($p<0.02$).

*Leitz, Wetzlar, Germany.

Concentrations of IgG and IgA: Table II shows mean concentrations of IgG and IgA in nasal secretions and sera in each group. Mean concentrations of IgG in nasal secretions from groups of chronic rhinosinusitis and allergic rhinitis were significantly ($p<0.001$) greater than the mean value of the group of normal subjects. There was no significant difference between mean concentrations of IgG in sera of each group.

Mean concentrations of IgA in nasal secretions from groups of chronic rhinosinusitis and allergic rhinitis exceeded significantly ($p<0.001$) the mean value of the group of normal subjects.

Table Ⅱ Concentrations of IgG and IgA in Nasal Secretions and Sera

			Normal Subjects	Chronic Rhinosinusitis	Allergic Rhinitis
Number of Case			40	47	74
IgG	N.S.	mg/100ml	A1 33(28-40)*	B1 81(67-97)	C1 72(62-85)
		IgG/T.P.(%)	A2 4.6(3.8-5.6)	B2 6.0(5.0-7.3)	C2 8.2(7.1-9.5)
	Sera	mg/100ml	1860(1750-1970)	1780(1650-1910)	1800(1680-1920)
		IgG/T.P.(%)	25.0(23.3-26.7)	24.8(22.8-26.8)	25.3(23.5-27.1)
IgA	N.S.	mg/100ml	A3 60(47-75)	B3 104(82-132)	C3 117(96-142)
		IgA/T.P.(%)	A4 8.1(6.3-10.5)	B4 7.5(5.9-9.7)	C4 13.3(11.3-15.6)
	Sera	mg/100ml	250(226-274)	247(205-287)	250(232-268)
		IgA/T.P.(%)	3.3(3.0-3.6)	3.0(2.7-3.3)	3.5(3.2-3.8)
IgA/IgG		N.S.	A5 1.8(1.4-2.3)	B5 1.3(1.1-1.6)	C5 1.7(1.5-1.9)
		Sera	0.14(0.13-0.15)	0.13(0.12-0.14)	0.15(0.14-0.16)

*±2SE of Mean.

A1<B1($p<0.001$), A1<C1($p<0.001$).

A2<B2($p<0.05$), A2<C2($p<0.001$), B2<C2($p<0.01$).

A3<B3($p<0.001$), A3<C3($p<0.001$).

A4>B4($p<0.001$), A4<C4($p<0.001$), B4<C4($p<0.001$)

A5>B5($p<0.01$), B5<C5($p<0.02$).

Table Ⅲ Concentrations of SC and Lactoferrin in Nasal Secretions

	Normal Subjects	Chronic Rhinosinusitis	Allergic Rhinitis
Number of Case	40	47	74
SC	42	30	42
μg/ml	(28-63)*	(22-41)	(33-55)
Lactoferrin	A 173	B 72	C 188
μg/ml	(128-234)	(50-105)	(148-239)

*±2SE of Mean.

A, C>B($p<0.001$).

Concentrations of SC and Lactoferrin in Nasal Secretions: Results are exhibited in Table III. Mean concentrations of SC in the group of chronic rhinosinusitis was relatively low when compared with that in groups of allergic rhinitis and normal subjects. However, this difference was not significant.

The mean concentration of lactoferrin in the group of chronic rhinosinusitis was lower than that in the groups of allergic rhinitis and normal subjects. There was a statistically significant difference between these values ($p<0.001$).

Immunofluorescence studies: Little difference in the staining pattern obtained with the five specific antibody conjugates was apparent between specimens of these nasal and paranasal sinus mucosae and polyps.

Figure 4 represents a common staining pattern with anti-IgA conjugate, showing many IgA forming cells in the interstitial connective tissue between glands, heavily diffuse staining throughout the subepithelial connective tissue, and scattered cytoplasmic deposits in the epithelial layer cells. IgA forming cells are also distributed beneath the epithelial layer (Fig. 5A) and less frequently around vessels (Fig. 5B). The most common immunocytes observed in the nasal specimens were IgA forming cells, while IgG and IgM forming cells were rarely present. Although the number of IgA immunocytes varied between different specimens, generally fewer cells were observed in severely diseased mucosae than specimens showing mild inflammatory reactions. However, it was evident that relatively fewer IgA forming cells were present in the maxillary sinus mucosa obtained from patients without paranasal sinusitis. Figure 6 is an immunofluorescent photograph of a maxillary sinus mucosa treated with anti-IgA conjugate, showing IgA forming cells approximating the basement membrane of glandular epithels in which cytoplasmic staining for IgA are seen (Fig. 6A). Figure 6B exhibits IgA immunocytes close to the basement membrane of acini. However, these acinar cells do not react with anti-IgA conjugate. In some instances, IgA deposits were seen in the cytoplasm or intercellular space of epithelial cells, whereas no IgA forming cells exist near this epithelial layer. IgA deposits were frequently detected in lumens of glandular acini and ductules.

Whereas anti-SC conjugate does not react with any subepithelial connective tissue as well as infiltrating cells, bright fluorescent staining for SC is apparent in the cytoplasm of epithelial cells (Fig. 7A) and of glandular acinar cells (Fig. 7C). However, many epithelial and glandular acinar cells were not stained with anti-SC conjugate. The intensity of fluorescent staining with anti-SC varied considerably between acini in

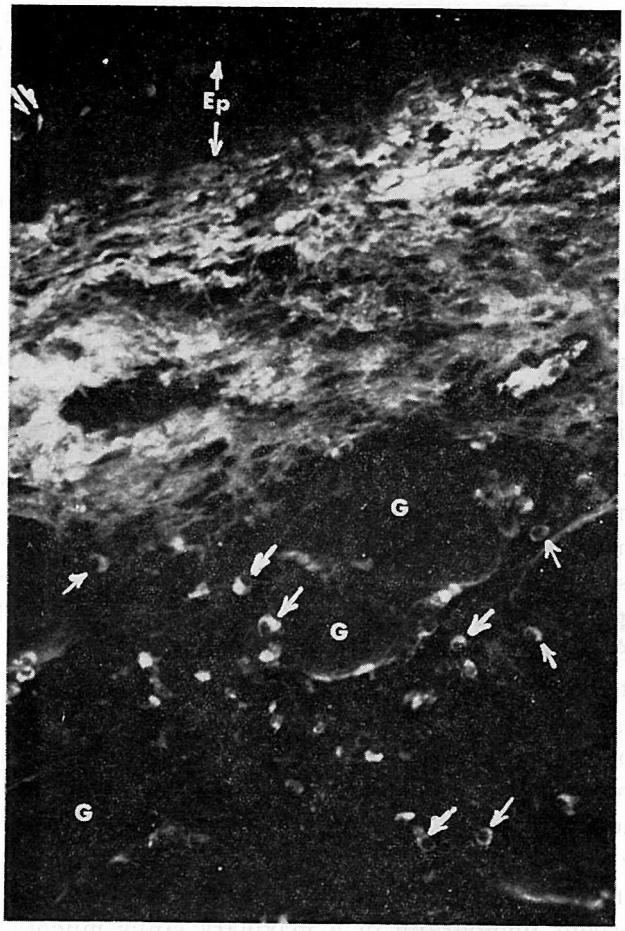

Figure 4. Immunofluorescence localization of IgA.
Section of a maxillary sinus mucosa from a patient with chronic rhinosinusitis showing IgA forming cells (single arrow) in the interstitium between glands (G), heavily diffuse staining throughout the subepithelial connective tissue and scattered epithelial (Ep) deposits of IgA (double arrows) (×125).

the same section as well as different samples. Figure 8 is a photograph of adjacent sections (of an ethmoidal sinus mucosa) treated with anti-IgA and anti-SC conjugates respectively showing brilliant fluorescence at nearly the same site of the epithelial layer. This finding suggests the secretion of secretory IgA.

Treatment with anti-IgG conjugate usually causes little or not epithelial or luminal staining in spite of heavily diffuse staining throughout the subepithelial connective tissue (Fig. 9A). However, in rare instances,

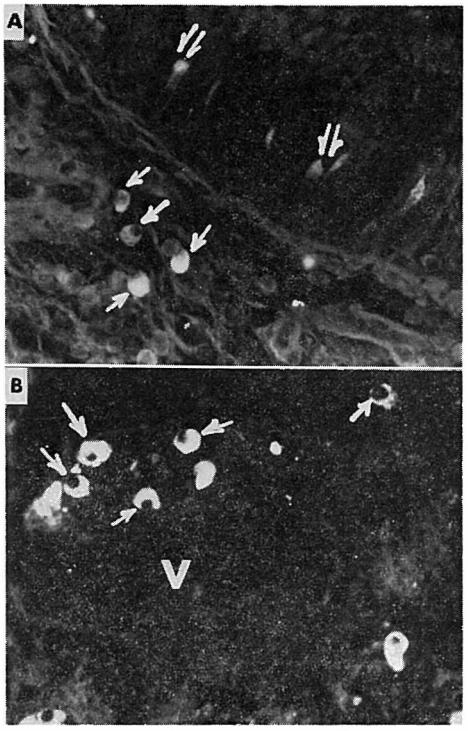

Figure 5. Localization of IgA forming cells.

A) Section of nasal polyp stained with anti-IgA (×312). Note that plasma-like cells positive for IgA (single arrow) are found scattered in the interstitium of subepithelial region, and double arrows indicate epithelial cells positive for IgA.

B) Section of nasal polyp treated with anti-IgA (×312)showing plasma-like IgA forming cells (single arrow) around vessels (V).

particularly severely diseased mucosa, heavy staining for IgG occurs all throughout the section as shown in Figure 9B. This finding suggests "spill-over" of serum elements to the lumen. IgG and IgM forming cells are rarely found mainly in the subepithelial region or around glandular elements as shown in Figure 9C. The number of IgG or IgM immunocytes was significantly smaller than that of IgA immunocytes (Fig. 9D).

Staining with anti-lactoferrin conjugate showed brlliant granular fluorescence only in glandular acini but not in the epithelial lining from the nasal specimens studied. The intensity and staining pattern of lactoferrin varied from one glandular structure to another in the same tissue section as well as different samples. Figure 10A presents one of the staining patterns which is characterized by strong staining in almost all

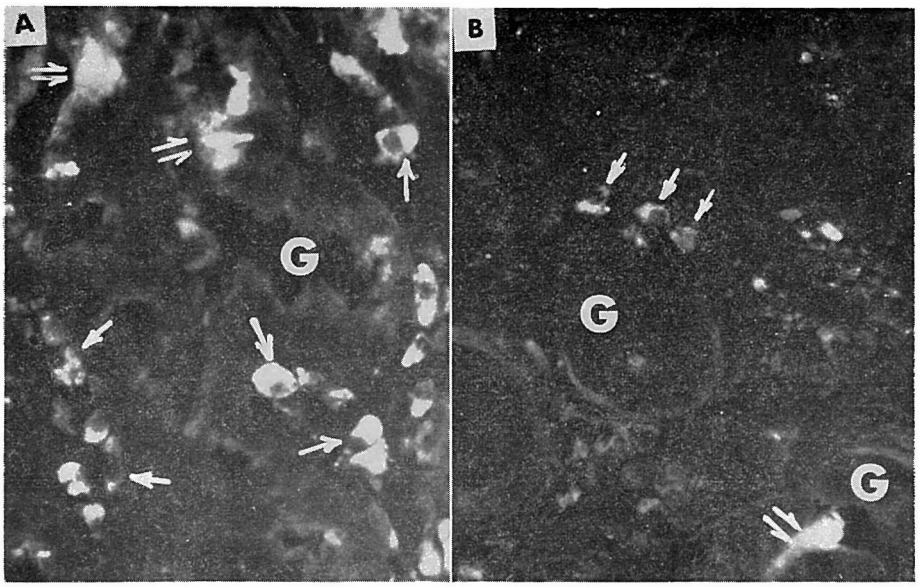

Figure 6. Immunofluorescence localization of IgA in glands and IgA forming cells.
A) Section of a maxillary sinus mucosa from a patient with chronic rhinosinusitis showing IgA forming cells (single arrow) close to the basement membrane of glandular acini and cytoplasmic deposits (double arrows) of IgA in the apical part of some glandular acinar cells (×312).
B) Section of a maxillary sinus mucosa obtained from a patient with chronic rhinosinusitis, showing IgA formingcells (single arrow) in the connective tissue between glands (G) which are not stained with anti-IgA in spite of IgA deposits (double arrows) in the glandular lumen (×312).

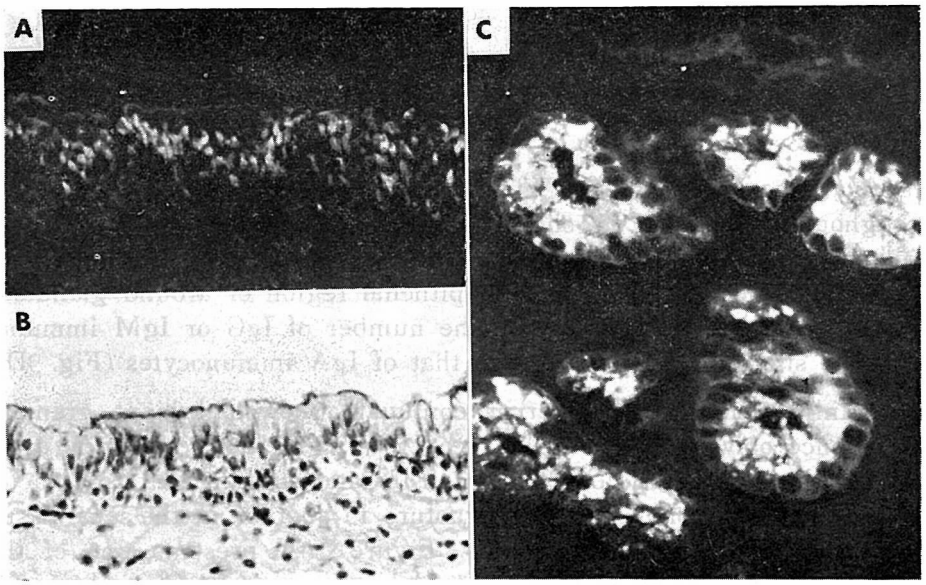

Figure 7. Immunofluorescence localization of secretory component (SC).
A) Section of nasal polyp representing cytoplasmic staining of SC in the epithelial cells (×125).
B) The same section as A) subjected with PAS-AB, showing that SC deposits are consistent with PAS-AB positive granular materials in the epithelial cells (×125).
C) Section of an inferior turbinate obtained from a patient with chronic rhinosinusitis exhibiting heavily diffuse staining of anti-SC in cytoplasm of glandualr acinar cells (×312).

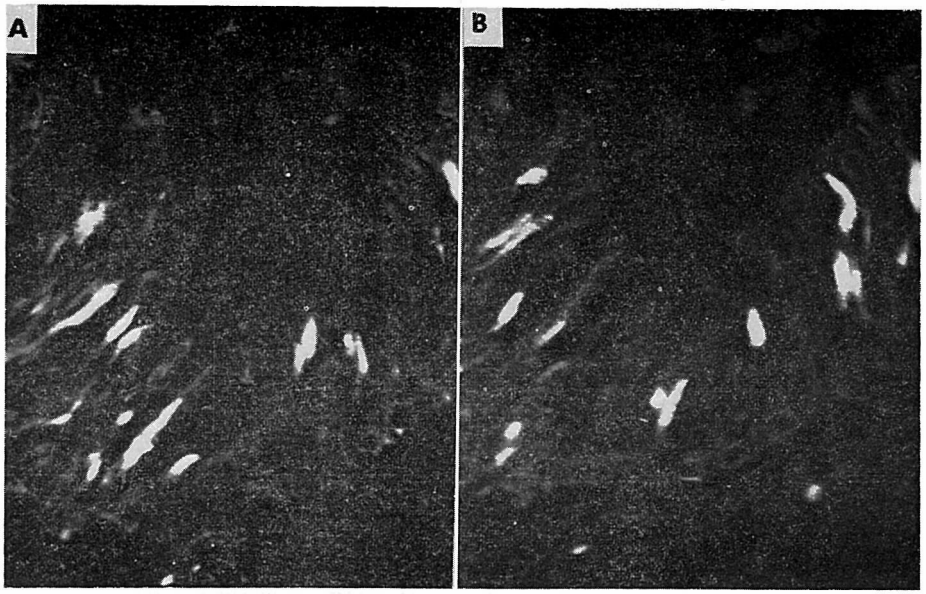

Figure 8. Immunofluorescence localization of IgA and secretory component (SC) in the epithelium.
Adjacent sections of an ethmoidal sinus mucosa treated with anti-IgA (A) and anti-SC (B) showing brilliant fluorescence at nearly the same site of the epithelial layer (×312).

the cells in each gland, while Figure 10B is the fluorescent photograph of a tissue section in which both positive and negative glandular structure for lactoferrin exist. In some glandular acinar cells the fluorescence was restricted to the apical portion of cytoplasm, while the localization of fluorescence in the base of cytoplasms was observed in some instances. A combination of both modes of the lactoferrin localization was also seen. As illustrated in Figure 11, which shows pictures of the same tissue section first stained with anti-lactoferrin conjugate for fluorescent light and next with PAS-AB for ordinary illumination, anti-lactoferrin fluorescence localizes correspondingly to PAS-AB negative glandular cells. Usually, PAS-AB positive glands were abundant in severely diseased mucosae and polyps.

The Immunohistochemical SC Affinity of IgA Immunocytes: Even though there were fewer positive cells than IgA immunocytes not binding free SC, positive immunocytes for the SC affinity test were detected in tissue sections of nasal specimens, including nasal polyps. The intensity of fluorescent staining in the SC affinity test varied between positive cells in the same section as well as different samples. Generally, the number of

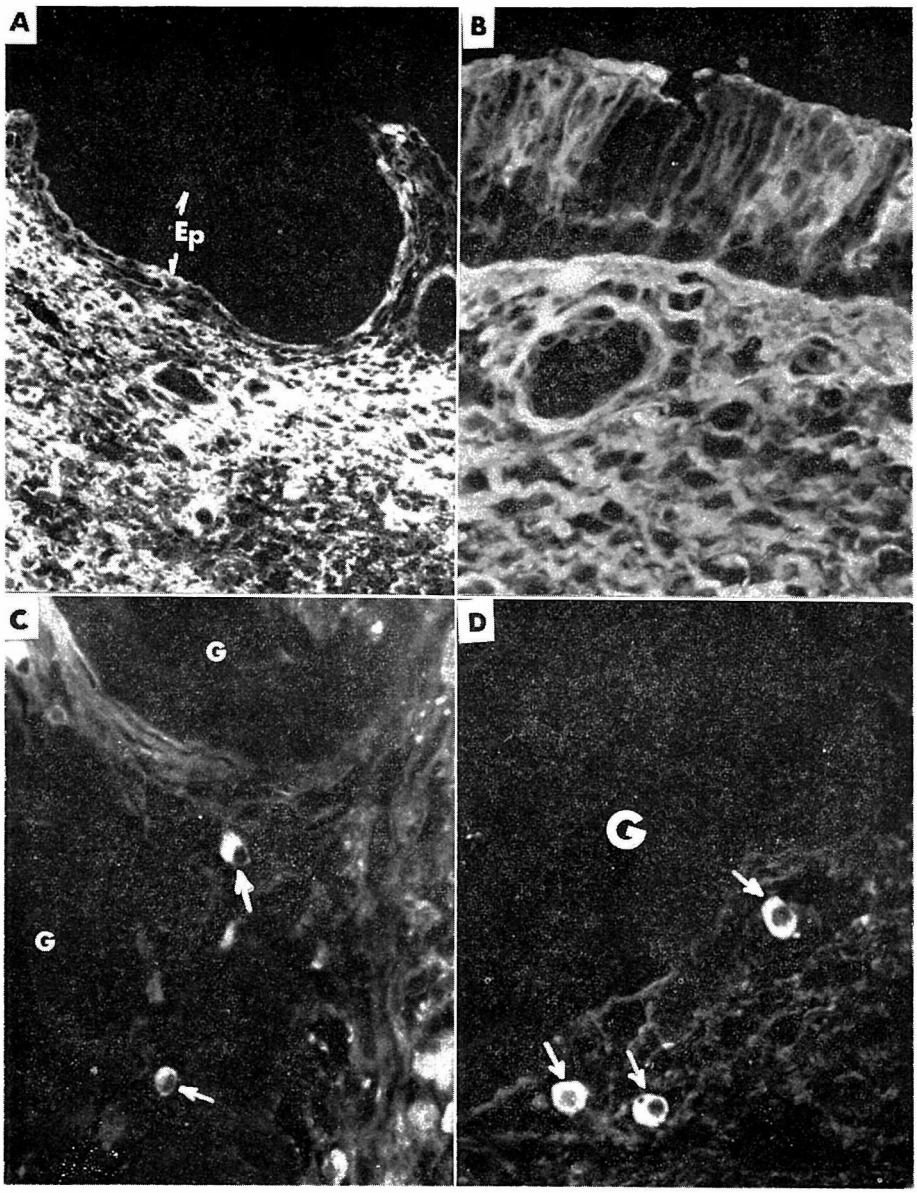

Figure 9. Immunofluorescence localization of IgG and IgM deposits and IgG- and IgM-forming cells.

A) Section of a maxillary sinus mucosa obtained from a patient with chronic rhinosinusitis, demonstrating that diffuse staining of anti-IgG is present in the subepithelial connective tissue, while the epithelium (Ep) is not stained (×312).

B) Section of nasal polyp showing that heavily diffuse staining is evidently found all over the section (×312). This finding suggests "spill-over" of serum elements into the lumen.

C) Section of a maxillary sinus mucosa obtained from a patient with chronic rhinosinusitis, showing IgG forming cells (single arrow) in the connective tissue between glandular acini (G) (×312).

D) Section of an ethmoidal sinus mucosa, showing IgM forming cells (single arrow) in the interstitium between the glands (G) (×312).

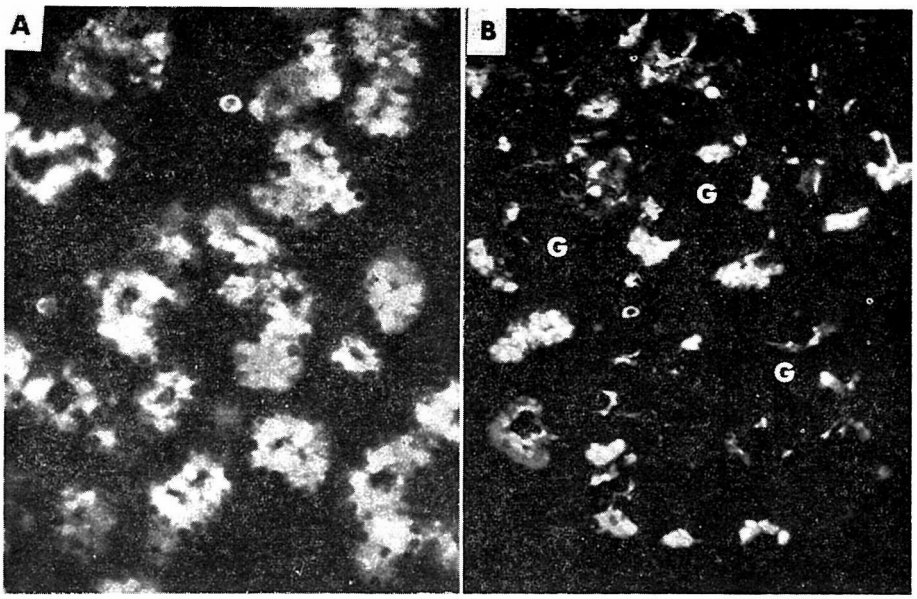

Figure 10. Immunofluorescence localization of lactoferrin.
A) Section of an ethmoidal sinus mucosa from a patient with chronic rhinosinusitis (×125). Note that course and speckled staining of anti-lactoferrin is observed in the cytoplasam of glandular acinar cells.
B) Section of a maxillary sinus mucosa form a patient with chronic rhinosinusitis, showing scattered lactoferrin deposits in the glandular cells (×125).

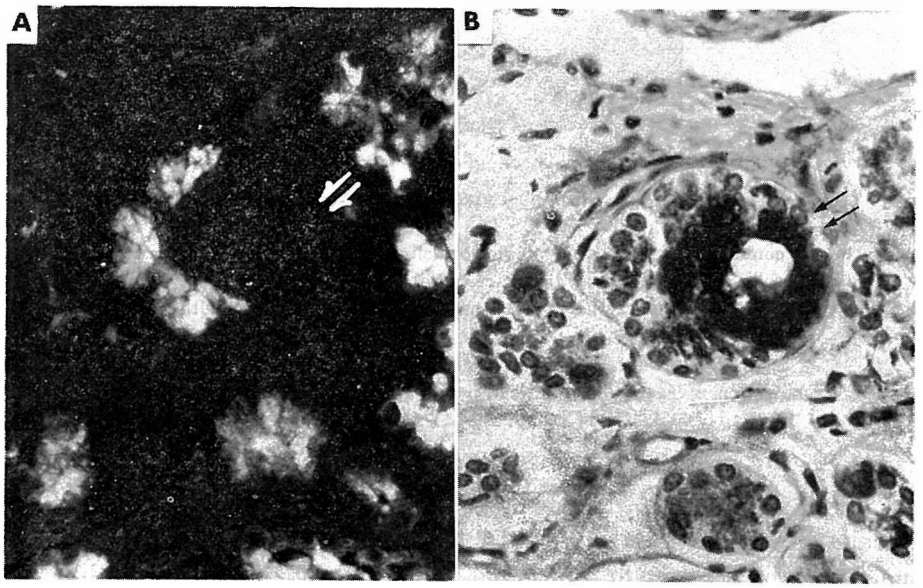

Figure 11. Photographs of the same section treated with anti-lactoferrin and consequently stained with PAS-AB.
A) Section of an inferior turbinate treated with anti-lactoferrin showing positive and negative (double arrows) glandular acinar cells (×312).
B) The same section subjected to PAS-AB staining, exhibiting strongly positive reaction (double arrows) (×312). This finding indicates that lactoferrin is localized in PAS-AB negative glandular cells.

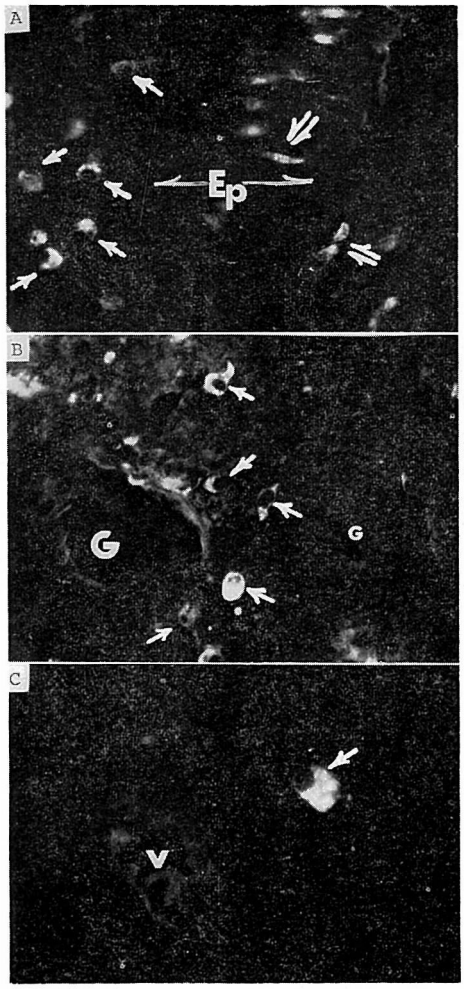

Figure 12. Immunohistochemical SC affinity test.

A) Section of a maxillary sinus mucosa from a patient without chronic rhinosinusitis, showing 10S IgA forming cells (single arrow) in the connective tissue beneath the epithelium (Ep). Some of the epithelial cells (double arrows) are reacted with anti-SC conjugate (×312).

B) Section of a maxillary sinus mucosa from a patient with chronic rhinosinusitis, showing 10S IgA forming cells (single arrow) in the interstitium between glandular acini (G) (×312).

C) Section of nasal polyp indicating 10S IgA forming cells (single arrow) around vessels (V) (×675).

IgA immunocytes positive for the SC affinity test was reduced in severely diseased mucosae. As can be seen in Figure 12, positive cells usually localizes close to epithelial layers or glands in which bright

fluorescent staining for SC is apparent in their cytoplasms. However, this finding was not constant. Positive cells, sometimes, are present near epithelial layers or glands which did not react with anti-SC conjugate (Fig. 12B). Immunocytes positive for SC affinity test are rarely observed around vessels (Fig. 12C).

DISCUSSION

There have been many studies dealing with immunoglobulins and other protein contents in nasal secretions[24)25)26)27)28)29)30)]. However, most investigators of these studies relied on materials obtained by instillation of saline into the nasal cavity and recovery of a part of the fluid with unknown amount of secretion. Protein determinants following saline irrigation can not indicate absolute values of concentrations, but only concentration ratios because the nasal secretion is diluted with unknown amount of saline. To avoid this matter, Dolovich et al.[31)] and Okuda et al.[32)] collected nasal secretions by sucking directly from the nasal cavity of patients with allergic rhinitis after stimulation of the secretion. The method for collection of nasal secretions employed in this study is based upon the adsorption of the secretion by weighed strips of filterpaper as originally described by Lorin et al[15)]. Although the secretion collected by this method is diluted, the absolute concentration of various protein components can be calculated.

In the present study the estimated mean total protein concentration in nasal secretions of 40 normal subjects was 733 mg/100ml. This value is relatively higher than that (636 mg/100ml) reported by Lorin et al[15)]. Results of this study showed that total protein contents in nasal secretions became elevated significantly in chronic rhinosinusitis. Mogi et al.[24)] reported, in their disc electrophoretic study of external secretions, that the electrophoretic pattern of purulent or mucopurulent nasal secretions is similar to that of corresponding sera while the pattern of glairy white secretions quite differed from that of sera. This evidence suggests that purulent or mucopurulent secretions contain considerable amounts of serum elements.

The mean IgA concentration of 60 mg/100ml for nasal secretions of the normal subjects, found in the present study, is in poor agreement with the mean value of 133 mg/100ml reported by Lorin et al.[15)], though the same method for collection of nasal secretions was adopted in both studies. Lorin et al. [15)] used a serum standard (7S) for IgA in nasal secretions and merely multiplied the results three times, since Bienenstock et al.[33)] suggested that the use of a serum standard (7S) for IgA

underestimates the levels of secretory IgA (11S) by approximately three times. In this study, a purified secretory IgA was employed as standard. Okuda et al.[34] obtained the mean IgA level of 73 mg/100ml for nasal secretions using secretory IgA as standard. As each sample of nasal secretions possesses both 7S serum type IgA and 11S secretory IgA in varying proportions, it is difficult to measure the exact amount of secretory IgA. However, it is evident in this investigation that IgA contents in nasal secretions were significantly elevated in chronic rhinosinusitis and allergic rhinitis.

It is now well known that secretory IgA molecule consists of two antigenically different protein units, IgA and secretory component (SC). Formerly, Rossen et al.[35] suggested that SC and IgA molecules are synthesized within the same plasma-like cell. However, subsequent studies [17)36)] as well as this work have denied this supposition by the fact that SC is only localized in the epithelial layer cells, glandular acinar cells, and in the lumen. IgA is produced by plasma cells present in the lamina propria, transported through the basement membrane to the epithelial layer, and then secreted to the lumen. IgA complexes with SC after reaching the epithelial zone. However, the exact location where complexing of IgA with SC occurs is still unsettled. Mogi[17] suggested that the conjugation could occur wherever they meet unless some inhibitor exists. Results of the present study showed that the most common immunocytes observed in the nasal specimens are IgA forming cells and distributed mainly beneath the epithelial layers, interstitial connective tissues between acini and less frequently around vessels. Lawton et al.[37] proposed a hypothesis that IgA is a primary stimulus for SC synthesis. However, our findings would not support this hypothesis because it was not constant evidence that positive stainings for SC in epithelium paralled with the presence of IgA forming cells existing close to the basement membrane of this epithelium. It has been reported that patients with selective IgA deficiency have normal or elevated levels of free SC in external secretions [38]. Mogi[17], in his immunoflourescent study, was of opinion that SC and IgA molecules are independently synthesized and that they are under independent control. Evidence is present in this study that although the number of IgA forming cells varied between different specimens, in general relatively fewer cells were observed in severely diseased mucosae and, contrary, in sound mucosae. Butler et al.[39] reported 7S IgA in nasal secretions being derived both from local production and by transudation from serum. In this study IgA contents in nasal secretions were elevvted in chronic rhinosinusitis. It is probable that serum IgA significantly contributes to the constitution of total IgA in nasal secretions

particularly from patients of chronic rhinosinusitis.

The present study demonstrated the existence of 10S IgA forming cells in the specimens of nasal tissue by the SC affinity test. However, the number of IgA immunocytes capable of binding with free SC was smaller than that of cells negative for the SC affinity test. It is also evidence that the number of 10S IgA immunocytes was reduced in severely diseased mucosae. On the basis of finding, obtained by the SC affinity test, that there is a preponderance of 10S IgA forming cells in the salivary glands and colon where secretory IgA is abundantly produced, Brandtzaeg[10] considered that immunocytes producing dimeric IgA (10S) seem to be characteristic of tissue containing SC producing epithels. Strober et al.[7] experienced case of a SC and secretory IgA deficiency patient. After careful examinations they suggested that SC provides a horming signal for IgA cells. However, Mogi[11] found that palatine tonsils, where SC is not synthesized, have dimeric IgA forming cells although not many. In the present investigation, as can be seen in Figure 12, 10S IgA forming cells usually localized close to epithelial lining or glands in which positive cells for anti-SC fluorescence are present. But this finding was not constant. It is still not clear whether or not these two types of cells belong to different cell lines or different development stages of the same line.

Lactoferrin is an antibacterial iron-binding protein in milk and other external secretions[12][13][14]. Masson et al.[12] and Tourville et al.[36] observed this protein existing in glands but not in epithelial linings of bronchial mucosa and parotid and submaxillary salivary glands. This study obtained similar findings. Lactoferrin was present in glandular acinar cells whereas any cells in the epithelial lining did not react anti-lactoferrin antibodies. As seen in Figure 11, serous cells produce this protein much more than mucous cells do. In the present study, it is noteworthy that lactoferrin in nasal secretions were reduced significantly in chronic rhinosinusitis. Moreover, PAS-AB positive glands were predominant in severely diseased mucosa. Nasal polyp is a resultant product of inflammatory process. Although this origin is still subject of controversy, Brandtzas et al.[41] and Bass et al.[42] reported no differences in the immunohistochemical charactesistics of nasal polyps and nasal mucosa. This study also failed to find obvious differences between nasal polyps and other mucosa of nasal specimens except that glands in polyps negative for anti-lactoferrin staining are abundant.

In conclusion, results of the present study show that although IgA content in nasal secretions was elevated in chronic rhinosinusitis, the

number of IgA immunocytes capable of binding SC was smaller than that of IgA immunocytes not binding SC. Moreover, lesser 10S IgA immunocytes were seen in specimens of severely diseased mucosa than in specimens of mild inflamed tissue. This evidence suggests that the function of local mucosal immunity is limited in the nasal and paranasal sinus cavities of patients with chronic rhinosinusitis, and that antibodies originating from the plasma contributes significantly to the constitution of IgA in nasal secretions.

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ACKNOWLEDMENT……We thank Prof. Toru Sekitani for his critical reading of this manuscript and we are grateful to Dr. Goro Mogi for his kind guidance and review of this manuscript.