Immunoglobulin M: Local Synthesis and Selective Secretion in Patients with Immunoglobulin A Deficiency

Abstract. *Local synthesis seems to be decisive for the selective secretion of 19S immunoglobulin M into parotid secretions. The "transport piece" is apparently not involved in the secretion of immunoglobulin M, for there is no association between the two components. The possible significance of the normal association of transport piece with secreted immunoglobulin A remains to be clarified.*

The major immunoglobulin of several external secretions is of the immunoglobulin A (IgA) class. It contains, however, an antigenic group that is not associated with the IgA of serum (*1*). A selective transport of IgA into the secretions may depend on this particular part of the molecule, tentatively termed the transport piece (*2*), the α_s-chain (*3*), or the T-chain (*4*). The transport piece seems to be produced by many kinds of secretory epithelial cells. Plasma cells that apparently synthesize IgA are commonly found adjacent to these epithelial cells (*1*, *5*, *6*); hence at least some of the secreted IgA is thought to be of local origin.

It has been claimed (*7*) that local synthesis and secretion of IgA may be normal despite a systemic deficiency of IgA. We were not able to substantiate this claim, as the results we now report indicate. On the other hand, local synthesis and selective secretion of immunoglobulin M (IgM) was demonstrated in patients with deficiency of IgA. Moreover, the secretion of IgM was independent of the transport piece, although the latter was synthesized by the patients.

Parotid secretions were collected bilaterally, with Curby caps, from the Stensen's ducts of four patients who had no detectable IgA in the serum, three patients with small amounts of immunoglobulins in the serum, and three healthy adults. The secretions were concentrated 3 to 40 times at 4°C with dried acrylamide cylinders (*8*) or 100 times by lyophilization; the content of immunoglobulin components was examined qualitatively by double diffusion and immunoelectrophoresis in agar gel (*9*), and quantitatively by single radial immunodiffusion (*10*).

Antiserums were obtained from rabbits immunized with immunoglobulin G (IgG) or IgM from normal human serum, or with IgA from human colostrum and parotid secretions. Activities against light polypeptide chains were removed as follows. Antiserum to IgG was adsorbed with pepsin-digested IgG [$F(ab')_2$]; antiserum to IgM was adsorbed with IgG; and antiserum to IgA was either adsorbed with $F(ab')_2$ alone or in addition with parotid secretion from patients with hypogammaglobulinemia—the latter to remove activity against transport piece. Antiserum to transport piece was obtained by adsorbing antiserum to colostral IgA or to parotid IgA with normal human serum until activities against serum IgA were completely removed. Antiserum to light chains was prepared by immunizing rabbits with $F(ab')_2$.

The concentrated secretions were chromatographed on Sephadex G-100 or G-200 equilibrated with 0.1*M* tris-HCl buffer (*p*H 8) containing 1.0*M* NaCl. Blue dextran, human albumin [Cohn fraction V (*11*)], and IgG were used as markers. The fractions were tested by radial and double diffusion before and after concentration.

Nasal mucosa was obtained by biopsy from two of the patients (G.B. and B.L.) deficient in IgA, and parotid tissue and gastric mucosa were obtained from one of them (B.L.). Parallel studies were carried out on tissues from subjects with normal amounts of immunoglobulins; six nasal, seven parotid, and eight gastric samples were included. The specimens were processed for localization of immunoglobulins by immunofluorescence (*6*, *12*). Orange-fluorescing IgG-containing plasma cells were distinguished from green-fluorescing IgA- or IgM-containing cells by double tracing, with the use of the red Kodak Wratten filter No. 23A and the green filter No. 57A (*13*).

The only significant immunoglobulin detectable in normal parotid fluid was IgA (Table 1). The secretions from the hypogammaglobulinemic patients were virtually devoid of immunoglobulins, but contained free transport piece, as described by South *et al.* (*2*). We found no IgA in the secretions of the patients entirely deficient in serum IgA. However, significant amounts of IgM were present in these secretions, and also IgG was readily detectable in two of them (Table 1). In all of them the ratio of IgG to IgM was markedly decreased compared with the ratio of IgG to IgM in the serum of the same patient (Table 1). Hence, the secretion of IgM cannot be explained by mere transudation of serum proteins, which would rather favor an increase in the ratio of IgG to IgM. The change of this ratio in the opposite direction indicates that some active or selective local process is involved in the secretion of IgM. Transudation may account for the IgG, however, which occurred in significant amounts only in the secretions of the two subjects with high concentrations of IgG in the serum (Table 1).

The transport piece was detected in the parotid fluids of the patients deficient in IgA. A selective secretion of IgM could therefore be mediated by this "piece," perhaps depending on its association with the secreted IgM, like its

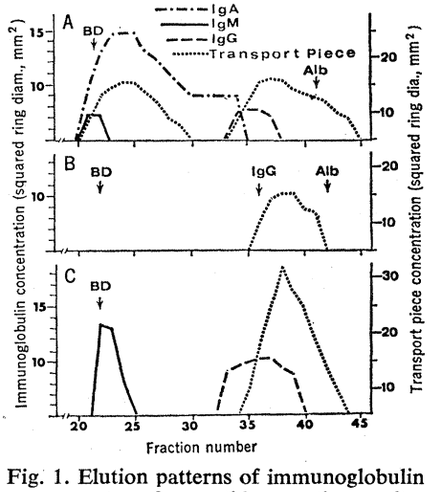

Fig. 1. Elution patterns of immunoglobulin components of parotid secretions after chromatography on Sephadex G-200. Column size, 1.5 by 88.0 cm; upward, flow, 1.5 ml cm^{-2} hr^{-1}. Fractions, 2.2 ml. Samples: (A) 100 ml of pooled normal secretion (concentrated 40 times); (B) 20 ml of secretion from hypogammaglobulinemic patients (concentrated 30 times); and (C) 60 ml of secretion from an IgA-deficient patient (concentrated 40 times). Distribution of IgA, IgM, IgG, and transport piece was determined by radial immunodiffusion with specific antiserums. The squared diameters (d^2) of the precipitin rings were used as concentration estimates. The relation between concentration and d^2 was not determined for transport piece. Values for immunoglobulins could be obtained from individual standard curves; for example, 12 mm^2 indicated on the average 1.35, 1.27, and 3.60 mg of IgG, IgM, and IgA per 100 ml, respectively. Elution positions (arrows) of the markers added to the samples were determined by absorbancy at 622 nm for blue dextran (*BD*), by absorbancy at 280 nm and by radial immunodiffusion for albumin (*Alb*), and by radial immunodiffusion for IgG.

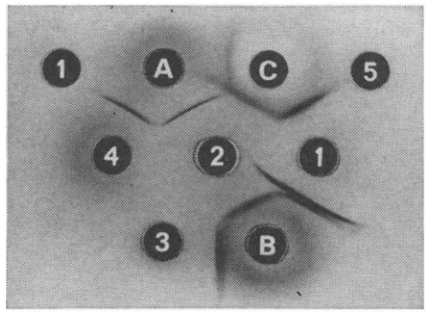

Fig. 2. Immunochemical analysis of parotid secretions concentrated 40 times. Antigens: *1*, pooled normal secretion; *2*, secretion from a patient lacking IgA; *3*, secretion from a hypogammaglobulinemic patient; *4*, pooled normal human serum (NHS); and *5*, NHS diluted 1 : 4. Antiserums: *A*, specific goat antiserum to IgM (anti-μ-chain); *B*, rabbit antiserum to parotid IgA adsorbed with F(ab')$_2$ (specific for α-chain and transport piece); and *C*, rabbit antiserum to parotid IgA adsorbed with NHS (specific for transport piece; contains human IgM among other serum proteins).

normal association with external IgA. However, no evidence for an association between transport piece and IgM or IgG could be obtained from chromatography, on Sephadex G-100 or G-200 (Fig. 1), of parotid fluids from patients deficient in IgA. The piece in these fluids behaved like the free transport piece in the secretions from the hypogammaglobulinemic patients, whereas in the normal secretion IgA-associated transport piece as well as small amounts of free piece were present.

Further attempts were made to demonstrate possible differences between the transport piece in IgA-deficient secretions and the free piece present in secretions from hypogammaglobulinemic and normal individuals. The first fraction obtained in the purification of parotid IgA on diethylaminoethyl cellulose (*1*) contained most of the free transport piece of normal secretion. The molecular radius (*14*) of that piece was compared by gel chromatography on Sephadex G-100 with the radii of those in the secretions of hypogammaglobulinemic and IgA-deficient patients. The three radii were not significantly different, the mean radius being 43.0 Å (standard deviation 0.13) with reference to human albumin (36.1 Å). Neither was there any difference in immunoelectrophoretic mobility; the transport piece migrated in all instances like parotid IgA in agar gel. A recent report (*15*) suggested that free transport piece contains light chain determinants. This could not be confirmed for the free piece from any of the three sources by testing fractions with an antiserum to F(ab')$_2$ in radial immunodiffusion after chromatography on Sephadex G-100 or G-200.

The immunochemical analysis (Fig. 2) supported the foregoing results. The free transport piece in normal secretion formed a reaction of identity with the precipitin line of the transport piece in IgA-deficient secretion; the latter line, moreover, joined the line of the free piece in the hypogammaglobulinemic secretion. These reactions were produced with an antiserum active against transport piece as well as against specific IgA determinants (α-chain). When the latter activity was removed by adsorption with normal human serum, both the free transport piece and the parotid IgA that contained attached transport piece were precipitated within the same line, which in turn joined the line of the transport piece in the IgA-deficient secretion. The latter line crossed the precipitin line of the IgM in this secretion, which is further evidence for lack of association between the two components. A reaction of identity occurred between the IgM in normal human serum and that in the IgA-deficient secretion. Since an antiserum against serum IgM was used, the result does not exclude the possibility that secreted IgM contains antigenic determinants not associated with serum IgM. However, no evidence for an increase in the molecular weight of secreted IgM could be obtained; it had sedimentation properties similar to the 19*S* IgM in serum (*16*).

Our results demonstrate that the transport piece does not act as a carrier protein for IgM, and another explanation has to be sought for the selective secretion of the latter in states of IgA deficiency. Also, there is still no definite evidence for a function of the

Table 1. Immunoglobulin content (in milligrams per 100 ml) of serum and parotid secretion determined by single radial immunodiffusion. Reference solutions of IgG contained protein isolated from normal serum; concentrations were calculated from the dry weight. Reference solutions of serum IgA contained 98 percent pure protein from pooled normal human serum; concentrations were determined by a microbiuret method with a standard curve based on weighed amounts of IgG. Protein concentrations of reference solutions of isolated parotid IgA were also determined by the biuret method with the same standard curve. The mean of values appearing in the literature served as a reference for the concentration of IgM in pooled normal human serum. Patient J.H. (age, 44 years) had hemorrhagic diathesis; patient B.L. (age, 53 years) had nasal polyposis; and patients G.B. and L.J. (age, 13 to 14 years) had ataxia telangiectasia.

Subjects	Serum			Ratio IgG: IgM	Parotid secretion			Ratio IgG: IgM*
	IgG	IgM	IgA		IgG*	IgM	IgA	
J.H.	3284	81	0*	40.5	0.21	0.87	0*	0.24
B.L.	2423	178	0*	13.6	.12	.35	0*	.35
G.B.	1439	105	0*	13.7	.02	.77	0*	.02
L.J.	1279	252	0*	5.1	.02	.21*	0*	.08
Normal	1230†	111†	328†	11.1	.04‡	.03*‡	3.93‡	1.40

*Figures calculated from quantitative values of concentrated samples. †Pooled from 100 healthy adults. ‡Pooled from three healthy adults.

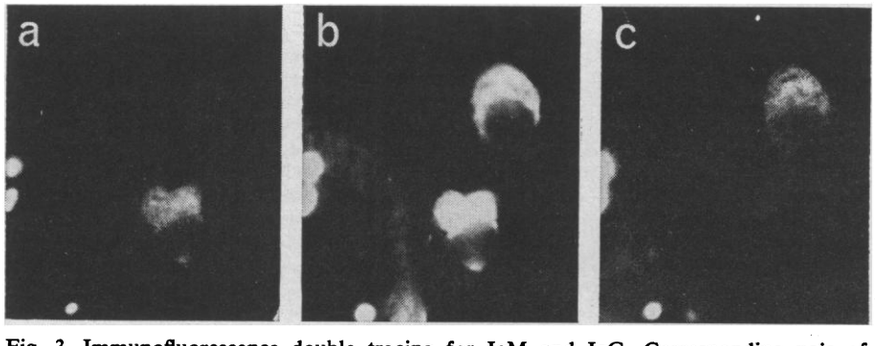

Fig. 3. Immunofluorescence double tracing for IgM and IgG. Corresponding pair of antiserums labeled with fluorescein isothiocyanate and rhodamine B isothiocyanate. Parotid specimen from a patient deficient in IgA. The same field of the section photographed through different filters: (a) an IgM-containing green plasma cell observed through the 57A filter; (b) the same IgM cell as well as an IgG-containing plasma cell seen through the K 430 filter; and (c) only the IgG-containing red cell observed through the 23A filter. Acinus with three yellow granules appears in the lower left corner because of autofluorescence. The ground substance and basement membrane zones of the connective tissue do not exhibit specific fluorescence since diffusible immunoglobulins were removed by washing in saline (*20*). Original magnification × 600.

transport piece in the normal selective secretion of IgA. On the other hand, local synthesis and consequently increased concentration of IgA in the connective tissue ground substance adjacent to secretory epithelia may be decisive for the normal secretion of this immunoglobulin (*6, 17*). Synthesis of IgA normally occurs in the parotid gland as well as adjacent to many other secretory epithelia, as judged from the presence of IgA-containing plasma cells (*1, 5, 6, 17*). Therefore we studied the occurrence of immunoglobulins in the parotid gland of a patient deficient in IgA. In contrast with normal parotid glands, the plasma cells and the connective tissue ground substance adjacent to the acini were devoid of IgA. However, sections with IgM- and IgG-containing plasma cells in numbers as high as 45 and 35, respectively, were commonly encountered throughout this parotid specimen (Fig. 3); the corresponding values for normal specimens did not exceed 5. Immunoglobulin M and IgG were also readily detectable in the connective tissue ground substance of the IgA-deficient patient. His gastric mucosa likewise contained a higher number of IgM-cells than the other gastric specimens and did not contain detectable IgA (*17*).

Other investigators (*1, 2, 18*) have noted that IgG and, particularly, IgM may occur in the parotid secretions of patients lacking IgA. Furthermore, Crabbé and Heremans (*19*) found an unusual high number of IgM-containing plasma cells in the intestinal mucosa of two such patients. This supports the foregoing results. The selective secretion of IgM in IgA-deficient patients may be explained at least in part by enhanced local synthesis of IgM; but such local synthesis does not seem to occur adjacent to all secretory epithelia, in contrast with the normal local synthesis of IgA. There was no consistently increased number of IgM-containing plasma cells in the two nasal specimens from patients deficient in IgA. Hence, IgM may not be regarded as a general compensatory secretory immunoglobulin in IgA-deficiency states, although it may function as such in some secretions. Immunoglobulin G may occur in the secretions of IgA-deficient patients on account of transudation from serum, in addition to enhanced local synthesis. The factors stimulating local synthesis of IgM and IgG adjacent to some secretory epithelia in the IgA-deficient patients remain unknown; the basis for the normal local synthesis of IgA also remains unknown.

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"Tactile" Stimulus Intensity: Information Transmission by Relay Neurons in Different Trigeminal Nuclei

Abstract. *Comparison of the information transmitted about the intensity of a steady "tactile" stimulus applied to facial skin by single trigemino-thalamic neurons in nucleus oralis and nucleus caudalis indicates that little information loss occurs at the medial lemniscal synaptic relay (nucleus oralis), but that it is gross within the nucleus caudalis.*

The parallel medial lemniscal and anterolateral components of the spinal somatic sensory pathways, although activated by "tactile" stimulation of the skin, do not contribute equally to sensory discriminative functions (*1*). This is true also for the homologues of these pathways in the brainstem trigeminal complex. Section of the spinal dorsal column, for example, produces among other things inability to locate or estimate the intensity of a "tactile" stimulus although its occurrence is appreciated. Conversely, spinal anterolateral tractotomy (or the analagous trigeminal tractotomy) increases slightly the threshold for detecting the stimulus, but does not significantly impair the subject's capacity to locate the stimulus and estimate its intensity.

Neural mechanisms which could account for these considerable functional differences between the medial lemniscal and anterolateral systems include (i) differing inputs from the cutaneous mechanoreceptors to the two systems, (ii) quantitative differences in synaptic linkage of primary neurons with second-order relay neurons in the two systems, with more effective transfer of information in the medial lemniscal relay, and (iii) differences in synaptic organization in the diencephalon, or some combination of these. Structural and functional differences in the diencephalon are established for the two pathways (*1*), but analyses of the static functional properties of single second-order neurons in these pathways have not previously demonstrated differences sufficient to account for their different contributions to tactile sensibility (*2*).

We have used some statistical methods of information theory (*3*) to compare information transmission by single trigemino-thalamic neurons within the trigeminal nucleus oralis (lemniscal) and nucleus caudalis (anterolateral) (*4*) concerning the intensity of a steady mechanical indentation of the skin of the face.

Unanesthetized cats, immobilized with intravenously administered gallamine triethiodide, were used for all experiments. Primary neurons were studied in the decerebrate animal by