THE IgA SYSTEM

I. Studies of the Transport and Immunochemistry of IgA in the Saliva*

By MARY ANN SOUTH,‡ M.D., MAX D. COOPER,‡ M.D., FRANK A. WOLLHEIM,§ M.D., RICHARD HONG, M.D., and ROBERT A. GOOD,|| M.D.

(From the Pediatric Research Laboratories, Variety Club Heart Hospital, and the Arthritis Unit, Department of Medicine, University of Minnesota, Minneapolis)

The mechanism of resistance to upper respiratory infections is poorly understood. Agammaglobulinemic patients, who are relieved in large part of lobar pneumonia, septicemia, meningitis, and recurrent urinary tract infections by gamma globulin, have persistent sinorespiratory tract infections, gastroenteritis, ileocolitis, and exudative enteropathy. This observation led us to consider the system involved in immunologic defense of the mucous surfaces in these patients.

Tomasi and coworkers (1) demonstrated that IgA is the predominant immune globulin in saliva and colostrum despite the fact that IgA is a minority component of the serum immunoglobulins. Tears (2), gastrointestinal secretions (2), tracheobronchial washings (3), and nasal secretions (4) have since been shown to contain IgA as the predominant immunoglobulin. Tomasi (5) has presented evidence that the IgA in the saliva and colostrum are alike but differ from serum IgA in that they have a higher sedimentation coefficient, 11S rather than the 7S of serum IgA, and that they have an antigenic determinant not present in serum.

These observations raise certain important questions. Why is IgA concen-

trated in mucous secretions, particularly as compared with IgG, the major serum immunoglobulin and a molecule of a size comparable to serum IgA? Is the IgA of secretions a locally produced unique type of IgA, basically different from serum IgA; or is there a mechanism for selective transport of a common IgA molecule into the secretions which is reflected in the immuno-chemistry of salivary IgA?

It is the purpose of this paper to report findings which shed light on these questions. We have observed that IgA can be selectively transported into saliva from serum, and that salivary IgA has immunochemical characteristics suggesting that it is composed of two separate immunochemical entities. It appears that salivary IgA differs from serum IgA only in that an additional protein is attached to the IgA molecule in its passage into the secretions. This additional protein can occur independently of IgA in agammaglobulinemic saliva and in saliva of many normal children. Although we have not directly demonstrated any transport role for this IgA-attached protein, we have found it operationally useful to call it the "transport piece".

Materials and Methods

Collection of Samples.—Parotid fluid (referred to hereafter as "saliva") was chosen as the secretion for study, since it could be easily obtained in large volumes from all our subjects, and since prior studies of this secretion had been most complete. Collections were made by means of a Curby cap (6), aided by stimulation with sour candy (Regal-Crown imported sour fruits, Murray Allen Imports, Inc., New York). Saliva samples were concentrated by negative pressure dialysis. Serum and saliva samples were tightly capped and kept frozen at −20°F until analyzed.

Antiserum.—Antiserum against human IgA was prepared in goats using IgA isolated from colostrum. In each instance the antiserum was absorbed with serum from a person lacking IgA. This antiserum gave a single line when reacted in immunoelectrophoresis and in gel diffusion against normal human serum. It gave a reaction of identity against colostrum and normal saliva, i.e. a single line, with spurring over the serum IgA line (see Fig. 1).

Immunochemical and Physicochemical Studies.—IgA levels were determined by a modification of the method of Heremans as previously described (7) using radial diffusion in agar with incorporated antiserum. IgG and IgM quantitations were performed with the Oudin tube technique (8). The antisera were prepared in rabbits by immunizing with several IgA myeloma proteins and with polyclonal IgM, prepared from a pool of rheumatoid arthritis sera (7). Antiserum against human IgG, prepared in monkeys, was obtained commercially (Immunology Incorporated, Glen Ellyn, Illinois, lot Cyn 34-1). Pure normal immunoglobulins prepared from serum as described (7) were used as standards. The standard error with both methods was ±10%. Since the rate of diffusion in agar is dependent upon molecular size, and since the standards were prepared with IgA purified from serum, all the apparent IgA values in saliva, as compared to serum IgA values, are falsely low, but the comparison among different saliva samples is valid. The sensitivity of the methods was: for IgA, 1 to 2 mg/100 ml; for IgM, 2 to 5 mg/100 ml; and for IgG, 5 to 10 mg/100 ml.

Double diffusion in gel was done by the method of Ouchterlony (9). Immunoelectrophoresis was done by the micromethod of Scheidegger (10). Salivary IgA was isolated by the method of Tomasi (5). The complement-fixing activity of isolated salivary IgA, aggregated by heating at 63°C for 30 min, was tested by the method of Mayer (11).[1]

---

[1] This test was kindly performed by Dr. Henry Gewurz.

The IgA in normal saliva was reduced by the method of Fleishman et al. (12). The saliva was incubated with 2-mercaptoethanol 0.2 M in 0.3 M trihydroxy-methylamino-methane HCl buffer, pH 8.2, at room temperature for 1 hr. Iodoacetamide was added in equimolar amounts and the mixture allowed to stand at 0°C for 1 hr. The saliva was then dialyzed against sodium borate buffer, pH 8.0.

Infusion Studies.—Six agammaglobulinemic patients were selected for infusion studies. They ranged in age from 8 to 41 yr and were of both "acquired" and congenital types. None had detectable IgA in serum or saliva. A total of 1 to 2 liters of fresh frozen plasma was in-
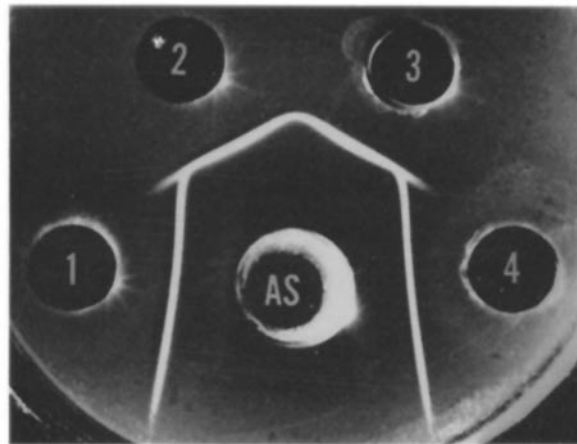

FIG. 1. Antigenic relationship of serum and salivary IgA, illustrated by immune diffusion in gel. AS, antiserum against human colostral IgA prepared in goats; wells 1 and 4, serum from a normal person; well 2, saliva from a normal person; and well 3, IgA isolated from saliva. All saliva samples in this and subsequent illustrations were parotid secretions and were concentrated about X25. Colostral and salivary IgA contain an antigenic determinant not present in serum IgA, as illustrated by the spurring of the salivary IgA line over the serum IgA line. This extra component is referred to in the text as "transport piece".

fused into each of these patients during an interval varying from 1 to 3 days. Specimens of serum and saliva were collected before the infusions and each day following the last infusion for a period of 5 days. The serum specimens were analyzed to quantitate the levels of IgA attained by the infusion, and the saliva was analyzed to discover any IgA, IgG, or IgM transferred from the infused plasma into the saliva.

RESULTS

When IgA isolated from saliva[2] was tested by Ouchterlony analysis with an antiserum containing antibodies to colostral IgA, it was partially identical with serum IgA and it had an extra antigenic determinant as previously demonstrated by Tomasi (5) (Fig. 1).

Isolated salivary IgA, aggregated by heat, did not fix complement. Similar positive control experiments carried out with heat-aggregated IgG produced a

---

[2] Saliva refers to parotid secretions throughout these results.

sharp drop in the concentration of whole complement activity in the standard hemolytic assay.

Studies of Infusion of Agammaglobulinemic Patients with Normal Serum.— Table I presents the findings of the infusion studies. Only 1 subject (M.L.) attained a serum level of more than 100 mg% IgA, and IgA appeared in this subject's saliva at the collections on the 1st, 3rd, and 4th days after infusion. The serum/saliva ratio was approximately 1000/1. There was no detectable IgM or IgG by Oudin tube or Ouchterlony techniques in any of these saliva samples or samples from any of the other transfused patients, so the transport of IgA was selective. Contamination of the samples with serum could not account for the presence of IgA since this source would have supplied IgG and IgM as

TABLE I

Infusion of Agammaglobulinemic Patients with Normal Plasma

Agammaglob- ulinemic recipient	Amount of serum infused	Peak serum level of IgA	Appearance of IgA in saliva	Appearance of IgG and IgM in saliva
	cc	*mg %*		
Congenital sex-linked (M.L.)	1600	150	+++*	0
Congenital sex-linked (T.G.)	1000	75	+‡	0
Congenital "sporadic" (J.K.)	1600	88	0§	0
"Acquired" (L.L.)	1000	80	0§	0
Congenital sex-linked (T. L.)	2000	90	0§	0

* Indicates appearance of 0.1 mg of IgA per 100 ml saliva on 3 different days.

‡ The individual specimens collected on 5 successive posttransfusion days were pooled and concentrated X 225.

§ Each sample of saliva was concentrated 50 to 100 times prior to analysis.

well. Because of difficulty in obtaining cooperation from this child, only small amounts of saliva could be obtained, and extensive physicochemical analysis of the secreted IgA could not be carried out. Fig. 2 compares immunoelectrophoretic patterns of normal saliva, and the saliva of this agammaglobulinemic patient before and after infusion. The antiserum used was specific for serum IgA. The IgA which appeared in the saliva after infusion produced an immunoelectrophoretic pattern similar to the normal salivary IgA.

Another agammaglobulinemic patient showed a trace of IgA in the saliva following infusion of normal serum. Although an IgA line was observed in a pooled and concentrated posttransfusion specimen, the IgA level was too low to be accurately measured; still this sample contained no detectable IgG or IgM and, as in the prior study, supported the concept of selective transport of IgA into the parotid secretions.

Studies of the Secretion-Specific Gamma A Determinant, "Transport piece".—

An untreated agammaglobulinemic patient's saliva, when reacted in agar diffusion with antibody against purified colostral IgA, gave a distinct precipitin band. By contrast, no precipitin line formed when the same saliva specimen was reacted with antibody against purified serum IgA. This precipitin band was subsequently shown to give a reaction of partial identity with the precipitin
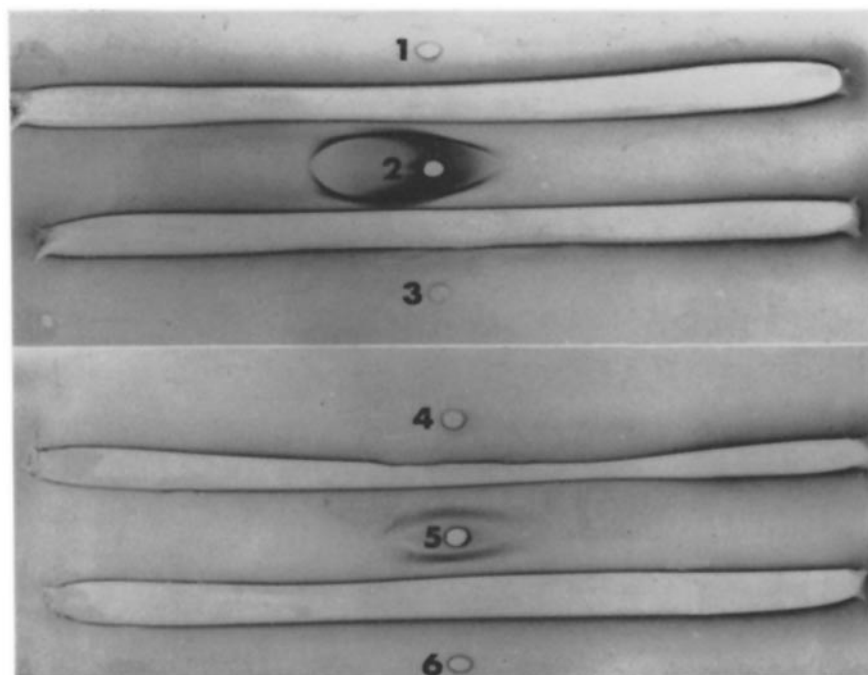

Fig. 2. Immunoelectrophoresis of saliva from a normal person and from an agamma-globulinemic patient before and after infusion with normal plasma. Wells 1 and 3 are empty; well 2, saliva from a normal person; wells 4 and 6, saliva of M. L. before plasma infusions; and well 5, saliva of M. L. after plasma infusion. All the troughs contain serum from rabbits hyperimmunized with purified IgA myeloma globulin. The cathode is at the left. After intravenous infusion of fresh whole plasma (1600 ml) IgA appeared in the saliva collected from this agammaglobulinemic patient on 3 successive days.

band formed when the antiserum against colostral IgA was reacted with normal salivary IgA, but complete nonidentity to the reaction produced with normal serum IgA (Fig. 3). This is the antigenic determinant originally described by Tomasi et al. (5)[3] as attached to salivary and colostral IgA globulin, and is referred to as the "transport piece" in this and previous reports (13, 14). This

---

[3] This relationship was confirmed with antiserum kindly sent to us by Dr. Tomasi.
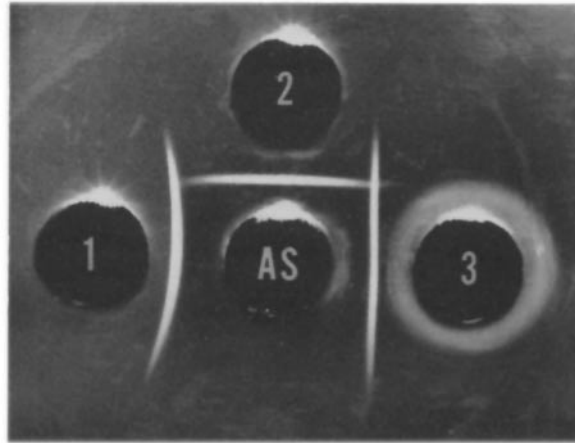
FIG. 3. Antigenic relationship of transport piece to IgA, illustrated by immune diffusion in gel. AS, antiserum against human colostral IgA prepared in goats; well 1, saliva from a normal person; well 2, saliva from an agammaglobulinemic person; and well 3, serum from a normal person. The transport piece, occurring independently in the agammaglobulinemic saliva, is partially identical to normal salivary IgA but nonidentical to serum IgA. Note also the character of the normal salivary IgA line. Its curve indicates that it is composed of larger molecules than is the serum IgA globulin.
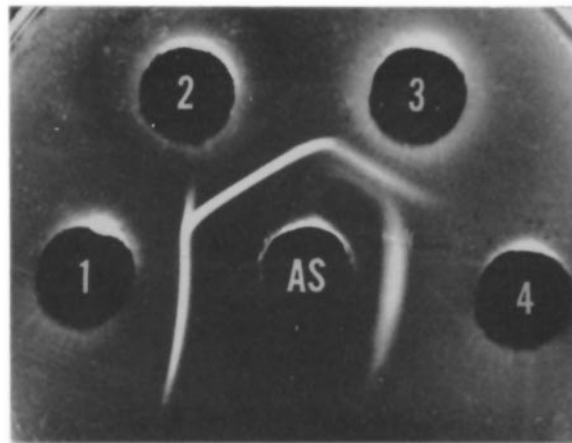

FIG. 4. Dissociation of the salivary IgA from the transport piece by mercaptoethanol reduction, shown by immune diffusion in gel. AS, antiserum against human colostral IgA prepared in goats; well 1, saliva from a normal person; well 2, serum from a normal person; well 3, saliva from a normal person reduced with 2-mercaptoethanol; and well 4, saliva from an agammaglobulinemic patient. After incubation with 0.1 M mercaptoethanol, salivary IgA is dissociated into two components, one antigenically identical to serum IgA and the other antigenically identical to the free transport piece as it occurs in the saliva of agammaglobulinemic patients.

substance has subsequently been demonstrated in the saliva of each of 18 agammaglobulinemic patients studied to date, 2 patients with ataxia-telangiectasia who lacked IgA in serum and saliva, a healthy adult with the anomaly described by Rockey, Hanson, Heremans, and Kunkel (15) who had no IgA in serum or saliva[4] and a 12-hr-old infant before the first feeding, with no demonstrable IgA in serum or saliva. In more than 100 determinations we have not found any saliva sample which lacks the transport piece, although concentrating the saliva sample is usually necessary for its demonstration.
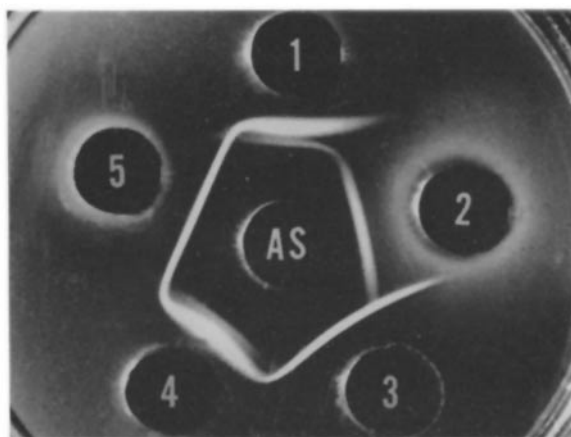
Fig. 5. Occurrence of free transport piece in the parotid secretions of a child, illustrated by immune diffusion in gel. AS, antiserum against human colostral IgA prepared in goats; wells 1 and 4, saliva of a child; well 2, saliva of an agammaglobulinemic patient; well 3, saliva of a normal adult; and well 5, serum of a normal adult. The child's saliva contains free transport piece, represented by the bands nearest the center well, which is antigenically identical to the transport piece in the agammaglobulinemic patient's saliva. Newborn babies, although they have no IgA in saliva, have transport piece.

In the reduced and alkylated form the normal salivary IgA is dissociated to form two distinct bands when reacted against anticolostral IgA, as had been demonstrated by Tomasi and associates (5). One of these lines is identical with the serum IgA line, and the other is identical with the transport piece line in agammaglobulinemic saliva (Fig. 4). The transport piece has also been found "free" in normal saliva, most frequently in children's saliva (Fig. 5).

Fig. 6 compares the immunoelectrophoretic patterns of the agammaglobulinemic saliva and normal saliva. The free transport piece is shown to migrate in the $gamma_1$ region. The transport piece determinant is demonstrated in both normal saliva and agammaglobulinemic saliva, but the IgA determinant in the normal saliva only.

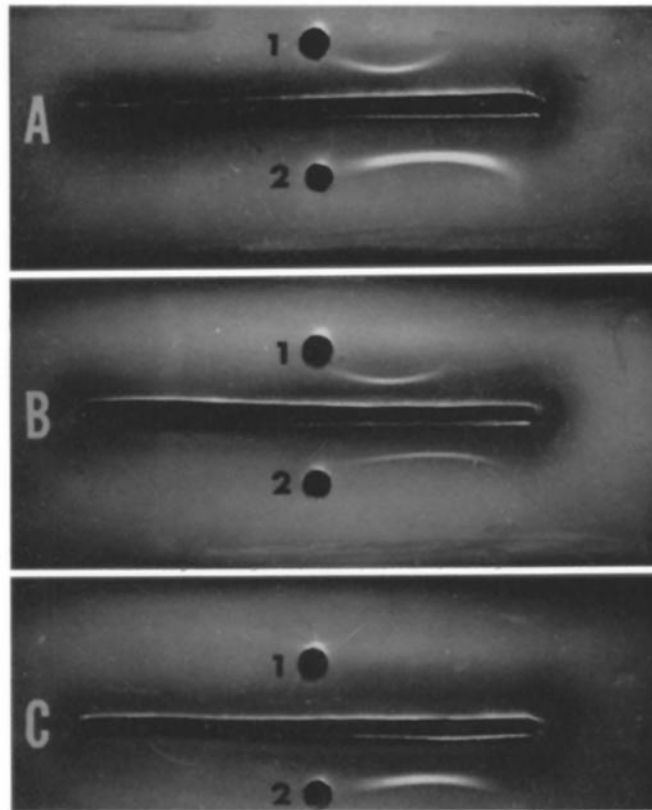[4] Patient of Dr. Douglas Heiner, Salt Lake City.

FIG. 6. Immunoelectrophoretic properties of free transport piece. Well 1, saliva from an agammaglobulinemic patient; well 2, saliva from a normal person; trough A, antiserum against human colostral IgA prepared in goats; trough B, the same antiserum absorbed with lyophilized normal serum; and trough C, the same antiserum absorbed with lyophilized agammaglobulinemic saliva. The cathode is at the left. The transport piece, a gamma$_1$-migrating protein, is attached to the IgA in normal saliva but occurs independently in the agammaglobulinemic patient's saliva. Serum IgA, not illustrated here, has a similar migration.

DISCUSSION

These observations are of interest from several points of view. First, we have been able to confirm and extend the discoveries of Tomasi and associates which show clearly that IgA in the saliva and colostrum is immunochemically different from IgA in the serum. The salivary IgA can be dissociated into two antigenically distinct components by treatment with 2-mercaptoethanol. One of the components appears immunochemically identical to serum IgA. The other component is immunochemically identical to a protein, the "transport piece", found in the saliva of patients completely lacking both serum and salivary IgA.

Thus far the IgA component has been found only in combination with the transport piece component in the saliva of children and adults. However, the transport piece is present also in an unbound form in the saliva of most children and some adults. It may be that transport piece is present in free form in young children's saliva because of the gradual increase of IgA production during childhood. The IgA serum levels rise slowly with age (16–18) and reach average adult levels around puberty (7). It could well be that children have an excess of transport piece because their formation of the IgA component of the salivary IgA is relatively deficient when compared to that of older children and adults.

Perhaps of greatest interest in these studies is the finding that patients with agammaglobulinemia and ataxia-telangiectasia who do not synthesize the serum IgA nonetheless possess the transport piece in saliva. Thus, from these patients the transport piece can be isolated and studied without need to consider its immediate relationship to the serum IgA determinant. Further, agammaglobulinemic patients, lacking a plasma cell system for synthesizing IgA (19) as well as the other immunoglobulins, present a strong argument for the concept that the transport piece is formed in cells different from those responsible for production of the IgA and the other immunoglobulins. This is of particular interest because the transport piece travels electrophoretically as gamma$_1$ globulin.

Immunohistochemical studies previously carried out on salivary glands are consonant with these findings, since Tomasi and associates (5) showed that the serum IgA component could be identified by the fluorescent antibody technique in the plasma cells of the salivary gland, whereas the additional component immunochemically related to salivary IgA but not to serum IgA was detectable only in the acinar cells adjacent to collecting ducts (perhaps the ductal epithelial cells) of the salivary gland.

In these studies we have further shown that IgA can be specifically transported into saliva of patients with agammaglobulinemia, an observation which can be taken as support for the concept that the IgA component in saliva is the same as that in serum, and is derived also from the plasma cells. The transport of IgA from serum to saliva in man is not surprising, since antibodies administered intravenously had been shown to appear in the secretions of the mouse (20), rabbit (21), and guinea pig (22), although these antibodies were not known to be of the IgA type. In our studies it is particularly important to emphasize that the transport of IgA into saliva occurred in the absence of transport of the other two immunoglobulins, IgG and IgM, even though the latter two components were present in the serum in higher concentrations after plasma infusions into agammaglobulinemic patients than was IgA. These observations suggest the existence of a specific transport mechanism for IgA. Such a transport mechanism might, indeed, involve the transport piece, with production of the transport piece by the epithelial cells, reception of the IgA

from the plasma cells by specific combination with the transport piece, and subsequent secretion of the combined molecule into the lumen of the salivary duct.

The infusion studies in the agammaglobulinemic patients stress another important point, namely that high concentrations of IgA are necessary to achieve such transport. Indeed, in our experiments transport of IgA from serum to saliva was observed in only 2 of 5 patients: in pooled posttransfusion samples of 1 child, but in 3 of 5 posttransfusion samples in the patient with the highest serum IgA level. In keeping with these observations, other investigators (5, 23) failed to demonstrate this transportation after infusion of relatively small amounts of I^{131}-labeled IgA. Is the need for high concentrations at the epithelial cell surface the reason for such large numbers of plasma cells in the lamina propria of the gut, and for their presence in the interstitial tissue of the salivary glands and mammary glands? Local production of IgA might be expected to deliver high concentrations of this globulin directly to the epithelial cells for subsequent combination with transport piece and delivery into the secretions. In this regard, it is of interest that Crabbé (24) has shown that an average of 80% of the plasma cells of the intestinal lamina propria are IgA-producing cells, a much higher proportion of IgA producers than is seen in lymph nodes and spleen (25, 26). Hochwald et al. (27) showed that C^{14}-labeled amino acids are incorporated directly into IgA in salivary gland as well as mammary gland tissue cultures. Evidence of local specific antibody production in many species has been reviewed by Pierce (28). This evidence, including the observations of antibody appearing earlier in secretions than in serum, or in higher titer, or occasionally exclusively in secretions, speaks strongly for local antibody production.

Heat aggregated IgA and IgA antibodies from serum do not utilize complement (29–31). In our study, aggregated salivary IgA also failed to show any complement-fixing activity. Since saliva and intestinal contents are, by pH and salt concentration, anticomplementary, a complement-requiring antibody system would be of no biological advantage in these fluids. The IgA group of antibodies function either alone or with the aid of biologically active substances other than complement. The transport piece might furnish such aid, or might serve either to stabilize the IgA molecule or in some other way make it better able to act in the milieu of the mucous surfaces.

The experiments of nature represented by patients lacking IgA: the agammaglobulinemics treated only with IgG, and ataxia-telangiectasia patients, support the concept that this system has biologic significance. The rare healthy persons with no IgA in serum or secretions seem to have been able to compensate by delivery of both IgG and IgM into the secretions, as reported by Rockey and associates (15) and Tomasi and associates (5), and as found in 1 person by

us.[5] These persons may present a key to greater understanding of the whole local protective process.

SUMMARY

1. Five patients with congenital or acquired agammaglobulinemia, lacking detectable IgA in serum or saliva, were transfused with 1 to 2 liters of normal plasma. In 2 of these patients IgA was demonstrated in parotid saliva collected after transfusion, but in none of the 5 was salivary IgG or IgM found. This observation indicates the selective transport of IgA into saliva.

2. The observation by others of an immunochemical difference between serum and salivary IgA globulin was confirmed. In contrast to serum IgA, salivary IgA is attached to a protein having antigenicity which migrates as a gamma₁ globulin. We have termed this protein component "transport piece".

3. The transport piece has been found in an unbound form in the saliva of persons completely lacking IgA: agammaglobulinemic patients, ataxia-telangiectasia patients, a healthy person lacking IgA, and a newborn infant. Free transport piece still occurs in the normal child's saliva after IgA production begins. By adulthood there is usually no free transport piece in the saliva.

4. Heat-aggregated salivary IgA, like heat-aggregated serum IgA, does not fix complement.

5. Our findings offer support for the view that there is a distinct local antibody system for the protection of the mucous surfaces.

BIBLIOGRAPHY

1. Tomasi, T. B., Jr., and Zigelbaum, S., The selective occurrence of gamma₁ₐ globulins in certain body fluids, *J. Clin. Invest.*, 1963, **42**, 1552.
2. Chodirker, W. B., and Tomasi, T. B., Jr., Gamma-globulins: Quantitative relationships in human serum and nonvascular fluids, *Science*, 1963, **142**, 1080.
3. Keimowitz, R. I., Immunoglobulins in normal human tracheobronchial washings, *J. Lab. and Clin. Med.*, 1964, **63**, 54.
4. Remington, J. S., Vosti, K. L., Lietze, A., and Zimmerman, A. L., Serum proteins and antibody activity in human nasal secretions, *J. Clin. Invest.*, 1964, **43**, 1613.
5. Tomasi, T. B., Jr., Tan, E. M., Solomon, A., and Prendergast, R. A., Characteristics of an immune system common to certain external secretions, *J. Exp. Med.*, 1965, **121**, 101.
6. Curby, W. A., Device for collection of human parotid saliva, *J. Lab. and Clin. Med.*, 1953, **41**, 493.

---

[5] Patient of Dr. Douglas Heiner.

7. Wollheim, F. A., and Williams, R. C., Jr., Immunoglobulin studies in six kindreds of patients with adult hypogammaglobulinemia, *J. Lab. and Clin. Med.*, 1965, **66**, 433.

8. Wollheim, F. A., and Williams, R. C., Jr., Studies on the macroglobulins of human serum. I. Polyclonal immunoglobulin class M (IgM) increase in infectious mononucleosis, *N. England J. Med.*, 1966, **274**, 61.

9. Ouchterlony, Ö., Diffusion-in-gel methods for immunological analysis, *Progr. Allergy*, 1958, **5**, 1.

10. Scheidegger, J. J., Une micro-méthode d'immuno-électrophorèse, *Internat. Arch. Allergy and Appl. Immunol.*, 1955, **7**, 103.

11. Mayer, M. M., Complement and complement fixation, *in* Kabat and Mayer's Experimental Immunochemistry (E. A. Kabat, editor), Springfield, Charles C Thomas, 2nd edition, 1961, 209.

12. Fleishman, J. B., Pain, R. H., and Porter, R. R., Reduction of gamma globulins, *Arch. Biochem. and Biophysics, (Suppl. 1)*, 1962, 174.

13. South, M. A., Wollheim, F. A., Cooper, M. D., and Good, R. A., Selective transport of IgA and its biologic significance in human saliva, *Fed. Proc.*, 1965, **24**, 502.

14. South, M. A., Wollheim, F. A., Warwick, W. J., Cooper, M. D., and Good, R. A., Local deficiency of immune globulin A in the saliva of patients with chronic sinopulmonary disease, *J. Pediat.*, 1965, **67**, 941.

15. Rockey, J. H., Hanson, L. A., Heremans, J. F., and Kunkel, H. G., Beta 2A-aglobulinemia in two healthy men, *J. Lab. and Clin. Med.*, 1964, **63**, 205.

16. Roth, N., Sur semiquantitativen Erfassung der beiden Serum-immun-globuline Beta$_{2A}$ und Beta$_{2M}$ in Neugeborenen- und Kindesalter, *Ann. Paediat.*, 1962, **199**, 548.

17. Huntley, C. C., and Lyerly, A., Immune globulin determinations in allergic children, *Am. J. Dis. Child.*, 1963, **106**, 545.

18. West, C. D., Hong, R., and Holland, N. H., Immunoglobulin levels from the newborn period to adulthood and in immunoglobulin deficiency states, *J. Clin. Invest.*, 1962, **41**, 2059.

19. Good, R. A., Agammaglobulinemia: an experimental study, *Am. J. Dis. Child.*, 1954, **88**, 625.

20. Fazekas de St. Groth, S., and Graham, D. M., Studies in experimental immunology of influenza. X. Passive immunity and its enhancement, *Australian J. Exp. Biol. and Med. Sc.*, 1954, **32**, 369.

21. Schubert, O., Diphtheria antitoxin in human and animal saliva, *Travaux de L'Institut d'Hygiène Publique de L'État Tchécoslovaque*, 1938, **2**, 45.

22. Burrows, W., and Ware, L. L., Studies on immunity to Asiatic cholera. VII. Prophylactic immunity to experimental enteric cholera. *J. Infect. Dis.*, 1953, **92**, 164.

23. Steihm, E. R., Vaerman, J. P., and Fudenberg, H. H., Immune globulin survival studies following transfusion, American Pediatric Society, 75th Annual Meeting, Philadelphia, May, 1965.

24. Crabbé, P. A., Carbonara, A. O., and Heremans, J. F., The normal human intestinal mucosa as a major source of plasma cells containing gamma A-immunoglobulin. *Lab. Invest.*, 1965, **14**, 235.

25. Mellors, R. C., and Korngold, L., The cellular origin of human immunoglobulins (γ_2, γ_{1M}, γ_{1A}), *J. Exp. Med.*, 1963, **118**, 387.
26. Bernier, G. M., and Cebra, J. J., Frequency of distribution of alpha, gamma, kappa, and lambda polypeptide chains in human lymphoid tissues, *J. Immunol.*, 1965, **95**, 246.
27. Hochwald, G. M., Jacobson, E. B., and Thorbecke, G. J., C^{14}-amino acid incorporation into transferrin and beta$_{2A}$-globulin by ectodermal glands in vitro, *Fed. Proc.*, 1964, **23**, 557.
28. Pierce, A. E., Specific antibodies at mucous surfaces, *Vet. Rev. and Annotations*, 1959, **5**, 17.
29. Heremans, J. F., Vaerman, J. P., and Vaerman, C., Studies on the immunoglobulins of human serum. II. A study of the distribution of anti-Brucella and antidiphtheria activities among gamma$_{SS}$, gamma$_{1M}$, and gamma$_{1A}$-globulin fractions, *J. Immunol.*, 1963, **91**, 11.
30. Ishizaka, T., Ishizaka, K., Borsos, T., and Rapp, H. J., C'1 fixation by human isoagglutinins: fixation of C'1 by gamma G and gamma M but not by gamma A antibody, submitted for publication.
31. Ishizaka, K., Ishizaka, T., Lee, E. H., and Fudenberg, H., Immunochemical properties of human gamma A isohemagglutinin. I. Comparisons with gamma G- and gamma M-globulin antibodies, *J. Immunol.*, 1965, **95**, 197.