*A MODEL FOR COLOSTRAL IGA**

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Tomasi[1] first demonstrated that normal serum immunoglobulin relations do not obtain in mucous surface secretions and that IgA, which is only a minor component of the serum immunoglobulins, is the major antibody component of the saliva, colostrum, urine, tears, bile, and small intestinal secretions. Work by others[2–4] extended these findings to other body fluids, and it is now generally accepted that IgA is the major antibody system of the mucous surfaces which open to the exterior. It has also been shown that the polymeric forms of the surface antibody IgA possess an antigenic fragment lacking in serum IgA. The observation that this fragment is secreted by agammaglobulinemic patients and that it is easily separable from the polymeric form suggests that a relatively simple modification of serum IgA in the glandular tissue might result in the formation of an antibody specifically adapted for surface function.[5] There is ample clinical evidence suggesting the importance of IgA as a unique antibody system of mucous surfaces. Thus, agammaglobulinemic patients continue to suffer from chronic progressive pulmonary infection despite administration of large amounts of IgG. Lack of salivary IgA (and by inference, bronchial IgA) in ataxia-telangiectasia is also associated with sino-bronchitis,[6] and persistent steatorrhea accompanies lack of IgA-producing cells in the intestine.[7] Recent studies in our laboratory now permit us to propose a reasonable model for colostral IgA antibody. This model suggests that minor structural adaptations relate the serum and colostral antibodies.

Methods.—Antisera: The antisera employed were prepared in goats and rabbits by the injection of appropriate antigens incorporated in Freund's complete adjuvant. Colostral preparations enriched in IgA, serum IgA, or light chains prepared from IgG served as antigens. An antiserum directed only against the specific antigenic determinant of colostral (or salivary) IgA was prepared by absorption of the colostral antibody by serum and a colostral preparation which had been depleted of IgA.

Reduction and alkylation was performed essentially as described by Fleischman *et al.*[8] Protein solutions at concentrations of 15–20 mg/ml in 0.3 *M* Tris HCl buffer, pH 8.2, were reduced with 0.3 *M* redistilled 2-mercaptoethanol (2-ME). Iodoacetamide was recrystallized from hot water and equimolar amounts of a 1 *M* solution were added to the reduced protein mixture.

Aqueous solutions of guanidine hydrochloride were absorbed repeatedly with charcoal until the absorbance at 280 mμ was 0.05 or less. The molarity of solutions used was determined by refractometry.[9]

Gel filtration was performed in Sephadex® G-200 or G-100. In experiments in which upward flow was used, density stabilization was accomplished by following the sample solution with a small volume (15–25 ml) of eluting solution of slightly greater density (usually 5.5 *M* guanidine) before starting the final system.[10]

Amino acid analyses were performed on the Beckman automatic amino acid analyzer.

Colostrum samples were obtained during the first 24 hr postpartum. Samples containing large amounts of IgA as determined by qualitative examination of immunoelectrophoretic analyses were pooled, and purification of colostral IgA was accomplished as previously described.[11]

Sedimentation studies and molecular weight determinations were performed in the Beckman model E ultracentrifuge using schlieren or interference optics. Plates were measured in a Nikon comparator at 10–50× magnification. Molecular weights were determined by the Yphantis

meniscus depletion method.[12] A partial specific volume of 0.74 was assumed. The molecular weight measurements were made in 5 *M* guanidine solutions and calculations were performed on a Control Data 1604 computer.[13]

Heavy and light chains were prepared in 1 *M* propionic acid as described by Fleischman *et al.*[8] IgG isolated by DEAE chromatography[14] and the protein from the major peak of the saline-borate Sephadex® column which was re-reduced and alkylated were used as starting materials (see *Results*).

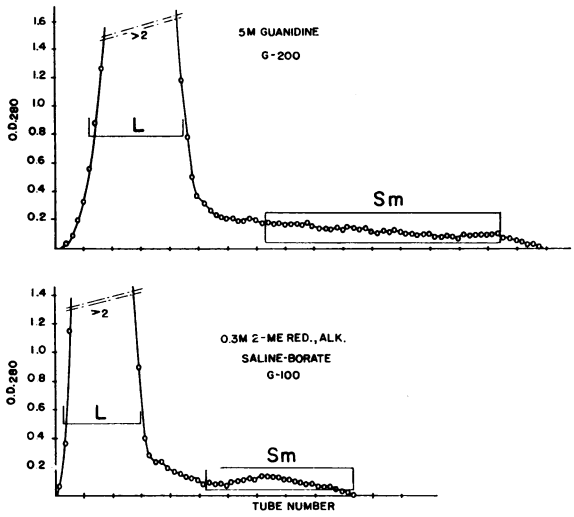

FIG. 1.—Elution patterns from gel filtration. Fractions pooled for analysis are encased in rectangles or enclosed in brackets. *Sm*, small fragment; *L*, large fragment.

Results.—When the isolated colostral IgA was dialyzed against 5 *M* guanidine and subsequently filtered over Sephadex® G-200, a long trailing peak was formed (Fig. 1). This fraction constituted approximately 10 per cent of the protein applied to the column. Similar results were obtained with a reduced alkylated preparation filtered over Sephadex® G-100 in saline-borate buffer, pH 8.0. Aliquots of the major peaks and of the small components were pooled and analyzed by Ouchterlony and chemical analyses. The small fragments released by the two treatments were shown to be antigenically identical, as were the larger fragments obtained by the two methods. When the small components were tested by Ouchterlony analysis with saliva from an agammaglobulinemic subject, a reaction of partial identity was seen using anticolostral antibody (Fig. 2).

The distinct antigenic determinants of the small fragments were shown to be due to light chain antigenic groups by the following experiments: If light chains prepared from IgG were reacted in diffusion experiments with saliva from agammaglobulinemic subjects, a reaction of partial identity was seen (Fig. 3*a*). Furthermore, when light chains of IgG were added to agammaglobulinemic saliva, the distinct antigenic determinants of the small fragment were seen to identify with the light chains (Fig. 3*b*). Using an antiserum directed only against light chains resulted in a continuous line of identity when tested against the small fragments obtained by either treatment, the saliva of an agammaglobulinemic, and light chains derived from either IgG or colostral IgA (Fig. 3*c*).

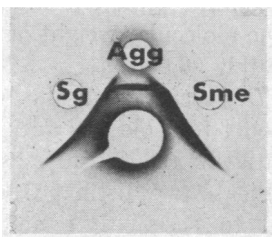

FIG. 2.—Antigenic relations of chromatographic fractions and agammaglobulinemic saliva. Antiserum against whole colostrum has been placed in center well. Partial identity of small fragments with agammaglobulinemic saliva is shown. *Sg*, small fragment prepared by guanidine; *Sme*, small fragment prepared by 2-ME treatment; *Agg*, agammaglobulinemic saliva.

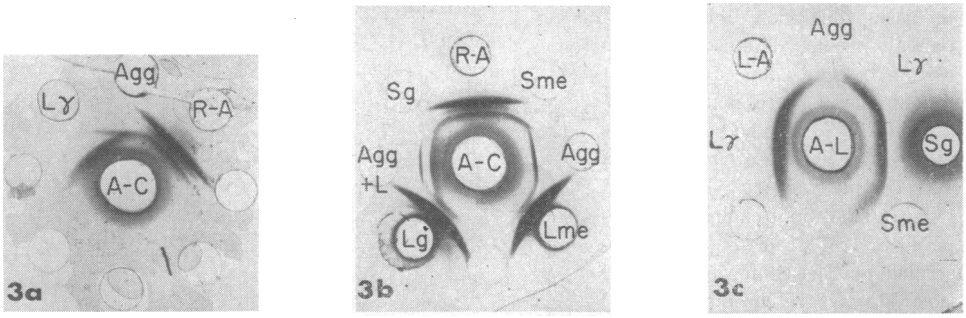

FIG. 3.—Relation of small fragment and transport piece to L chains. (*a*) L chains of IgG show partial identity with transport piece of agammaglobulinemic saliva which is in turn related to the small fragment released by 2-ME. *Lγ*, L chains of IgG; *Agg*, agammaglobulinemic saliva; *R—A*, reduced, alkylated colostrum; *A—C*, antiserum to colostrum. (*b*) L chains of IgG added to agammaglobulinemic saliva account for the antigenic specificity of the small fragment but also show partial identity to some determinants present in the transport piece of agammaglobulinemic saliva. *Lg*, large fragment prepared from guanidine column; *Agg + L*, agammaglobulinemic saliva to which L chains have been added; *Sg*, small fragment prepared from guanidine column; *Lme*, large fragment prepared by 2-ME method. Other symbols as in (*a*). (*c*) The identity of light chain preparations of IgG and colostral IgA and the presence of L chain determinants in the small fragments of colostral IgG are shown. *Lγ*, light chains of IgG; *L—A*, light chains of colostral IgA; *A—L*, antiserum directed against IgG L chains. Other symbols as in (*a*) and (*b*).

The large fragments showed reactions of identity by Ouchterlony analysis with serum IgA (Fig. 4). To examine further the nature of the large fragments in relation to serum immunoglobulins, we re-reduced an aliquot of a pool of the major peaks obtained from Sephadex® chromatography of colostral IgA reduced with 0.3 *M* 2-ME. Following re-reduction, re-alkylation, and dialysis against 1 *M* propionic acid, the protein was chromatographed on Sephadex® G-100 equilibrated with propionic acid. An elution pattern characteristic of heavy and light chains was obtained. The mass ratio of protein in the two peaks was 3:1 (H:L). The material from the second peak was compared to light chains obtained from IgG. In their amino acid composition, antigenicity (Fig. 3*c*), and behavior in acrylamide electrophoresis (Fig. 5), the light chains obtained from the colostral IgA were indistinguishable from the light chains of IgG.

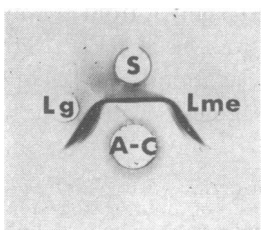

FIG. 4.—The antigenic identity of the large fragments prepared by guanidine and 2-ME to serum IgA is shown. *Lg*, large fragment prepared from guanidine column; *S*, serum IgA; *Lme*, large fragment prepared from 2-ME column.

Following reduction with 0.3 *M* 2-ME and alkylation with equimolar amounts of iodoacetamide, the sedimentation coefficient of the original material decreased from 11*S* to 6.9*S* as determined in saline-borate buffer, pH 8.0.

The approximate molecular weight of the large fragment obtained by 5 *M* guanidine treatment determined by the meniscus depletion method of Yphantis[12] was 510,000. The molecular weight of the small fragment was approximately 50,000.

Discussion.—On the basis of these data, a reasonable model for colostral IgA may be proposed. The original antigenic relationships as defined by Tomasi and South[5,11] indicate two distinct fragments, one antigenically identical to serum IgA and another which is secreted by agammaglobulinemic patients in the absence of detect-

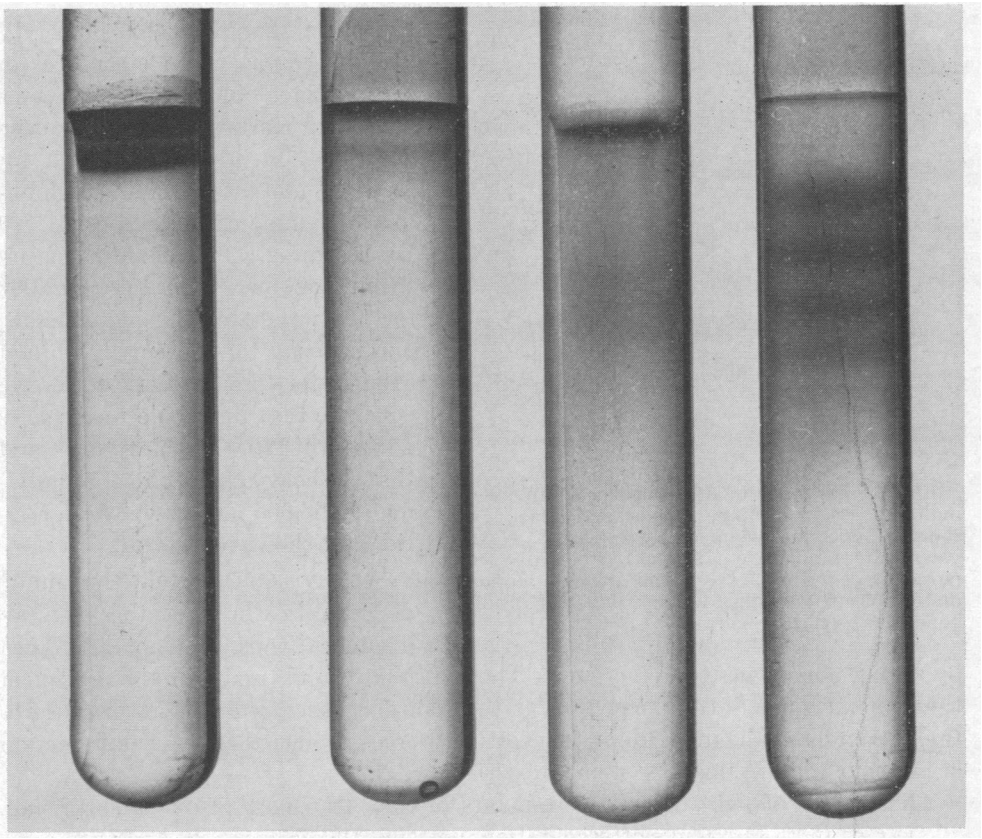

FIG. 5.—Acrylamide electrophoretic patterns of colostrum preparations. *From left to right:* untreated colostrum, large fragment, small fragment, light chains of colostral IgA. Light chains of IgG showed an identical banding pattern.

able immunoglobulins (transport piece). Since the pieces are separated by 5 *M* guanidine, they appear to be bonded noncovalently. The decrease in size of the whole molecule upon reduction with 0.3 *M* 2-ME at neutral pH is reminiscent of the response of IgM to similar treatment and suggests the formation of monomeric units. Since the disruption of the polymeric structure is also associated with the release of the transport piece, it appears that the monomers are maintained by weak disulfide bonds in a configuration complementary to that of the transport piece.

The virtual identity of the light chains of the colostral IgA to the light chains of IgG is consistent with present concepts of immunoglobulin structure. The light chains of IgM have been shown by Chaplin *et al.*[15] to be identical in amino acid composition with those of IgG. Our data extend these observations to the IgA system. Bernier's[16] data for light chains derived from a gamma-A myeloma show some differences from the IgG light chains, but the myeloma was of the lambda type and hence lacked the proportion of kappa chains characteristic of a normal immunoglobulin light chain population. Since by three criteria—amino acid composition, antigenicity, and acrylamide electrophoretic banding—the light chains from colostral IgA are identical with IgG light chains, one may assume a molecular weight in

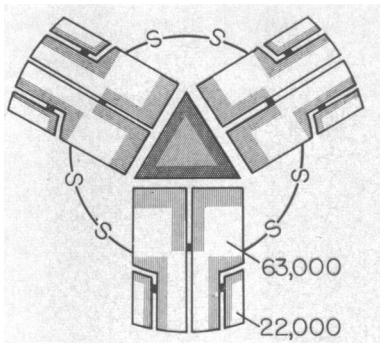

FIG. 6.—Proposed model of colostral IgA. *S—S* indicates weak disulfide bonds and black bars show stronger interchain disulfide bonds. Noncovalent interactions are indicated by the striped and cross-hatched areas. Numbers indicate approximate molecular weights. The number of disulfide bonds has been assumed.

the same range (viz., 22,000).[17-19] The basic four-chain structure for the immunoglobulins appears to be a consistent feature and has now been described, in addition to IgG, for IgM[20, 21] and also for immunoglobulins of nonmammalian species.[22, 23] By assigning a four-chain structure to our monomeric unit, taking 22,000 for the weight of the light chains, and utilizing the measured value of 510,000 for the polymeric form, we conclude that the whole molecule is a trimer of 170,000 mol wt units, and that the heavy chains are of approximately 63,000 mol wt. These relations are shown in Figure 6.

We envision three four-chain units of serum IgA held together by weak disulfide bonds, i.e., not reinforced by strong noncovalent interactions. These disulfide bonds maintain the relation of the monomers in a fit complementary to the transport piece, which is in turn held to the remainder of the molecule by noncovalent forces. Since there is a small fraction of 17*S* colostral IgA, higher polymers exist.

The finding of light chain determinants in the "transport piece" is surprising. That they are not artifacts of preparation, i.e., that they are not cleaved from associated IgA molecules, is evident from their detection in the fragment secreted by agammaglobulinemic patients who lack detectable amounts of immunoglobulins in their saliva. It is tempting to postulate that the light chain determinants in the transport piece provide specificity for the transport phenomenon. Perhaps mutation of genes controlling light chain synthesis has resulted in a fragment adapted for traversing membranes.

The elucidation of the interrelations of the two antigenic fragments of the colostral IgA system provides insight into the adaptation of antibody for surface activity. An attractive hypothesis is that serum IgA becomes polymerized and attached to a small protein fragment in glandular tissues. This modification allows secretion onto the mucous surfaces, and, in addition, the large size may enhance efficiency of the antibody. The necessity of polymeric structure to full IgM activity is well known. These notions as well as the final proof of the model are capable of scientific test.

Summary.—We propose a structural model for colostral IgA based upon immunochemical analyses of the whole molecule and the dissociation products formed by cleavage of noncovalent or disulfide bonds. The molecule appears to be a trimer of serum IgA linked by weak disulfide bonds. These disulfide bonds maintain the relation of the monomers in a fit complementary to the transport piece, which is in turn held to the remainder of the molecule by noncovalent forces. When the transport piece is removed from the polymer, L chains can be obtained in the usual man-

ner from the remainder. These L chains are identical with those obtained from IgG. The transport piece contains L chain determinants.

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