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The Nature of the Polymeric Serum IgA In Man[1] (39061)

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Analyses of molecular forms of IgA isolated from normal human serum led several investigators to conclude that 80–90% of human serum IgA occurs in a monomeric form. The remaining 10–20% is made up of smaller amounts of IgA dimers (10 S) and higher polymers (13 S and 15 S) (for a review see 1). In a previous study (2) we used three markers specific for polymeric IgA (the capacity to bind secretory component *in vitro*, the presence of the J chain, and reactivity with an antiserum that recognized only the polymeric form of IgA) in an attempt to show that human bone marrow could be regarded as a major source of both monomeric and polymeric serum IgA in myeloma patients as well as in normal individuals. In the bone marrow of normal persons, the number of cells that displayed positive reactions with markers for polymeric IgA was proportionate to the amount of polymeric IgA in normal human serum, as would be expected from reports in the literature. However, no appreciable reactivity with any of the three markers was observed in the sera of normal persons from our group (2). The discrepancy between our results and the data given in the literature prompted us to reinvestigate the nature and the amount of the polymeric IgA in normal human serum.

Materials and Methods. Three markers (Table I) were used to determine the presence of polymeric IgA in sera from six healthy adults, in isolated polymeric serum IgA fractions from five other healthy adults, and in pooled sera from 80 blood donors.

Polymeric IgA from the individual serum samples was prepared in two ways. In four instances, 6 ml of each serum were applied on a Sephadex G-200 column (2.6 × 100 cm, upward flow, in phosphate-buffered saline, pH 7.2). Fractions, of the first peak, that contained IgA were concentrated by ultrafiltration (Amicon Corp., membrane PM-30) and applied on a Sepharose 6B column. After elution, fractions of the second major peak were concentrated and tested for the presence of IgA, IgM, and IgG by the double immunodiffusion technique. All fractions were principally composed of IgA, but small amounts of IgM were also present.

Three ml of each of two serum samples (one from an individual and one from the serum pool) were applied on a Sephadex G-200 column (2.5 × 96 cm; buffer, 0.05 *M* Tris–HCl in 0.1 *M* KCl; pH, 7.5). Fractions from the front of the elution profile of IgA (estimated by the double diffusion technique) were pooled and concentrated. To remove IgM, these fractions were passed over an immunoadsorbent consisting of an insolubilized globulin fraction of an anti-human-IgM serum (3). Analytical ultracentrifugation showed that the samples contained only two broad peaks with sedimentation coefficients of 10 S and 21 S. Only IgA and α_2 macroglobulin were detected immunochemically in these fractions. All samples were tested at protein concentrations from 2 to 10 mg/ml.

Results. When sera from healthy adults were tested for the presence of polymeric IgA, no positive reactions were observed with the anti-dimer IgA serum and no SC binding to IgA was observed *in vitro*. The sensitivity of these techniques would allow detection of serum polymeric IgA when present in concentrations above 0.18 mg/ml (approx. 9% of the total IgA). To detect lower concentrations of polymeric IgA and to eliminate the possibility that excess monomeric IgA might interfere with precipitation of the dimer by the anti-dimer IgA serum (2), further experiments were performed with isolated polymeric IgA preparations. Any possible erroneous evaluations of the J chain and SC

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TABLE I. TEST MARKERS FOR THE DETECTION OF DIMERIC (POLYMERIC) HUMAN IgA.[a]

Test marker	Antiserum	Technique
Dimeric determinants of IgA	Sheep anti-dimer IgA No.: 716	Ouchterlony double radial immunodiffusion and immunoelectrophoresis
Presence of J chain	Rabbit anti-human J chain No.: 4	Polyacrylamide gel electrophoresis (15) and immunoelectrophoresis after reduction of the IgA by 0.2 M mercaptoethanol in 9 *M* urea (pH = 8.6)
Binding of SC *in vitro*	Sheep anti-SC No.: 716 Rabbit anti-SC No.: 462	Immunoelectrophoresis after incubation of the IgA samples with free SC from human milk

[a] Schematic description summarizing data from (2, 9).

binding tests were eliminated, because IgM was either absent from the preparations or present in only trace amounts.

Three of the four preparations obtained by the first procedure displayed no positive reaction with the anti-dimer IgA serum and no J chain was detected. The fourth showed traces of J chain and a very weak reaction with the anti-dimer IgA serum. All four samples showed some SC binding *in vitro* (that would account for 1–5 % of the isolated IgA). Two other preparations of polymeric IgA obtained by the second procedure gave no positive reaction with any of the three markers.

Discussion. The results of this study indicate that the sera of healthy adults contain minimal amounts of true dimeric IgA that is characterized by the presence of a J chain (4, 5) and of antigenic determinants specific for dimers (3, 6, 7), as well as by its ability to bind the SC (8–10). Kownatzki (11) analyzed normal human serum for its J chain content and concluded that this polypeptide was primarily associated with IgM. Techniques are not yet available to exactly quantitate the level of true dimeric IgA, however, from our results, it can be estimated as lower than 1 % of the total serum IgA. From our experiments, it could not be ascertained whether the remainder of the IgA eluted from the Sephadex G-200 column, in the position of polymeric IgA, represents aggregates formed *in vivo* or whether it represents *in vitro* aggregates elicited by the preparatory techniques.

If we concur that less than 1 % of the total serum IgA is actually in dimeric form and if we consider that the bone marrow contains an appreciable number of cells capable of producing dimeric IgA, a question arises as to the fate of this polymeric IgA. It is unlikely that such polymers would be specifically degraded upon reaching the blood pool. It seems reasonable to postulate that IgA polymers originating in the bone marrow are transported by the blood to a common preexcretory pool located in the interstitial subepithelial fluid at the secretory sites. This might occur in the same manner described by Heremans (12) as occurring in dogs. These IgA polymers, along with locally produced dimeric IgA, may be subsequently transferred through the epithelial wall into secretions. A feasible model for such a selective epithelial IgA transport was proposed by Brandtzaeg (13). This possibility is supported by the demonstration of Coelho et al. (14) that the predominant IgA paraprotein detected in the saliva of patients with IgA myeloma is the polymer bound to the SC and not the monomer. The low level of dimeric IgA present in the serum under normal conditions implies that this clearing mechanism is very efficient. However, evidence indicates that pathological conditions at the secretory sites may result in increased levels of the dimeric and the secretory IgA in serum.

Summary. The nature and the amount of polymeric IgA in sera from normal, adult humans was investigated by means of three markers that selectively differentiate polymeric IgA: the capacity to bind secretory component; the presence of the J chain; and the reactivity with an antiserum that recognizes only the polymeric form of IgA. The results demonstrated that the sera of healthy adults contain only minimal amounts (less than 1 % of the total serum IgA) of the true dimeric IgA. These conditions are valid for healthy individuals only. Pathological con-

ditions at the secretory sites may result in increased values for the dimeric as well as for the secretory IgA in serum.

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