SOME PROPERTIES OF ANTISERA TO SERUM AMYLOID A PROTEIN (SAA): INHIBITION OF PRECIPITATION BY COMPLEXING OF SAA TO ALBUMIN*

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Previous studies by us (1) and by Anders et al. (2) have demonstrated that the amyloid A (AA) protein is derived from the amino terminal region of a larger serum precursor known as the SAA protein, which has a mol wt of about 11,000–14,000 and exists in serum a higher molecular weight complex with a mol wt estimated to be 85,000 (3) or 200,000 (4). Though this complex has not been isolated, recombination studies of SAA liberated by Sephadex filtration in 10% formic acid with several serum proteins implicate binding to serum albumin and self-association in the formation of the complex.1

Quantitation of the SAA protein has been accomplished only with antisera to the AA protein (5), and levels have therefore had to be expressed as equivalents of AA protein. During the isolation of SAA from serum it became apparent that the radioimmunoassay underestimated the actual amount of SAA in serum by a factor of 10 or more (1). Hence, antisera to the SAA protein were prepared to obtain a better means of quantitation and also to study the nature of the SAA protein. The present report presents the properties and some of the unusual features of these antisera, demonstrates the reason why they cannot be used to quantitate SAA in serum, and points to the possibility that similar problems may arise in quantitating other proteins that exist in serum as complexes.

Materials and Methods

SAA and AA. SAA from two patients, Cot and Wea, with myeloma and macroglobulinemia and high concentrations of the SAA protein were isolated as described (1). AA proteins were the same as those used in previous studies (5).

Antisera. Four rabbits were immunized with SAA in complete Freund’s adjuvant in the footpads and boosted every 2–4 wk in the back. Two of the rabbits responded in 2 mo, one after 6 mo, and the fourth failed to produce detectable antibodies before death 8 mo after the start of immunization.

Immunologic Methods. Double diffusion in agar was done as described by Ouchterlony (6).

Results

Three of the rabbits produced antibodies after 2–6 mo of immunization. The antisera gave a single sharp precipitin band which showed a reaction of identity....

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1 Rosenthal, C. J., and E. C. Franklin. Manuscript in preparation.
with the two SAA preparations employed (Fig. 1a). They failed to react, however, with four preparations of AA protein and with sera that gave good precipitin lines with antisera to the AA protein and therefore contained large amounts of SAA protein. Since in the purification of SAA the proteins had been subjected to 10% formic acid, it seemed possible that this measure could have induced some changes in the antigenic determinants. Treatment of the sera or AA proteins with 10% formic acid followed by neutralization failed to induce reactivity with the antisera to SAA.

Since SAA exists in serum complexed to itself and to serum albumin, it seemed possible that the complex was not reactive with anti-SAA because the determinants were masked. To test the possibility, a solution of SAA (1 mg/ml) was mixed either with an equal volume of saline, human serum albumin ranging in concentration from 80–0.65 mg/ml or human gamma globulin (HGG) (80–10 mg/ml), and tested by double diffusion in agar. As shown in Fig. 1b, the addition of HGG even at the highest concentration did not diminish or alter the precipitin line of SAA. In contrast HSA at concentrations greater than 10 mg/ml completely abolished precipitation and in concentrations as low as 1.25 mg/ml it diminished the intensity. Furthermore, when the mixture was allowed to diffuse from a well adjacent to pure SAA, it shortened the precipitin line of SAA (Figs. 1a and b). Similar conclusions can be drawn from immunoelectrophoresis analyses in which addition of albumin to SAA abolished its reactivity.
Discussion

Three points warrant discussion. Firstly, it would appear that the carboxyl terminal region unique to SAA and not shared with AA contains the more potent antigenic determinants, since all three responding rabbit antisera failed to react with the AA protein. Previous studies have indicated that AA is a weaker antigen than SAA since fewer rabbits responded to it and generally required more prolonged immunization before antibodies appeared.

Secondly, though minor differences in the behavior of certain molecules have been noted when the protein is tested alone or complexed to another protein, for example free light chains compared to IgG (7), haptoglobin, and haptoglobin-hemoglobin complex (8), there is no precedent for the complete elimination of antigenic activity of one molecule when bound to another. An analogous situation may exist in the case of antibodies to idiotypic determinants of immunoglobulins whose reactivity can be largely blocked when the antigen binding site has reacted with the corresponding haptene (9). Since here the antibody appears to be directed against a small region of the antigen, it seems possible that the behavior of SAA could be due to the fact that the binding site is the same as the antigenic determinant(s) or that binding causes a change in conformation which blocks access of the antibody to the antigenic region of the molecule.

Thirdly, this unusual feature precludes the use of these antisera in detecting and quantitating SAA in serum, since the concentration of albumin is significantly greater than that needed to block reactivity. Although this degree of inhibition was not noted with antisera to the AA protein used in the immunoassay to quantitate SAA, some degree of binding of the AA protein to serum proteins has been previously observed by us (10). Hence, these findings may explain why the radioimmunoassay underestimates the amount of SAA, in serum by a factor of 10 or more (1) and also the observation of Sipe et al. (11) that the apparent yield of SAA increases with each purification step. Whether similar problems will arise in the future with other molecules that exist in serum complexed to carrier proteins remains to be determined and should be considered in developing immunoassays for hormones, vitamins, and other molecules that exist in serum as complexes. Furthermore, the presence of antibodies in the immunized rabbits would have been missed had pure antigen not been used in testing.

Summary

Three potent rabbit antisera to human serum amyloid A protein (SAA) appear to be directed exclusively to the carboxyl terminal region not shared with the tissue amyloid A protein. Since binding to albumin completely blocks the reaction of these antisera with the antigen, and since SAA exists in serum complexed to albumin, the anti-SAA cannot be used to detect or quantitate this serum component. The possibility that similar problems will be encountered with immunoassays for molecules that exist complexed to other proteins is discussed.

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