Immunological Relationships of Serum α-Macroglobulins in the Human, Rat, and Rabbit[1] (35933)

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Two immunologically and electrophoretically distinct serum α-macroglobulins, α_1M and α_2M, have been described in normal rabbits (1–3), while only an α_2M has been identified in man (4). However, the relationship of the human α_2M to the rabbit α-macroulins is not clear. The amount of α-macroglobulin in serum increases during the nephrotic syndrome in man (5, 6) and during acute inflammation in the rabbit (7) and rat (8). We now present immunological evidence that: (i) rabbit α_1M corresponds to human α_2M and to a normal protein in the rat; (ii) in rats with an acute inflammation, the protein (presumably α_2M) (8) which appears in the serum in relatively large amounts is antigenically related, but not identical, to the protein present in normal rats which is homologous to rabbit α_1M and to human α_2M; (iii) rabbit α_2M is antigenically related to a protein in the rat which does not increase during acute inflammation; (iv) rabbit α_2M has no homologue in human serum that has as yet been identified.

Materials and Methods. The preparation of purified rabbit α_1M and α_2M has previously been described (3, 9, 10). In the present studies, the two rabbit αM were separated from each other by starch block electrophoresis and isoelectric focusing with ampholytes of pH 4–6; in both procedures, α_1M was more anodal than α_2M. Antisera were prepared in goats by repeated injections of the proteins emulsified in Freund's adjuvant. The goat antirabbit α_1M required absorption with rabbit IgG. Monospecific goat antirabbit α_2M was produced by absorption of a combined antirabbit α_1M and α_2M with purified α_1M (3). Goat antihuman α_2M (Hyland, Costa Mesa, CA, Lot. No. 8218M002A1) and rabbit antihuman α_2M (Behringwerke AG., Marburg-Lahn, Germany, Batch No. 1467 BN) were obtained commercially. Serum was obtained from healthy adult rats and humans. Serum from a rat, injected subcutaneously with turpentine and cottonseed oil 48 hr earlier, was kindly provided by Miss Stella Cha. Immunoelectrophoresis in agar gel was performed by the method of Scheidegger (11). Agar gel double diffusion was performed by the method of Ouchterlony (12).

Results. The results of immunoelectrophoresis of normal rabbit serum (NRS) and normal human serum (NHuS) against goat antisera to human α_2M and rabbit α_1M are shown in Fig. 1. Antirabbit α_1M gives a single precipitin arc in the α region with NRS and NHuS; the arc with NRS is slightly more anodal than the arc with NHuS. Antihuman α_2M also gives a single precipitin arc in the α region with NRS and NHuS; again the arc with NRS is slightly more anodal than the arc with NHuS. The position of these NRS and NHuS arcs corresponds with the position of the precipitin arcs developed with a goat antiserum to both rabbit α_1M and α_2M. The goat antirabbit α_2M antiserum did not react with human serum. These results indicate that human α_2M is antigenically related to rabbit α_1M. This is further illustrated by the double diffusion reactions in Fig. 2. Both the antirabbit α_1M and the antihuman α_2M produce single precipitin bands which coalesce when reacted against human serum or against purified rabbit α_1M. Reactions of partial identity are seen in the reactions of either

[1] This work was supported in part by U.S. Public Health Service Grants AI-09241 and AI-07043.

[2] In partial fulfillment of the requirements for the Doctor of Philosophy Degree in the Graduate College. Supported by a postdoctoral fellowship from the Schweppe Foundation.

[3] Recipient of U.S. Public Health Service Research Career Development Award AI-28687.

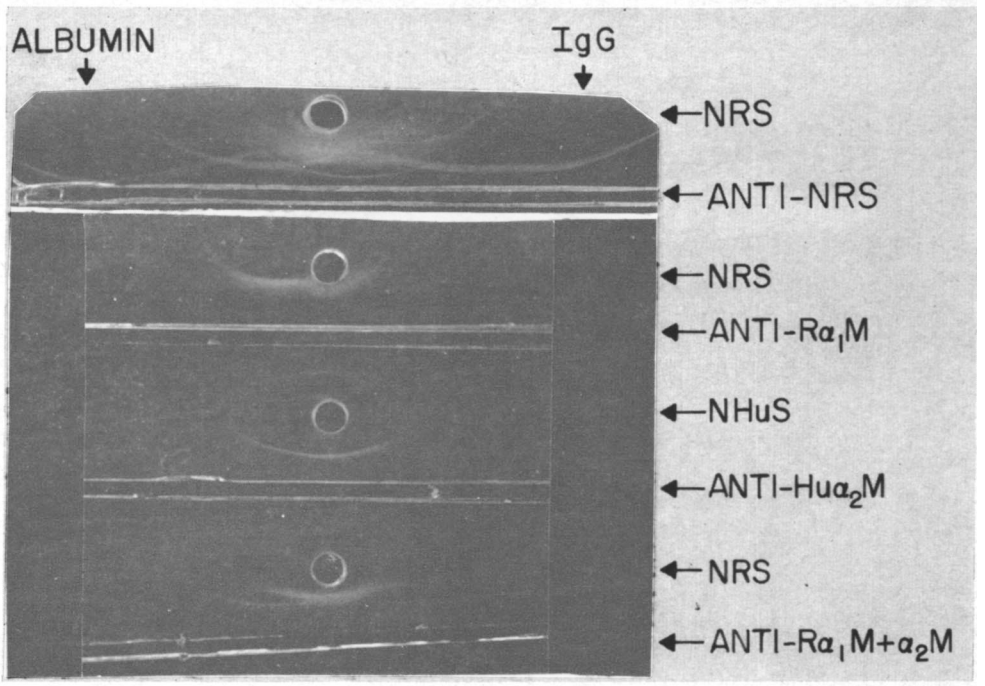

FIG. 1. Immunoelectrophoresis in agar gel: anode to the left; NRS = normal rabbit serum; ANTI-NRS = goat antinormal rabbit serum; ANTI-Rα_1M = goat antiserum to rabbit α_1M; NHuS = normal human serum; ANTI-Rα_1M + α_2M = goat antiserum to rabbit α_1M and rabbit α_2M.

antiserum with rabbit and human serum; a spur appears with the homologous antigen. Figure 3a demonstrates that rabbit α_1M and α_2M are immunologically distinct, as seen by the reaction of nonidentity, *i.e.*, the crossing of the α_1M band by the α_2M band.

In normal rat serum, a protein antigenically related to human α_2M and rabbit α_1M is demonstrated by the reaction of partial identity observed with the rabbit antihuman α_2M (Fig. 3b), as well as by a similar reaction with the goat antirabbit α_1M (not illustrated). A protein in normal rat serum antigenically related to rabbit α_2M is demonstrated

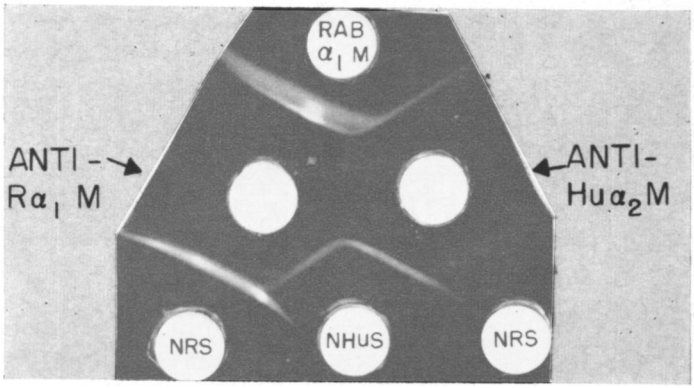

FIG. 2. Ouchterlony plate showing relationship of rabbit α_1M and human α_2M: (top) RAB α_1M = purified rabbit α_1M; (middle) ANTI-Rα_1M = goat antiserum to rabbit α_1M; ANTI-Huα_2M = goat antiserum to human α_2M; (bottom) NRS = normal rabbit serum; NHuS = normal human serum.

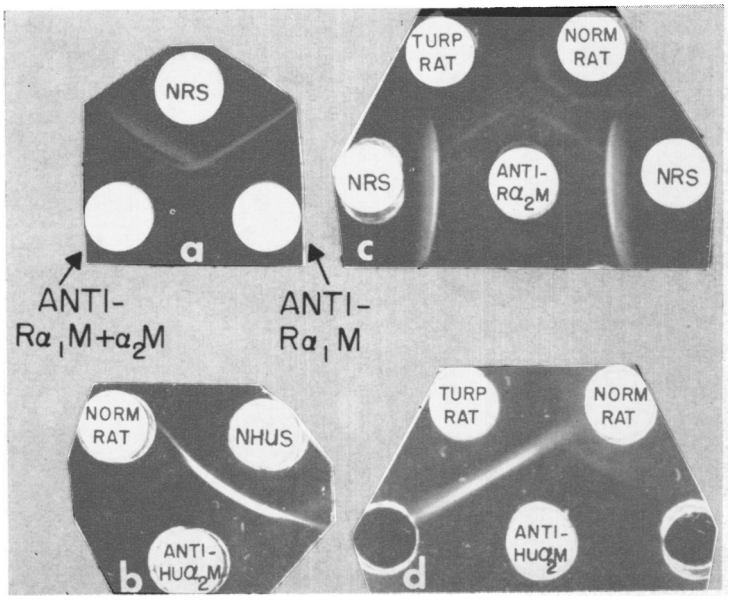

FIG. 3. Ouchterlony plates: NRS = normal rabbit serum; NORM RAT = normal rat serum; TURP RAT = turpentine injected rat serum; NHuS = normal human serum; ANTI-Rα_1M = goat antiserum to rabbit α_1M; ANTI-Rα_2M = goat antiserum to rabbit α_2M; ANTI-Rα_1M + α_2M = goat antiserum to rabbit α_1M and rabbit α_2M; ANTI-Huα_2M = rabbit antiserum to human α_2M (Behringwerke); (a) reaction of nonidentity of rabbit α_1M and rabbit α_2M; (b) reactions of rabbit antiserum to human α_2M with normal human serum and normal rat serum; (c) reactions of goat antiserum to rabbit α_2M with normal rabbit serum and with normal and turpentine injected rat serum; (d) reactions of rabbit antiserum to human α_2M with normal and turpentine injected rat serum.

by the reaction of partial identity with the goat antirabbit α_2M (Fig. 3c); this protein was also present in the serum of a rat traumatized by the injection of turpentine 2 days earlier, as indicated by the reaction of identity. On the other hand, the serum of the traumatized rat reacted with the antihuman α_2M to give a dense precipitin band with a spur extending past the precipitin band formed with normal rat serum (Fig. 3d); this indicated that the protein formed in the traumatized rat possessed antigenic determinants lacking in the normal rat protein.

Discussion. Rabbit and human αM have similar chemical and physical properties. Rabbit α_1M and α_2M have sedimentation coefficients of 16–20 S (1, 13); rabbit α_2M has a molecular weight of 815,000 (13). Human α_2M is 19 S, has a molecular weight of 820,000, and like the rabbit αM (1, 13) is approximately 91% protein and 9% carbohydrate (14–16). Human α_2M and rabbit α_1M and α_2M could not be distinguished from each other by electron microscopy (17), and all three have been found to bind trypsin (18–21).

Despite the similarities between rabbit and human α_2M, the two proteins appear unrelated in their antigenic properties. The two α_2M do not share any antigenic determinants detectable by reactions in agar gel, as observed in the present experiments and previously by Picard *et al.* (13). Furthermore, whereas we have found that rabbit α_2M allotypes are controlled at a complex autosomal locus (3, 10), Berg and Bearn have described an X-linked human α_2M locus (22). Additionally, human α_2M has been found to contain zinc (23), while rabbit α_2M contains nickel (24).

Human α_2M and rabbit α_1M, while not identical electrophoretically, are homologous proteins, since in Ouchterlony plates: (i) each protein gives a reaction of identity when

reacted with antihuman α_2M and antirabbit α_1M; and (ii) a reaction of partial identity occurs when each antiserum is reacted with both proteins. This agrees with reports of others who noted that human α_2M and rabbit α_1M are chemically similar, but not identical (1, 20).

Heim (8) reported that no reaction occurred in agar gel between normal rat serum and a Behringwerke rabbit antihuman α_2M. In our studies, we did observe a precipitin reaction between normal rat serum and a similar antiserum from Behringwerke. Such a discrepancy may be explained by the fact that, in the present experiments, wells were filled up to seven times, remaining filled for at least 6 hr, thus increasing the sensitivity of the test. James (25), while studying the reactions of a rabbit antihuman α_2M with sera from various species, also noted a weak precipitin reaction with rat serum.

Antirabbit α_2M reacted against a protein which was present in normal rat serum, and unchanged after turpentine injection. An α_1M has been reported by others as exhibiting these properties in the rat (26).

Our immunological evidence supports the studies of De Vonne *et al.* (27), who concluded that rabbit α_1M behaved similarly to human α_2M and to rat α_2M as an acute phase reactant in inflammation, while rabbit α_2M did not. We must note, however, that others have observed that human α_2M does not appear to be an acute phase reactant as it is not elevated in rheumatoid arthritis (28–30) and pancreatitis (31). Heim (8) observed the *de novo* appearance of an α_2M which reacted with rabbit antihuman α_2M in a rat challenged with endotoxin. We observed that turpentine induced inflammation in the rat resulted in the appearance of a substance immunologically related, but not identical, to the normally present homologue of human α_2M and rabbit α_1M. The precipitin reaction of the antihuman α_2M antiserum was more dense and gave a spur with this antigen, when compared with the protein in normal serum. This would suggest that new antigenic determinants are added to the αM molecule during inflammation, and may be related to the observation of Got *et al.* (7) that the sialic acid content of rabbit α_1M increases during inflammation. Hence, when αM levels are determined by immunoassay, a change in the strength of a reaction may indicate either an antigenic or a quantitative change in the αM, or possibly both.

In this study, we have demonstrated that a rabbit α_1M is related to human α_2M and to an acute phase reactant present in normal rats. As the electrophoretic mobility of immunologically related αM may differ among species (32, 33) and in pathological states (34), it is suggested that reactions with antihuman α_2M antisera be performed to help differentiate between the two types of α-macroglobulins existing in rabbits, rats, and perhaps other species.

Summary. Rabbit serum contains two immunologically distinct α-macroglobulins, α_1M and α_2M, while only an α_2M has been described in human serum. Rat serum contains an α_2M which, like rabbit α_1M, increases in acute inflammation. Antisera to these macroglobulins cross-react with serum proteins in different species. Using such antisera, we have shown: (i) rabbit α_1M corresponds to human α_2M and to a normal protein in the rat; (ii) the α_2M that appears in rat serum in relatively large amounts during acute inflammation is antigenically related, but not identical, to a protein present in normal rats, which is homologous to rabbit α_1M and to human α_2M; (iii) rabbit α_2M is antigenically related to a protein in the rat which does not increase during acute inflammation; (iv) rabbit α_2M has no homologue in human serum that has as yet been identified.

Note added in proof. Ohlsson has recently reported the presence of two αM's in dog serum that are both immunologically related to human α_2M [Ohlsson, K., Biochim. Biophys. Acta **236**, 84 (1971)]. Our goat anti-rabbit α_2M and goat anti-human α_2M both react with dog serum; however, the anti-rabbit α_2M does not react with human serum and does not cross-react with the anti-human α_2M in reactions with rabbit serum. Furthermore, the anti-human α_2M does not react with purified rabbit α_2M, which seems to be immunologically unrelated to either of Ohlsson's proteins.

We thank Dr. H. Gerhard Schwick of Behringwerke AG., Marburg-Lahn, Germany, for his kind

advice, and Dr. Samuel Nerenberg for his gift of the commercial antisera.

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Received June 14, 1971. P.S.E.B.M., 1971, Vol. 138.