Allotypy of High Density Lipoprotein of Rabbit Serum

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ABSTRACT A common antigenic polymorphism of high density lipoprotein (HDL) in rabbit serum is described. The presence or absence of an antigen termed HI 1 appears to be controlled by autosomal dominant inheritance. The polymorphism should be a useful tool in the study of serum lipoproteins, particularly since genetic polymorphisms within the low density lipoprotein are already known in several species. The HI polymorphism may make the rabbit more useful for model studies of serum lipoproteins in health and disease.

Early in this century Schütze (1, 2), working with iso-immunization of rabbits, produced the first evidence of homospecific antigens in the serum of mammals. In 1956, Oudin (3) confirmed these results and extended the studies considerably. He introduced the term “allotype” and demonstrated that the “allotypes” he was working with represented different antigenic specificities of the γ-globulin of the rabbits and that they were genetically determined. In recent years, individual antigenic differences have been revealed in the serum of a number of other animal species.

In 1961, Allison and Blumberg (4), using an antiserum from a multiply transfused patient, revealed an allotype in human serum. The antigenic variation was in the β-lipoprotein. Since then, several genetically determined antigens contained in the β-lipoproteins of normal serum have been found (for review see refs. 5–7). Allotopy of β-lipoprotein of rabbit serum has been demonstrated independently by Albers and Dray (8) and by Kelus (9).

The main lipoproteins in the serum of mammals can be separated into low density lipoprotein (LDL) and high density lipoprotein (HDL), having β and α mobility, respectively, on electrophoresis. Whereas common antigenic variants of serum β-lipoprotein (or LDL) have been found in several species, the situation is quite different for α-lipoprotein (or HDL). To our knowledge, no genetic polymorphism of HDL has been demonstrated in any species, although the significance of some earlier observations in this regard is not quite clear.

In order to establish model systems for immunogenetic and other studies of the serum lipoproteins, we recently immunized rabbits with serum from other rabbits, hoping to obtain antibodies to inherited lipoprotein antigens. One of the rabbits produced an antibody which revealed an apparent allotypic specificity of HDL. In this paper we report the first experiments with this allotype.

Abbreviations: HDL and LDL, high and low density lipoproteins, respectively.

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MATERIALS AND METHODS

Normal rabbit sera

For immunization purposes, a pool was made of equal volumes of serum from each of 19 rabbits of five different strains. Individual serum samples from all the rabbits were kept for subsequent tests.

In addition, sera from a total of 90 adult albino rabbits of the local strain, random bred in a closed colony, were tested against the precipitin-containing antiserum. Information on the sex of the animals was available for 61 of these rabbits.

Finally, we tested sera of 4–6 week old offspring of eight matings where both parents were available for testing, and of four matings where only the mothers could be tested.

Immunization of rabbits

Two albino rabbits of the local strain, each weighing approximately 3.5 kg, and two gray rabbits, weighing approximately 6 kg, were each immunized initially with 2 ml of serum from the pool, mixed with 1 ml of Freund’s complete adjuvant (Difco Laboratories, Detroit), distributed on foot pads and several intramuscular and subcutaneous sites, as well as with 2 ml of serum intravenously. On the 17th day this procedure was repeated, although no injection was given in the foot pads. On the 24th, 25th, and 26th days of the immunization course, each animal was given 2 ml of serum from the pool intravenously. Thus, each animal had received a total of 14 ml of whole serum from the pool. One of the animals died, so that only three animals remained to be bled, 5 days after the last injection.

Gel diffusion tests

Agar purum (Behringwerke AG, Marburg) was used for double diffusion tests, which were conducted as described previously (10). Ion agar no. 2 (Oxoid Ltd., London) was used for immunoelectrophoresis.

The slides were stained with Oil Red O or Sudan Black as lipid dyes, and Thiazine Red R as a protein stain (all dyes were from G. T. Gurr Ltd., London). Esterase activity in the precipitin bands was demonstrated by the method of Uriel (11).

Other methods

Lipoprotein electrophoresis was carried out in agarose (l’Industrie Biologique Française S.A., Gennevilliers) gel at pH 8.6 on polyester film. Lipoproteins of different density classes were prepared from rabbit serum by flotation in the ultracentrifuge (12). γ-globulin of rabbit serum was prepared by ion-exchange chromatography on DEAE-cellulose (13) (Whatman DE 32).
EXPERIMENTS AND RESULTS

Analysis of sera of immunized rabbits

Sera of the three animals that survived the immunization course were tested in double-diffusion experiments against the individual sera of the pool. Serum from only one of the immunized albino rabbits (R 56) contained a precipitin, which formed a precipitin band with all individual sera of the pool, except with serum from R 56 itself. Later, several nonreacting sera were found. Examples of reactions are shown in Fig. 1. The precipitin band could be stained with protein or lipid dyes and esterase activity could be demonstrated in the line.

Identification of the reacting component of immune serum

Serum from R 56 was subjected to immuno-electrophoresis, and the antibody trough was filled with serum of the pool. A precipitin line was observed only in the γ region, indicating that the precipitin of rabbit immune serum formed part of the γ-globulins.

Immune serum from R 56 was subjected to ion-exchange chromatography on DEAE-cellulose. The first chromatographic peak contained γ-globulin exclusively. Only material from this peak possessed precipitating capacity toward the pool of rabbit sera. We concluded that the precipitin of immune serum R 56 is a γ-globulin.

Serum from R 56 obtained before immunization possessed no precipitating capacity. Thus, the precipitin in serum from R 56 is an antibody.

Characterization of the reacting component in normal rabbit sera

Samples of the serum pool and of individual sera of the pool were subjected to immuno-electrophoresis with immune serum from R 56 in the antibody trough. A precipitin arch appeared in the α2-globulin region, indicating a reacting component with electrophoretic mobility slightly lower than that of albumin (Fig. 2, lower part). The precipitin band had the same staining properties as in the double-diffusion experiments. When samples of the serum pool or individual rabbit sera were tested in immuno-electrophoresis against a donkey antiserum to rabbit serum proteins (Behringwerke AG, Marburg), two precipitin bands stainable with lipid dyes were found. These lines corresponded to the immunoelectrophoretic positions of β- and α-lipoproteins. The precipitin arch observed in immuno-electrophoresis when serum from R 56 was used as an antiserum corresponded to the second of these (Fig. 2). This suggests that the antigen of immune serum R 56 is an α-lipoprotein.

Serum lipoproteins of different density classes were prepared from a rabbit serum that reacted with antiserum R 56. The lipoprotein fractions obtained were tested against the antiserum (Fig. 3). A strong precipitin line appeared against the HDL fraction (1.063–1.21 g/ml). Only traces of reaction were observed with the LDL fraction (1.019–1.063 g/ml), with the very low density (<1.019 g/ml) lipoprotein fraction, and with the infranate obtained by centrifugation at the density 1.21 g/ml. Thus, the antigen has the density of HDL. Reactions between purified HDL from a positive reactor and purified γ-globulin from antiserum R 56 are shown in Fig. 4.

Additional studies on antiserum R 56

Since rabbit R 56 apparently had produced antibody to a component of HDL present in the other individual rabbit sera of the pool, we determined whether serum from R 56 itself contained HDL, both by lipoprotein electrophoresis and by immuno-electrophoresis. Serum R 56 did contain HDL, with an electrophoretic mobility apparently identical with that of the lipoprotein in the other rabbit sera. Thus, the antigenic difference between the HDL of serum R 56 and that of the other rabbit sera was not reflected in any demonstrable difference in electrophoretic mobility.

Since isimmune sera may contain antibodies to more than one antigen, antiserum R 56 was absorbed, by an intrabasin absorption technique, with each of the individual sera belonging to the pool, and each time tested against all the sera of the pool. Absorption with one antigen-containing serum always removed all precipitating capacity. The antiserum therefore appears to be monospecific for an antigen present in some, but not all, rabbit sera.
Antiserum R 56 was tested against a panel of 48 normal human sera. No cross-reactions appeared in this experiment.

Notation
The antigen revealed by antiserum R 56 has in our laboratory provisionally been designated HI 1, where HI is an abbreviation for High density lipoprotein.

Occurrence of the HI 1 antigen in the local strain of albino rabbits
To study the occurrence of the HI 1 antigen in a population of albino rabbits, we tested sera from a total of 90 animals of the local albino strain. Of the 90 sera, 22 were found to lack the HI 1 antigen and 68 to possess it. Thus, almost 76% of the albino rabbits in this closed colony were positive with respect to the HI 1 antigen.

Table 1 shows the distribution of positive and negative sera for the part of the material for which information as to the sex of the animals was available. This comparison gave no indication of an influence of sex on the presence or absence of the HI 1 antigen.

It seemed reasonable to assume that the HI 1 antigen was genetically determined. Evidence supporting or rejecting this hypothesis was sought in a study of the HI 1 types of parents and offspring. At the present time we can report the results obtained in eight planned matings. These preliminary breeding data are summarized in Table 2.

In our first breeding experiments, we have given preference to matings that would provide critical information as to the hypothesis of a dominant mode of inheritance for the HI 1 antigen, i.e., matings where both parents lack the antigen. It can be seen from Table 2 that we have studied 21 offspring of four such matings, and found all to lack the HI 1 antigen. Thus, no exception was found to the hypothesis of a dominant mode of inheritance.

The one mating between a positive male and a negative female rabbit resulted in both positive and negative offspring (the two male offspring were negative).

All 11 offspring were positive from three matings where both parents were positive.

Table 1. Distribution of the HI 1 serum antigen between the two sexes

<table>
<thead>
<tr>
<th></th>
<th>Antigen present</th>
<th>Antigen absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>16</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Females</td>
<td>32</td>
<td>9</td>
<td>41</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>13</td>
<td>61</td>
</tr>
</tbody>
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\[ \chi^2 = 0.0251; 0.80 < P < 0.90. \]

Table 2. Breeding data on the HI 1 antigen of rabbit serum

<table>
<thead>
<tr>
<th>Phenotype of parents</th>
<th>Mother</th>
<th>Father</th>
<th>Litter</th>
<th>Progeny</th>
<th>Phenotype of offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neg.</td>
<td>Neg.</td>
<td>4</td>
<td>6</td>
<td>0 21</td>
</tr>
<tr>
<td></td>
<td>Neg.</td>
<td>Pos.</td>
<td>5</td>
<td>5 21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pos.</td>
<td>Pos.</td>
<td>5</td>
<td>5 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neg.</td>
<td>Pos.</td>
<td>1</td>
<td>8 2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Pos.</td>
<td>Pos.</td>
<td>3</td>
<td>4 11 11 0</td>
<td></td>
</tr>
</tbody>
</table>

* Neg. = HI 1 antigen absent; Pos. = HI 1 antigen present.
In addition to these eight matings, we have tested the offspring of four matings in which serum from the fathers was not available for testing. Three of the mothers were positive. They had a total of 15 offspring, which all possessed the HI 1 antigen in the serum. The fourth mother lacked the HI 1 antigen, but all her seven offspring were positive, and must have inherited the antigen from the father, if the hypothesis of a dominant mode of inheritance holds true. On this assumption, the five males among the offspring must have received the HI 1 antigen by male-to-male transmission.

The high number of positives among the tested offspring is evidence that the HI 1 antigen manifests itself early in life.

**DISCUSSION**

The evidence that the precipitin in serum of R 56 is an antibody appears to be firm. The ultracentrifugal characteristics and electrophoretic mobility of the reacting component of normal rabbit sera, together with the staining properties of the precipitin bands, seem to prove that the corresponding antigen is part of the HDL of normal rabbit serum. As a result of the immunization procedure, rabbit 56 has apparently produced antibody to an HDL antigen that is absent from its own serum, although the rabbit possesses "common" HDL.

The most likely interpretation of this finding is that the HI 1 antigen is part of a genetic polymorphism of rabbit HDL. There was no ambiguity in the reactions: any given serum was either positive or negative. This clear-cut bimodal distribution is consistent with the notion that HI 1 is part of a genetic polymorphism.

The limited breeding data support this view. The occurrence of 21 negative and no positive individuals among the offspring of matings where both parents were negative represents a highly significant deviation from the overall prevalence of HI 1 in this closed colony, and is consistent with the hypothesis of a dominant mode of inheritance for the HI 1 antigen. An X-linked dominant mode of inheritance is considered unlikely from the distribution of HI 1 in the two sexes, and the occurrence of both positive and negative female offspring of a positive male and a negative female, as well as the possible observation of male-to-male transmission, excludes this possibility. Although the breeding data are scarce, they permit the conclusion that the HI 1 antigen is probably governed by an autosomal dominant mode of inheritance. On this assumption, rabbits possessing the HI 1 antigen are heterozygous or homozygous for a gene that can conveniently be called HI 1. Those in whose serum no HI 1 antigen is demonstrable presumably lack the HI 1 gene.

To our knowledge, this polymorphism has not been demonstrated previously. Kasukawa and his coworkers (14) reported the occurrence of antibodies to serum antigens following skin-grafting in rabbits. One isoantigen had lipoprotein characteristics. However, according to the illustrations published by these authors, the lipoprotein they observed had a slower migration rate than the HDL variant observed by us. Dray and Young (15), in their early studies of iso-precipitins in rabbits, mentioned one isoantigen, electrophoretically located in the α-region. This antigen was not investigated further, and the authors did not suggest that it was a lipoprotein. It too seems to show a slower electrophoretic mobility than the antigen described here.

We are not aware of any report of a common genetic polymorphism of HDL in any other species.

The observation of a genetic polymorphism of rabbit HDL appears promising for future genetic studies. At present, no information is available on the relation between the gene loci involved in the synthesis of the main serum lipoprotein in mammals. Since allotypes have already been observed in the LDL of rabbit serum, linkage studies between the polymorphisms of the two main classes of serum lipoproteins should now be possible.

In rabbits, the interesting phenomenon of allelic exclusion has been demonstrated for the LDL allotypes (16). If the product of an allele to H1 can be demonstrated, it may be possible to investigate whether this genetic mechanism applies to the allotypes of HDL as well.

The serum lipoproteins are of particular interest because of their connection with atherosclerotic disease, and a vigorous study of their structure and function is called for. With allelic markers on both main classes of serum lipoproteins, the rabbit may become more useful for several kinds of model study. If a common polymorphism of HDL, similar to that in the rabbit, should be found in man, a new tool would be available for the study of human serum lipoproteins in health and disease.

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