HEAVY AND LIGHT CHAIN ALLOTYPIC MARKERS ON RABBIT HOMOCYTOTROPIC ANTIBODY*

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Antibody which gives passive cutaneous anaphylaxis (PCA) reactions in the species in which it is produced has been termed homocytotropic antibody (HCA) by Becker and Austin (1). Although it was believed for some time that the rabbit did not produce homocytotropic antibody (2), Zvaifler and Becker (3) demonstrated the presence of these antibodies in rabbits. The electrophoretic mobility, molecular size, and chemical sensitivities of rabbit HCA are similar to those of skin-fixing antibodies demonstrated in rats (4), dogs (5, 6), and man (7). They are different from those of homologous antibody eliciting PCA in guinea pigs (8–10) and mice (11). Lindqvist (12) has shown that rabbit HCA formed against tetanus toxoid represents a distinct class of rabbit antibody, likely the rabbit counterpart of human reaginic antibody (IgE) (7).

The immunoglobulin classes[1] of the rabbit, IgG, IgM, IgA, and presumably homocytotropic antibody, are distinguished by antigenic differences residing in part at least in the Fc portion of the heavy (H) chain. Allotypic markers (13) of the group a specificities (14) present on the Fd portion of IgG (γ-chain) have been shown to be present also on IgM (μ-chain) (15, 16) and IgA (α-chain) (17). Thus, each heavy chain comprises an Fc region characteristic of its class (α, γ, or μ) and a second region bearing a group a marker common to all of these classes. Light chain markers of the group b specificities present on rabbit κ-chains are also common to these immunoglobulin classes, but this is expected since the various classes share light chains.

The purpose of the present study is to determine whether the groups a and b allotypic markers are found on rabbit HCA. We will demonstrate that rabbit homocytotropic antibody bears allotypic markers characteristic of both heavy and light chains, and that the major portion of the antibodies bears these genetic markers.

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[1] The immunoglobulin molecules and fragments are named following the recommendations of a committee of the World Health Organization (18). For those allotypic specificities which have been assigned to a definite locus (14) the initial A has been omitted, e.g., a1 in lieu of Aa1. If the locus is undefined, the specificity is indicated as A followed by the number, e.g., A11.

Materials and Methods

Preparation of Homocytotropic Antibody.—Rabbits were immunized by footpad injection of 0.5 ml (0.12 ml/footpad) of a mixture containing 19 parts Amphojel® (aluminum hydroxide gel, Wyeth Laboratories, Inc., Philadelphia, Pa.) and 1 part 2% solution in saline of recrystallized egg albumin (Sigma Chemical Co., St. Louis, Mo.).

The rabbits were bled by heart puncture 15 days after the initial injection. The blood was allowed to stand several hours at room temperature and centrifuged. The serum was stored in the cold.

Passive Cutaneous Anaphylaxis.—Passive cutaneous anaphylaxis (PCA) assays were performed as follows. The back of a young female albino rabbit was denuded with an electric clipper and marked off into areas. Dilutions of the immune sera (0.2 ml/area) were introduced intradermally, producing small blebs which disappeared in about an hour. After a minimum of 72 hr the rabbit was injected intravenously with 1.0 ml of a 2% ovalbumin solution containing 25 mg Evans blue dye (Eastman Organic Chemicals, Rochester, N.Y.) in saline. 30 min later the rabbit was killed by a blow behind the head, and the skin from the test area was removed. The inner vascularized region was stripped off, and the skin was stretched and allowed to dry at room temperature. Spots were scored as described under Results.

Anti-Allotype Immunoadsorbent.—Anti-allotype sera were prepared by the method of Oudin (19). Rabbits lacking an allotypic determinant were injected with ovalbumin-specific precipitates from rabbits bearing this determinant.

Immunoadsorbent was prepared by treatment of the anti-allotype sera with ethyl chloroformate by the method of Avrameas and Ternynck (20). We are indebted to Dr. Leonard Herzenberg for furnishing details for application of this procedure to anti-allotype sera.

The anti-allotype immunoadsorbent (AAIA) was conditioned before use by incubation with pooled rabbit serum containing all known allotypic determinants except the one against which it was directed. This was done to minimize the possibility of removal of HCA by antibody directed against genetic determinants other than those of groups a or b. For example, allotypic specificities such as A11 (21) and A12,[2] although normally detected only by hemagglutination techniques, will bind to AAIA directed against them. (Steward, Kindt, and Todd. Unpublished data.)

Inhibition of PCA Reactions with AAIA.—Dilutions of sera with antiovalbumin HCA were incubated 1 hr with AAIA in ratio of two parts diluted serum to one part solid AAIA. Following centrifugation the supernatant was reincubated with fresh AAIA. These steps were repeated until the allotype determinant in the serum could no longer be detected by interfacial precipitin test. Usually 3 or 4 incubations were required. Control incubations of serum with AAIA directed against specificities not present in the serum being tested were carried out in all cases. The supernatants from the adsorbed serum and from the controls were then assayed by PCA in duplicate rabbits.

RESULTS

Formation of HCA.—A group of ten rabbits was immunized with ovalbumin. Bleedings from these animals 15 days after initial injection were assayed for HCA by passive cutaneous anaphylaxis. Five of the animals gave PCA response at dilutions of 1:20 or greater, two gave no response at dilutions higher than 1:10, and three rabbits gave no reaction in the PCA assay even when undiluted serum was applied to the test animal. Sera from three rabbits showing the

[2] Mandy, W. J., and C. W. Todd. Manuscript in preparation.

strongest responses were chosen to represent each group a (H chain) allotype in the experiments described below.

Scoring PCA Data.—Fig. 1 shows a skin from a PCA test rabbit. The spots are rated by size and intensity of color. The spot *A15* is rated double positive (++); *A30, A60, A120, B15, B30* are positive (+); *B60* is positive/negative (±); and all others are negative (−). Similar ratings are given spots for the data tabulated below.

TABLE I

Light Chain Allotypic Markers on HCA

Treated* with	HCA from rabbit	
	12-06‡ (a2, b4)	12-35‡ (a3, b5)
Nothing	++	++
Anti-b4 AAIA	−	+
Anti-b5 AAIA	+	−

* Diluted (1:15) sera were adsorbed until the allotypic marker under study was not detectable by interfacial precipitin test.

‡ Spots compared at 1:15 dilution.

TABLE II

Heavy Chain Allotypic Markers on HCA

Treated* with	HCA from rabbit		
	11-75‡ (a1, b5)	12-06§ (a2, b4)	12-35§ (1 (a3, b5)
Nothing	++	++	++
Anti-a1 AAIA	−	+	++
Anti-a2 AAIA	+	−	++
Anti-a3 AAIA	+	++	−

* Diluted (1:15) sera were adsorbed until the allotypic marker under study was not detectable by interfacial precipitin test.

‡ Spots compared at a dilution of 1:30.

§ Spots compared at a dilution of 1:15.

Light Chain Allotypes of Rabbit HCA.—The series of spots on the rabbit skin shown in Fig. 1 indicate that treatment of antiovalbumin serum from rabbit 12-35 (a3, b5) with anti-b5 AAIA removes its ability to give positive PCA reactions at the concentration used (series C). Reactions caused by similar dilutions of the same serum are shown after no treatment (series A) and treatment with anti-b4 AAIA (series B). The B series was incubated with fresh anti-b4 AAIA as many times as had been necessary to incubate the C series with anti-b5 AAIA to remove all traces of b5 as detected by interfacial precipitin tests. The fact that the serum treated with anti-b4 retains activity rules out the possibility of HCA removal by handling or nonspecific adsorption except

for the slight difference seen between the B series and the untreated serum of the A series. In all cases these nonspecific losses were small compared to activity removal by the specific anti-allotype reagents.

Table I presents the data obtained with two rabbits, one of which (12-06) carries the b4 light chain allotype and the other (12-35) carries the b5 light chain allotype. These data show that the anti-b4 AAIA is active in removing the HCA from the serum of rabbit 12-06 (a2, b4), while it has no effect on the PCA reactivity of the serum from 12-35 (a3, b5). Conversely, the anti-b5 AAIA is active in removing the HCA from the serum of rabbit 12-35 while it has no effect on the PCA reactivity of the serum from 12-06. We conclude from these experiments that the light chains of rabbit HCA bear the group b allotypic markers b4 and b5.

Heavy Chain Allotypes on HCA.—Table II presents results from a similar experiment with three rabbit antisera having antiovalbumin activity in PCA assays. These rabbits each bear one of the three known group a (H chain) allotypic specificities. The data of Table II indicate that each of the group a specificities may be present on rabbit HCA. They further show that a major portion of the molecules involved in the response to the PCA assay bear these H chain allotypic markers.

DISCUSSION

Immunoadsorbent prepared by treatment of anti-allotype serum with ethyl chloroformate has removed HCA from the sera of rabbits carrying the specificity against which the anti-allotype serum is directed. This has been demonstrated for both group a and group b allotypic specificities. Similar removal has not been obtained using immunoadsorbents for allotypic specificities not present in the rabbit from which the serum containing the HCA was obtained.

Initial attempts employing untreated anti-allotype sera to effect the removal necessitated considerable dilution of the serum being tested because of the relative weakness of anti-allotype sera. At these dilutions preimmune sera from the rabbits in which the anti-allotype sera were prepared also eliminated PCA activity. This was not simply the result of dilution since goat serum employed in equal quantity did not eliminate the PCA reaction. These findings are considered a reflection of the complex nature of the fixation reaction. The injected HCA presumably competes successfully with a large excess of autologous HCA for skin fixing sites; yet it can not compete with equal success with nonautologous HCA injected with it at the same site.

Two distinct kinds of antibody that sensitize homologous skin have been reported. The first, present in the mouse (11) and guinea pig (8–10), is a 7S, heat-stable antibody with an electrophoretic mobility in the fast IgG region. The second, present in rats (4), man (7), and dogs (5, 6), is larger than 7S and heat labile. It is electrophoretically faster than IgG and has been desig-

nated IgE (7). Rabbit HCA is more similar to the second type, although there is some question as to the heat lability of this antibody (3, 12).

Zvaifler and Becker (3) found rabbit HCA against dinitrophenylated bovine serum albumin present only in the primary response. Subsequently this antibody has been found in secondary responses to tetanus toxoid by Lindqvist (12) and to ovalbumin. (Kindt and Todd. Unpublished data.) Certain parasites (22, 23) have been shown to elicit extremely potent HCA responses in the rabbit in response to primary and occasionally secondary inoculations. With the exception of these parasites, it appears that all rabbits do not make detectable HCA to a given antigen, and that some antigens give stronger responses than others. The response in the rabbit is thus similar to the allergic response in man. Whether this is a genetically determined characteristic in the rabbit has not been reported.

Although rabbit HCA to a hapten was earlier reported (24) to be of the IgA class, recent evidence (12, 25, 26) indicates that at least most HCA in the rabbit is in a distinct class. The behavior of the antiovalbumin HCA activity reported here is similar to that described by Lindqvist (12) and appears to be the counterpart of IgE as described in the human (7) and canine (5, 6) species.

The detection of light chain allotypes of the b group indicates that the light chain component of rabbit HCA is similar to that of the other immunoglobulin light chains. The fact that the activity to the PCA assay is so effectively removed by anti-b4 or anti-b5 indicates that most of the light chains come from the κ-class, which bears the group b specificities, as has been the case with other rabbit immunoglobulins studied.

The presence of group a allotypic markers on the heavy chains of HCA represents an extension of the finding that these genetic markers are common to all other rabbit immunoglobulins thus far described. Explanations for the presence of both the class specific (α, γ, ϵ,[3] or μ) and the allotype bearing portions of the rabbit heavy chains must take into account that the allotypic specificities are determined by the primary structure (27, 28, 29) and are not due to prosthetic groups (such as a carbohydrate). The argument is not satisfying that a common ancestral remnant of a gene present prior to evolution of class differences codes for the allotypic specificities in individual class-specific genes, because it necessitates an explanation involving three independent evolutionary paths to the rabbit H chain genes, one for each group a specificity. To dismiss the markers shared by the immunoglobulin classes as closely linked, but otherwise unrelated is not valid because of serologic studies showing great similarity of the class-shared markers (30, 31). Genetic models based on these observations are discussed in greater detail elsewhere (30, 32).

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[3] The H chain of rabbit HCA is here designated ϵ, since it seems very likely that this antibody in the rabbit is the counterpart of IgE in man (7).

SUMMARY

Evidence has been presented that rabbit homocytotropic antibody prepared against ovalbumin bears allotypic markers of both group a (heavy chain) and group b (light chain). This was shown by specific removal of activity in passive cutaneous anaphylaxis assays by anti-allotype immunoadsorbents.

The homocytotropic antibody has properties which indicate that it belongs to a new class of rabbit immunoglobulin. These results demonstrate that this newly described class possesses the allotypes of both groups a and b, as has been previously shown for other rabbit immunoglobulin classes.

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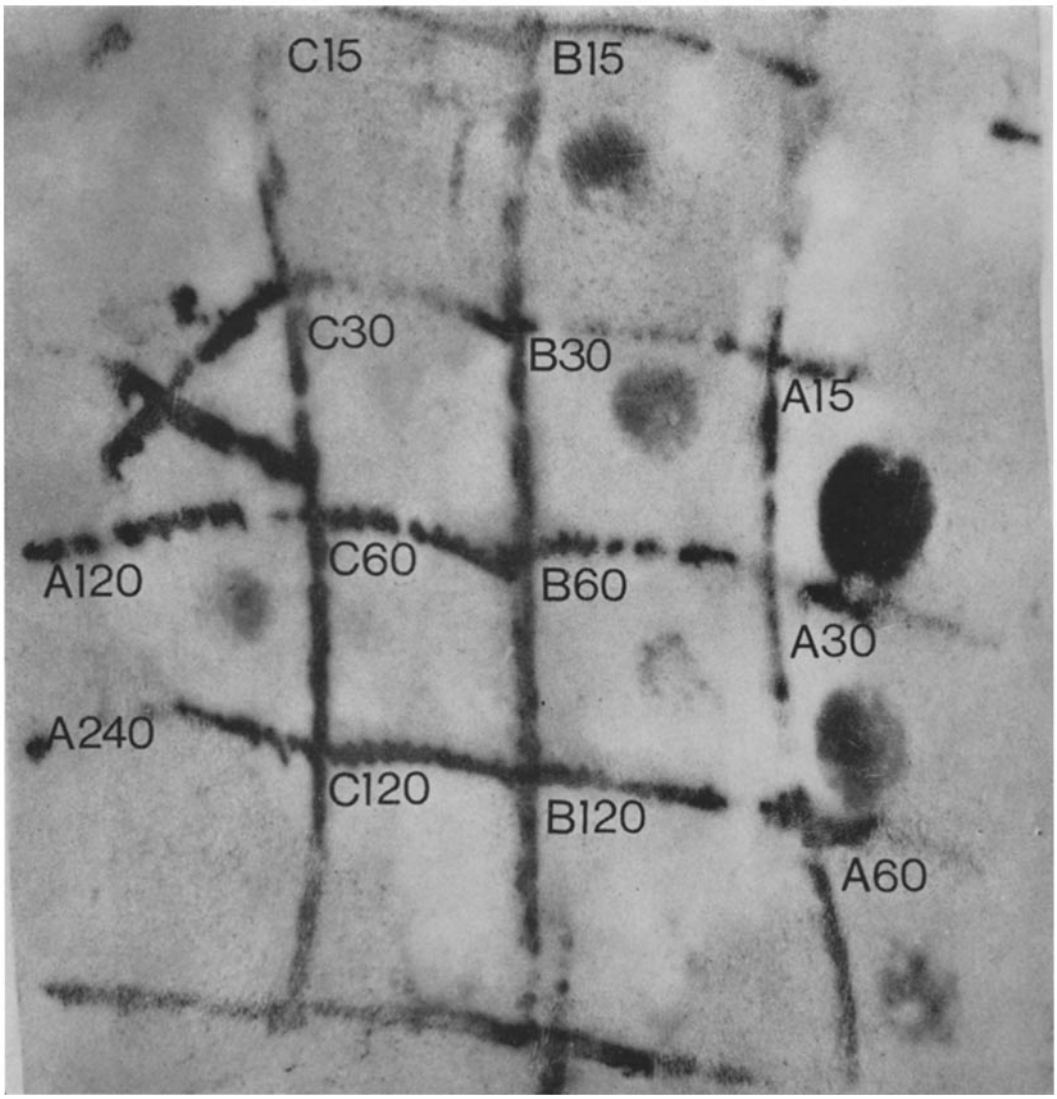

FIG. 1. Skin from PCA test rabbit 21-96. Each area was injected intradermally with 0.2 ml of rabbit 12-35 (a3, b5) antiovalbumin. *A*, no treatment; *B*, treated with anti-b4 immunoabsorbent; *C*, treated with anti-b5 immunoadsorbent. The numbers are reciprocals of the dilutions in saline. ¾ actual size.