

THE INHERITANCE OF ANTIBODY V REGIONS IN THE RABBIT: LINKAGE OF AN H-CHAIN-SPECIFIC IDIOTYPE TO IMMUNOGLOBULIN ALLOTYPES*

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Idiotypic inheritance was first demonstrated (1) in partially inbred rabbits immunized with streptococcal carbohydrate antigens. Several subsequent investigations in both the rabbit and the mouse have confirmed this finding and have suggested that idiotypes are linked to the H-chain allotypes (2, 3) and associated with specific L-chain subgroups (4). However, in studies on rabbit antiproteus, antisalmonella, and antipneumococcal antibodies, no evidence that idiotypic specificities are genetically transmitted could be obtained (5-8).

Difficulties in detecting idiotypic inheritance may be attributable to a variety of factors. For example, if a large repertoire of genes encoding idiotypes exists in the genome, antibodies with a particular idio type may not be selected in a given response, or may be expressed only for a brief period of time (9-12). Perhaps more important is the fact that most idiotypes require a specific H and L combination for their expression (7, 13-15). Therefore, in order for idiotypic inheritance to be detected simultaneous expression of genes at two unlinked loci is required (16). If a given idio type could be serologically dissected into its respective H and L components, then detection of the product of one variable region gene would no longer require the concomitant expression of the other.

This report describes the preparation of an anti-idio type antiserum specific for the H chain of an homogeneous antibody isolated from rabbit 4135 (4135 Ab). Sera from related and unrelated rabbits were tested for presence of idiotypic determinants identical to or cross-reactive with the 4135 H-chain idio type (HI_d)¹ by their ability to inhibit the homologous binding reaction between radiolabeled 4135 Ab and insolubilized anti-HI_d. This analysis revealed that HI_d was inherited and linked to the a3 allotype present in the H-chain allogroup J. Furthermore, one rabbit with phenotype E,H produced HI_d-reactive antibodies that expressed both C_H and V_H region allotypes characteristic of the J allogroup.

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¹ *Abbreviations used in this paper:* 4135 Ab, a homogeneous antistreptococcal antibody with allotype a3/b4; HI_d, the heavy chain-specific idio type of 4135 Ab; HAS, *N*-hydroxysuccinimide-activated Sepharose; GalNAc, *N*-acetylgalactosamine; PBS, 0.15 M NaCl buffered to pH 7 with 0.05 M KPO₄ with 0.05% NaN₃.

Materials and Methods

General. Techniques for preparation of streptococcal vaccine, immunization of rabbits, antibody quantitation, isolation of IgG, preparation of anti-allotype sera, and isolation of an homogeneous antistreptococcal antibody with allotype a3/b4 from the transfusion plasma of rabbit 4135 (a²a³/b⁴b⁴) have been previously described (17-19).

H- and L-Chain Separation and Recombination. Antibodies were separated into heavy and light chains by mild reduction and alkylation followed by chromatography on Sephadex G-75 in 10% acetic acid according to the principles of Fleischman et al. (20). Recombination of H and L chains was performed as previously described (21).

Preparation of Idiotypic Antisera. Rabbit 4371 (a²a³/b⁴b⁴) was immunized with Group C streptococcal vaccine (17), and its antibodies were isolated utilizing an immunoadsorbent column containing *p*-aminophenyl- α -*N*-acetylgalactosamine coupled to Sepharose 2B as previously described (22).

4135 homogeneous heavy chains were recombined with 4371 pooled antistreptococcal light chains, and 1 mg of the hybrid molecules was mixed with 1 ml of Group C streptococcal vaccine in sterile saline. The resulting mixture was emulsified in an equal volume of incomplete Freund's adjuvant and injected subscapularly back into rabbit 4371 at monthly intervals. Idiotypic antisera against intact 4135 antibody was prepared in an allotypically matched rabbit (4107) as previously described (23). The rabbits were bled monthly after the third injection.

Preparation of 4135 Fab fragments. 4135 Fab fragments were prepared by the method of Porter (24).

Radioiodination. In most instances, purified antibody and IgG preparations were radiolabeled by the iodine monochloride method (25). Occasionally, samples were radioiodinated by the lactoperoxidase method as described by David (26).

Radioimmunoassay. Insolubilized antisera were routinely prepared by coupling whole sera to *N*-hydroxysuccinimide-activated Sepharose (HAS) as described by Gottlieb et al. (27). In the case of anti-HId, 2.2 mg of purified antibody, obtained from 20 ml of 4371 antiserum, was insolubilized on 1 g of HAS. Binding curves were constructed using 0.25 μ g of radiolabeled antigen and various dilutions of the HAS-anti-HId in a total vol of 200 μ l. Inhibition analyses were performed using concentrations of antisera which bound 50% of the radiolabeled antigen. HId cross-reactions were measured by the ability of undiluted, heat-inactivated sera to inhibit the homologous binding reaction between the HAS-anti-HId and 4135 Ab. Latent group a allotypes were quantitated as described previously (28).

Isoelectric Focusing. Analytical electrofocusing in polyacrylamide gel (pH 5-8) was carried out on an LKB Multiphor apparatus as described in LKB Application Note 75. The gels were stained for 12 h with 0.01% Coomassie Brilliant Blue and destained in distilled water, ethanol, acetic acid (6:3:1).

Isolation of Anti-HId. The anti-idiotypic components specific for the 4135 H chain were isolated from 4371 idiotypic antiserum on an immunoadsorbent column prepared by coupling 25 mg of 4135 Ab to 50 ml of CNBr-activated Sepharose 4B (29). In a typical isolation, 20 ml of serum was applied to the column at room temperature. After extensive washing with phosphate-buffered saline (PBS), the bound antibody fraction was eluted with 3 M NH₄SCN in PBS. This fraction was dialyzed against PBS and then passed through a second column containing 10 ml of Sepharose 4B to which 20 mg of pooled rabbit IgG was coupled. This column removed rheumatoid factors and antibody denatured by the NH₄SCN. The antibodies were next passed through a column containing 20 mg of 4135 L chains bound to 40 ml of Sepharose 4B to remove any activity for the L chains of 4135 Ab. A yield of 2.2 mg of antibody was obtained. This material was designated anti-4135 HId.

Isolation of HId-Positive Molecules. A specific anti-HId immunoadsorbent column was prepared by binding 1.3 mg of purified anti-HId to 40 mg of HAS. The gel suspension was poured into a previously acid-washed and silicized glass column (ID, 0.5 cm). A rabbit IgG column containing 10 mg of IgG was similarly prepared, and both columns were washed with PBS containing 1% bovine serum albumin. Radiolabeled IgG (10 mg) from the serum of an HId-positive rabbit was passed through the IgG column, and the unbound fraction was applied to the anti-HId column. After washing with PBS, the bound antibodies were eluted in 1 ml of 3 M NH₄SCN, dialyzed against PBS, and assayed for their reactivity with various insolubilized antisera.

Typing for d11/d12 by Sucrose Density Gradient Centrifugation. Purified ¹²⁵I-labeled HId-

positive antibody (1 μg) was incubated with 0.2 ml of an anti-d-allotype serum for 2 h at 37°C. The mixture was cooled to 4°C overnight and layered over a linear 5–20% (wt/vol) sucrose density gradient in PBS. Centrifugation was carried out in a Beckman Model L5-65 ultracentrifuge (Beckman Instruments Inc., Fullerton, Calif.) with a SW27 rotor for 24 h at 27,000 rpm at 4°C. The gradient was collected in 0.5-ml fractions which were counted for radioiodine and analyzed spectrophotometrically for protein content. In a negative reaction, a single radioactive peak was observed in the position of IgG as determined by calibration runs. In a positive reaction, faster sedimenting components appeared, corresponding to soluble immune complexes formed between the anti-allotype serum and the antibody in question. The protein content of the antiserum was used as an internal reference. The anti-d allotype sera were kindly provided by Dr. William J. Mandy.

Results

Homogeneous H chains from 4135 Ab were isolated and recombined with L chains from an antistreptococcal antibody pool isolated from the serum of rabbit 4371. The recombinant molecules were then injected into the L-chain donor (4371) with the expectation that only the H chains would be immunogenic. The idiotypic antiserum was insolubilized on HAS according to Gottlieb et al. (27) and tested for its ability to bind intact 4135 Ab. The HAS antiserum reacted with radiolabeled 4135 Ab preparation but bound less than 40% of the antibody at the highest concentration of anti-HId used. Therefore, the antibodies specific for 4135 Ab were enriched by isolation on a specific immunoabsorbent column.

Isolation and Characterization of Anti-HId Components. The specific antibody components were isolated from whole antiserum on an affinity column consisting of 4135 Ab coupled to Sepharose 4B. The eluted fraction was insolubilized on HAS and its specificity determined by radioimmunoassay. Fig. 1 depicts the binding curves of 4135 Ab, 4135 H-chain recombinant, 4135 L-chain recombinant, and a3b4 IgG to the isolated antibody preparation. The recombinant IgG molecules used in these assays were prepared from (a) 4135 H chain and a pool of b4 L chains, and (b) 4135 L chains and a pool of a3 H chains. As seen in Fig. 1, the HAS-anti-HId bound the intact Ab and the 4135 H-chain recombinant equally well, but bound neither the L-chain recombinant nor the a3b4 IgG preparation. HAS-anti-HId also bound 4135 Fab fragments, but not 4135 L chains.

As shown in Table I, the homologous binding reaction between HAS anti-HId and either 4135 Ab or 4135 H-chain recombinant was completely inhibited by 4135 Ab, 4135 H-chain recombinant, and 4135 Fab. However, 4135 L, 4135 L-chain recombinant, a3/b4 IgG, and 4135 preimmune serum were unable to inhibit these reactions. The idiotypic nature of the binding between HAS-anti-HId and 4135 Ab was verified by the fact that a digalactosamine hapten isolated from Group C streptococcal carbohydrate (22) inhibited this reaction.

The degree of contamination of the 4135 H recombinant with intact 4135 Ab was determined using an idiotypic antiserum (4107) which reacted with the intact 4135 Ab and not with the isolated chains. A standard inhibition curve was constructed using increasing amounts of unlabeled 4135 Ab to inhibit the homologous binding reaction between HAS-4107 and 0.25 μg of radiolabeled 4135 Ab. Addition of 100 μg of the 4135 H-chain recombinant gave an inhibition value of 10%. This value reflects a maximum of 0.15% contamination with the intact 4135 Ab.

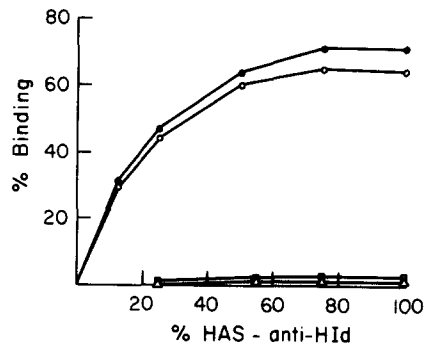


FIG. 1. Binding of radiolabeled 4135 Ab (●), 4135 H-chain recombinant (○), 4135 L-chain recombinant (□), and a3/b4 IgG (Δ) to isolated anti-HId antibodies insolubilized with HAS. The 100% level of HAS-anti-HId corresponds to 100 μ l of the reagent prepared from 2.2 mg of isolated anti-HId and 1 g of HAS in a vol of 35 ml.

Inheritance of HId. With the specificity of the HAS-anti-4135 HId for the V_H region of 4135 Ab established, a search for H-chain idiotypes identical to or cross-reactive with that of 4135 was initiated. Cross-reaction was determined by the ability of Group C antistreptococcal sera to inhibit the homologous binding reaction between 4135 Ab and the HAS-anti-HId. Sera obtained 27–29 days after the first streptococcal immunization were used as inhibitors in the screening procedure. Serum samples from 70 related rabbits and 43 unrelated rabbits were tested. As shown in Table II, 43% of the sera from related rabbits immunized with Group C streptococcal vaccine gave inhibition values greater than 10%, while only 8% (2/24) of sera from unrelated rabbits gave significant inhibition. Furthermore, neither Group A antisera nor preimmune sera inhibited the homologous binding.

None of the sera tested completely inhibited the binding reaction between 4135 and anti-HId. To determine whether this incomplete inhibition reflected the presence of antibodies idiotypically identical to 4135 H in concentrations too low to yield complete inhibition, dilutions of HId-positive sera were tested as inhibitors. It was found in 9 of 10 sera tested that inhibition values were not reduced by diluting the sera 1:4. This indicates that the incomplete inhibition was due to partial idiotypic identity of the antibodies in these sera to 4135 HId rather than to low concentrations of identical idiotypes. The IgG fraction from the one serum (4231) that did not reach a plateau value was concentrated and used as an inhibitor. A plateau value of 62% was reached with a threefold concentration of the 4231 IgG.

It was next considered whether the same determinants were detected in all the HId-positive sera tested. Mixtures containing equal amounts of two sera were tested as inhibitors to determine whether additive values could be obtained. Table III lists the inhibition values given by all possible binary mixtures of six HId-positive sera. The six sera tested could be placed into three distinct groups based on inhibition values obtained in this experiment. Group I included sera 2897 and 3551, Group II, 2996, 4351, and 4539, and Group III, 4231. Sera within a single group gave the same level of inhibition, either singly or in mixtures. Mixtures containing a Group II serum with a serum from either

TABLE I
Inhibition of the Homologous Idiotypic Reaction Between 4135 Ab or H-Chain Recombinant and Anti-HId by Various Inhibitors

Antigen	Inhibitor	Concentration of inhibitor	Percent inhibition
		<i>mg/ml</i>	
4135 Ab	4135	1.0	95.4
	4135 H recombinant*	1.0	91.8
	4135 Fab	1.0	94.7
	3-O- α -GalNAc GalNAc‡	2.0	39.4
	4135 L recombinant*	1.0	3.6
	4135 L	4.0	2.4
	3,4 IgG	1.0	2.0
	4135 preimmune serum	undiluted	1.8
4135 H recombinant	4135 Ab	1.0	96.0
	4135 L recombinant*	1.0	3.1
	4135 L	4.0	4.1

* Recombinant molecules were prepared from 4135 H or L chains and L or H chains from a3/b4 IgG.

‡ Hapten prepared from Group C carbohydrate (22).

Group I or III gave inhibition values that were approximately equal to the sum of the values obtained for the individual sera. In fact, the mixture of a Group II plus a Group III serum gave nearly complete inhibition of the binding reaction. The combination of a Group I and a Group III serum gave an inhibition value equal to that obtained for the Group III serum alone.

Linkage of HId to an a3 Allgroup. The inheritance of HId was tested using related rabbits with all possible combinations of the group a allotypes. However, as shown in Fig. 2, all but one of the cross-reactive sera were from rabbits expressing allotype a3, which was the allotype of the proband Ab 4135.

Because rabbit H chains of various classes express different allotypes that are controlled by closely linked genes inherited as sets called allgroups (30), it was possible to examine the relationship between HId and the different allgroups within the family. Extensive allotyping revealed that within the extended family of 4135, three distinct allgroups contained the a3 allotype – "I", "J", and "J'" (see Fig. 3 legend). The allgroup J'² arose from a crossover in rabbit 2663 (see dotted arrow, Fig. 3) and has the same V_H allotype genes as J, but differs with regard to its C_H allotypes (31). It was found, as illustrated in Fig. 2 and 3, that all but one of the strongly cross-reactive a3-positive sera were from rabbits with allgroups designated J or J'. It therefore appears that HId is linked to the specific a3 gene or genes contained in the allgroups J or J'. All of the related rabbits were homozygous for the b4 allotype and, therefore, information concerning linkage of the HId to the b allotypes could not be obtained.

Molecular Association of the HId and the a Allotype. Because the majority of rabbits typed positive for HId were heterozygous with respect to their a

² This allgroup is currently under investigation by Dr. W. C. Hanly and Dr. A. Gilman-Sachs. The notation "J'" is a tentative assignment pending more detailed information concerning the IgM allotypes.

TABLE II
Detection of 4135 HIid in Related and Unrelated Rabbits

	Antigen	Number studied	Inhibition of the 4135 HIid reaction*			Percent positive
			10%	5-10%	5%	
			(+)	(±)	(-)	
Unrelated rabbits	Group A	19	0	2	17	0
	Group C	24	2	3	19	8
Related rabbits	None‡	11	0	0	11	0
	Group C	70	30	26	14	43

* HIid cross-reactions were detected by inhibition of the binding reaction between radiolabeled 4135 Ab and HAS-anti-HIid. Inhibition values greater than 10% were considered positive.

‡ Sera from rabbits that produced HIid upon immunization.

allotypes, the molecular relationship between HIid and the group a allotypes could not be determined from these results alone. To determine the group a allotypes of molecules bearing HIid, a specific anti-HIid immunoabsorbent column was prepared. IgG was isolated from immunized rabbits with allotypes a^1a^3 and a^2a^3 , radiolabeled, and passed through a pooled IgG column to remove nonspecifically binding molecules. The effluent from this column was then applied to the anti-HIid column, and the HIid-positive molecules were eluted and tested for their ability to bind various antisera. The data in Table IV indicate that all molecules that bound to the anti-HIid column carried the a3 allotype. The capacity of the column and the effects of the elution procedure on the molecules were determined using 4135 Ab as a control. A 50 μg sample of this antibody was applied to the column, eluted, and assayed for binding activities. The entire 50 μg sample was recovered and binding to HAS-anti-a3 was reduced only 7% when compared to pre-elution binding values. When the IgG fraction from a rabbit that showed negative results in the inhibition assay (2460) was placed on the anti-HIid column, the effluent contained too few counts to permit quantitation. This result indicates a complete concordance between inhibition of the homologous HIid binding assay and the ability to bind to the anti-HIid immunoabsorbent column.

Association of HIid with Latent a3 Allotype in Rabbit 4232 (a^1a^2/b^4b^4). An analysis of the HIid-positive components in the serum of rabbit 4232, which produced a significant amount of HIid yet lacked the a3 allotype, was next conducted. It was found, using a quantitative radioimmunoassay (28) for the allotype a3, that detectable concentrations of the a3 allotype were present in two different bleedings from this rabbit. The bleeding used in the HIid assay contained 3 $\mu\text{g}/\text{ml}$ of latent a3 allotype. Other bleedings from this animal were tested for HIid (Fig. 4), and a correlation was observed between the presence of latent a3 allotype and the presence of HIid.

Further evidence that the same molecules expressing the latent a3 allotype also express HIid was provided by experiments in which HAS-anti-allotype sera were used to adsorb molecules containing the a3 allotype. As depicted in Fig. 5, two sequential adsorptions with HAS-anti-a3 removed 85% of the latent a3 allotype along with the majority of the HIid activity. In the reciprocal experi-

TABLE III
Inhibition of the Reaction between 4135 Ab and Anti-HId by Binary Mixtures of Six HId-Positive Antisera

Inhibitor serum 2	Inhibitor serum 1					
	2897	3551	2996	4351	4539	4231
	(%)	(%)	(%)	(%)	(%)	(%)
2897	28					
3551	30	27				
2996	53	58	34			
4351	60	52	35	35		
4539	59	57	34	35	34	
4231	60	66	88	89	82	62

*All inhibitors were added at concentrations previously determined to give maximum inhibition. Values shown are percent inhibition as averaged from four replicate experimental determinations.

ment, adsorption with anti-HId removed 54% of the latent allotype activity. Similar adsorptions with anti-a1 and anti-a2 did not affect the levels of HId in the 4232 serum (Fig. 5).

Antiserum from 4232 was fractionated on an immunoabsorbent column consisting of *p*-aminophenyl- α -*N*-acetylgalactosamine coupled to Sepharose. The column was washed with PBS and eluted sequentially with *N*-acetylgalactosamine, *N*-acetylgalactosamine in 2 M sodium chloride, and 5 M guanidine hydrochloride. Five protein peaks were observed using this elution procedure: two from the PBS elution and one from each of the other eluants. These five peaks were concentrated, dialyzed, and assayed for latent a3 allotypes and for HId. Fraction 4 was significantly enriched with molecules bearing the latent allotype as well as those bearing HId (Table V). This fraction was then radiolabeled, and the specific HId antibodies were isolated and allotyped. HAS-anti-a3 sera bound 64% of the purified preparation, whereas both HAS-anti-a1 and HAS-anti-a2 reacted with less than 10% of the purified molecules.

Typing for d Allotypes. The isolated, radiolabeled, HId-positive molecules from 4232 ($a^1a^2/d^{12}d^{12}$) were typed for constant region markers (d allotypes) by sucrose density gradient centrifugation in the presence of anti-d11 or anti-d12. As shown in Fig. 6, soluble immune complexes were formed between 4232 radiolabeled HId-positive antibodies and the anti-d11 antiserum. When the purified antibodies were incubated with anti-d12 serum, the radioactive peak appeared in the position observed in calibration runs for IgG. In similar experiments, 4135 Ab likewise reacted only with anti-d11 antibodies. In contrast, the HId-positive, a3-positive molecules isolated from the crossover rabbit (2663), which expressed the J' allogroup (see Fig. 3), were found by this method to express only d12 determinants.

Discussion

The present report documents the properties of an H-chain-specific idiotypic antiserum (anti-HId) prepared using a method that should prove generally applicable to the production of other chain-specific idiotypic antisera. The anti-HId antiserum was used in an immunogenetic study within an extended rabbit

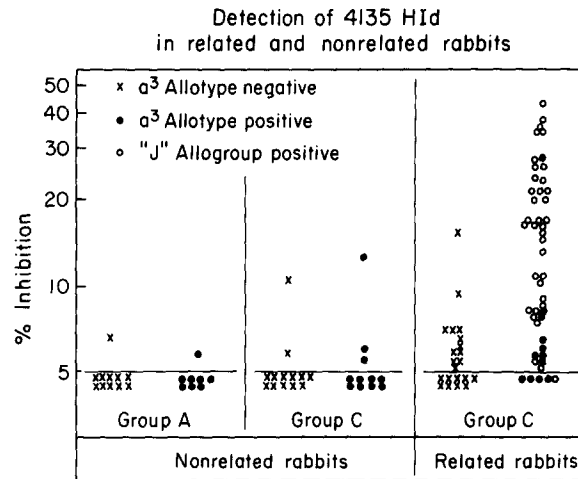


FIG. 2. Detection of the HI_d in related and nonrelated rabbits immunized with Group A and Group C streptococcal vaccine. All preimmune sera tested gave inhibition values less than 5%. The open circles refer to rabbits with either the "J" or "J'" allogroup.

family to determine inheritance and linkage patterns of HI_d. This discussion will treat first the preparation and properties of anti-HI_d and then the genetic implications of the linkage data obtained by its use.

Antiserum specific for the H chain of homogeneous antibody 4135 was elicited by immunizing an allotypically matched rabbit with hybrid immunoglobulin containing 4135 H chain and an L-chain pool from the rabbit to be immunized. A procedure of this type was necessary because rabbit idiotypic antisera normally recognize only determinants dependent on a specific H-L combination. Chain-specific idiotypic antibodies have in rare instances been detected in rabbit antisera (32), although chain specificity is the normal case in idiotypic antisera against human myeloma proteins with lambda L chains (13). Recently, the occurrence of H- and L-chain-specific idiotypes for mouse antibodies to streptococcal Group A carbohydrate has been described (33).

The use of L chains isolated from the rabbit in which the antiserum was prepared eliminated the possibility that L-chain-specific components would be raised, but did not preclude the possibility that the interaction of 4135 H chain with the pooled L chains would elicit antibodies recognizing new sets of combination determinants. Two factors may have enhanced the relative antigenicity of H-chain-specific determinants. First, because the L chains used were heterogeneous, the concentration of any one H-L pair may have been too low for the combination determinants to be effectively antigenic. Second, because tetrameric recombinant molecules were not specifically isolated, the immunogen very likely contained a mixture of polymeric molecules. It is possible that H chains presented in the altered state of polychain complexes were the effective immunogen.

Two of the properties of the anti-HI_d antiserum are of particular interest. First, examination of the HI_d-reactive antibodies by isoelectric focusing indicated restricted heterogeneity in the preparation (M. L. Yarmush. Unpublished data). A similar degree of restriction has been noted in anti-idiotypic antibodies

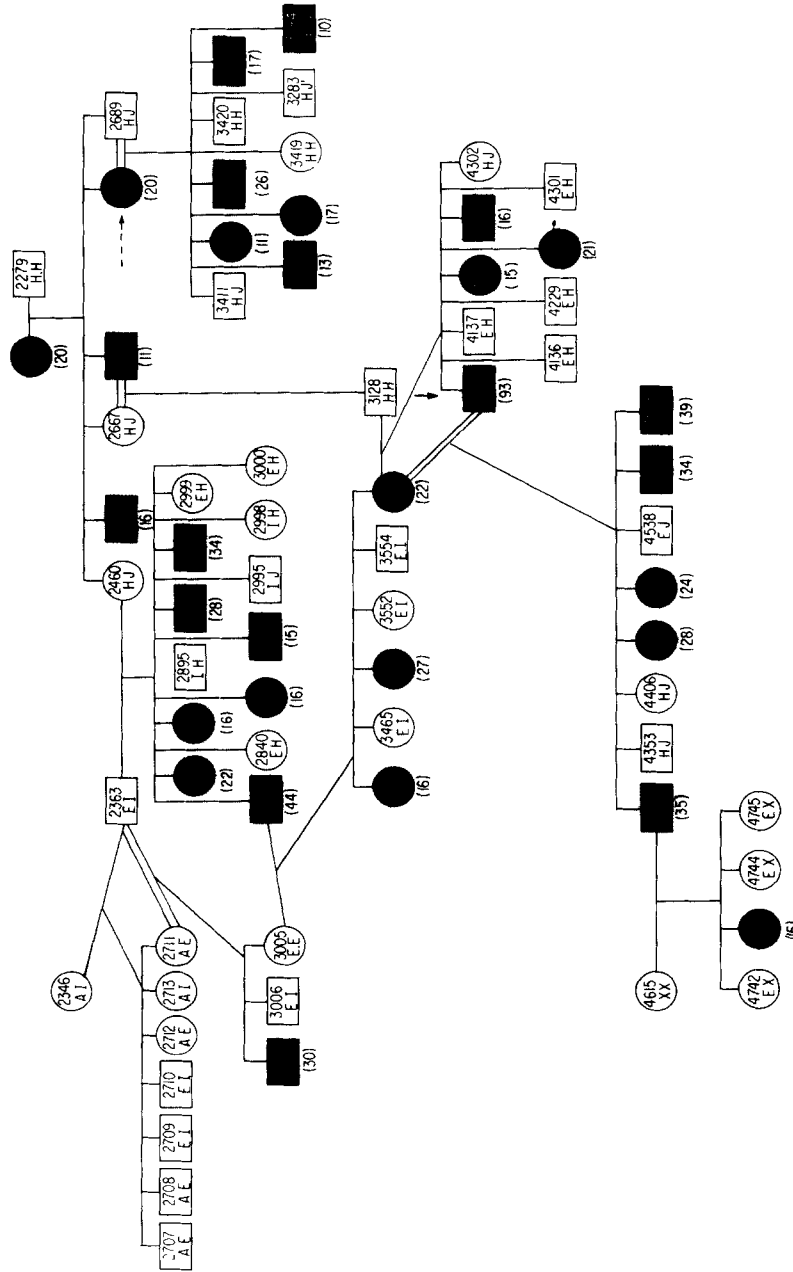


FIG. 3. Pedigree of rabbit 4135 (solid arrow) showing rabbits positive (black background) and negative (white background) for the H1d. Allogroups are indicated beneath the rabbit number and the percent inhibition of the homologous binding reaction between 4135 Ab and the HAS-anti-H1d is shown in parentheses for all positive rabbits. Complete allogroup typing of representative members of the family was done by W. Carey Hanly. The five H-chain allogroups present in this family are indicated by single letter notations: A (a¹, x⁻, x⁻, n⁸¹, f⁷³, g⁷⁴, d¹², e¹⁵), E (a¹, x⁻, y⁻, n⁸¹, f⁷⁰, g⁷⁵, d¹², e¹⁵), H (a², x³², y³³, n⁸², f⁶⁹, g⁷⁷, d¹², e¹⁵), I (a³, x³², y⁻, n⁻, f⁷¹, g⁷⁵, d¹², e¹⁵), J (a³, x³², y⁻, n⁻, f⁷², g⁷⁴, d¹¹, e¹⁵), and J' (a³, x³², y⁻, n⁸², f⁷², g⁷⁴, d¹¹, e¹⁵) (30). Undetermined allogroups are designated by the single letter notation "X".

TABLE IV
Serologic Characterization of Isolated HIid-Positive Molecules

Rabbit	Group a allo- types	Amount eluted*	Percent binding			
			Anti-a1	Anti-a2	Anti-a3	Anti-HIid
		μg				
4539	1, 3	11.8	3	6	63	59
4545	2, 3	13.5	8	5	59	56
4231	2, 3	7.3	4	6	69	49
4351	1, 3	9.7	7	3	71	62
2996	1, 3	10.9	7	8	68	60
4135*	3	50.0	4	3	73	71
2460‡	2, 3	<1.0	—	—	—	—

* 10 mg of radiolabeled IgG was applied to the column. In the case of 4135 Ab, 50 μg was used.

‡ This serum was HIid negative by radioimmunoassay.

directed against an L-chain idiotype (21) as well as antibodies directed against group a allotypes (34). Second, inheritance of antistreptococcal antibodies bearing determinants cross-reactive to HIid was demonstrated in the present study by the ability of sera from rabbits related to 4135 to inhibit the homologous binding reaction between HAS-anti-HIid and 4135 Ab. Only the L-chain idiotype studied earlier (21) yielded similar results. In both the human (35, 36) and the rabbit (6) it has previously been found that the only molecules capable of inhibiting an homologous idiotypic binding reaction are other antibodies isolated from the individual producing the proband antibody.

The inability of the test sera to completely inhibit the reaction between anti-HIid and 4135 Ab indicates that no V_H region completely identical to that of antibody 4135 was produced by a related rabbit. It is noteworthy, however, that values approaching 100% inhibition could be obtained using certain mixtures of HIid-positive sera. The fact that the six test sera examined by the mixed inhibition experiment could be divided into three discrete groups (Table III) indicates that a relatively small number of determinants are recognized by the anti-HIid. This results further indicates the feasibility of obtaining, by absorption, antibodies directed against a very restricted number or possibly a single determinant of a homogeneous antibody. It is tempting to speculate that these procedures could yield antibodies directed against determinants contained within a single hypervariable region. Genetic studies employing such reagents could provide evidence to support theories of antibody diversity which call for multiple gene inserstions in the V region (37, 38).

The inheritance pattern of HIid within the extended family of 4135 was examined to determine factors associated with its expression. An indication that a small number of genes determine the expression of HIid was given by the lack of correlation between HIid positivity and the degree of relationship between the test animals and the proband, 4135. When the covariance method was used to calculate the coefficients of relationship between 4135 and those rabbits tested for HIid, values ranging from 0.06 to 0.85 were obtained. However, no correlation between degree of HIid cross-reactivity and these values was observed. This negative result indicates that HIid expression is not a

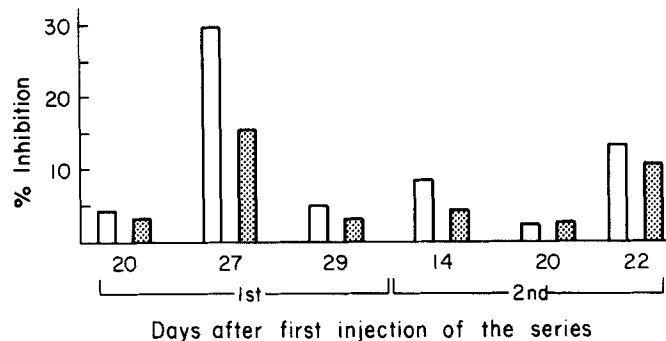


FIG. 4. HId activity (shaded area) and latent a3 allotype activity (unshaded area) in six serum samples of rabbit 4232. Latent allotype activity is represented as the percent inhibition of the binding of a radiolabeled a3/b4 IgG preparation to HAS anti-a3 sera. HId activity is represented as the percent inhibition of the homologous binding reaction between 4135 Ab and the HAS-anti-HId.

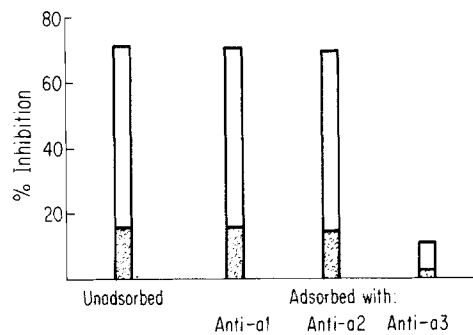


FIG. 5. HId activity (shaded area) and latent allotype activity (unshaded area) in 4232 serum after absorption with HAS antiallotype antisera. HId and latent allotypes were measured as in Fig. 4.

polygenic phenomenon. If it were a polygenic trait, the degree of relationship would show a positive correlation with the number of responders and intensity of the HId response.

Examination of the pedigree of rabbit 4135 (Fig. 3) clearly indicates that the presence of the a3 allotype linked to the J allogroup is the major factor determining expression of the HId. Included among the rabbits screened in this study were those carrying the J' allogroup, which resulted from a crossover within the H-chain allotype genes (31). Whether the crossover occurred between the variable and constant region genes, as first thought, or between C_H genes encoding IgM allotypes and those encoding C_γ and C_α allotypes is presently under study (Hanly and Gilman-Sachs, unpublished). It is certain, however, that the a3 allotype in the resulting allogroup is associated with the d12 allotype rather than d11. Several rabbits with the J' allogroup showed positive HId reactions. Isolation of these cross-reactive antibodies and typing for the d allotypes revealed that they were positive for allotype d12. This finding supports the linkage of HId to these V_H genes and furthermore indicates that idiotypic and allotypic genes involved in the crossover were able to integrate with the new C_γ genes to synthesize these HId-positive H chains. Rabbits possessing the I allogroup,

TABLE V
 Fractionation of Antistreptococcal Antibodies from Serum 4232 on an
 Immunoabsorbent Column*

Fraction	Buffer used for elution	Total protein mg	Percent inhibition	
			a3	HId
1	PBS	241.2	6	2
2	PBS	1.4	3	4
3	0.5% GalNAc	4.2	4	3
4	0.5% GalNAc (2 M NaCl)	2.8	41	24
5	5 M guanidine-HCl	5.6	10	4

* The tabulated data were obtained from an analytical fractionation of 3 ml of serum. Fractions were concentrated to 1 ml before use as inhibitors of HId and latent allotypic assays (Fig. 4).

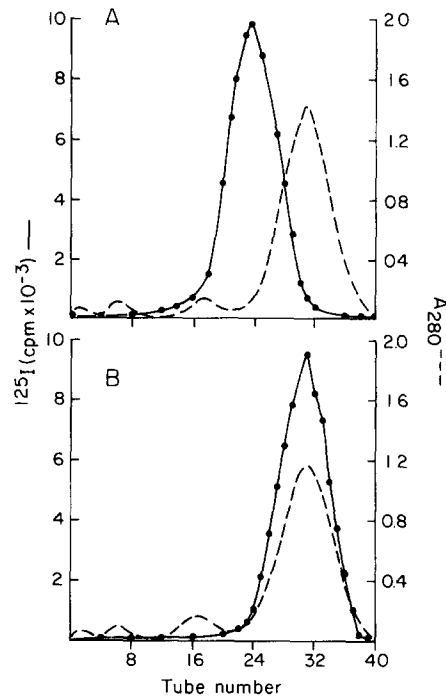


FIG. 6. Sedimentation profiles of radiolabeled 4232 HId-positive molecules in the presence of anti-d11 serum (A) and anti-d12 serum (B). Solid lines represent the ^{125}I activity; dashed lines, serum protein.

which contains the same V region allotypes as does the J allogroup, however, produced no more HId cross-reactive antibody than rabbits that lacked the a3 allotype (see Fig. 3).

Two sera were found which were apparent exceptions to the absolute linkage of HId to the J allogroup. One of these was from rabbit 4232 ($a^1a^2/d^{12}d^{12}$) which lacked a3 as a nominal allotype but was HId positive. Isolation of HId-positive antibodies from 4232 serum, however, yielded an antibody preparation which was exclusively a3/d11, characteristic of the J allogroup. Strosberg et al. (39)

have earlier reported the simultaneous appearance of a group *a* and a group *b* latent allotype in the sera of a rabbit immunized with *Micrococcus lysodeikticus*. The latent allotype d11 has also been found in these sera (W. J. Mandy and A. D. Strosberg. Unpublished data). However, no data on the molecular associations of this C_H allotype with the latent a2 allotype are presently available. Expression of H chains with latent a3 and d11, as found for rabbit 4232, provides evidence that the appearance of a latent allotype is not an isolated and independent event but may entail expression of linked genetic material which may be absent from the nominal serotype. The precise role of the idiotype in this set of events remains to be determined. The coordinate expression of two allotypes from an allogroup not present in the nominal genotype indicates a need to re-evaluate molecular interpretations of latent allotype and models for the regulation of coordinate gene expression in immunoglobulin chain biosynthesis.

Summary

Anti-idiotype antibodies specific for the H chain of an homogeneous antistreptococcal antibody (4135 Ab) were prepared by injection of recombinant molecules consisting of the H chains from 4135 Ab and L chains isolated from the injected rabbit. The antibodies prepared in this fashion (anti-HId) were specific for the V_H region of 4135 Ab. Using this preparation in an inhibition of binding assay, sera from rabbits related and unrelated to 4135 were screened for the presence of the 4135 HId. It was found that about 45% of the related rabbits, when immunized with streptococcal Group C vaccine, produced antibodies with a cross-reactive idiotype, while less than 10% of similarly immunized unrelated rabbits produced molecules bearing HId. The expression of HId was linked to the a3 allotype present in the H chain allogroup J. Antibody molecules bearing the HId determinant were isolated from heterozygous (a^1a^3 and a^2a^3) rabbits and shown to express the a3 allotype.

One rabbit lacking the a3 allotype produced significant amounts of antibodies expressing HId. These antibodies were found to express both V_H and C_H allotypes characteristic of the J allogroup, although neither allotype was found in a preimmune IgG sample from this rabbit.

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