Allelic Exclusion of Light Chain Allotypes in Rabbit IgM Cold Agglutinins* (33485)

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The kappa and lambda types of light polypeptide chains were both found in single IgM molecules of human cold agglutinins (1, 2). On the other hand, the b_4 and b_5 light chains of rabbits heterozygous for the light chain allotypes b^4b^5, are not found in the same IgG molecule (3)[1] The human kappa and lambda chains are both present in all normal individuals and are controlled by genes which are not allelic whereas the b_4 and b_5 light chains of the rabbit are allelic. Since the IgM molecule consists of five 7S subunits (4) and presumably has 10 light chains compared to two light chains in IgG, the question arose whether subunits having different light chains, b_4 or b_5, are assembled into a single IgM molecule in b^4b^5 heterozygous rabbits. This paper presents evidence that single IgM cold agglutinin molecules of b^4b^5 heterozygous rabbits carry either the b_4 or b_5 light chain but not both.

Materials and Methods. The IgM cold agglutinins were produced in rabbits of known genotype (b^4b , b^5b^5, b^4b^5) by injection of heat killed *Listeria monocytogenes 4b* (5). The cold agglutinins were isolated by thermal elution with barbital buffered saline from sensitized autologous red blood cells, followed by passage through Sephadex G-100, equilibrated with borate buffer, pH 8.4, to remove contaminating hemoglobin (5). The eluted cold agglutinin gave a single precipitin band characteristic of IgM by immunoelectrophoresis and double diffusion analysis using goat antirabbit whole serum and goat antirabbit IgM (6). Previous work has shown that the eluted cold agglutinins gave a single peak characteristic of IgM by sedimenation in the ultracentrifuge. The cold agglutinins were trace-labeled with either ^{131}I or ^{125}I (approximately one atom of iodine per molecule of protein) (7) and the free iodine was removed by passage through Sephadex G-25. The labeling efficiency was 70% and precipitation with 10% trichloroacetic acid yielded less than 1% of nonprotein bound radioactive iodine. Because of the possible damaging effect of labeling, the ^{131}I and ^{125}I tagged cold agglutinins were each incubated with washed autologous red blood cells at 4° and eluted from these erythrocytes as described above.

The IgG of the b^4b^5 heterozygote rabbit was isolated by the batch method using DEAE Sephadex with phosphate buffer (pH 6.5, 0.01 *M*) (8). Antiserum to rabbit IgM was prepared in goats immunized with purified rabbit cold agglutinins as previously described (7). The allotype antisera were produced by immunization with b4 and b5 immunoglobulins as previously described (9).

The amount of each allotype present in the cold agglutinin preparation was determined by using successive addition of allotypic antiserum to assure complete precipitation of each allotype (3). Separate portions of ^{131}I- and ^{125}I-labeled cold agglutinin were precipitated by anti-b_4, anti-b_5 or anti-IgM in antibody excess. After the first precipitation, a second and third precipitation was carried out using the same antiserum plus a known amount of unlabeled carrier IgM of the appropriate allotype added to the supernatant of the previous precipitation. The precipitates from the three precipitin reactions were combined and counted in a well-type scintillation counter connected to a spectrometer

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[1] Allotypes Ab^4 and Ab^5 are abbreviated to b_4 and b_5.

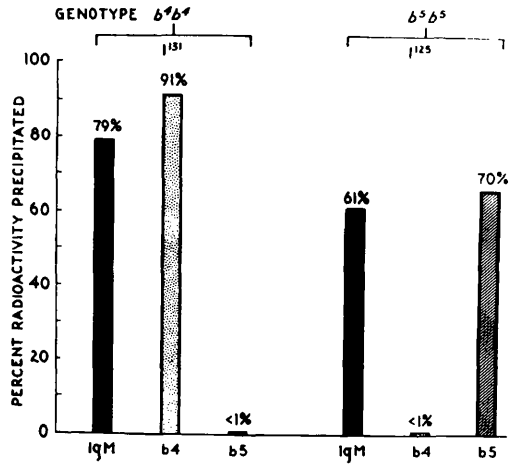

FIG. 1. Precipitation of radiolabeled b^4b^4 and b^5b^5 cold agglutinins by antisera to: IgM, b_4 and b_5.

using the optimal setting for each isotope. The combined supernatant fluids were counted in the same way.

The cold agglutinin from the b^4b^5 heterozygous rabbit was labeled with ^{125}I. The allotype distribution was analyzed by first completely precipitating one allotype in three successive precipitations as outlined above. After the complete precipitation of one allotype, an aliquot of the combined supernatant fluids was analyzed by three successive precipitations with the other allotypic antiserum. This was carried out in two ways: (i) the anti-b_4 followed by the anti-b_5, and (ii) the anti-b_5 followed by the anti-b_4.

Following the three precipitations, the pooled supernatant fluids and the combined precipitates were counted. Artificial mixtures of ^{125}I-labeled b_5 and ^{131}I-labeled b_4 cold agglutinins were also analyzed to rule out the possibility of aggregation of b_4 and b_5 molecules when mixed in solution. After correction for free iodine and for counting interference of the two isotopes, the results were expressed as percentage radioactivity precipitated.

Results. The amount of b_4 and b_5 allotype precipitated from cold agglutinins of b^4b^4 and b^5b^5 homozygous rabbits with antiallotypic antisera is shown in Fig. 1: 91% of the b_4 molecules were precipitated by anti-b_4; 66% of the b_5 molecules were precipitated by anti-b_5. Less than 1% of nonspecific precipitation occurred with each antiserum. The difference between the amount of b_4 and b_5 precipitin is consistent with the known observation that individual rabbits vary in the percentage of light chains controlled by the *b* locus; the range being from 65 to 90% (3).

In an artificial mixture of these same solutions (Fig. 2), 96% of the b_4 molecules and 70% of the b_5 molecules were precipitated with the specific antiserum. This is in close agreement with the analysis before mixing (Fig. 1) indicating lack of coprecipitation which might have occurred if there had been aggregation of the b_4 and b_5 molecules. Also, as before, there was less than 1% nonspecific precipitation.

The anti-IgM precipitated 79% (Fig. 1) and 77% (Fig. 2) of the b_4 molecules and 61% (Fig. 1) and 59% (Fig. 2) of the b_5 molecules. A possible explanation for the smaller percentage of molecules precipitating with antiserum to IgM compared to the anti-b_4 and anti-b_5 is the probability that the anti-IgM is directed to a heavy chain subclass of IgM whereas the antisera to the allotypes are directed toward light chain determinants present on either subclass.

In the heterozygote (Fig. 3), essentially the same amount of b_4 IgM (50 vs 47) and of b_5 IgM (40 vs 35) was precipitated by the respective antiserum regardless of the order in which they were precipitated. The percen-

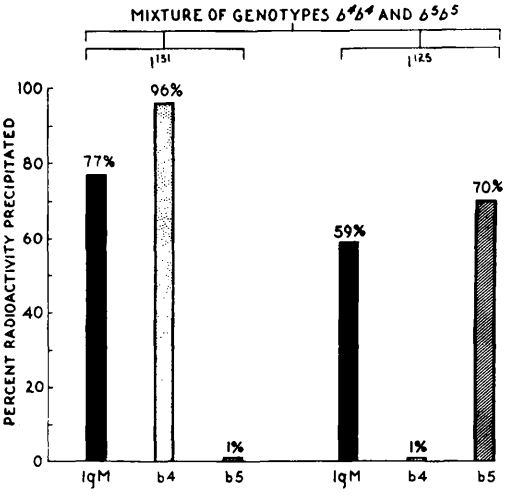

FIG. 2. Precipitation of mixtures of radiolabeled b^4b^4 and b^5b^5 cold agglutinins by antisera to: IgM, b_4 and b_5.

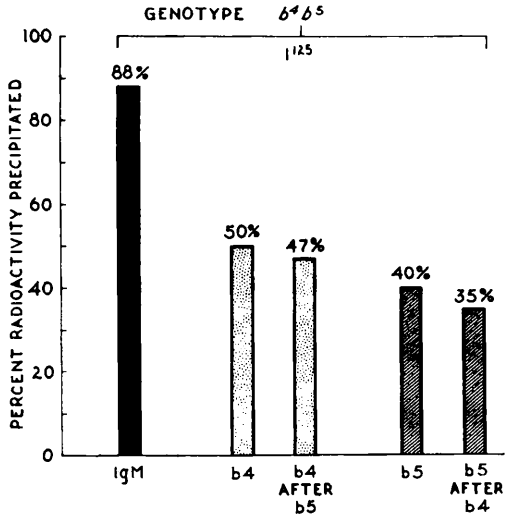

FIG. 3. Sequential precipitation of radiolabeled b^4b^5 cold agglutinins by antisera to: IgM, b_4 and b_5.

tage of molecules precipitated by the anti-IgM (88%) equaled the sum of those precipitated by the anti-b_4 and anti-b_5 in either order (85 and 87%).

The IgG isolated from the serum of the heterozygous rabbit was similarly analyzed for b_4 and b_5 content: 52% of the molecules were b_4; 34% were b_5. This is similar to the results reported recently by Hoyer and Mage who observed that the ratio of the b_4 to b_5 molecules in heterozygous rabbits was the same in IgG and IgM (10).

Discussion. The observation that the amount of b_4 or b_5 IgM cold agglutinins of b^4b^5 heterozygous rabbits that can be precipitated by anti-b_4 or anti-b_5 is independent of the order of precipitation indicates that essentially all the molecules have either b_4 or b_5 but not both allotypic markers. Nevertheless, the present data does neither exclude a small percentage of hybrid molecules nor the possibility that the cold agglutinins may not be representative of the total IgM population.

The finding that the b_4 or b_5 light chain markers are not on the same IgM is the same as that for IgG (3). It is of some interest that it was recently reported (11) that allelic allotypes do indeed appear on the same molecule of rabbit α_2 macroglobulin. That allelic exclusion occurs in the synthesis of IgG and IgM but not in the synthesis of α_2M molecules may have special significance for the biologic function of these proteins.

The cold agglutinins produced in humans have been shown to contain kappa and lambda chains on the same molecule (1, 2). However, the genes for the production of these two types of chains are not allelic. The experiment in man, analogous to that presented for the b_4 and b_5 allotypes in rabbits would be to determine whether the allelic Inv genetic markers of kappa chains are found in the same cold agglutinin molecules.

Summary. The IgM cold agglutinins were produced by intravenous injection of heat killed L. monocytogenes 4b into rabbits heterozygous (b^4b^5) for the light polypeptide chains of immunoglobulins. The radiolabeled cold agglutinins were analyzed for the b^4 and b_5 allotypes. The percentage of b_4 and b_5 light chains precipitable was independent of the order of precipitation with anti-b_4 and anti-b_5 indicating that the cold agglutinin IgM molecules carry either the b_4 or b_5 light chain but not both.

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