-AN ALLOTYPIC SPECIFICITY PRESUMABLY OF THE a SERIES IN RABBIT IMMUNOGLOBULINS, DIFFERENT FROM a1, a2 and a3.

P.-A. CAZENAVE, C. BREZIN and J. ROLAND

Service d'Immunochimie Analytique, Institut Pasteur, 28 rue du Dr.Roux, 75015 Paris, France.

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SUMMARY. - From the serum of a wild rabbit lacking all the known allotypic specificities of the a series, IgG showing an allotypic specificity named A100 has been isolated and antisera against it prepared in domestic rabbits. The determinants responsible for the A100 allotypic specificity are present both on IgG and IgM. They are located on the heavy chain and the Fab fragment of IgG.

Evidence for the genetic determinism of A100 suggests that it is the product of a new allele at the a locus.

Three allotypic specificities of rabbit immunoglobulins of the a series, designated as a1, a2, and a3 have been described (1-5). They are apparently controlled by three allelic genes at the a locus (4). It was shown that these specificities are carried by IgG, IgM, IgA and IgE (reviewed in 6). Furthermore, it seems that the determinants responsible for these specificities are located on the variable part of the heavy chains (reviewed in 7).

Sera of wild rabbits Oryctolagus cuniculus have been tested for the allotypic specificities of the a series (8). The serum of one of them (WR2) lacked all the a series specificities. Two domestic rabbits with $a(1^-2^-3^-)$ phenotype had already been described (4). The IgG of the wild rabbit WR2 injected into domestic rabbits having different allotypic genotypes raised precipitating antibodies against an allotypic specificity named A100. The localisation of this allotypic specificity on rabbit IgG molecules and evidence for its genetic determinism are described in this paper.

MATERIAL AND METHODS

Animals: The 21 wild rabbits came from the area of Rambouillet in France and

were kindly supplied by Michel Seman. The domestic rabbits were Bouscat Giant and Normandy Grey.

Immunoglobulin preparations. The rabbit sera, after removal of the β -lipoproteins by precipitation with dextran sulfate (9), was gel filtrated on Sephadex G200. IgM was purified from the excluded fraction. IgG was isolated from the second peak by chromatography on diethyl-amino-ethyl-cellulose (10).

Anti-allotypic sera. The preparation of anti-a1, anti-a2 and anti-a3 immune sera has been described in a previous paper (11). The anti-A100 sera were prepared by injecting $A100^-$ domestic rabbits (DR 238: $a(1^+2^-3^+)$, DR 260: $a(1^-2^-3^+)$, DR 267: $a(1^+2^+3^-)$) with IgG purified from the serum of wild rabbit WR2 following the technique used previously (11).

Fragments and chains of IgG. The Fab and Fc fragments of IgG WR2 were prepared by papain hydrolysis (12). The heavy and light chains were separated by reduction of IgG with dithiothreitol, alkylation with iodoacetamide, and gel filtration on Sephadex G200 in the presence of 5M guanidine (13).

Antigen-antibody reactions and their inhibition. These reactions were carried out by precipitation in a liquid medium at the interface between two solutions, and by the method of immunochemical analysis in gels (14) using the technique of double diffusion in a cell with parallel walls (15).

Reactions of allotypes and anti-allotypic antibodies were also studied using ^{125}I-labelled IgG and insoluble polymers of anti-allotypic immune sera (16). IgG of different allotypic specificities were labelled with ^{125}I by the chloramine T method (17). Insolubilisation of the antibodies was performed by polymerization of anti-allotypic antisera with ethyl chloroformate (18) with some modifications (19): when the serum in presence of ethyl chloroformate becomes very "lactescent", the suspension is diluted in an acetate buffer 0.1M, pH 4.5 (5ml of buffer per ml of immune serum). Agitation is maintained for one hour. The suspension is then homogenized in very small particles.

RESULTS

Among the 21 wild rabbits tested for their allotypic specificities, WR2 with phenotype $a(1^-2^-3^-)$ provided the immunizing IgG. Sera of two other wild rabbits, WR5 and WR10, were precipitated by the three anti-A100 (anti-WR2) immune sera and by antisera against specificities of the a series, a2 and a3 respectively. Three hundred domestic rabbits tested were $A100^-$.

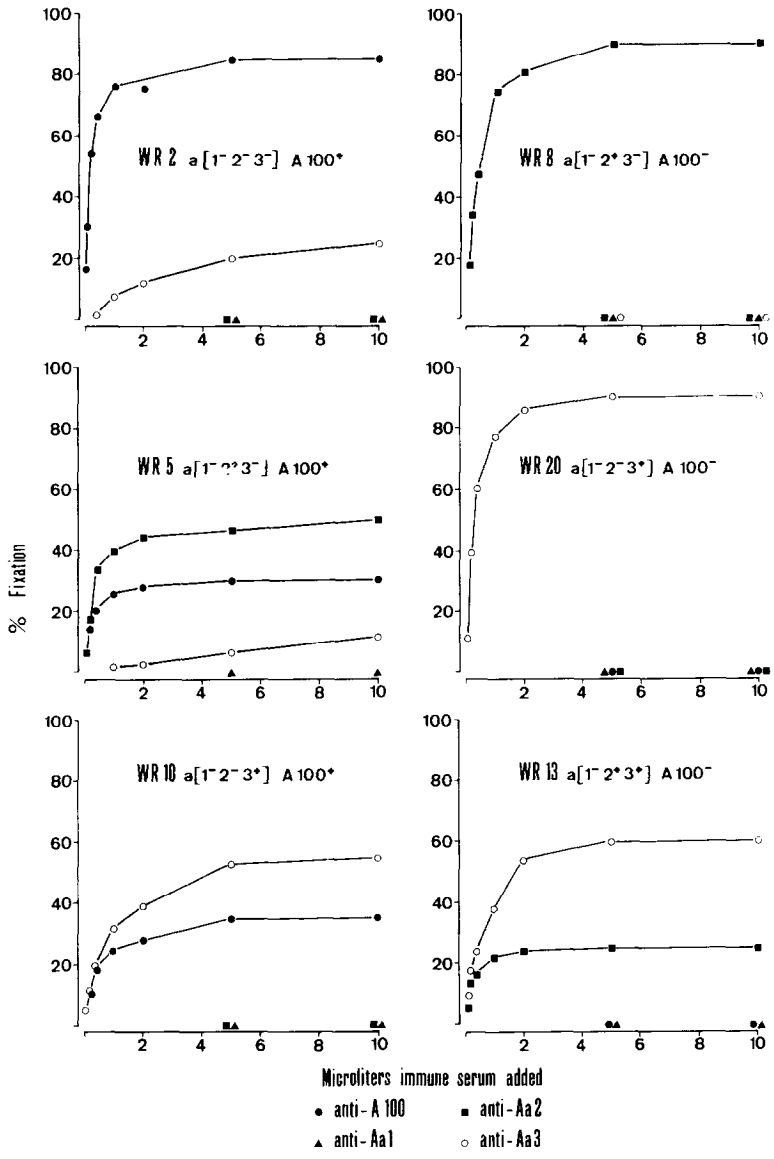

Figure 1. Binding curves of ^{125}I- labelled IgG of 6 wild rabbits to cross-linked anti-allotypic sera. Increasing volumes of cross-linked immune sera were added to a constant amount of ^{125}I-labelled IgG (about 20000 cpm).

The binding curves of ^{125}I-labelled IgG from these three A100^{+} rabbits and from A100^{-} controls (WR8, WR20, WR13) to insolubilized anti-allotypic sera are shown in figure 1 and the results summarized in table I. These results suggest that WR2 is homozygous for A100, WR5 and WR10 being heterozygous: 85%

Table I

Per cent of ^{125}I-labelled IgG from the sera of wild rabbits adsorbed to 5µl of polymerized anti-allotypic immune sera.

Labelled IgG		Polymer of anti-allotypic immune sera			
N°	Génotype	anti-a1	anti-a2	anti-a3	anti-A100 DR 267
WR2	$a(1^-2^-3^-)$ $A100^+$	0	0	20	85
WR5	$a(1^-2^+3^-)$ $A100^+$	0	48	10	30
WR10	$a(1^-2^-3^+)$ $A100^+$	0	0	55	35
WR8	$a(1^-2^+3^-)$ $A100^-$	0	85	0	0
WR20	$a(1^-2^-3^+)$ $A100^-$	0	0	85	0
WR13	$a(1^-2^+3^+)$ $A100^-$	0	25	60	0

of IgG WR2 adsorbed to the polymer of anti-A100 immune serum, while only 30% of IgG WR5 and 35% of IgG WR10 adsorbed to the same polymer. The IgG of WR5 and WR10 were adsorbed to anti-a2 and anti-a3 antisera in a proportion incompatible with homozygosity for the a specificity considered: 48% for a2 and 55% for a3. This interpretation is in good agreement with the fact that the IgG of $A100^-$ wild rabbits WR8 (homozygous a2/a2), WR20 (homozygous a3/a3) and WR13 (heterozygous a2/a3) exhibit percentages in the range expected with the quantitative effect observed in domestic rabbits (4). The partial adsorption of WR2 IgG to the anti-a3 immune serum polymer is explicable by the cross-reaction between the A100 allotypic pattern and the antibodies against the a3 allotypic pattern (20).

The above data seem to indicate that the A100 allotypic specificity belongs to the a series. This hypothesis is in agreement with the presence of the A100 allotypic specificity on IgM of $A100^+$ rabbits. Thus, the anti-A100

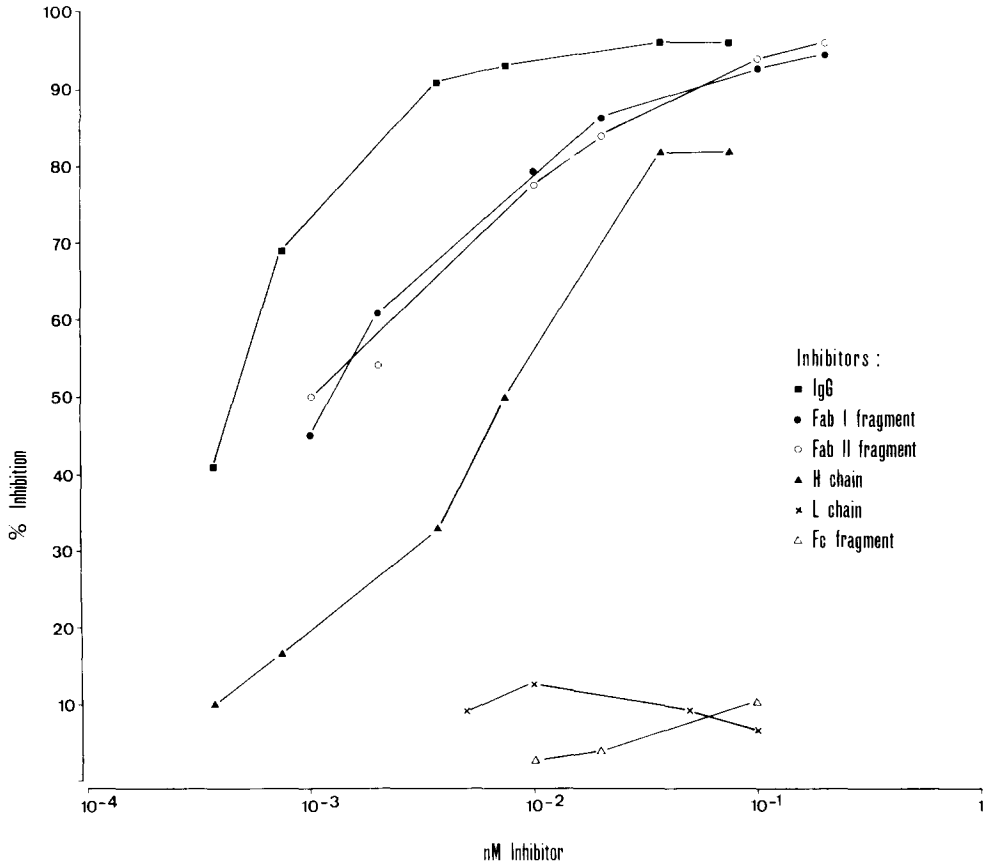

Figure 2. Inhibition, by FabI, FabII and Fc fragments, by H and L chains and by unlabelled IgG WR2, of the binding of ^{125}I-labelled IgG WR2 to cross-linked anti-A100 immune serum (DR 267).

immune sera do not distinguish by precipitation in gel medium IgG from IgG isolated from an A100^{+} serum. Furthermore, A100^{+} IgM inhibits the adsorption of A100^{+} IgG to insolubilized anti-A100 immune sera.

The localization of the A100 allotypic pattern on IgG subunits also corroborates these data. The results are illustrated in fig.2. The binding of ^{125}I IgG WR2 to the polymerized anti-A100 immune sera was inhibited by the FabI and FabII fragments of WR2, while the binding was not inhibited by the Fc fragment. The binding was inhibited by the heavy chain but not by the light chain preparation, demonstrating that A100 allotypic specificity is carried by the Fd fragment of IgG.

DISCUSSION

From the data presented we can conclude that the allotypic specificity A100, carried both by IgG and IgM, is located on the Fd fragment of the heavy chain.

Since no progeny study has been made to date, our conclusion concerning the genetics of the A100 specificity rests upon several arguments, none of which constitutes a proof in itself, but which taken together provide good presumptive evidence of its genetic determinism.

First, the A100 specificity was detected in a wild rabbit lacking all the known specificities of the a series. Second, none of the rabbits possessing the A100 allotypic specificity possessed two allotypic specificities of the a series. Third, the quantitative differences observed in comparing IgG from the different wild rabbits is in good agreement with the observation (4) that in the case of two allotypic specificities determined by two alleles, the concentration of molecules with the specificity considered is larger in the serum of homozygous rabbits than in the serum of heterozygous rabbits.

In the light of these results we think that A100 is a specificity determined by an allele at the a locus of rabbit immunoglobulins. In a subsequent paper we will give further evidence in favor of this hypothesis, the most important of which concerns the cross-reaction between the A100 allotypic pattern and the antibodies directed against the a3 allotypic pattern (20).

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