COMPARISON OF THE IDIOTYPY OF ANTIBODIES SYNTHESIZED BY RABBITS IMMUNIZED FIRSTLY WITH A FRAGMENT OF HUMAN SERUMALBUMIN AND SECONDLY WITH WHOLE SERUMALBUMIN .

Pierre-André Cazenave

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

Received May 18,1973

SUMMARY: Two rabbits were immunized with the "inhibitor" fragment of human serumalbumin and were then injected with whole serumalbumin. The same idiotypic determinants or very similar determinants with a different molecular distribution were detected on: A) Anti-"inhibitor" antibodies obtained after the first immunization (against "inhibitor), b) Anti-"inhibitor" antibodies obtained after the second immunization, c) Antibodies combining with serumalbumin but not with the "inhibitor" fragment obtained after the second immunization.

Idiotypy of antibodies (1,2) is their property to possess antigenic specificities, idiotypic specificities , peculiar to antibodies synthesized against a given antigen by given individual or group of individuals. The first observations of idiotypy of antibodies were made in man (3) and in the rabbit (4) .

In the rabbit, idiotypic specificities can be similar for antibody fractions having different functions toward the same antigen against which they are directed (5-7).More particularly, idiotypic specificities can be similar for antibodies directed against different determinants of the same protein antigen (8,9).In contrast, they are not the same for antibodies against two non cross-reacting antigens as has been shown: a) when the two antigens are injected successively into the same individual, whatever the interval of time (5,10),and b) when the two antigens are injected simultaneously (8).

The antibodies whose idiotypy are compared in the present study were obtained after two successive immunizations with two antigens recently employed in another experiment (9).These antigens have

Copyright © 1973 by Academic Press, Inc.
All rights of reproduction in any form reserved.

a special kind of cross-reactivity in that the first antigen, used for the primary immunization, is a fragment of the second antigen (human serumalbumin) which is injected into the same rabbits in a second immunization. The first antigen is the "inhibitor" fragment of human serumalbumin with a molecular weight of 11,000 which is obtained by the degradation of albumin with rabbit spleen cathepsin D (11,12).

MATERIAL AND METHODS

1. Antigens and immunizations . The preparations of human serumalbumin (HSA) were those employed elsewhere (9). The "inhibitor" fragment (I) (11,12) was generously given by Dr. C.Lapresle.

Rabbits n° 422 and 423 were immunized with 0.5 mg fragment I emulsified in complete Freund's adjuvant. Sera collected 4 to 7 weeks after immunization were pooled and designated S_1. Five months later, the same rabbits were injected with 2 mg HSA emulsified in the same adjuvant. Sera collected 3 to 8 weeks later were pooled and designated S_2.

2. Antibody isolation. All the antibodies studied were, without exception, taken from the second peak following filtration of the sera on Sephadex G-200. Anti-HSA antibodies were isolated on an HSA polymer insolubilized with glutaraldehyde (13). Anti-HSA antibodies were isolated on an HSA polymer insolubilized with glutaraldehyde (13). Anti-I antibodies were isolated with fragment I coupled to diazotized para-amino-benzyl cellulose (PAB-I)(14) ,either from pool S_1(anti-(I)S_1 or from pool S_2 (anti-I)S_2 .Antibodies which combine with HSA,but not with fragment I,were isolated from pool S_2 absorbing anti-HSA antibodies,isolated on poly-HSA with PAB-I (anti-(SA-I)S_2) . Antibodies were eluted from their immunoadsorbant with 0.1 N HCl.

3. Anti-idiotypic immunizations . Anti(I)S_2 antibodies from rabbit 422 and anti-(I)S_1 from rabbit 423 were polymerized with glutaraldehyde (13) and were employed separately in two different anti-idiotypic immunizations schedules (see table I) as was used in anti-idiotypic immunization against anti-para-amino-benzoate antibodies (15).

4. Antigen-antibody reactions. These reactions were carried out by precipitation in a liquid medium at the interface between two solutions and by method of immunochemical analysis in gels (16) using the technique of double diffusion in a cell with parallel walls (17).

Table 1

Results of anti-idiotypic immunizations

(Reactions in liquid medium at the interface)

Immunizing material	N° of rabbits	422 NS*)	422 S_1	422 S_2	423 NS*)	423 S_1	423 S_2	9 anti-HSA sera	anti-I S_1	anti-I S_2	anti-(SA-I) S_2
									422		
422 anti-I S_2	728	0	+	+	0	0	0	0	+	+	0
	757	0	+	+	0	0	0	0	+	+	0
	761	0	++	++	0	0	0	0	++	++	0
										423	
423 anti-I S_1	656	0	0	0	0	0	0	0	0	0	0
	758	0	0	0	0	++	++	0	++	++	0
	760	0	0	0	0	0	0	0	0	0	0

+ and ++ represent the degree of intensity of the precipitation reaction , 0 no reaction.

*) : serum obtained from rabbits n° 422 and 423 before immunization.

A comparison of the precipitation in gel of three anti-idiotypic sera n° 728, 757 and 761 with anti-(I) antibodies from rabbit n° 422 using a cell with parallel walls (double diffusion) , shows that they precipitate the same idiotypes. Serum n° 761, which had the greatest precipitating power, was employed for studying the idiotypy of antibodies from rabbit n° 422 .

Antigen-antibody reactions were also studied by passive hemagglutination, fragment I being coupled to sheep red blood cells with glutaraldehyde (18).

In order to study, by precipitation, anti-HSA antibodies which were not precipitated by anti-idiotypic antibodies but which were able to combine with these antibodies, the fractions were polymerized by glutaraldehyde in such a way as to obtain soluble polymers (9,19).

RESULTS

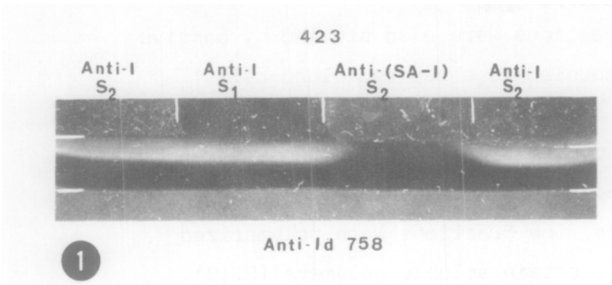
The results of the anti-idiotypic immunizations are summarized in table I.

The specificity of the anti-(SA-I)S_2 antibodies was confirmed by passive hemagglutination. Even at concentration of 500 µg/ml these antibodies did not agglutinate red blood cells coupled with fragment I whereas agglutination occurred at a concentration of 0.07 µg/ml of anti-I (S_1 and S_2).These results were obtained with antibodies isolated from both sera n° 422 and 423 .

A comparative study of the idiotypy of anti-(I)S_1 ,anti-(I)S_2 and anti-(SA-I)S_2 antibodies.

The precipitation reactions in a liquid medium are summarized in table I.

A comparative reaction of the anti-idiotypic serum n° 758 with the three antibody fractions anti-(I)S_1, anti-(I)S_2 isolated from serum n° 423 , is shown in fig.1. No precipitation zone is seen in front of the anti-(SA-I)S_2 fraction.A precipitation zone is continuous in front of the layers containing anti-(I)S_1 and anti-(I)S_2 fractions showing that at least one idiotype is common to these two fractions. When the anti-idiotypic serum was absorbed, in a liquid medium by the anti-(I)S_1 antibodies, the supernatant no longer precipitated the anti-(I)S_2 antibodies. Conversely, when the anti-idiotypic serum was absorbed with anti-(I)S_2, the supernatant no longer precipitated the anti-(I)S_1 antibodies. The anti-(SA-I)S_2 antibodies completely inhibited the precipitation of both fractions anti-(I)S_1 and



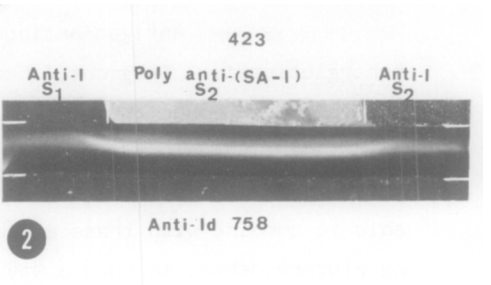


Fig. 1 : Reaction , in an agar gel, in a cell with parallel walls (double diffusion in one dimension) of anti-idiotypic rabbit serum n° 758 (anti-Id 758, lower layer) with the three antibody fractions isolated from the serum of rabbit n° 423 (upper layers): anti-(I)S_1 antibodies (1mg/ml), anti-(I)S_2 antibodies (1.5 mg/ml) and anti-(SA-I)S_2 antibodies (1 mg/ml). The white dashes indicate the interfaces between the various layers. At least one idiotype, shown by a continuous zone of precipitation in front of anti-(I)S_1 and anti-(I)S_2, is common to both fractions. The anti-(SA-I)S_2 fraction is not precipitated by the anti-idiotypic serum. However, the photograph suggests that this fraction inhibits the precipitation of anti-(I)S_2 and more particularly of anti-(I)S_1 by the antiserum. This inhibition, confirmed in liquid medium (see results), suggests that certain anti-SA-I) antibodies possess idiotypic determinants, e.g. x, y, z, distributed on different molecules and which, therefore, are not precipitated by anti-idiotypic serum, whereas certain antibodies from the anti-(I)S_1 and anti-(I)S_2 fractions which have the same determinants, or determinants x', y', z' related to x, y, z, gathered on the same molecule , are precipitated by the anti-idiotypic serum. This hypothesis has been proven using soluble polymers of the anti-(SA-I)S_2 fraction (see fig. 2).

Fig. 2 : Reaction, in conditions similar to those of Fig. 1, of the anti-idiotypic serum n° 758 (anti-Id 758, lower layer) with the three antibody fractions isolated from rabbit n° 423 (upper layers): anti-(I)S_1 antibodies (1 mg/ml), anti-(I)S_2 antibodies (1.5 mg/ml) and the soluble polymers solution of anti-(SA-I)S_2 antibodies (1mg/ml) . The precipitation zone is continuous in front of the three upper fractions .

---

anti-(I)S_2 by the anti-idiotypic serum. The precipitation of fraction anti-(I)S_2 by the anti-idiotypic serum in proportions close to equivalence was completely inhibited by an amount of anti-(SA-I)S_2 antibodies which was 4 times the amount of fraction anti-(I)S_2.

Similar results were obtained with antibodies from rabbit n° 422.

The soluble polymers of the anti-(SA-I)S_2 fraction (poly anti-(SA-I)S_2 from rabbit n° 423 were precipitated in liquid medium by the anti-idiotypic serum n° 758 . The reaction, in gel, of the anti-idiotypic serum n° 758 with the 3 fractions anti-(I)S_1, anti-(I)S_2 and poly anti-(SA-I)S_2 was compared and is shown in fig.2.There is a continuous

precipitation zone in front of the three upper fractions. When the anti-idiotypic serum was absorbed, in a liquid medium, with the solution of poly anti-(SA-I)S_2, the supernatant no longer precipitated either the anti-(I)S_1, or the anti-(I)S_2 fractions.

Similar results were obtained with antibodies from rabbit n° 422 .

DISCUSSION

The idiotypic similarity between anti-(I)S_1 and anti-(I)S_2 antibodies was to be expected since it had already been shown in a study of the idiotypy of antibodies against Salmonella typhi that the idiotypic specificities of antibodies produced after long interval of time against the same antigen material were the same (6).

The idiotypic similarity between the anti-(I)S_2, the anti-(SA-I)S_2 and the anti-(I)S_1 antibodies would just confirm the observation already made on anti-fibrinogen (8) and on anti-HSA antibodies (9) if the immunizations preceding bleedings S_1 and S_2 had been carried out with HSA; comparison of fig.1 and fig.2 shows yet another example of a different molecular distribution of the same, or of very similar, idiotypic determinants (8,9). What is particularly surprising about this similarity, and especially between anti-(I)S_1 and anti-(I)S_2, is that the former appeared after immunization against fragment I, which had no cross-reactivity with the region (SA-I) against which the anti-(I)S_2 antibodies were directed. The hemagglutination titers show that this similarity is not due to contamination between the two fractions.

The idiotypic similarity between anti-(I)S_1 and anti-(SA-I)S_2 antibodies raises an important theoretical problem. Several hypotheses were discussed in a recent paper (9) to explain the differences in the molecular distribution of the same (or very similar) idiotypic determinants in different antibody fractions. The idiotypic si-

milarity between anti-(I)S_1 antibodies appearing after immunization against fragment I and anti-(SA-I)S_2 antibodies appearing after a second immunization with HSA suggests that there is a transfer of information by some unknown mechanism, from the cells responding to primary stimulation with fragment I to those cells which are about to synthesize anti-(SA-I) antibodies. This raises a question about the specificity of memory cells.

ACKNOWLEDGEMENTS: I thank Dr.Jacques Oudin for his continual encouragement and helpful discussion during this work. I thank Dr. Claude Lapresle for his gift of fragment I, and Dr.Hugh Miller for his help with the translation of this article . This work was supported by a grant of the Délégation Générale à la Recherche Scientifique et Technique (Convention n°6700605O1) and a grant of the Centre National de la Recherche Scientifique (ER 67) to Service d'Immunochimie Analytique of Institut Pasteur (Chef de Service Dr.J.Oudin) where it has been done .

REFERENCES

1. OUDIN,J., J.Cell.Physiol., 67 Suppl.1, 77 (1966)
2. OUDIN,J., Proc.Roy.Soc. B, 166, 207 (1966)
3. KUNKEL,H.G., MANNIK,M., and WILLIAMS,R.C. Science,140, 1218 (1963)
4. OUDIN,J. , et MICHEL,M., Compt.Rend.Acad.Sc., 257, 805 (1963)
5. OUDIN,J. et MICHEL,M., Compt.Rend.Acad.Sci., 268, 230 (1969)
6. OUDIN,J. and MICHEL,M., J.Exp.Med., 130, 619 (1969)
7. OUDIN,J. and CAZENAVE,P.A., Proc.Nat.Acad.Sci.,USA, 68, 2616 (1971)
8. CAZENAVE,P.A. et OUDIN,J., Compt.Rend.Acad.Sc., 276, 423 (1973)
9. CAZENAVE,P.A., FEBS Letters (in press)
10. OUDIN,J., and MICHEL,M. J.Exp.Med., 130, 595 (1969)
11. LAPRESLE,C., et WEBB,T. Ann.Inst.Pasteur, 99, 523 (1960)
12. WEBB,T. and LAPRESLE,C.Biochem.J., 91, 24 (1964)
13. AVRAMEAS,S., and TERNYNCK,T. Immunochemistry, 6, 53 (1969)
14. CAMPBELL,D.H. , LUESCHER,E. and LERMAN,L.S. Proc.Nat.Acad.Sci.USA, 37, 575 (1951)
15. DAUGHARTY,H., HOPPER,J.E., MAC DONALD,A.B. and NISONOFF,A. J.Exp.Med., 130, 1047 (1969)
16. OUDIN,J. Compt.Rend.Acad.Sc., 222, 115 (1946)
17. OUDIN,J. ,Ann.Inst.Pasteur, 89, 531 (1955)
18. AVRAMEAS,S., TAUDOU,B., and CHUILON,S. Immunochemistry, 6, 67 (1969)
19. CAZENAVE,P.A., manuscript in preparation .