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Identification of Rabbit Allotypic Specificity A1 by an Isoprecipitin Produced by Immunization with Antigen-Antibody Complexes. (28649)

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The sera of guinea pigs and rabbits immunized with various antigens have beeen shown to agglutinate sheep cells sensitized with rabbit amboceptor and human Group O Rh positive erythrocytes sensitized with incomplete Rh antibodies. These antigens have included ferritin, rabbit red blood cells, *Escherichia coli, Proteus OX 19,* and autologous denatured γ-globulin, most of which antigens had been precipitated or agglutinated with their specific antisera(1-6). It has been postulated that the agglutinating factor thus produced in animals was an "anti-antibody" formed by the immunized animals against antibody globulin altered during formation of the antigen-antibody complexes, and that this might be considered comparable to the agglutination activating factor (rheumatoid factor) observed in human rheumatoid arthritis (2,4,6-8). During the course of attempts to produce rheumatoid factor-like activity in rabbits by immunization with specific immune agglutinates(9-11), a new isoprecipitin for a rabbit γ-globulin allotype was found. This report describes the method by which the isoprecipitin was obtained and characterized.

Materials and methods. Rabbits were obtained from closed colonies of the Laboratory Aids Branch, Nat. Inst. of Health. New Zealand White rabbits with allotypic specificity A4 but not A5, and Flemish Giant rabbits with both allotypic specificities A4 and A5 were used.

Type A4 donor rabbits were immunized with *Salmonella typhosa* "H" antigen* to produce high titer antiserum (1:10,000 to 1:12,000), using standard techniques(12). Anti-*S. typhosa* "H" antiserum obtained from individual rabbits was mixed with fresh *S. typhosa* "H" antigen in proportions insuring a large excess of antibody, as determined by prior titrations. 1.0 ml of a 1:10 dilution of antiserum in 0.85% NaCl was added to 1.0 ml of *S. typhosa* "H" antigen containing 1 $\times$ 10^9 organisms, and the mixtures were incubated at 50-52°C for 18 hours. The resultant agglutinates were recovered by centrifugation, washed 6 times with saline, and resuspended in saline to a final volume of 1.0 ml.

Type A4 recipient rabbits were injected with 1.0 ml of resuspended agglutinate emulsified with 1.0 ml of complete Freund's adjuvant (0.85 ml Bayol F, 0.15 ml Arlacel A, 0.4 mgm *Mycobacterium butyricum*) as follows: intradermally in the footpads, nuchal region, and back; subcutaneously in the nuchal region and back; and intramuscularly in the legs. After 6 weeks, additional injections of 2.0 ml each of saline-resuspended agglutinates (without adjuvant) were given intraperitoneally twice a week for a total of 17 booster doses, with intervals for rest and bleeding, as noted in Table I.

Sera were tested for flocculation of bentonite particles coated with human γ-globulin (B.F.T.)(9), and for anti-typhoid antibodies by standard agglutination techniques(12). Sera and their euglobulin fractions (obtained by ammonium sulphate precipitation and dialysis) were tested for agglutination of

* Kindly furnished by Col. A. S. Benenson, Walter Reed Army Inst. of Research.

TABLE I. Bentonite Flocculation Titers, Sensitized Sheep Cell Agglutination Titers (of Whole Serum and Euglobulin Fractions), and Anti-Typhoid Titers in Rabbits Immunized with Typhoid-Rabbit Anti-Typhoid Complexes.

	IMM 1×*					IMM 8×				IMM 5×	IMM 4×		
Time:	0	4 wk				10 wk				13 wk	17 wk		
Rabbit No.	BFT	BFT	SSCA Whl	SSCA Eug	Typh	BFT	SSCA Whl	SSCA Eug	Typh	BFT	BFT	SSCA Whl	SSCA Eug
16	0	0	0	0	640	8	64	0	10,240	8	8	512	8
17	0	0	0	0	320	0	0	0	20,480	0	32	0	0
18	0	0	0	0	640	32	128	0	10,240	32	64	1024	32
19	0	0	0	0	2560	0	0	0	20,480	0	4	0	0

* Immunization.

sheep red cells sensitized with rabbit amboceptor (S.S.C.A)(11). The sera were tested for isoprecipitins by double diffusion in agar gel and by immunoelectrophoresis(13).

Results. Table I shows the bentonite flocculation, sensitized sheep red cell agglutination, and anti-typhoid agglutination titers of four of the immunized rabbits. As shown in Table I, the sera of 2 of these rabbits, Nos. 16 and 18, reacted in moderate titer with rabbit amboceptor sensitized sheep cells and in low titer with human γ-globulin coated bentonite particles; the sera of rabbits Nos. 17 and 19 failed to react, except for developing low bentonite flocculation titers after 17 weeks. All animals developed very high anti-typhoid titers, as might have been expected after such a course of immunization.

The post-immunization sera of rabbits 16 and 18 were tested by double diffusion in agar gel plates with sera of individual rabbits to ascertain whether the BFT and SSCA titers observed were due to isoprecipitins rather than to "anti-antibodies." Antiserum 18 (center well) reacted with 4 of the rabbit sera to give precipitin bands (Fig. 1). Precipitin reactions were observed with the serum of: 1) rabbit 58, donor of the typhoid antiserum; 2) rabbit 99, "donor" of the amboceptor used in the sheep cell agglutination test; 3) rabbit 17, pre-immunization; and 4) rabbit 19, pre-immunization. Two pre-immunization sera, from rabbits 16 and 18, did not give precipitin reactions with antiserum 18. In another experiment (not shown), antiserum 16 was tested similarly and gave the same results as antiserum 18. Thus, rabbits 16 and 18 had developed precipitating antibodies to a serum constituent present in 4 rabbits but absent in 2 (Fig. 1).

The post-immunization sera of rabbits 16 and 18 were tested by immunoelectrophoresis with γ-globulin fractions of sera of individual rabbits, in an attempt to identify these precipitating antibodies. The precipitin reactions of rabbit antiserum 16 in an immunoelectrophoretic plate are shown in Fig. 2; a sheep anti-rabbit γ-globulin system is included for reference. Antiserum 16 reacted with the γ-globulin of pre-immunization rabbit serum 17. Rabbits 16, 17, 18, and 19 had been shown to have allotypic specificity

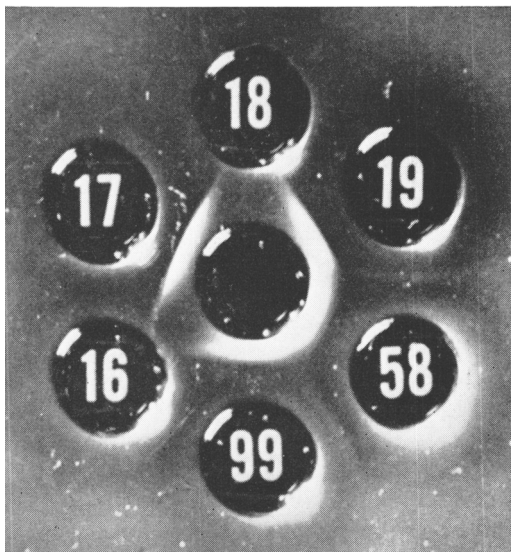

FIG. 1. Double diffusion agar gel—precipitin reaction between Rabbit No. 18 post-immunization serum (center well) and Rabbits No. 17 and 19 pre-immunization sera, as well as Rabbit No. 58 antiserum donor and Rabbit No. 99 amboceptor "donor." No reaction with Rabbit No. 18 or Rabbit No. 16 pre-immunization sera.

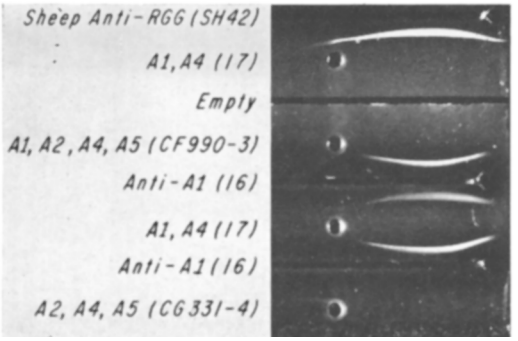

FIG. 2. Immunoelectrophoretic pattern showing reaction of rabbit anti-A1 antiserum with sera from rabbits containing A1 allotype. The sera containing allotype A1 also contain other allotypes (A2, A4, or A5); these do not react with anti-A1. A reference sheep anti-rabbit γ-globulin (RGG) system is shown for comparison. The individual rabbits from which normal sera and anti-sera were obtained are indicated within parentheses.

A4 (formerly called II), and to lack allotypic specificity A5 (formerly called I)(14). Therefore, antiserum 16 identified a new allotypic specificity which originally was designated L, and later was given the new notation A1(15). In the system shown in Fig. 2, antiserum 16 reacted with the γ-globulin of a rabbit (CF990-3) with both A4 and A5 allotypic specificities, and did not react with a second rabbit (CG331-4) having both A4 and A5. Antiserum 18 gave identical results (not shown). It was previously shown that γ-globulin allotypes A4 and A5 are controlled by allelic genes(14). The appearance of A1 (L) in A4-A5 heterozygous rabbits suggests that allotypic specificity A1 is controlled at a second gene locus. In another experiment, 20 A4-A5 heterozygous rabbits were tested for the presence of A1; A1 was present in 8 and absent in 12 of these rabbits, consistent with the concept that A1 is determined at a second locus.

Discussion. A new γ-globulin allotypic specificity, identified by means of the antisera produced in rabbits Nos. 16 and 18 immunized with typhoid-rabbit anti-typhoid complexes, was found to be present in the sera of rabbits Nos. 17, 19, 58, and 99, as well as in 8 of 20 other rabbits in our colony, and was originally designated L. This was subsequently found to correspond with Type E of Dubiski and Kelus(15), and Type *b* of Oudin(16), all of which were later given the new designation of Type A1(15).

The finding of these antisera facilitated the investigation of new allotypic specificities, during which it was found that A1, A2 and A3 are controlled by 3 autosomal alleles at the *a* locus(17).

The reaction of the post-immunization sera of rabbits Nos. 16 and 18 with sensitized sheep cells is explained by the finding of an isoprecipitin in these immune sera against γ-globulin allotype A1 present in the serum of rabbit No. 99 (the donor of the amboceptor used to sensitize the sheep cells) and by the finding of allotype A1 in the serum of rabbit No. 58 (the donor of the typhoid antiserum used in the immunization of rabbits Nos. 16, 17, 18, and 19). The absence of reaction of the post-immunization sera of rabbits Nos. 17 and 19 with sensitized sheep cells is explained by the fact that these rabbits possessed the same γ-globulin allotype A1 in common with rabbits Nos. 58 and 99, and therefore did not form antibodies against it. Thus, production of an isoprecipitin for the allotypic specificity A1, rather than production of "rheumatoid-like factor," accounted for the positive "rheumatoid factor" reaction with sensitized sheep red cells. However, as can be seen from the above results, the sensitized sheep cell agglutination test is suitable for detection of antibodies to allotypes, and could provide a simple and convenient technique for testing of sera for the presence of anti-allotype antibodies. Conceivably, the bentonite flocculation test could be similarly employed for rapid detection of such antibodies if rabbit γ-globulin of defined genotype were used for coating the bentonite particles.

The reaction, of the post-immunization sera of rabbits 16 and 18 and of the sera obtained after prolonged immunization of rabbits 17 and 19, with human γ-globulin coated bentonite particles requires explanation. It is possible that rabbit anti-γ-globulin allotype A1 antibodies cross-react with components of pooled human γ-globulin. On the other hand, prolonged immunization with typhoid-rabbit anti-typhoid antibody complexes may have given rise to a different type of anti-γ-globulin antibody (present in post-immunization

sera 17 and 19), capable of reacting with human rather than with rabbit γ-globulin(3).

Summary. A new isoprecipitin for a rabbit γ-globulin allotype was found during attempts to produce rheumatoid factor-like activity in rabbits by immunization with specific typhoid-anti-typhoid complexes. This allotypic specificity, originally designated L, was subsequently found to correspond with Type E of Dubiski and Kelus, Type *b* of Oudin, and and was later reclassified Type A1 of Dray *et al.* This new antiserum facilitated the immunochemical study of allotypic specificities A1, A2, and A3, which are controlled at the *a* locus. The sensitized sheep cell agglutination reactions observed with the sera of some of the immunized rabbits appeared to be due to the isoprecipitin for the allotypic specificity A1 reacting with its antigen in the rabbit amboceptor used. The bentonite flocculation reactions observed with the sera of the immunized rabbits may be due to cross-reactions of the rabbit isoprecipitin and human γ-globulin, or to production of a different anti-γ-globulin antibody which reacts primarily with human γ-globulin.

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In vivo Absorption of Amprolium and Its Competition with Thiamine. (28650)

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The coccidiostat amprolium* [1-(4-amino-2-n-propyl-5-pyrimidinylmethyl)-2-picolinium chloride • HCl] is a weak anti-metabolite of thiamine. It is capable of producing thiamine deficiency in chicks(1), adult chickens, and eggs(2) but only when added at levels in excess of 700 ppm to diets containing approximately 5 to 6 ppm thiamine. Parenteral and oral administration of the vitamin overcomes the vitamin deficiency(1,2). Apparently the primary site of action is in the intestine, because hens administered amprolium orally deposited less thiamine and less drug in yolk than those given comparable doses parenterally, and less thiamine was absorbed in the presence of amprolium from hen's ligated loops, *in situ*(3). Data presented here, obtained by the ligated-loop technic, on absorption of drug in chicks, show that amprolium is absorbed preferentially from the duodenum of the chick and corroborate data from studies with hens showing that amprolium interferes with thiamine absorption.

* Obtained from Merck & Co., Inc., Rahway, N. J.

Methods. Ligated segments of the digestive tract were prepared in male and female White