

ISOLATION AND IMMUNOCHEMICAL CHARACTERIZATION OF RABBIT 7S ANTI-IgG WITH RESTRICTED HETEROGENEITY*

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Rabbits immunized intravenously with Groups A, A-variant, and C streptococcal vaccines produced 19S and 7S anti-IgGs in addition to antibodies to streptococcal carbohydrates (1). 19S anti-IgG, as detected by hemagglutination of rabbit erythrocytes coated with rabbit IgG, is produced in every immunized rabbit. Over one half of all immunized rabbits tested using a coprecipitation test produced 7S anti-IgG and in eight antisera, the concentration of 7S anti-IgG exceeded 5 mg/ml.

In the studies reported here it has been shown that the 7S anti-IgGs isolated from several antisera had exceptional molecular uniformity, as determined by various electrophoretic procedures, and had specificity for the Fc piece of IgG. The idiotypic properties of the anti-IgGs have been examined by anti-idiotypic serum to the homogeneous 7S anti-IgGs. In one case an idiotypic cross-reaction was observed between the 7S anti-IgG and the 19S anti-IgG in the same rabbit antiserum. An idiotypic cross-reaction between IgG and IgM rabbit antibodies with the same specificity and isolated from the same antiserum has also been reported by Oudin and Michel (2). More recently an idiotypic cross-reaction was observed between the IgG and IgM myeloma proteins isolated from the serum of the same patient (3, 4).

Materials and Methods

Preparation of Streptococcal Vaccines and Antigens, Immunization of Rabbits, Quantitative Precipitin Analysis for Antibodies to Streptococcal Carbohydrates, and Electrophoretic Methods.—These procedures have been described previously (1, 5-8).

Rabbit Antisera to Streptococcal Carbohydrates.—Group A, A-variant, and Group C antisera were employed in these studies.

Measurement of IgM and IgG.—The radial diffusion technique described by Mancini et al. (9) was used for the measurement of IgM and IgG. The influence of 19S and 7S anti-IgGs on the diffusion rate of IgM and IgG was assessed in a previous paper (1).

Measurement of 19S and 7S Anti-IgGs.—These methods were previously described (1). The 19S anti-IgG in an antiserum was measured by agglutination of rabbit red blood cells

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coated with IgG. The 7S anti-IgG was quantitated with a coprecipitation assay that is based on the ability of 7S anti-IgG to coprecipitate with antigen-antibody complexes.

Isolation of the Rabbit IgG Fraction from Antistreptococcal Antisera.—The antisera were dialyzed against 0.02 M potassium phosphate buffer, pH 7.2, and then applied to a DEAE column equilibrated with the same buffer. The fall-through peak that contained the IgG was concentrated and immediately dialyzed against phosphate-buffered saline. The IgG fraction was stored in concentrations of 5 mg/ml of phosphate-buffered saline at 4°C.

Radioactive Labeling of 7S Anti-IgGs.—Isolated 7S anti-IgGs were labeled with ^{125}I according to the method of McFarlane (10).

Immunoabsorbents.—Isolation of antibodies to the streptococcal group carbohydrates employed immunoabsorbent columns containing carbohydrate antigen coupled to Sepharose. The preparation of the immunoabsorbent and the procedure for its use were described (11, 12).

Isolation of 7S anti-IgG employed an immunoabsorbent column of rabbit Fr II coupled to Sepharose using the CNBr procedure published by Axen et al. (13).

Isolation of 7S Anti-IgG.—The immunoabsorbent column procedure for the isolation of 7S anti-IgG was carried out at 4°C. In the first step the anticarbohydrate antibody was removed from the serum with the carbohydrate immunoabsorbent column. 5 ml of antistreptococcal antisera was applied to a 2.5×25 cm column equilibrated with phosphate-buffered saline, pH 7.2. The fall-through serum was devoid of anticarbohydrate antibody. The absorbed antibody was eluted from the column by lowering the pH of the elution buffer with a gradient maker containing 0.1 N sodium acetate buffer, pH 4.0, and phosphate-buffered saline.

The fall-through peak from the carbohydrate immunoabsorbent column was concentrated, dialyzed against phosphate-buffered saline, and then applied to a second immunoabsorbent column (2×20 cm) containing rabbit IgG coupled to Sepharose that was also equilibrated with buffered saline. 7S anti-IgG was retarded on this column, but was subsequently eluted without a change in elution buffer after the bulk of the serum proteins. The 7S anti-IgG is devoid of 19S anti-IgG because the latter was bound to the column. Recovery of the 19S anti-IgG from the column by elution was abandoned because this procedure resulted in loss of antibody activity. The isolated anticarbohydrate antibodies and the 7S anti-IgG preparations were concentrated and stored in aliquots at 4°C in phosphate-buffered saline.

Specificity of the 7S Anti-IgG.—Fragments of IgG were obtained by enzymatic digestion for use in serologic studies to determine the antibody specificity of the 7S anti-IgG. The IgG was hydrolyzed by pepsin at pH 4.5 according to the method described by Nisonoff et al. (14). The fragments were separated by gel filtration on Sephadex G-150 equilibrated with phosphate-buffered saline, pH 7.2. The first component off the column was the $\text{F}(\text{ab}')_2$. This component did not react with goat antiserum to rabbit Fc gamma when analyzed by Ouchterlony analysis. The second component off the column contained the Pep-III' fragment. This was further purified by gel filtration on a column of Sephadex G-75. The Pep-III' fragment showed one predominant band when analyzed by sodium dodecyl sulfate (SDS)¹-polyacrylamide gel electrophoresis (15). This fragment, according to Utsumi and Karush (16), has a mol wt of 27,000 and an s rate of 2.4 S. It is a dimer of 12,000 mol wt subunits that can be dissociated in SDS. The fragment is located near the C-terminal end of the Fc piece.

In the inhibition of precipitation experiments, 10 μg of radiolabeled 7S anti-IgG was incubated for 1 h at 37°C with various inhibitors, including IgG, $\text{F}(\text{ab}')_2$, and Pep-III'. Subsequently, 2 μg of Group A streptococcal carbohydrate and 60 μg of anti-Group A antibody were added, and the reaction volume was adjusted to 0.1 ml with phosphate-buffered saline. The tubes were mixed, incubated at 4°C for 18 h, and centrifuged. 50 μl were removed with an Eppendorf (Brinkmann Instruments, Inc., Westbury, N. Y.) volumetric pipette. This

¹ Abbreviation used in this paper: SDS, sodium dodecyl sulfate.

sample, plus the 50 μ l sample remaining in the initial tube, were counted for radioactivity. The radioactivity of the precipitate was calculated from these two values. When inhibitor was not added, approximately 30% of [125 I]7S anti-IgG precipitated with antigen-antibody complexes under these experimental conditions.

The same inhibition experiments have also been carried out with the F(ab')₂ fragment of [125 I]7S anti-IgG. In these experiments 10 μ g of radiolabeled F(ab')₂ was added to the reaction mixture instead of the intact 7S anti-IgG.

Preparation of Anti-Idiotypic Antisera.—Antisera to isolated 7S anti-IgG components were prepared in Rockefeller strain guinea pigs. Each animal received 5 mg of pooled rabbit IgG (Pentex Biochemical, Kankakee, Ill.; Fr II) intravenously in order to render them tolerant to common IgG determinants following the method described by Henney and Ishizaka (17). On the same day 100 μ g of purified 7S anti-IgG mixed with complete Freund's adjuvant was injected into the footpads of each animal. 4 wk later the guinea pigs were bled out. The antisera were passed through immunoabsorbent columns containing pooled rabbit IgG coupled to Sepharose by the cyanogen bromide method (13). In some instances, when the anti-idiotypic antisera contained traces of antibodies to serum components, other than immunoglobulins, they were subsequently passed through a second immunoabsorbent column prepared from Sepharose and whole rabbit serum by the cyanogen bromide method.

RESULTS

Isolation of 7S Anti-IgG.—The isolation procedure for 7S anti-IgG consists of two preparation steps employing two different immunoabsorbent columns. The isolation of the 7S anti-IgG from a Group C streptococcal antiserum R3387 is depicted in Fig. 1. In the first preparation step, the antistreptococcal antibody is removed from the antiserum with an immunoabsorbent column containing streptococcal Group C carbohydrate coupled to Sepharose. Anticarbohydrate antibody binds to the column and can be eluted by lowering the pH of the elution buffer (pool II). The fall-through component (pool I), containing the 7S anti-IgG and all other serum proteins, was concentrated and applied to a second immunoabsorbent column prepared from rabbit IgG coupled to Sepharose. On this column, 7S anti-IgG was retarded and eluted after the other serum proteins. This elution (pool III) was achieved without any change of the elution buffer because of the low binding affinity of the 7S anti-IgG for the column. The microzone electrophoretic patterns of antiserum R3387 and the isolated anticarbohydrate antibody (pool II) and the 7S anti-IgG (pool III) are depicted in the upper right corner of Fig. 1. The electrophoretic mobility of the 7S anti-IgG corresponds to the major monodisperse component in the whole antiserum. In this antiserum, the anticarbohydrate antibody (pool II) is polydisperse.

7S anti-IgG was isolated from four antistreptococcal antisera that contained between 6 and 14 mg of 7S anti-IgG per ml. In Table I are listed the concentrations of 7S anti-IgG in the four antisera as detected by the coprecipitation test (1), and the amount of purified 7S anti-IgG obtained from each antiserum. The yield of 7S anti-IgG is expressed as the percent of the material present in the whole antiserum. The percent yields ranged from 13 to 46%. These differences probably depend on the relative binding affinity of the 7S anti-IgG to the immunoabsorbent column. All of these isolated 7S anti-IgGs were free of other

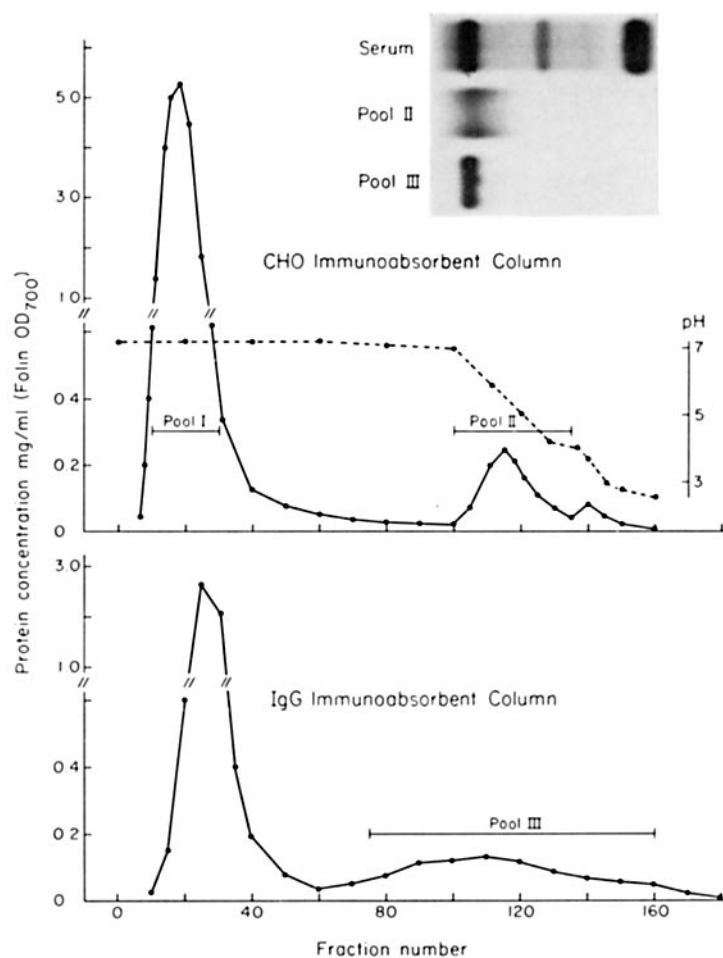


FIG. 1. Isolation of 7S anti-IgG. *Upper panel:* chromatography of antistreptococcal antiserum R3387 on a carbohydrate immunoabsorbent column. Pool I exclusion peak; pool II anti-Group C antibody. *Lower panel:* chromatography of pool I on IgG immunoabsorbent column. Pool III 7S anti-IgG. *Insert:* microzone electrophoretic pattern of antiserum R3387, antistreptococcal antibody (pool II), and 7S anti-IgG (pool III).

serum proteins. All preparations gave a single IgG precipitin arc on immunoelectrophoresis when tested with goat antiserum to whole rabbit serum. They did not precipitate with group-specific streptococcal carbohydrate.

Molecular Homogeneity of 7S Anti-IgGs.—All four isolated 7S anti-IgG preparations gave a homogeneous pattern on microzone electrophoresis. In Fig. 2 are presented two examples of purified 7S anti-IgGs and the patterns of the antisera from which they were isolated. In one case, the 7S anti-IgG is a prom-

inent major component in the IgG region of the electrophoretic pattern of the whole serum, whereas in the other, the 7S anti-IgG is submerged from view by the broad band of polyclonal gamma globulins.

Because of the antibody activity of the 7S anti-IgG, other IgG components may be bound to the purified preparations. It is unlikely, however, that this is the case because several of the final anti-IgG preparations had electrophoretic

TABLE I
Recovery of 7S Anti-IgGs from Streptococcal Antisera by the Immunoabsorbent Column Procedure

Serum no.	Total IgG in serum	7S anti-IgG in serum*	7S anti-IgG recovered	Percent Yield
	mg/ml	mg/ml	mg	%
R3387	30	14.0	5.4	39
R4057	25	6.5	3.0	46
R3439	35	10.0	2.4	24
R3416	31	9.2	1.2	13

* These values were determined by the coprecipitation test.

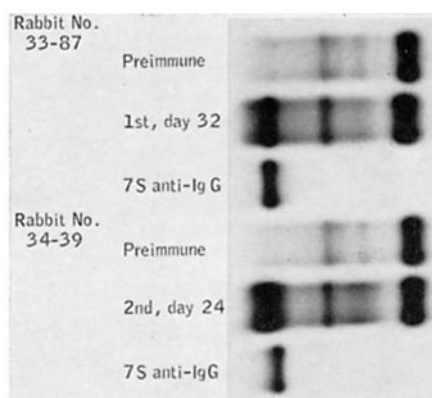


Fig. 2. Microzone electrophoretic pattern of anti-Group C antisera R3387 and R3439 and of the 7S anti-IgGs isolated from these sera.

homogeneity when tested with various methods. Depicted in Fig. 3 are the alkaline urea disk electrophoretic patterns of four reduced and alkylated 7S anti-IgGs, as well as an IgG pool. The light chain pattern of the 7S anti-IgGs of R3387 and R4057 showed an especially high degree of homogeneity, which suggests that other serum IgG was not bound to any appreciable extent to the 7S anti-IgG.

There was no evidence that the allotype of the immunized rabbit has an influence on the 7S anti-IgG response. 7S anti-IgGs were isolated from three rabbits that were allotype a₂, 3/b₄. Two of the isolated 7S anti-IgGs were

a2/b4, and one was a3/b4. The exclusion of the second group *a* allele in the isolated 7S anti-IgGs also argues against the presence of other serum IgG components in the purified preparations.

The Binding Site of 7S Anti-IgG.—The binding site of 7S anti-IgG was localized to the F(ab')₂. This was shown in experiments that employed the coprecipitation test and analytical ultracentrifugation.

The coprecipitation test is based on the ability of 7S anti-IgG to react with antigen-antibody complexes (1). Isolated 7S anti-IgG could not be used to prepare F(ab')₂ for these experiments because the antibody activity was unstable. For this reason, F(ab')₂ was prepared from the isolated IgG fraction of the antisera, a procedure which preserved the antibody activity. Presented in Table II are the results from using the IgG fractions and the F(ab')₂ preparations of four antisera in the coprecipitation test. From 18 to 42% of the total IgG in the four antisera was anti-IgG. 9–22% of the F(ab')₂ preparations were anti-IgG. The preparation of F(ab')₂ fragments reduces but does not eliminate

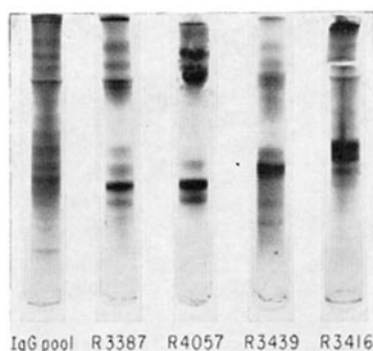


FIG. 3. Urea disk electrophoresis of reduced and alkylated 7S anti-IgGs and pooled rabbit IgG.

TABLE II

Detection of 7S Anti-IgG in the IgG Fraction of Streptococcal Antisera and in the F(ab')₂ Prepared from the IgG Fraction

Antistreptococcal serum	Recovery of the sample by the coprecipitation test*	
	Isolated IgG	F(ab') ₂ from isolated IgG
	%	%
R3387	42	22
R4057	34	18
R3439	18	9
R3416	26	14

* The percent recovery indicates the proportion of the total sample that is 7S anti-IgG and F(ab')₂, respectively.

the anti-IgG activity. These results indicate that the binding site is on the $F(ab')_2$ portion of the anti-IgG.

Ultracentrifugation experiments offered further proof that the binding site of the 7S anti-IgG was on the $F(ab')_2$. In these experiments, the $F(ab')_2$ fragments of the isolated 7S anti-IgGs were mixed with a pool of rabbit IgG and subsequently examined in the analytical ultracentrifuge. In Fig. 4 are depicted the sedimentation patterns of $F(ab')_2$ of isolated 7S anti-IgG of serum R3387 and of pooled rabbit IgG: both individually and together after mixing. The mixture of $F(ab')_2$ and IgG sedimented in one symmetrical peak with an s rate larger than that of the IgG, an indication that the $F(ab')_2$ formed a complex with IgG. In order to assess the stoichiometry of the complex formation, the sedimentation patterns were analyzed for a series of different mixtures

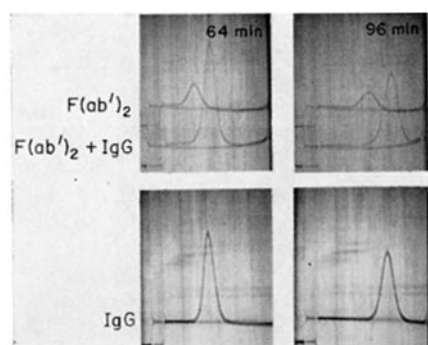


FIG. 4. Complex formation between $F(ab')_2$ of 7S anti-IgG and pooled rabbit IgG. *Upper panel:* the upper portion depicts the schlieren pattern of $F(ab')_2$; the lower portion that of the mixture between $F(ab')_2$ and IgG. *Lower panel:* schlieren pattern of IgG alone. Solvent: phosphate-buffered saline, pH 7.2. Temperature 20°C, speed 56,400 rpm. Pictures taken after 64 and 96 min.

in which the concentration of $F(ab')_2$ was kept constant, whereas the concentration of pooled IgG was increased. 4 mol of IgG pool were needed to bind 1 mol of $F(ab')_2$ from the isolated 7S anti-IgG of serum R3387 at 20°C. This ratio was even higher for the $F(ab')_2$ fragments of the other isolated 7S anti-IgGs. The requirement of a molar excess of IgG over $F(ab')_2$ in these binding studies may be explained by the low binding affinity of the 7S anti-IgGs. However, the specificity of the 7S anti-IgG might also influence the molar ratio between IgG and $F(ab')_2$ in these experiments. Recent data suggest that a 7S anti-IgG may have activity for only a fraction of the IgG pool used for complex formation.

Specificity of the 7S Anti-IgG for the Fc Piece.—Two experiments revealed that the 7S anti-IgG reacted with the Fc piece of the IgG antibodies to streptococcal carbohydrates that were present as complexes with the carbohydrate antigen. In the first experiment, a comparison was made between the reactivity of the 7S anti-IgG with Group A streptococcal antigen-antibody complexes

and with complexes prepared with antigen and $F(ab')_2$ of streptococcal antibody. These two kinds of complexes at equal molar concentrations were allowed to form in the presence of the four antisera containing 7S anti-IgG. The amount of 7S anti-IgG that coprecipitated with either type of complexes is listed in Table III. Of serum R3387, no 7S anti-IgG precipitated with the $F(ab')_2$ complexes, whereas 14 mg of 7S anti-IgG per ml serum precipitated with complexes prepared with native antibody. This result indicates that the 7S anti-IgG has specificity for an antigenic site(s) on the Fc portion of the IgG molecule. Of the other three sera used in this experiment, small amounts of IgG, maximally 1.8 mg/ml for serum 3416, precipitated with $F(ab')_2$ complexes. The small amount of IgG that coprecipitated with the $F(ab')_2$ complexes may indicate that a minor component of the 7S anti-IgGs has specificity for the $F(ab')_2$ portion of the IgG molecule. It is more likely, however, that this minimal reaction is due to the so-called agglutinators, as first described by Osterland

TABLE III
Coprecipitation of 7S Anti-IgG with Streptococcal Antigen-Antibody Complexes Formed with Either Native IgG Antibody or the $F(ab')_2$ of the Antibody

Serum no.	Coprecipitation of 7S anti-IgG with streptococcal ag/ab complexes	
	Complexes prepared with native streptococcal antibody	Complexes prepared with $F(ab')_2$ of streptococcal antibody
	mg/ml	mg/ml
R3387	14.0	0
R4057	6.5	0.4
R3439	10.0	1.5
R3416	9.2	1.8

et al. (18) for man and later by Mandy for rabbits (19). Agglutinators are IgG antibodies that react with antigenic sites of $F(ab')_2$ that have been exposed by enzymatic cleavage of IgG.

The specificity of the antibody-binding site was further analyzed by inhibition of the coprecipitation reaction with either intact IgG or IgG fragments. ^{125}I -labeled 7S anti-IgG isolated from serum R3387 was used. 2 μg of Group A streptococcal carbohydrate, 60 μg of anti-Group A antibody, 10 μg of [^{125}I]7S anti-IgG, and an inhibitor were allowed to interreact in a reaction volume of 0.1 ml. In controlled tests without addition of an inhibitor, 30–40% of the [^{125}I]anti-IgG coprecipitated with antigen-antibody complexes. Shown in Fig. 5 is the inhibition of the coprecipitation reaction by pooled rabbit IgG and a peptide termed Pep-III' (16), the largest peptide obtained from the Fc region of IgG by pepsin digestion. It was used since the rabbit Fc piece is insoluble at the concentrations required in these inhibition experiments.

Both IgG and Pep-III' inhibited the coprecipitation reaction, whereas rabbit $F(ab')_2$ had no inhibitory effect. At equal molar concentrations, IgG was a

somewhat more effective inhibitor than Pep-III'. Complete inhibition of coprecipitation was only achieved with approximately a 10–15-fold molar excess of soluble IgG over the amount of IgG present in the antigen-antibody complex. It is, therefore, apparent that 7S anti-IgGs can be detected even in hypergammaglobulinemic antisera with the coprecipitation test provided that there is less than a 10–15-fold excess of IgG components over the amount of antibody added to the test system to form complexes.

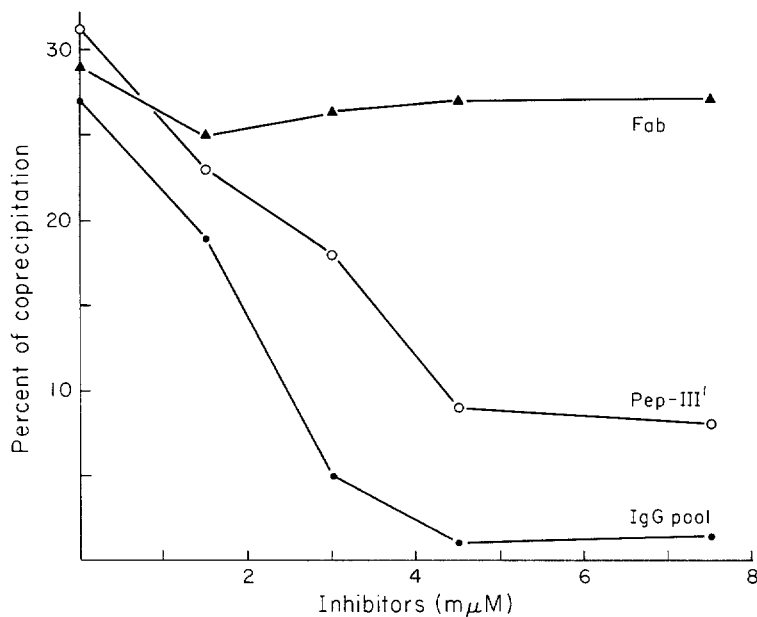


FIG. 5. Inhibition of coprecipitation of 7S anti-IgG R3387 by IgG and the IgG fragment Pep-III'.

Shared Idiotype between 7S and 19S Anti-IgGs.—In a previous report it was emphasized that the antisera of all rabbits hyperimmunized with streptococcal vaccines produced 19S anti-IgG while 7S anti-IgG occurs in more than one-half (1). Studies were undertaken therefore to determine if the idiotype detected in the homogeneous 7S anti-IgG was also present in the 19S anti-IgG isolated from the same hyperimmunized rabbit.

Heterologous anti-idiotypic sera were produced in guinea pigs to homogeneous 7S anti-IgG isolated from rabbits R3387 and R4057. The anti-idiotypic antisera were absorbed with a pool of rabbit IgG coupled to Sepharose. In double diffusion in agar, the monoclonal 7S anti-IgG and the $F(ab')_2$ prepared from it gave a precipitin band with the anti-idiotypic serum, but an IgG pool gave no reaction. No idiotypic cross-reactions were seen among 20 streptococcal antisera tested that contained 7S anti-IgG.

It was first necessary to isolate the IgM fraction of a streptococcal antiserum in order to show that it contained a component that had an idiotypic cross-reaction with the 7S anti-IgG. The IgM and IgG fractions of the antistreptococcal antisera were initially isolated with a Sephadex G-200 column. The IgM component was recycled through G-200 Sephadex and further purified by sucrose density ultracentrifugation. Immunological testing indicated that the IgM preparations were free of IgG. No precipitin line was observed in immunodiffusion in agar between a high titer anti-Fc gamma antiserum and the IgM preparations employed at various concentrations.

As shown by the immunoelectrophoretic pattern in Fig. 6, the anti-idiotype

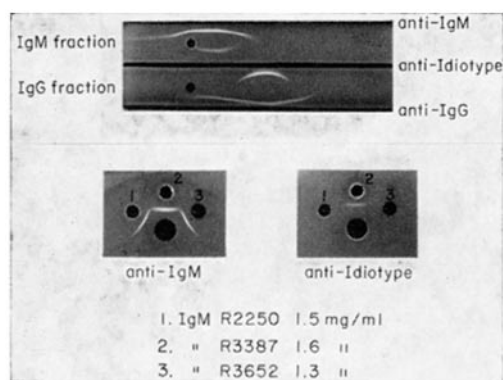


FIG. 6. Shared idiotype between 7S and 19S anti-IgG of antiserum R3387. *Upper panel:* immunoelectrophoresis of the IgM and IgG fraction of antiserum R3387. The middle trough contains anti-idiotype serum raised against 7S anti-IgG R3387. *Lower panel:* Ouchterlony analysis of IgM fractions of three antisera (R2250, R3387, and R3652) containing high titers of 19S anti-IgG with the anti-idiotype serum.

serum to 7S anti-IgG from R3387 reacted not only with the IgG fraction but also with the IgM fraction. It should be noted that both the IgG and the IgM fractions gave a limited symmetrical precipitin arc with the anti-idiotype serum. The IgM fraction, after absorption with an immunoabsorbent of IgG-coated Sephadex beads, no longer reacted with the anti-idiotype serum. This finding is an indication that the component in the IgM fraction that reacts with the anti-idiotype serum is an antibody with anti-IgG activity.

Ouchterlony double diffusion was employed to demonstrate serological similarity between isolated 7S anti-IgG and the component in the IgM fraction that reacts with the anti-idiotype serum. This is shown in Fig. 7. The precipitin band between 7S anti-IgG and the anti-idiotype serum merges with the precipitin band between the anti-idiotype serum and the IgM fraction. Both the 7S anti-IgG and the IgM fraction were isolated from the same rabbit.

Some human 19S anti-IgGs are known to precipitate in agar immunodiffusion

with complexes of human IgG or IgG of other species such as rabbit or horse (20). Experiments were therefore designed to exclude the possibility that the precipitin line between the IgM fraction of R3387 and the anti-idiotypic antiserum that is seen in Fig. 6 (top frame) is due to reactions of 19S anti-IgG with either complexes of rabbit or guinea pig IgG. This possibility is not ruled out by the absorption of the anti-idiotypic sera with solid immunoabsorbents because it is conceivable that they contain small amounts of complexes consisting of rabbit IgG and guinea pig anti-IgG antibody. 19S anti-IgG might react with these trace amounts of complexes and form precipitin bands in agar double diffusion. If this were the case, precipitin lines should also be seen between the anti-idiotypic antiserum and IgM fractions of other antistreptococcal antisera that have similarly high 19S anti-IgG titers. As shown in Fig. 6 (lower frame) this did not occur. Two IgM preparations from immunized rabbits R2250 and R3652 give no reaction with the anti-idiotypic serum. Negative results were

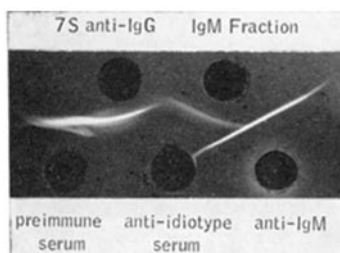


FIG. 7. Shared idiotypicity between 7S and 19S anti-IgG of antiserum R3387. The upper left well contains isolated 7S anti-IgG, and the upper right well the IgM fraction of the same antiserum. The anti-idiotypic serum was raised against 7S anti-IgG R3387.

also seen with IgM preparations from three other antisera. All five of these IgM preparations were used in concentrations similar to that employed for the IgM preparation that reacted with the idiotypic antiserum. All IgM preparations had similar 19S anti-IgG activity as detected by hemagglutination (titer 1/10,240 to 1/20,480). This result excludes the possibility that the precipitin reaction seen here is due to a nonspecific reaction between 19S anti-IgG and rabbit IgG complexes with guinea pig IgG in the anti-idiotypic serum.

A precipitin reaction between 19S anti-IgG and guinea pig IgG complexes in the anti-idiotypic serum had to be examined as another possible cause of the reaction of the anti-idiotypic antiserum and the IgM fraction of R3387. Such a reaction could have occurred if the anti-idiotypic antiserum contained small amounts of a precipitating antibody specific for a minor serum component other than the 7S anti-IgG. Small amounts of complexes that form in this way might have precipitated with 19S anti-IgG through interaction with guinea pig antibody. To exclude this possibility, IgM fractions of six different antistreptococcal antisera with 19S anti-IgG hemagglutination titers of 1/10,240 to 1/20,480 in-

cluding R3387 were analyzed for their ability to precipitate in agar with guinea pig IgG complexes. For this purpose, guinea pigs were immunized with Group A streptococcal vaccine and the antibody was isolated by the immunoabsorbent columns. Complexes between the Group A carbohydrate and guinea pig antibody were formed at equivalence and in antigen excess. These complexes were used at various concentrations in immunodiffusion experiments with the different IgM fractions using an agar concentration of 0.5%. In no case was a precipitin line seen between the IgM fraction and these antigen-antibody complexes.

As a result of these control experiments, it appears that the IgM fraction of serum R3387 contains a 19S anti-IgG that has the same idiotypic determinants as the homogeneous 7S anti-IgG that was isolated from this same serum.

DISCUSSION

The majority of rabbits immunized intravenously with Groups A, A-variant, and C streptococcal vaccines produce 7S anti-IgG in addition to antibodies to the group-specific carbohydrate. Since 7S anti-IgGs may occur at serum concentrations as high as 15 mg/ml, they can be isolated and characterized immunochemically. The two major obstacles in the isolation procedure that limit the recovery of 7 S anti-IgG from antisera are the low binding affinity of 7S anti-IgGs and the loss of antibody activity during purification. The low binding affinity of 7S anti-IgG for rabbit IgG is apparent from the loose binding to an IgG immunoabsorbent column and from the stoichiometry of complexes formed between the $F(ab')_2$ fragment of 7S anti-IgG and rabbit IgG. Ultracentrifugation of immune complexes revealed that 4 mol or more of IgG were required to bind 1 mol of the $F(ab')_2$ fragments of 7S anti-IgG.

Loss of anti-IgG activity was seen during the purification of 7S anti-IgG by DEAE fractionation at low ionic strength. Other experiments employing zone electrophoresis on agar for the purification of 7S anti-IgG also revealed a decrease in the antibody-binding capacity. Purification by an immunoabsorbent consisting of IgG coupled to Sepharose has given the best yields of active 7S anti-IgG.

A striking feature of the 7S anti-IgGs isolated from two different rabbit antisera was their molecular uniformity. Both migrated in one narrow band on microzone electrophoresis. In each case one major light chains band was observed when analyzed on urea polyacrylamide gel disk electrophoresis. 7S anti-IgGs are distinct from the serum agglutinators (18, 19). 7S anti-IgGs react with the Fc portion of intact IgG, whereas the agglutinators interact with a portion of IgG that becomes exposed after treatment with different enzymes. Nevertheless, the presence of small amounts of agglutinators, especially in antisera R3439 and R3416, is suggested by the fact that some IgG coprecipitated with complexes formed between the Group A carbohydrate and $F(ab')_2$ fragments of anticarbohydrate antibody.

Anti-IgGs interact with rabbit IgG in soluble form or with IgG in antigen-antibody complexes. By virtue of this interaction, anti-IgGs may interfere with precipitation reactions and may lead to ambiguity in the detection of allotypic and idiotypic specificities by quantitative assays (T. J. Kindt and V. A. Bokisch, personal communication).

An idiotypic cross-reaction was detected between the 7S and 19S anti-IgGs of the same antiserum with anti-idiotypic sera prepared in guinea pigs immunized with isolated 7S anti-IgG. This idio type was not detected in 20 other 7S anti-IgGs tested. This finding is in agreement with those of Oudin and Michel (2), who demonstrated an idiotypic cross-reaction between IgG and IgM anti-salmonella antibodies from the same rabbit. Idiotypic cross-reactions between an IgG and IgM protein from the same myeloma serum were described simultaneously by Penn et al. (3) and Wang et al. (4). These findings, together with our observation of an idiotypic cross-reaction between 7S and 19S anti-IgGs, support the concept that IgM and IgG are products of the same clone of cells.

The homogeneity of 7S anti-IgGs suggests that some antisera with predominantly one type of 7S anti-IgG may have a high degree of specificity for certain antigenic sites on the Fc portion of IgG. Preliminary experiments (J. W. Chiao, V. A. Bokisch, R. M. Krause, personal communication) involving rabbit antisera with high titers of 19S anti-IgG show indeed that these anti-IgGs, like human rheumatoid factors, can be used to detect genetic markers on the Fc portion of the rabbit IgG molecules.

Little is known about the biologic functions of anti-IgGs that occur during hyperimmunization of animals or in certain disease states in man. It is conceivable that they assist in the elimination of antigen-antibody complexes from circulation by enlarging the size of the complex. Anti-IgG deposits have been detected in the glomeruli of patients who had 19S anti-IgGs (21). An inhibitory effect of 19S anti-IgGs on complement independent phagocytosis of antibody-coated bacteria was demonstrated by Messner et al. (22). Notkins described a regulatory effect of anti-IgGs on immune cytolysis (23). It was shown that 19S anti-IgG enhanced lysis of virus-infected cells in the presence of low levels of antiviral antibody. In the presence of high concentrations of antiviral antibody, cell lysis was inhibited by 19S anti-IgG. A possible regulatory function of 19S anti-IgG on the immune response is suggested by experiments of King et al. (24) who showed that anti-IgG can stimulate DNA synthesis of peripheral lymphocytes. Most experiments with anti-IgGs have employed patient material, a fact which placed certain restraints on the experimental design. Therefore, rabbits that produce large amounts of 19S and 7S anti-IgGs during immunization with streptococcal vaccines may be a useful experimental model in future studies on the biologic properties and functions of anti-IgGs.

SUMMARY

7S anti-IgGs were isolated from four rabbit antistreptococcal antisera by the use of immunoabsorbent columns. The isolated proteins were of restricted molecular heterogeneity; they formed a monodisperse band on microzone electrophoresis and had a limited number of light chain bands when analyzed on urea polyacrylamide gel. The binding site of the 7S anti-IgGs was detected

in the $F(ab')_2$ portion of the molecule. The binding site has antibody specificity for the Fc portion of IgG. For one 7S anti-IgG the combining site on the Fc portion could further be defined. A pepsin fragment of Fc, described as Pep-III', was a potent inhibitor of the coprecipitation of 7S anti-IgG with antigen-antibody complexes.

An idiotypic cross-reaction was detected between the 7S and 19S anti-IgGs isolated from the same rabbit with anti-idiotypic sera prepared in guinea pigs. This idiotypic specificity was not detected in the 7S anti-IgGs of 20 other rabbits.

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