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Antiglobulins and Cryoglobulins in Rabbits Producing Homogeneous Streptococcal Antibodies

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Summary. After immunization with group B or group C streptococcal vaccines, antiglobulins and cryoglobulins were found in the sera of rabbits producing homogeneous antibodies. The cryoglobulins contained antiglobulin activity and homogeneous streptococcal antibodies. The antiglobulins reacted with both rabbit and human IgG. Precipitating antibody activity to rabbit IgG lacking sialic acid was identified in one cryoglobulin.

A rabbit that produced two homogeneous antibodies to group B streptococci produced a third homogeneous immunoglobulin without anti-streptococcal antibody activity after deliberate immunization with rabbit IgG. The appearance of the new homogeneous immunoglobulin coincided with re-stimulation of antiglobulin formation. The possibility is discussed that antiglobulin antibodies may be responsible for the clonal proliferation of plasma cells that leads to homogeneous immunoglobulin production.

INTRODUCTION

The antibody response even to simple haptenic determinants is normally heterogeneous (Haber, 1968). However, electrophoretically monodisperse (homogeneous) IgG antibodies can regularly be elicited in rabbits to group-specific streptococcal carbohydrates (Krause, 1970; Herd and Spragg, 1972), and pneumococcal polysaccharides (Kimball, Pappenheimer and Jaton, 1971), when rabbits are hyperimmunized with whole bacterial cells. Homogeneous antibodies have also been elicited by the dinitrophenyl hapten conjugated to the single sulphydryl group of the enzyme papain (Brenneman and Singer, 1970). It has been suggested that homogeneous antibody production, when it occurs, is due to the simple chemical nature of the antigenic determinants involved (Brenneman and Singer, 1970), or to a limited number of antibody genes in homogeneous responders (Eichmann, Braun and Krause, 1971). This communication shows that high titres of antiglobulin antibodies and cryoglobulins are found in rabbits producing homogeneous streptococcal antibodies and suggests that antiglobulins play a role in determining homogeneous antibody production.

MATERIALS AND METHODS

Immunization of rabbits

Rabbits bred at Monash University were immunized with either group B (*Streptococcus agalactiae*) or group C (*S. equi*) vaccines as previously described (Herd and

Spragg, 1972). Rabbits given a secondary or tertiary immunization were allowed to rest 3–7 months between the injection regimes.

Streptococcal vaccines used to immunize rabbits are indicated; 210-B refers to rabbit 210 immunized with group B streptococci.

Rabbit 275-B was given multiple site intradermal injections of 20 mg b^9b^9 IgG emulsified in 1 ml Freund's complete adjuvant (Difco).

Immunochemical studies

Cryoprecipitates were isolated by centrifugation, washed twice with cold saline and solubilized by adjusting to pH 4·5 in unbuffered saline.

Immunoelectrophoresis was carried out in 1 per cent Ionagar (Oxoid), 0·06 M Veronal, pH 8·6. Anti-whole rabbit serum was obtained from Hyland.

Zone electrophoresis, purification of rabbit IgG and streptococcal group specific carbohydrates, and anti-streptococcal agglutination titrations have previously been described (Herd and Spragg, 1972).

Absorption of antiserum. Antiserum 275 (0·2 ml) was absorbed twice with 0·05 ml of packed *Proteus vulgaris* OX19 cells which had been treated with 1·0 ml anti-*Proteus* serum produced in a b^9b^9 rabbit and also absorbed twice with an equal volume of packed streptococcal vaccine cells.

Neuraminidase treatment. Sialic acid was removed from IgG by treatment with *Vibrio cholerae* neuraminidase (Calbiochem) using 25 units/mg protein in 0·1 M Tris-maleate, 0·001 M $CaCl_2$, pH 5·6, 37°, 1 hour.

Antiglobulin activity

Anti-rabbit globulin activity was measured by haemagglutination of 1 per cent haemolysin-treated sheep red blood cells (Kabat and Mayer, 1961) after antisera were absorbed twice with one-fourth volume of normal sheep cells. Only one cryoglobulin (530-C) showed agglutinating activity for normal sheep cells. Cryoglobulins were tested at protein concentrations ranging from 1 to 10 mg/ml and titres expected from a 10 mg/ml solution were calculated.

Anti-human globulin activity was tested by Dr Maurice Cauchi (Monash University) by agglutination of globulin-coated latex particles (Hyland).

RESULTS

CRYOPRECIPITATION OF RABBIT ANTI-STREPTOCOCCAL ANTISERA

All of twenty-five rabbits immunized with group-specific streptococcal vaccines produced antiserum that precipitated to some extent after freezing or storage at 4°. After secondary immunization precipitation of antiserum was apt to occur even at room temperature prior to refrigeration. Microscopic examination of the cryoprecipitates after Gram-staining showed no microbial contamination. Immunoelectrophoresis showed that the cryoprecipitates were immunoglobulins (Fig. 1). Thus, the precipitates were judged to be cryoglobulins.

ANTIBODY ACTIVITIES OF SERA AND CRYOGLOBULINS

All immune sera and cryoglobulins tested showed anti-rabbit globulin activity in passive haemagglutination tests (Table 1). Anti-human globulin activity was also found in 11/24

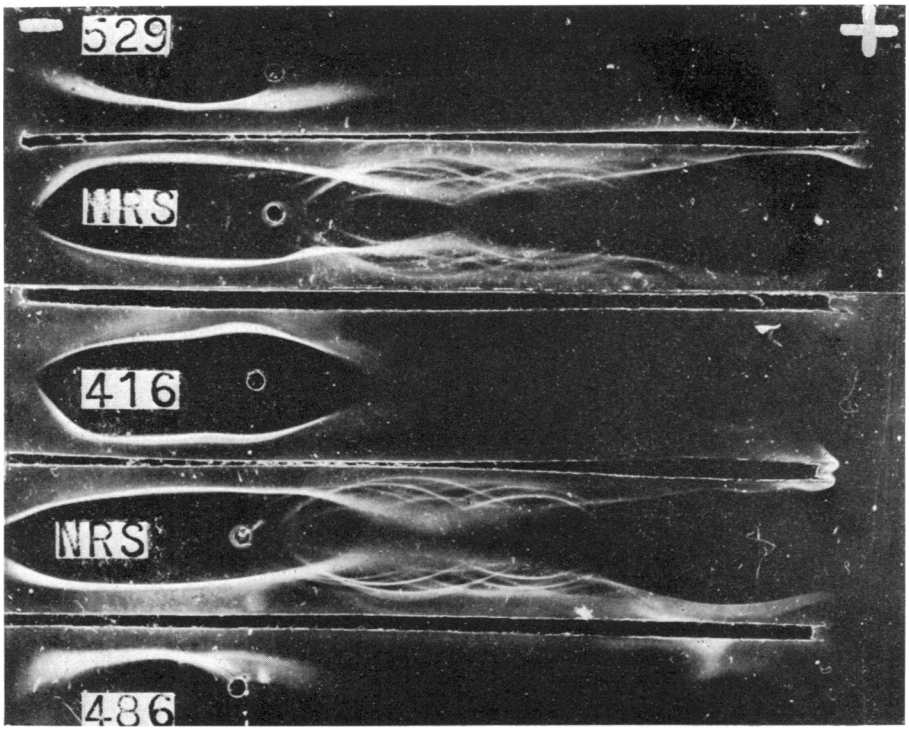

FIG. 1. Immunoelectrophoresis of three cryoglobulins (529-C, 416-B, and 486-B) and normal rabbit serum (NRS) against anti-whole rabbit serum.

immune sera and 7/12 cryoglobulins. None of the sera or cryoglobulins showed precipitation in gel diffusion with normal IgG. However, one cryoglobulin (210-B) showed precipitating antibody activity for rabbit IgG treated to remove sialic acid (Fig. 2). Attempts to demonstrate the same antibody specificity in the serum of 210-B were unsuccessful.

All cryoglobulins contained agglutinating antibody for the streptococcal vaccine used for immunization (Table 1). The cryoglobulins also contained precipitating antibodies to homologous streptococcal carbohydrates (Fig. 2). Zone electrophoresis of cryoglobulins showed the presence of homogeneous immunoglobulin bands similar to those in the antisera (Fig. 3).

STIMULATION OF A HOMOGENEOUS IMMUNOGLOBULIN BY IMMUNIZATION WITH IgG

A homozygous b^4b^4 rabbit, number 275, produced two homogeneous antibodies after primary immunization with group B streptococci. One month after streptococcal immunization was discontinued rabbit number 275 was given intradermal injections of b^9b^9 IgG for production of anti-allotype serum. When antiserum was taken 1 month later homogeneous streptococcal antibodies were disappearing but a strong band of slow electrophoretic mobility was now seen in the anti-b^9 serum, indicating the production of a new homogeneous immunoglobulin (Fig. 4). This immunoglobulin seemed unlikely to be anti-allotype antibody because the serum had only weak precipitating activity for b^9 IgG. Repeated

TABLE 1

ANTIBODY TITRES FOR RABBIT GLOBULIN AND STREPTOCOCCAL VACCINES

Anti-streptococcal antisera		Antiglobulin titre*		Homologous streptococcal agglutinin titre	
		Serum†	Cryoglobulin (10 mg/ml)	Serum†	Cryoglobulin (10 mg/ml)
Primary immunization	210-B	8192	Nt	32768	Nt
	275-B	2048	Nt	16384	Nt
	277-B	2048	Nt	16384	Nt
	283-B	4096	Nt	16384	Nt
	416-B	512	Nt	8192	Nt
	485-B	4096	1900	16384	114
	486-B	2048	Nt	16384	Nt
	393-C	8192	Nt	65536	Nt
	394-C	1024	Nt	16384	Nt
	529-C	2048	160	32768	393
	530-C	2048	1280	32768	320
	532-C	2048	89	32768	44
	560-C	2048	31	16384	62
	561-C	4096	94	32768	25
Secondary immunization					
	210-B	4096	180	3200	330
	233-B	2048	Nt	4096	Nt
	277-B	2048	Nt	4096	80
	392-C	4096	80	16384	320
	394-C	256	Nt	8192	40
	416-B	2048	46	8192	340
	486-B	2048	16	8192	512
Tertiary immunization					
	277-B	1024	113	4096	142

* Agglutination titres for rabbit globulin adsorbed to sheep red cells.
† Serum supernatant after removal of cryoprecipitate.
Nt, not tested.

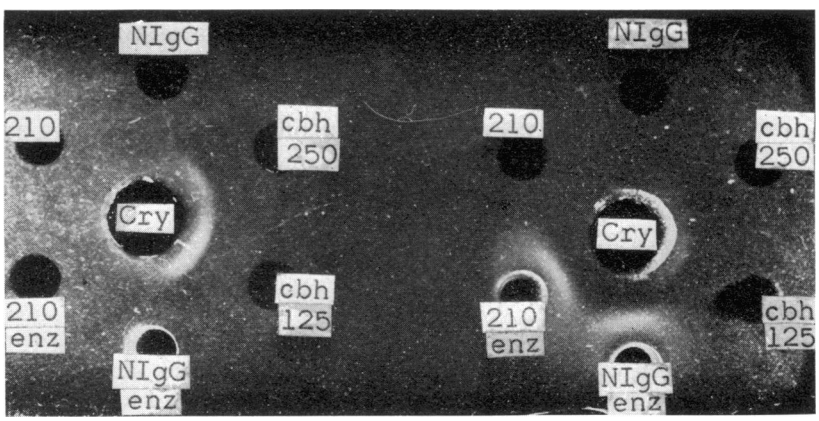

FIG. 2. Gel diffusion reactions of cryoglobulin 210-B reacting with group B carbohydrate at a concentration of 250 mg/ml (cbh 250) and 125 mg/ml (cbh 125), with normal rabbit IgG (NIgG), autologous IgG (210), and IgG treated with neuraminidase, either normal (NIgG enz) or autologous C210 enz). The cryoglobulin in the right central well cryoprecipitated from antiserum taken 3 weeks after immunization began, the cryoglobulin in the left central well was obtained after 5 weeks.

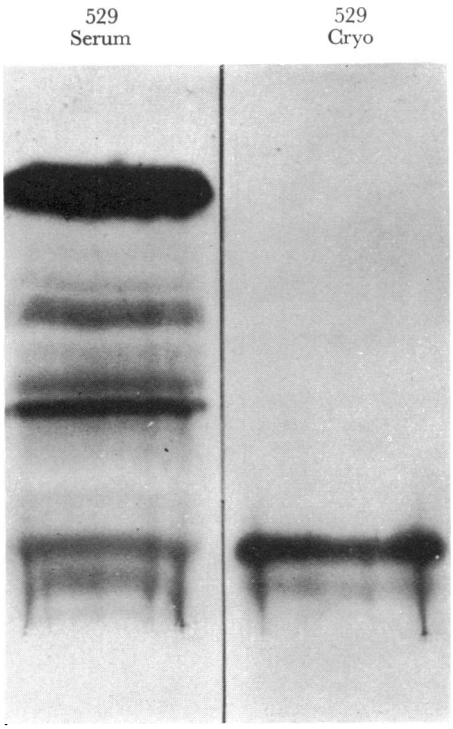

FIG. 3. Zone electrophoresis of the immune serum and cryoglobulin from rabbit 529-C showing that the cryoglobulin contains homogeneous streptococcal antibodies.

absorption with b^9 IgG or with group B streptococci failed to remove this component from the serum. Thus, the antibody activity, if any, of this component is obscure.

The injection of IgG into rabbit number 275 maintained a high antiglobulin titre but had no effect on the decreasing anti-streptococcal antibody titre (Table 2). This rabbit developed unusually severe intradermal reactions at the sites of injection of the b^9 IgG and shortly after anti-b^9 serum was taken, it died of unknown causes.

DISCUSSION

Cryoglobulins and antiglobulins were demonstrated in the sera of all rabbits producing homogeneous anti-streptococcal antibodies after immunization with killed streptococcal vaccines. The cryoglobulins appear to be immune complexes of antiglobulin antibody and homogeneous anti-streptococcal antibodies.

Antiglobulins frequently arise in rabbits hyperimmunized with bacterial vaccines (Bokisch, Bernstein and Krause, 1972; Williams, Mellbye and Kronvall, 1972) and in a variety of human clinical disorders (Goldberg, 1971). Factors suggested to render immunoglobulins autoimmunogenic are lack of sialic acid (Zinneman, Levi and Seal, 1968), alteration of carbohydrate composition by bacterial components (McIntosh, Kaufman, McIntosh and Griswold, 1972) or partial denaturation of globulin in the form of immune complexes (Williams *et al.*, 1972). The rabbit antiglobulins studied here reacted with heterologous rabbit and human IgG, showing that the antigenic determinant(s) is not

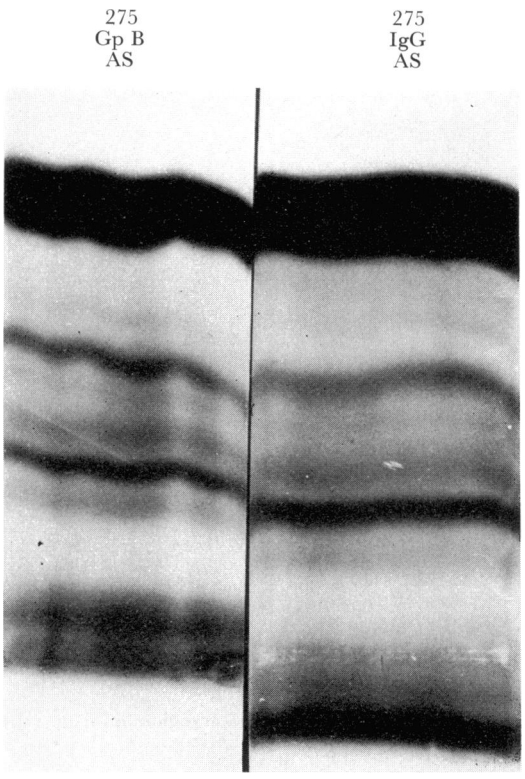

FIG. 4. Zone electrophoresis of primary immune serum to group B streptococci produced in rabbit number 275 (275 Gp B AS) and serum from the same rabbit after immunization with rabbit IgG (275 IgG AS).

TABLE 2

IMMUNIZATION SCHEDULE AND ANTIBODY TITRES FOR RABBIT NUMBER 275

Day	Injection	Antibody titre	
		Anti-group B vaccine	Antiglobulin
	ml Group B vaccine		
1, 2, 3	0·25, 0·25, 0·25	Nt	Nt
7, 8, 9	0·50, 0·50, 0·50	Nt	Nt
14, 15, 16	0·75, 0·75, 0·75	8192	256
21, 22, 23	1·0, 1·0, 1·0	16384	512
28	1·0	8192	2048
35		16384	2048
42		4096	1024
	mg IgG		
68	20	Nt	Nt
96		16	1024

Nt, not tested.

restricted to anti-streptococcal antibodies. However, a reaction with heterologous globulin was observed primarily in test systems where the globulin used as antigen was aggregated on the surface of red blood cells or latex particles. One antiglobulin was found with precipitating antibody activity for non-aggregated rabbit IgG. This antiglobulin was in the cryoglobulin produced by rabbit 210-B, and showed immunologic specificity for an antigenic determinant exposed by cleavage of sialic acid residues. Preliminary experiments in this laboratory (Raison, personal communication) have shown that purified anti-group carbohydrate antibody has less sialic acid than the non-antibody globulin in rabbits immunized with streptococci. The facts suggest that the antigenic stimulus for antiglobulin is denatured autologous IgG, and implicate an alteration to the carbohydrate residue in the denaturation process.

The finding that a new homogeneous immunoglobulin, without known antibody activity, was produced by a rabbit given deliberate secondary immunization with rabbit IgG suggests an autoimmune mechanism for homogeneous antibody production in these rabbits. It is clear that homogeneous antibodies have been induced in rabbits only by the simultaneous injection of antigens with substances that can stimulate antiglobulin production. That is, by injection of bacterial vaccines or papain, an enzyme whose proteolytic activity for IgG can render it autoimmunogenic (Williams and Kunkel, 1963). It is known that antiglobulins can suppress the synthesis of immunoglobulins as in allotype suppression (Mage, 1967) and can also stimulate lymphocyte proliferation (Singhal and Wigzell, 1971).

Rabbit homogeneous antibodies are similar to human myeloma proteins in that they arise from the clonal proliferation of plasma cells. Most untreated IgG myeloma patients produce anti-IgG antibody that is capable of stimulating autologous myeloma cells to undergo DNA synthesis and cellular proliferation (Abdou and Abdou, 1972). By analogy, in rabbits hyperimmunized with streptococci, antiglobulins may have a two-fold action in suppressing immunoglobulin synthesis by heterogeneous clones of antibody-forming cell precursors while stimulating proliferation of selected clones that secrete the homogeneous antibodies.

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