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Autoantibodies Detected in Rabbits Hyperimmunized with Group A, C, and G Streptococcal Vaccines

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Rabbits hyperimmunized with group A, C, and G streptococcal vaccines developed autoantibodies with affinities for different tissues (smooth muscle, cytoplasmic, and myocardial antibodies) and for autologous proteins (anti-albumin and anti-immunoglobulin antibodies). The presence of anti-albumin and smooth muscle antibodies, associated with a high level of immunoglobulin, suggests the development of hepatic disorders in the hyperimmunized rabbits.

Rabbits injected with nonviable group A streptococcal cells developed lesions in connective tissue and in the vascular system, changes in lymphatic tissue and spleen, and parenchymatous liver lesions. The proposed mechanism of streptococcal action refers to delayed- and immediate-type hypersensitivity, immune complex injury, and direct toxic effects resulting from degradation of cell walls after phagocytosis. On the other hand, a possible pathogenetic role in the initiation of tissue injury may be played by streptococcal antigens cross-reactive with tissues (heart, kidney) and the corresponding cytotoxic antibodies. The numerous studies concerning these problems have been comprehensively reviewed by Ginsburg (7, 8).

Rabbits immunized intravenously with streptococcal vaccine also develop antibodies against autologous immunoglobulin G (IgG) (2, 3) and cryoglobulins (9, 10).

Because the problem of the autoimmunity produced in rabbits by immunization with streptococci requires elucidation, it was of interest to follow the appearance of autoantibodies with affinities for tissues and autologous proteins (albumin, IgG) in rabbits hyperimmunized with group A, C, and G streptococcal cells. Moreover, our aim was to provide evidence for a possible pathogenetic role played by group C and G (which were less implicated than group A) streptococci in human pathology.

MATERIALS AND METHODS

Preparation of streptococcal vaccines and immunization of New Zealand white rabbits. Group A (Wheatley), C (Chestle), and G (Valente) (originating from Colindale, London, and Prague) streptococcal vaccines were prepared by growing cells in 1 liter of Todd-Hewitt medium for 24 h at 37°C. The cells were harvested by centrifugation, washed three times with 0.15 M saline, and suspended in 50 ml of saline containing 5% pancreatic extract. They were incubated for 24 h at room temperature, washed six times with saline, and inactivated at 56°C for 1 h. The vaccine contained 2×10^9 cells per ml. During week 1, the rabbits received 0.5 ml of vaccine intravenously; during weeks 2 through 4, they received 1.0 ml three times per week. Bleeding was done in week 5 (4 weeks after immunization began). The numbers of rabbits were 24 for group A, 23 for group C, and 28 for group G.

Measurement of IgG. IgG was measured by the radial diffusion technique described by Mancini et al. (14).

Measurement of anti-SRBC antibodies. Anti-sheep erythrocyte (SRBC) antibodies were measured by agglutination of nomal SRBC. Absorption with SRBC was performed for all sera having anti-SRBC activity.

Measurement of anti-IgG antibodies. Anti-IgG activity was measured by agglutination of SRBC coated with rabbit hemolytic serum or with rabbit IgG isolated by diethylaminoethyl-cellulose chromatography from the same serum.

Measurement of anti-streptococcal polysaccharide antibodies. Anti-streptococcal polysaccharide antibodies were measured by agglutination of SRBC coated with the streptococcal polysaccharides by the chromium chloride technique (1).

Anti-albumin antibodies. Anti-albumin antibodies were tested by immunodiffusion against rabbit albumin polymerized with glutaraldehyde. The polymerization was done in 0.1 M phosphate buffer, pH 6.8, using a protein concentration of 20 mg/ml and an albumin/glutaraldehyde weight ratio of 8:1 (12).

Autoantibodies. Autoantibodies with affinity for tissues were detected by indirect immunofluorescence, using sheep anti-rabbit immunoglobulin serum conjugated with fluorescein isothiocyanate.

RESULTS

After immunization of New Zealand white rabbits with group A, C, and G streptococcal

vaccines, the IgG levels were raised in comparison with the preimmune one (Fig. 1). At the same time, specific anti-polysaccharide antibodies were produced (Fig. 2).

The mean values found for IgG were 42 ± 5 (group A), 29 ± 2 (group C), and 30 ± 2 (group G) mg/ml, compared with the preimmune value of 16.8 ± 1.1 mg/ml.

The levels of anti-polysaccharide antibodies were 7.3 ± 0.4 (group A), 3.2 ± 0.4 (group C), and 12.3 ± 0.7 (group G). These values represent the $\log_2$ of the hemagglutination titer. No anti-streptococcal activity was found in the preimmune serum samples.

The levels of anti-IgG antibodies ($\log_2$ of the hemagglutination titer) were 2.8 ± 0.4 (group A), 4.8 ± 0.4 (group C), and 4.8 ± 0.3 (group G) (Fig. 3), compared with the preimmune level of 0.6 ± 0.1.

The anti-SRBC antibodies had titers of 1.4 ± 0.3 (group A), 6.0 ± 0.2 (group C), and 1.5 ± 0.2 (group G) (Fig. 4), compared with the preimmune titer of 0.7 ± 0.1.

In 38% of the rabbits immunized with group C streptococcal vaccine, anti-albumin antibodies were observed by immunodiffusion. Rabbits immunized with group A and G streptococcal vac-

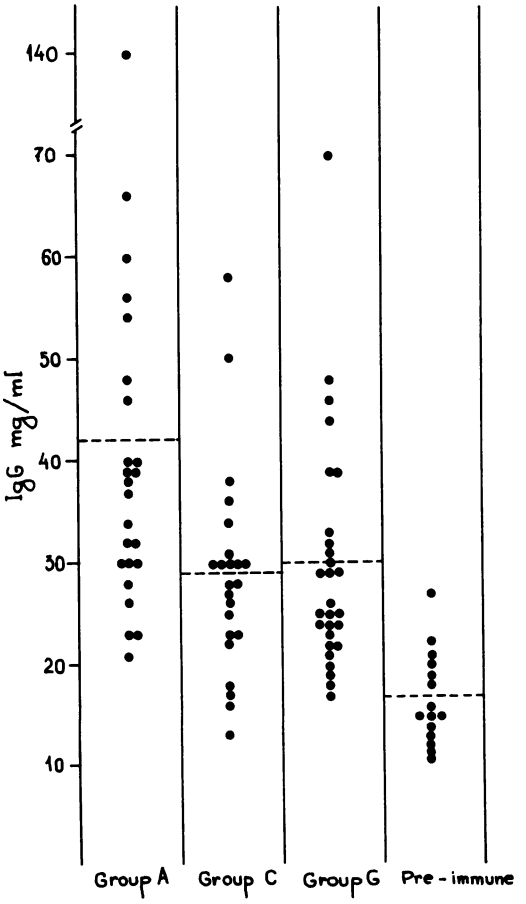

FIG. 1. *IgG level in rabbits hyperimmunized with group A, C, and G streptococcal vaccines. The representative data on 15 preimmune serum samples are shown. Mean values are indicated by dashed lines.*

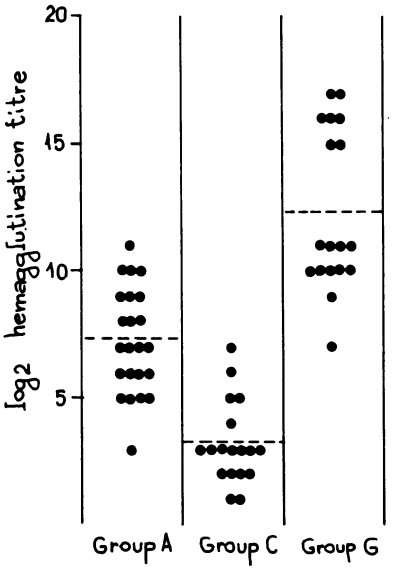

FIG. 2. *Level of anti-polysaccharide antibodies in rabbits hyperimmunized with group A, C, and G streptococcal vaccines, measured against the homologous group polysaccharide antigen. Mean values are indicated by dashed lines.*

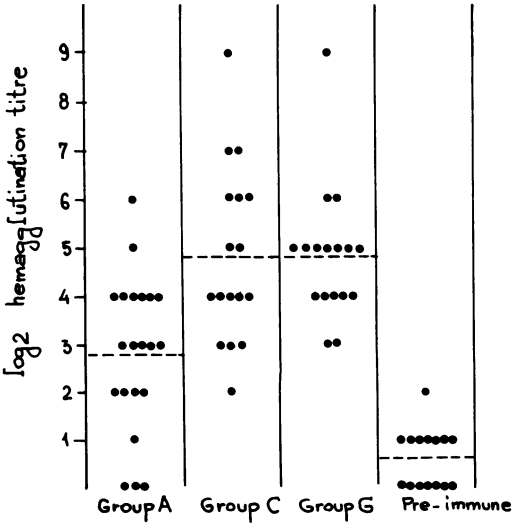

FIG. 3. *Level of anti-IgG antibodies in rabbits hyperimmunized with group A, C, and G streptococcal vaccines. The representative data on 15 preimmune serum samples are shown. Mean values are indicated by dashed lines.*

cines were not tested for the presence of anti-albumin antibodies.

The incidence of different kinds of autoantibodies, detected by immunofluorescence, is presented in Table 1. Rabbits with positive immunofluorescence in the preimmune serum were excluded. In all three groups of rabbits, smooth muscle, cytoplasmic, and myocardial antibodies were found.

Our results indicate a relatively high incidence of autoimmune phenomena in rabbits hyperimmunized with streptococcal vaccines. The frequency found for smooth muscle antibodies exceeds that associated with human chronic active hepatitis, reaching an incidence of 70%, as described in acute viral hepatitis (17). The tendency toward development of autoimmune phenomena is confirmed also by the presence of anti-IgG antibodies in all tested rabbits.

Comparing the IgG level (total IgG and IgG with certain specificity: anti-polysaccharide, anti-IgG, and anti-SRBC) with the presence or absence of anti-smooth muscle and anti-albumin antibodies, we found that the presence of these two kinds of autoantibodies was associated with a higher level of IgG, significant differences ($P < 0.05$) being recorded for the total IgG (group A), and anti-polysaccharide antibody (group C) levels (Table 2).

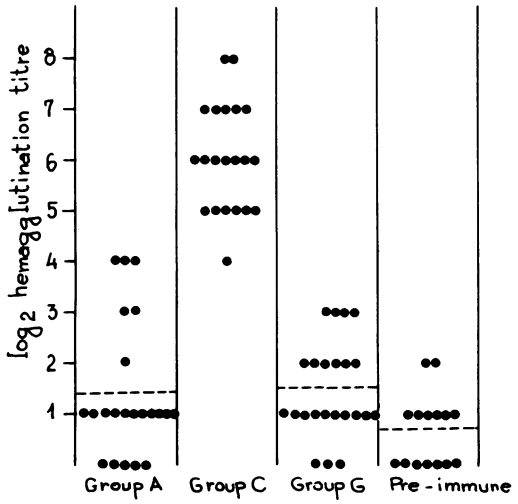

FIG. 4. *Level of anti-SRBC antibodies in rabbits hyperimmunized with group A, C, and G streptococcal vaccines. The representative data on 15 preimmune serum samples are shown. Mean values are indicated by dashed lines.*

DISCUSSION

Our data seem to indicate that autoantibody formation was stimulated by an intense specific immune response, especially in the anti-group C streptococcal sera. The simultaneous stimulation of specific and nonspecific immunoglobulins has been described for various antigens, as, for instance, against tobacco mosaic virus (20), insolubilized bovine serum albumin (5), and type

TABLE 1. *Autoantibodies detected by immunofluorescence in rabbits hyperimmunized with group A, C, and G streptococcal vaccines*

Vaccine group	Autoantibody (%)		
	Smooth muscle	Cytoplasmic	Myocardial
A	39.3	41.7[a]	30.7
C	50.0	13.6	45.5
G	67.8	42.9[a]	35.7

[a] Four rabbits from group A and one from group G showed mitochondrial antibodies. Sera were positive also in the complement fixation test with mitochondrial antigen, with titers between 1/16 and 1/128.

TABLE 2. *Correlation between autoimmune phenomena and the IgG level found in rabbits hyperimmunized with group A, C, and G streptococcal vaccines*

Vaccine group	Autoantibody[a]	IgG level			
		Total (mg/ml)	Anti-polysaccharide ($\log_2$ T[b])	Anti-IgG ($\log_2$ T)	Anti-SRBC ($\log_2$ T)
A	SMA+	50.3 ± 12.2*[c]	7.6 ± 0.5	3.4 ± 0.5	1.0 ± 0.2
	SMA−	37.5 ± 3.4*	7.1 ± 0.6	2.8 ± 0.5	1.6 ± 0.4
C	SMA+	29.4 ± 2.6	4.1 ± 0.6*	5.4 ± 0.7	6.3 ± 0.3
	SMA−	28.3 ± 3.4	2.3 ± 0.3*	4.3 ± 0.5	5.8 ± 0.4
	AAA+	29.9 ± 3.7	4.1 ± 0.5*	5.3 ± 0.7	6.7 ± 0.3
	AAA−	27.3 ± 2.5	2.3 ± 0.2*	4.6 ± 0.6	5.8 ± 0.4
G	SMA+	31.1 ± 2.9	12.8 ± 0.8	4.6 ± 0.2	1.6 ± 0.2
	SMA−	27.1 ± 3.4	11.5 ± 1.6	5.0 ± 0.6	1.3 ± 0.4

[a] SMA, Smooth muscle antibodies; AAA, anti-albumin antibodies; +, present; −, absent.
[b] T, Hemagglutination titer.
[c] *, Significant difference ($P < 0.05$).

SIII pneumococcal polysaccharides (4). In the case of pneumococcal polysaccharides, in spleen cell cultures, besides specific antibodies, some directed against different antigens (SRBC, various haptens) were found. Possible similar behavior of streptococcal and pneumococcal polysaccharides, at least in some aspects, could explain anti-SRBC antibody formation and, possibly, the autoantibody stimulation in rabbits hyperimmunized with streptococcal vaccine.

On the other hand, autoantibody formation may be due to possible cross-reactivity between streptococcal and hepatic antigens or to the release of hepatocellular antigenic components, since a similar frequency of smooth muscle antibodies is seen in human liver diseases (6). The presence of anti-albumin antibodies, detected in anti-group C sera, suggests the development of hepatic lesions in the animals hyperimmunized with streptococcal vaccines, since these autoantibodies appear to be a specific test for liver cell damage (11, 13). Hepatic lesions produced during streptococcal immunization have been described by other authors and reviewed by Ginsburg (7, 8), but in our case the presence of albumin antibodies is an index for liver cell dysfunction. These disturbances may be due to some toxic effect of the streptococcal vaccine and/or to the overloading of Kupffer cells induced by the intravenous administration of antigens (19). Also the presence of mitochondrial antibodies (in group A) and the histopathological aspect of the liver, characterized by the presence of a fibrotic process in the hepatic lobules and of lymphohistiocytic infiltrates in the portal spaces, support the idea of hepatic lesions produced in the hyperimmunized rabbits, which present similarities with those seen in human chronic active hepatitis.

The autoimmune phenomena appear to have different distributions, according to streptococcal group. Thus, rabbits hyperimmunized with group A and G streptococcal vaccines present a high incidence of cytoplasmic antibodies, contrary to results with group C.

Antibodies to heart were present in all three groups of rabbits. Lack of fluorescence with heart fibers was observed with another lot of anti-group A sera, this being difficult to explain because group A streptococci were the most implicated in the autoimmune phenomena affecting the heart (8).

A limitation of autoimmune phenomena in rabbits producing antibodies of electrophoretically restricted heterogeneity was noticed. Such rather homogeneous antibodies are produced by 5 to 20% of New Zealand red (16, 18) or New Zealand white (15) rabbits hyperimmunized with streptococcal vaccines.

Our results have shown that the hyperimmunization of rabbits with group A, C, and G streptococcal vaccines induces an immunological hyperreactivity manifested by a high level of IgG and specific antibodies and by autoimmune phenomena, characterized by a histological and serological pattern similar to that seen in human chronic active hepatitis. Our data appear to argue for a possible pathogenetic role played by group C and G streptococci, which are less implicated in human pathology than is group A.

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