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Enhancement Activity of Homologous Anti-Staphylococcal Sera in Experimental Staphylococcal Synovitis of Chicks: A Possible Role of Immune Adherence Antibodies

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Homologous anti-staphylococcal sera passively potentiated the development of experimental staphylococcal synovitis infection of chickens. These antisera obtained from chickens hyperimmunized with live *Staphylococcus aureus* were selected according to their immune adherence and agglutinating properties. While the agglutinins rose steadily for 11 weeks during the hyperimmunization schedule, the immune adherence antibody titers reached a peak at 8 weeks and dropped to almost zero 2 to 4 weeks later. The enhancement activity of these antisera was associated with a relatively high level of immune adherence antibodies and seemed not to be correlated with their agglutinin titers.

The enhancement phenomenon which was first observed by Kaliss (4) when homotransplanted tumors in host actively or passively immunized with constituents of the tumor outlived those transplanted in untreated animals has now a more general immunological significance. Voisin (10), who emphasized the importance and broad implications of this phenomenon, has proposed the term "immunological facilitation," which he defines as "The mechanism whereby an antibody (or an immune reaction) promotes the persistence of the corresponding antigen and the integrity of the cytological structures which support it, by preventing it from inducing or undergoing (or both) immune rejection." This definition includes the enhancing action of some antibody preparations on the survival time of homotransplanted nontumor tissues as well as their inhibiting action on the autoimmune reactions. Voisin also assumes that non-tissue antigens can be affected by the enhancement phenomenon. With bacterial antigens, it is possible to inhibit or delay the active formation of antibodies in animals treated with bacterial antigens and their corresponding antibodies (6, 9).

In a preliminary experiment (2), we showed first that chickens treated with an antiserum specific for the bursa of fabricius lymphocytes manifested an increased resistance of a *Staphylococcus aureus* challenge which induced, in the controls, a typical lethal staphylococcal synovitis, second that an homologous anti-staphylococcal serum had the opposite effect; it potentiated the development of the experimental staphylococcal synovitis of the chickens. These two observations let us suppose that enhancing antibodies could be involved in this experimental infection.

This work confirms the enhancing effect of homologous anti-staphylococcal sera in the experimental staphylococcal synovitis of the chicken and demonstrates a possible relationship between this enhancing activity and the immune adherence titer of the antisera.

MATERIALS AND METHODS

Bacterial strain. The *S. aureus* strain SL-27 used in these experiments was isolated from a human pneumonia case. Bacterial suspensions, washed twice, were prepared from cultures grown on nutrient agar for 5 h.

Preparation and characterization of the immune sera. Two groups of 45-day-old Leghorn chickens were hyperimmunized with the *S. aureus* strain SL-27. In the first group, four chickens were injected subcutaneously with 10^9 live cells mixed with complete Freund adjuvant. Four weeks later, we injected them intravenously, at weekly intervals during 2 months, with 10^7 live staphylococci. In the second group, four chickens were injected subcutaneously with 10^7 live *S. aureus* mixed with incomplete Freund adjuvant. Three weeks later, they received weekly an intravenous injection of 0.5×10^7 live staphylococci during 2 months. In both groups, the birds were bled before each intravenous injection for antibody titration.

Each antiserum was heated at 56 C for 30 min and

absorbed with human red blood cells. The agglutinins were titrated by a microagglutination test described by Williams and Whittemore (11). The immune adherence assays were done according to Nelson's technique (7) with a heated suspension of staphylococci as antigen, pooled guinea pig sera as complement, and washed human red blood cell suspensions as indicator particles. The tests were performed by using serial twofold dilutions of the antisera in microtiter plates (Cooke Engineering, Alexandria, Va.) by a micromethod (1).

Experimental synovitis in chicks. Intravenous injections of relatively small doses of coagulase-positive staphylococci may induce in chicks a subacute type of infection which cannot be prevented by crude staphylococcal antitoxin treatment (3). The pathognomonic symptoms are easily observable: lameness in one or more joints, mostly in the tibia-metatarsal joints, hobbling gait, dejection, and anorexia. The chicks may die within a few days.

Experimental synovitis in chicks treated with homologous anti-staphylococcal sera. In the first three experiments, the chicks were injected intravenously with a mixture of the antiserum and a subinfectious staphylococcal dose. This mixture was prepared at 4 C and was injected within 5 min after its preparation. Nonimmune serum or saline water were mixed with the bacteria and injected to the control chicks. In a fourth experiment, the antisera or a nonimmune serum were injected intraperitoneally 5 h before the intravenous inoculation of the staphylococcus suspension.

RESULTS

Antibody response of the chicken during the staphylococcal hyperimmunization. The antibody response of the chicken in the staphylococcal hyperimmunization of the first group (Fig. 1) was comparable to that of the second group (Fig. 2). Whereas the agglutinin titers increased during the immunization schedule until the 11th week, the immune adherence antibody titers reached a peak at the 8th week and declined thereafter to reach almost zero at the 12th week. The immune adherence antibody titers did not exceed 1:60, whereas the agglutinating antibodies reached a peak of 1:35, 886 at the 11th week.

Staphylococcal synovitis in chicks treated with anti-staphylococcal sera. For the experimental synovitis, we selected three antisera according to their immune adherence and agglutinating titers. These antisera were chosen to verify a previous observation (2) which seems to indicate a correlation between the immune enhancement activity of the anti-staphylococcal sera with their immune adherence titer. The first three experiments (Table 1) have been realized with the same antiserum (AS-A) showing a relatively high level of immune adherence antibodies and a high level of agglutinins. This serum was compared with either saline, nonimmune serum, or a serum having no immune adherence antibodies but a high agglutinating titer. In the first experiment, all of the chicks treated with the AS-A presented the pathognomonic symptoms of synovitis and 73% (8/11) died, whereas no chick inoculated with the bacteria mixed with saline died nor showed any clinical sign of the subacute infection. In the second experiment, 62.5% (5/8) of the chicks treated with the AS-A died from synovitis while only 10% (1/10) of those injected with the antiserum 3470 died. This antiserum had no detectable immune adherence antibody but a high agglutinating titer. In the third experiment, the chicks, being older, received a comparatively lower dose of bacteria. Treated with 0.2 ml of AS-A, only 40% (4/10) of these chicks died but all showed the clinical signs of synovitis, whereas only 20% of the chicks treated with the nonimmune serum had the clinical signs and none of them died. In the fourth experiment, the chicks were treated by the intraperitoneal route with an antiserum (AS-3492) having a higher immune adherence but a lower agglutinating titer than AS-A. All of these birds had the clinical signs of synovitis and 86% (6/7) died, whereas none of the controls

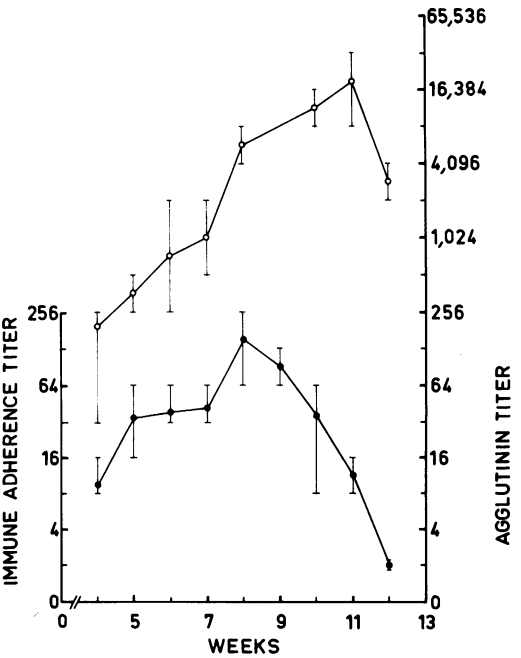

FIG. 1. *Antibody response of the chicken (group one) hyperimmunized with S. aureus. Immune adherence antibody titers, ●; agglutinating mean antibody titers, ○.*

died and only one of them showed the clinical signs.

DISCUSSION

Two schedules of hyperimmunization of the chicken with a staphylococcal strain have shown that whereas the serum agglutinins increase steadily during the first 11 weeks of the immunization, the immune adherence antibodies reach a peak at the 8th week, and then disappear within 2 to 4 weeks. Although we have not yet found any explanation for this unusual observation, we are immunizing rabbits with the same protocol to see whether this is particular to chickens. On the other hand, the maximal titer of the immune adherence reaction reached is relatively low compared with the agglutinating titers. This is in agreement with the observation of Kourilsky et al. (5), who measured the immune adherence and agglutinating antibodies in the serum of men infected with staphylococci: the immune adherence titers were 50 to 100 times lower than the agglutinating titers. We have shown (Table 1) that some homologous anti-staphylococcal sera could facilitate the development of the experimental staphylococcal synovitis in chicks. The passive enhancement activity of the sera could be demonstrated either when the antiserum was mixed with the staphylococci at 4 C and then injected intravenously as a mixture, or when the antiserum was injected intraperitoneally 6 h before the intravenous inoculation of the bacteria. This enhancement effect could be observed only with the antisera which had a high immune adherence antibody titer but seemed to be independent of their agglutinin content.

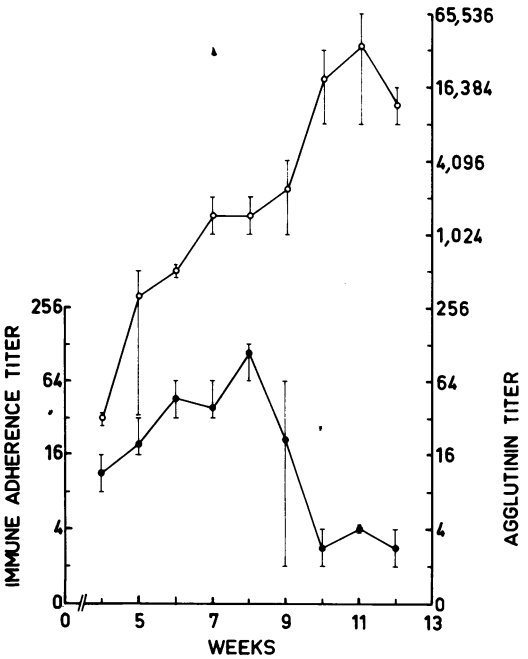

FIG. 2. *Antibody response of the chicken (group two) hyperimmunized with S. aureus. Immune adherence antibody titers, ●; agglutinating mean antibody titers, O.*

Thus the passive injection of special homologous anti-staphylococcal sera promotes the establishment of the synovitis in chickens. This paradoxical enhancement effect is emphasized by our preliminary observation (2). To prevent the production of circulating antibodies, we have treated chickens with an antiserum spe-

TABLE 1. *Experimental staphylococcal synovitis in chicks treated with homologous anti-staphylococcal sera*

Expt no.	Chick age (days)	Bacterial dose ($\times 10^6$)	Treatment[b]	Antiserum titer		Staphylococcal synovitis[a]	
				Immune adherence	Agglutinin	Clinical signs	Dead from
1	6	8.5	AS-A (0.1 ml)	64	16,384	11/11[c]	8/11[d]
			Saline (0.1 ml)			0/10	0/10
2	9	19	AS-A (0.1 ml)	64	16,384	7/8	5/8
			AS-3470 (0.1 ml)	0	32,768	3/10	1/10
			Saline (0.1 ml)			2/10	1/10
3	22	14	AS-A (0.2 ml)	64	16,384	10/10	4/10
			NS (0.2 ml)	2	16	2/10	0/10
			Saline (0.2 ml)			1/10	0/10
4	15	12	AS-3492 (0.1 ml)	128	8,192	7/7	6/7
			NS (0.1 ml)	2	16	1/8	0/8

[a] $P < 0.05$ for the first group versus other groups in each experiment.
[b] AS-A, AS-3470, AS-3492, chicken anti-staphylococcal sera; NS, chicken nonimmune serum.
[c] Number of chicks showing the pathognomonic symptoms of synovitis/number of injected animals.
[d] Number of chicks dead from synovitis/number of injected animals.

cific for lymphocytes of the bursa of fabricius. This antilymphocyte serum, which inhibited in vitro the production of antibody in a modified Cunningham assay for hemolysin-producing cells (8), protected 50% of the chickens challenged with a lethal dose of staphylococci, whereas the same bacterial suspension induced a lethal staphylococcal synovitis in all of the controls. According to these observations, the staphylococcal synovitis of the chick seems to be enhanced by antibodies.

It is important to recall that one can discriminate between two forms of the experimental staphylococcal infection in chicks: first the acute or toxic type; second the subacute or chronic type, characterized by a clinical osteoarthritis (3). The enhancement phenomenon reported here has been observed only in the subacute-type infection. In the toxic-type infection, on the contrary, the antitoxic antibodies have shown a protective effect. Furthermore, the inhibition of the production of humoral antibodies has promoted this toxic infection. Thus, the subcutaneous inoculation of a staphylococcal dose which induced an acute type of infection, killing within a few hours 40% of the normal chicks, has killed 70% of the bursectomized chicks (unpublished data).

To firmly establish the relationship between the immune adherence antibodies measured in vitro and their enhancement effect observed in promoting the development of the experimental synovitis of the chicken, we are now concentrating our efforts on the isolation and characterization of the immunoglobulin class responsible for this phenomenon.

This observation, that antibodies promote the development of a bacterial infection, seems to be comparable to those described with transplantation of tumors and normal homologous tissues. In both cases, the antisera protect the foreign cells against the reactions of the host. The mechanism by which a bacterial infection is enhanced is probably similar to the transplantation enhancement mechanism if we assume that an antibody (or an immune reaction) promotes the persistence of the corresponding antibody and the integrity of the cytological structures which support it (10).

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