Immune Response to Intracellular Parasites: Suppression by Antibody[1] (36121)

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The effectiveness of the serological methods described for diagnosis of congenital infection in the early newborn period depends upon the ability of the fetus in utero to form antibody in response to the infecting agent (1–4). Since the presence of antibody has been shown to suppress the immune response to vaccination in newborns (5–7), and since antibodies formed against an infectious pathogen in the mother during pregnancy may be transmitted via the placenta to the fetus before the organism crosses the placenta, we considered it of interest to study the possible suppressive effect that passively administered antibody may have on the immune response to an intracellular infection.

Materials and Methods. Animals. Mice of the (C57Bl/6 × DBA/2) F1 hybrid strain were used and were fed standard mouse chow and given water *ad libitum*.

Antigens. Sheep red blood cells (SRBC) were washed three times in phosphate-buffered saline (PBS), pH 7.2. Each mouse received 2×10^8 cells intraperitoneally.

Toxoplasma (RH strain) was harvested from peritoneal fluid of 3-day infected mice and washed three times in PBS. The sediment was suspended in PBS and forced through a 25-gauge needle to disrupt infected cells. Enough formalin to effect a 1% solution was then added and the suspension was stored at 4°, 18 hr. This antigen preparation is referred to as "formalin-killed *Toxoplasma* (FKT)." Before inoculation the suspension was washed twice in PBS and the organisms were counted in a Neubauer–Levy hemocytometer. Two tenths milliliter 4×10^8 organisms was injected in each mouse intraperitoneally.

Trophozoites from the less virulent C56 strain were harvested from peritoneal fluid of mice which had received an intraperitoneal inoculation of brain of chronically infected mice (cyst form). The peritoneal fluid was handled as described above for the RH strain. This preparation is referred to as live *Toxoplasma* (LT). Two tenths milliliter containing 4×10^4 live organisms was inoculated intraperitoneally into each mouse. Five days later the mice were given 40 mg/100 ml sulfadiazine in their drinking water. This was continued for 15 days to prevent death from the inoculum and to establish chronic infection.

Antibodies. Antiserum to SRBC was obtained from mice 10 days after they had received the last of three injections at 7-day intervals. The pool of antisera, collected from 23 mice, had a hemagglutinin titer of 1:4096. Antiserum against FKT was collected 15 days after the mice had received the second of two inoculations of FKT at 15-day intervals. A pool of serum from 23 mice had a titer of 1:4096 in the Sabin–Feldman dye test (DT). Antiserum against live *Toxoplasma* was collected from mice which had a chronic (30-day) C56 strain infection, 30 days after they had received a booster dose of live RH strain. The pooled sera of 19 mice had a titer of 1:16384 in the DT. Control antiserum was collected from mice which had been inoculated intraperitoneally three times with Hank's balanced salt solution (HBSS) at 7-day intervals. Ten days after the last inoculation, the mice were bled and the pooled sera of 16 animals was tested for hemagglutinins against SRBC and for anti-

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bodies to *Toxoplasma*. None were demonstrable.

Serology. Hemagglutination was performed using the microtiter method described by Sever (8). The DT was performed as described by Frenkel and Jacobs (9). Titers in both hemagglutination and dye test were converted to $\log_2$ and are presented as the average $\log_2$ titer of each group of at least three to four animals.

Results. Inhibition of the immune response to SRBC by passive immunization. Newborn mice were injected intraperitoneally with 0.05 ml of anti-SRBC serum or normal mouse serum. Six weeks later they were tested for hemagglutinins to SRBC and were found to be negative. The animals were challenged with 2×10^8 SRBC and the hemagglutinin response was followed in sera collected at 4- to 5-day intervals. As can be seen in Fig. 1, a depression in the immune response

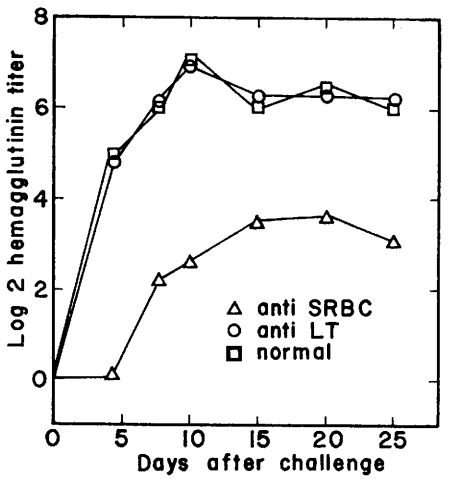

FIG. 1. Immune response of mice passively immunized with anti-SRBC (△), anti-LT (○), and normal (□) sera at birth and challenged with SRBC 6 weeks later.

was noted in those mice which received anti-SRBC serum when compared with the response of mice injected with normal mouse serum. The serological results obtained in mice given antisera against live *Toxoplasma* when newborns and challenged when 6 weeks old with SRBC are also shown in Fig. 1. The mice responded normally to this antigen, showing that the immunosuppressive effect of passive immunization was antigen specific in this system.

Inhibition of the immune response to Toxoplasma by passive immunization. Newborn mice were injected intraperitoneally with 0.05 ml of either antiserum formed against LT or normal mouse serum. Six weeks later they had no detectable antibodies in the DT and were challenged with 5×10^5 LT of the C56 strain. The results are shown in Fig. 2.

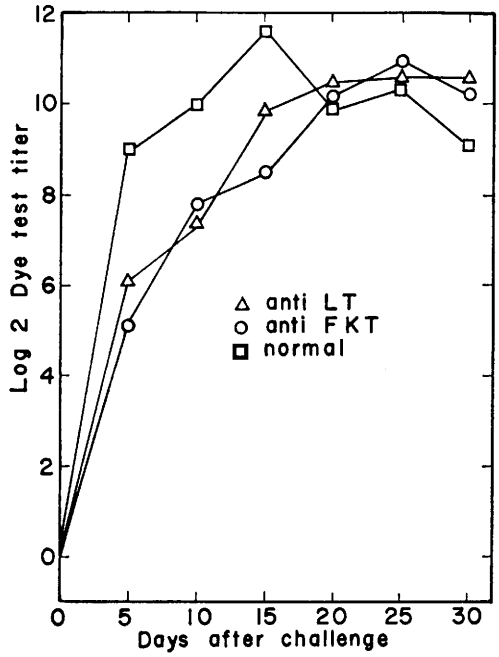

FIG. 2. Immune response of mice passively immunized with anti-LT (△), anti-FKT (○), and normal (□) sera at birth and challenged with 5×10^5 live *Toxoplasma gondii* organisms 6 week later.

Despite the absence of demonstrable antibody in the recipients at the time of challenge, an immunosuppressive effect was noted in that there was a slower induction period and an initially lower antibody response in mice which had received the anti-*Toxoplasma* serum. Similar results were observed in newborn mice that had been injected with antiserum against FKT and challenged either with dead (Fig. 3) or live *Toxoplasma* (Fig. 2) 6 weeks later. Nonviable *Toxoplasma* and antiserum prepared against them were employed as a parallel system to the SRBC model and to determine if either of these

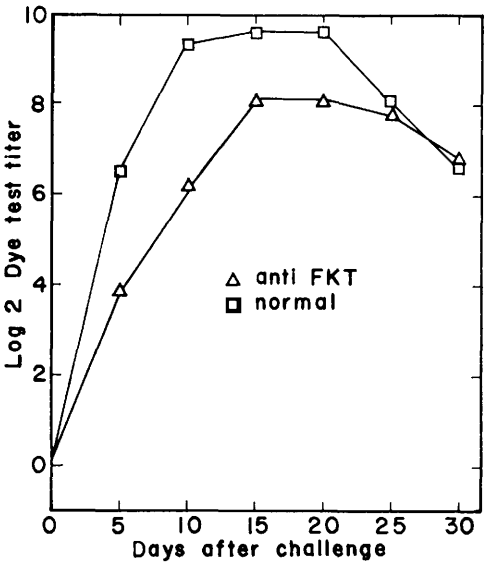

FIG. 3. Immune response of mice passively immunized with anti-FKT (△) and normal (□) sera at birth and challenged with formalin-killed toxoplasmas 6 weeks later.

parameters would effect results different from those in the LT model.

To determine whether a more marked suppression would be demonstrable if a smaller challenge inoculum of *Toxoplasma* was used, three groups of newborn mice were injected intraperitoneally with 0.05 ml of antisera produced against LT. Six weeks later they were challenged intraperitoneally with either 5×10^4, 5×10^3, or 5×10^2 live C56 strain *Toxoplasma* organisms. The immune response was quite similar to that observed when the challenge dose was 5×10^5. In Fig. 4 is a representative pattern of response obtained in a control and test group challenged with 5×10^2 LT. All mice challenged with LTs were treated with sulfadiazine to minimize deaths and allow sufficient time to observe the immune response.

Discussion. Our results demonstrate that passively administered antibody may suppress the immune response to intracellular pathogens. Very small amounts of passively administered antibody have been found to be immunosuppressive in a least three other systems: after challenge with SRBC (10); the immune response to *Escherichia coli* lipopolysaccharide in mice (11); and enhancement of ascites tumor PB8 (12). Uhr and Moller (13) have discussed the complexities of this form of immunosuppression and considered the accumulated data to imply that interaction with antigen is the first step.

In our study, modeled after that of Ryder and Schwartz (10), antigen was inoculated 6 weeks after the antisera had been administered to the newborns. At that time, none of the passively administered antibodies could be detected. Thus, all antibody found after the antigenic challenge was formed in response to the stimulus produced by the antigen. Ryder and Schwartz (10) concluded that by 6 weeks after injection of the same volume of antiserum as was used in the present studies, only 0.0002 ml would remain.

Passively administered anti-*Toxoplasma* antibody in amounts undetectable by usual serological means suppressed the normal response. The immunosuppression observed was not as marked as that in the SRBC system.

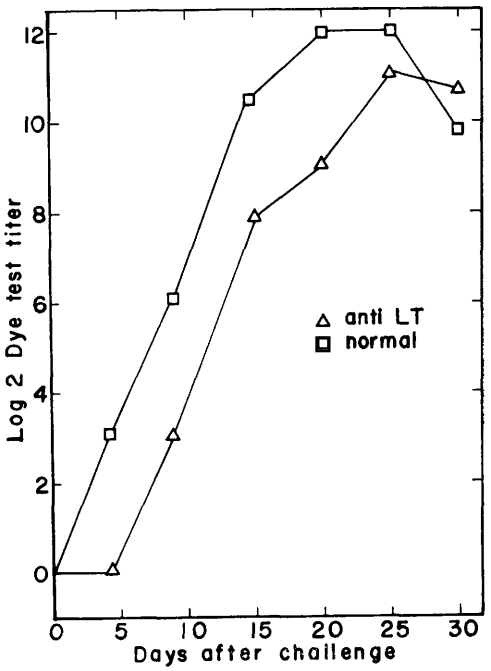

FIG. 4. Immune response of mice passively immunized with anti-LT (△) and normal (□) sera at birth and challenged with 5×10^2 *Toxoplasma gondii* organisms 6 weeks later.

If the inhibitory action of antibody is indeed on the macrophage, as has been suggested (10, 14), the presence of *Toxoplasma* inside these cells does not appear to disrupt the process. However, the increasing amount of antigen which is constantly being produced by the multiplying organisms appeared to overcome the suppressive effect. This "breakthrough" was not observed in the SRBC system or when dead *Toxaplasma* organisms were employed.

Toxoplasma has been isolated from humans and animals in whom antibody was not demonstrable (15, 16). Jacobs (17) suggested that the altered antibody response might be due to exposure of the host immune system to the parasite or parasite antigens during fetal life. He noted that some newborn mice from chronically infected mothers when injected with FKT did not form detectable antibodies. No parasites could be isolated from their tissues; they evidently had not been infected during fetal life and could not be considered tolerant. This unresponsiveness might be explained by our findings that even serologically undetectable amounts of antiserum against *Toxoplasma* are able to alter the normal immune response to killed parasites. The mice in Jacob's studies undoubtedly had received placentally transmitted antibody from their chronically infected mothers. Although in our experiments we noted suppression of antibody response, there was detectable antibody formation; this occurred in mice which had received only one dose of antiserum 6 weeks before, and after a challenge with live organisms or with a relatively large number of dead parasites. In nature, transplacental infection probably occurs with relatively few organisms. The suppressive effect of maternal antibodies in newborns might be much more impressive, especially when we consider that they may be receiving the suppressive antibody constantly through the fetal membranes or as is known to occur in some animals, continue to receive it through colostrum.

In another experiment Jacob's (17) isolated *Toxoplasma* from mice, with a negative serology, born of chronically infected mothers. He suggested that this finding may represent occasional instances of immune tolerance to a congenitally transmitted infectious agent or to antigens of the agent. Since the transport of immunoglobulin G from the mother to the fetus through the materno–fetal barrier occurs in mice, as has been demonstrated by Gitlin and Morphis (18), the unresponsiveness of the young might have been caused by the suppressive effect of this antibody. As the number of parasites reaching the young may be small and the parasite strains relatively avirulent (19), the antigenic stimulus could be sufficently low to be unable to overcome the suppressive effect of maternally transmitted antibody. The effectiveness of the maternal antibody in controlling the infection might conceivably result in encystment of *Toxoplasma* as well as immunosuppression. This might result in a newborn with *Toxoplasma* infection who has not formed his own antibody against the organism and in whom the persistence of the parasite may not serve as sufficient antigenic stimulus even after complete disappearance of maternal antibody.

Summary. Initial antibody formation in mice against a live intracellular parasite, *Toxoplasma gondii,* was suppressed by administration of antibody formed against both live and dead *Toxoplasma* organisms. The antiserum was administered to the mice 6 weeks before they were challenged with the live parasite. At the time of the challenge none of the administered antibody could be detected in the mice using the Sabin–Feldman dye test. These studies suggest that antibody may suppress immune responsiveness against intracellular pathogens.

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