Inability to Induce Tolerance to Bovine Gamma Globulin in Mice Infected at Birth with Lymphocytic Choriomeningitis Virus (34610)

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Immumological tolerance to heterologous gamma globulins can generally be induced in mice by pretreatment with protein which has been ultracentrifuged to eliminate aggregates (1). Two circumstances have been described in which mice with viral infections do not become tolerant after this treatment: (1) mice acutely infected with lactic dehydrogenase virus (LDV) (2, 3), and (2) New Zealand Black (NZB) mice (4–6), which contain murine leukemia viral antigens (7) and C-type virus particles (8).

The possiblity that interference with the induction of tolerance might be a more general phenomenon in persistent viral infection was studied in mice infected with lymphocytic choriomeningitis virus (LCM) and in AKR mice. LCM, an RNA virus as are LDV and murine leukemia virus, produces a life-long viremia in mice inoculated during the first day of life (9, 10). AKR mice, a strain in which virtually all develop leukemia (11, 12), have a life-long viremia with murine leukemia virus (12, 13). This report presents evidence that tolerance to bovine gamma globulin (BGG) could not be induced in 6-week-old mice with persistent LCM infection, but could be induced in AKR mice of similar age.

Materials and Methods. Pregnant C57Bl/6 mice were obtained from the NIH colony. Their offspring were treated according to the following schedule:

Day 1 (6–30 hr after birth). Infection. Normal mouse brain or LCM-infected mouse brain (0.03 ml), injected intracerebrally.

Day 42. "Tolerogen." Ultracentrifuged BGG (10 mg) or noncentrifuged egg albumin (2.5 mg), injected intraperitoneally.

Day 56. "Immunogen." Noncentrifuged BGG in complete Freund's adjuvant (Difco) (0.25 mg injected into each foot pad, total dose 1 mg BGG).

Day 81–88. Bleeding. Serum tested for antibody to BGG.

LCM virus was strain CA 1371, passaged in mouse brain. LCM-infected brain was prepared for injection as a 10% extract in veal-infusion broth; the inoculum contained about 10^5 mouse ID_{50}/0.03 ml, and was free of LDV. The LCM-infected and noninfected mouse colonies were housed in separate plastic isolators. At the conclusion of the first experiment, each mouse was tested for LCM viremia by intracerebral inoculation of blood into weanling mice; all control mice were free of virus and all LCM-injected mice had virus in titers of 10^3–$10^{4.5}$ ID_{50}/0.03 ml of blood. BGG (Armour Pharmaceutical) was prepared for induction of tolerance by ultracentrifugation in a Spinco 40 rotor at 40,000 rpm for 30 min. Egg albumin was used as a control in consideration of the possibility that large protein doses might nonspecifically lead to tolerance. Anti-BGG antibodies were measured by hemagglutination of BGG-coated, formalinized, tanned sheep erythrocytes as previously described (6).

Results. Two experiments in LCM-infected mice were performed, with results shown in Fig. 1. The mean hemagglutination titer 5–6 weeks after challenge for uninfected mice pretreated with ultracentrifuged BGG was 1:1.6, compared to 1:20 in LCM-infected mice pretreated with the identical material. The differences between the two groups were significant ($p < .05$ for each experiment, $p < .001$ for the combined experiments) com-

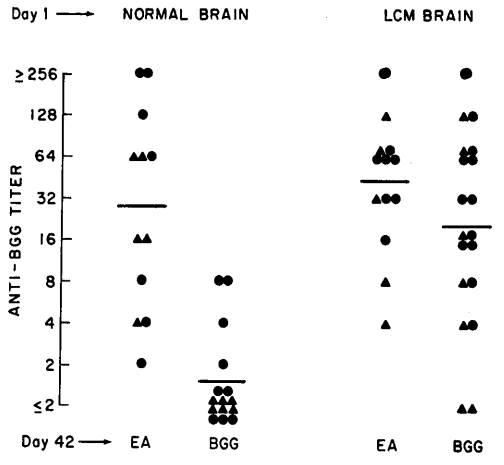

FIG. 1. Anti-BGG reciprocal titers in LCM-infected (LCM brain) and uninfected (normal brain) mice pretreated with ultracentrifuged BGG or noncentrifuged egg albumin (EA), challenged with BGG, and tested by hemagglutination. Different symbols indicate two separate experiments.

puted by the Mann-Whitney U test (14) and the Smirnov test (15). No significant differences were observed among the groups of uninfected and infected mice pretreated with egg albumin and infected mice pretreated with "tolerogen" (ultra-centrifuged BGG), although two mice in the last group made no detectable antibody.

The presence of viral antibody is detectable in late LCM infection (16), as in late LDV infection (17); antibody to the murine leukemia agent appears at 8 months in the NZB host (7). To study tolerance induction in the presence of a chronic viremia without evidence of antibody production, a group of 8-week-old AKR mice was treated with ultracentrifuged BGG, challenged, and tested as described in the above protocol. There was no interference with tolerance induction in these mice (Table I).

Discussion. These data indicate that LCM-carrier mice did not become tolerant to challenge with BGG after pretreatment with ultracentrifuged BGG. LCM virus, widely disseminated in chronically infected mice, could affect immunologic regulation through its presence in macrophages (18) and its capacity to destroy lymphocytes (19). Depression of the immune response to sheep red blood cells has been described in 10-day-old mice infected with LCM at birth; depression was not seen in 5-month-old mice treated in this manner (20). In the above experiment, 56-day-old LCM-infected and uninfected mice showed similar anti-BGG titers. Thus, chronic LCM infection in these mice caused neither immunodepression nor the higher antibody titers seen with LDV under certain conditions (2).

TABLE I. Tolerance Induction in AKR Mice.

	Pretreatment	
	BGG	EA
Number with titer >4	0/11	8/8
Mean anti-BGG titer	0	12

A common feature of the three conditions in which tolerance cannot be induced is the long-term presence of virus and viral antibody, as well as circulating immune complexes containing virus or viral antigens: LDV infection (17), NZB mice (7), and LCM infection (16). The virus, under the conditions described, causes minimal, if any, acute effects in the host. Chronic damage, such as the nephritis of New Zealand (21) and LCM-infected mice (16, 22), appears to result from the immune complexes rather than the agent itself. The host-virus relationships are one of a carrier state in which the virus continues to replicate while antibodies to the virus are formed.

Interference with tolerance induction does not appear explicable simply on the basis of chronic viral infection. AKR mice, which become tolerant to BGG (Table I), are infected with a virus which appears similar, if not identical, to that found in NZB mice (7, 8). However, New Zealand mice cannot be made tolerant to BGG (6). These mice show abnormal immune recognition of several additional antigens. They produce antibodies to vertically transmitted G + mouse leukemia antigen (7), and to BGG after pretreatment with ultracentrifuged protein (6), as well as autoantibodies to erythrocytes (23) and nucleic acids (24, 25). Since tolerance rather than immunity is the expected finding in all these cases, the aberrant response to BGG may be a consequence of a more generalized

genetic disorder of immunologic regulation in NZB mice rather than of viral infection. AKR mice, which contain G + antigen, develop neither autoantibodies nor anti-G + antibody (26), and are easily rendered tolerant to BGG. It has been reported that induced infection with murine leukemia viruses causes depression of antibody formation (27–30); naturally-occurring leukemia virus infection in AKR mice did not lead to alterations in tolerance induction.

Summary. Pretreatment with ultracentrifuged BGG did not lead to tolerance to this protein in 6-week-old C57Bl/6 mice with persistent LCM infection. Tolerance was induced in uninfected C57Bl/6 mice, and in AKR mice which have viremia with murine leukemia virus.

The authors thank Dr. J. Parker, Microbiological Associates, for expert maintenance of the LCM colony.

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Received Nov. 6, 1969. P.S.E.B.M., 1970, Vol. 133.