Rapid Loss of Tolerance Induced in Weanling NZB and B/W F_1 Mice

Abstract. *Young adult mice (30 to 43 days old) of autoimmune NZB and B/W F_1 strains failed to develop immunologic tolerance when treated with ultracentrifuged bovine gamma globulin and then challenged 12 days later. By contrast, weanling NZB and B/W F_1 mice (18 to 25 days) as well as weanling C3H mice (16 to 19 days) became tolerant and had no serum antibody 12 days after challenge. The C3H mice remained unresponsive, but NZB and B/W F_1 mice produced antibody between days 27 and 41. The rapid loss of previously established tolerance to foreign antigens could have its parallel in the loss of tolerance to autoantigens with subsequent development of lupus nephritis and Coombs' positive hemolytic anemia in these animals.*

After three months of age, NZB and NZB/NZW F_1 hybrid mice spontaneously develop an autoimmune disease characterized by Coombs' positive hemolytic anemia, lupus glomerulonephritis, hypergammaglobulinemia, positive lupus erythematosus cells, antinuclear factors, and antibodies to DNA.

The pathogenesis of these abnormalities is not known, but current work in several laboratories has led to the conclusion that the NZB mouse is unusually reactive immunologically even during the 1st week of life (*1*). We reported that 5- to 8-week-old NZB and B/W F_1 mice were not rendered tolerant to ultracentrifuged bovine gamma globulin (BGG) in doses which easily induced tolerance in C3H, NZW, and C57B1/6 control strains (*2*). This relative inability to induce tolerance to foreign antigens in young adult NZB and B/W F_1 mice suggested that autoimmunity in these animals might develop either as a lack or loss of tolerance to self-antigens. To explore this possibility, we attempted to induce tolerance to BGG in 2- to 3-week-old (weanling) NZB and B/W F_1 mice; we now report here the rapid loss of tolerance in these animals.

Tolerance was induced to ultracentrifuged BGG (Armour Pharmaceutical Company) (*2*). The BGG was centrifuged (Spinco-40 rotor) at 105,000*g* for 30 minutes. Control mice were injected with egg albumin (Worthington) prepared in a similar manner. They served as a check on the specificity of the tolerance so induced.

On day minus 12, five to seven weanling NZB (18 to 25 days of age), young adult NZB (43 days), weanling B/W F_1 (18 to 20 days), and young adult B/W F_1 (30 days) mice of either sex were injected intraperitoneally with either BGG (24.5 or 10.5 mg) or egg albumin (10.5 mg). Control weanling C3H mice (16 to 19 days) also received 10.5 mg of BGG intraperitoneally. Twelve days later, all animals were challenged (immunized) by injecting the footpads (day 0) with 1.0 mg of BGG in complete Freund's adjuvant. Mice were bled 12, 27, 41, and 72 days later. Serum samples were inactivated for complement, absorbed with sheep erythrocytes, and assayed for antibody to BGG by a hemagglutination (Microtiter) method (*2*).

The results of this experiment are shown in Table 1 and in Figs. 1 and 2. Twelve days after challenge, the weanling NZB and B/W F_1 mice were nonresponsive to challenge. However, the young adult mice (30 to 43 days), despite prior treatment, had antibody titers equivalent to the controls injected with egg albumin (*2*). The NZB and B/W F_1 control mice of all ages injected with egg albumin showed brisk antibody production when challenged with BGG.

On day 27 after challenge, both weanling NZB and B/W F_1 animals were still relatively unresponsive to BGG though some of them were beginning to show recovery from immunologic unresponsiveness as evidenced by production of small amounts of antibody. The data obtained at 41 and 72 days after challenge showed that this initial antibody response was of significance. Antibody titers in the weanlings were now only slightly below those in the treated young adult animals, with virtually all individual titers being greater than 3. By 72 days, all mice had recovered from their initial unresponsive state.

In contrast, C3H mice at 72 days were still completely tolerant to BGG; four-fifths of the mice had no detectable antibody whatsoever. Control C3H mice given egg albumin promptly made normal amounts of antibody to BGG.

This study demonstrates that NZB and B/W F_1 mice between the ages

Table 1. Average titers and numbers of mice having antibodies to BGG greater than 3, on days 12, 27, 41, and 72 after challenge. Titers are expressed as log to base 2.

Groups	Day 12		Day 27		Day 41		Day 72	
	Titers > 3 (No.)	Average titer	Titers > 3 (No.)	Average titer	Titers > 3 (No.)	Average titer	Titers > 3 (No.)	Average titer
			*Soluble bovine gamma globulin**					
NZB (18 to 25 days)	0/7	0.3	0/6	1.8	5/6	4.8	6/6	5.3
NZB (43 days)	6/6	4.2	5/5	5.4	5/5	7.2	5/5	6.8
B/W F_1 (18 to 20 days)	0/7	0.6	1/7	2.3	7/7	6.1	7/7	6.3
B/W F_1 (30 days)	6/6	5.8	6/6	6.1	6/6	7.2	6/6	7.5
C3H (16 to 19 days)	0/5	0.0	0/5	0.0	0/5	0.4	0/5	0.6
			Egg albumin†					
NZB controls (18, 43 days)	3/4	4.5	4/4	7.5	4/4	8.0	4/4	8.7
B/W F_1 controls (18 to 20 days)	4/4	5.0	4/4	6.8	4/4	9.0	4/4	8.3
C3H controls (16 to 19 days)	3/3	4.0	3/3	6.6	3/3	6.6	3/3	7.3

* All animals given either 10.5 or 24.5 mg of BGG intraperitoneally. The results were the same for both doses. † All animals given 10.5 mg of egg albumin intraperitoneally.

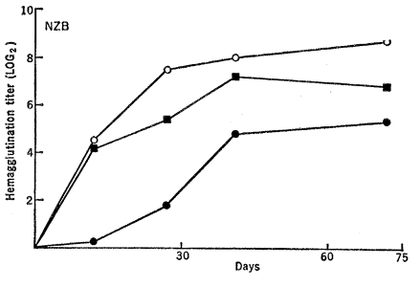

Fig. 1. Hemagglutination titers (log_2) for weanling and young adult NZB mice given either BGG or egg albumin. Open circles, 10.5 mg of egg albumin given at 18 or 43 days of age; solid squares, 10.5 or 24.5 mg of BGG given at 43 days of age; solid circles, 10.5 or 24.5 mg of BGG given at 18 to 25 days of age.

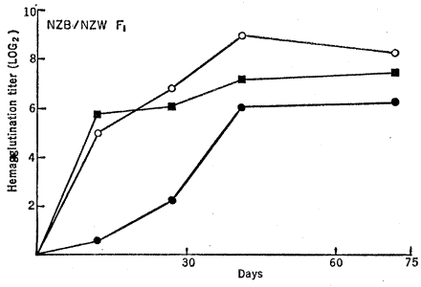

Fig. 2. Hemagglutination titers (log_2) for weanling and young adult NZB/NZW F_1 mice given either BGG or egg albumin. Open circles, 10.5 mg of egg albumin given at 18 to 20 days of age; solid squares, 10.5 or 24.5 mg of BGG given at 30 days of age; solid circles, 10.5 or 24.5 mg of BGG given at 18 to 20 days of age.

of 18 to 25 days are capable of becoming tolerant to large systemic doses of ultracentrifuged BGG, but that, once again, somewhat older mice of these same strains show a marked resistance to the development of immunological tolerance (*2*). A striking observation was the rapid loss of tolerance in the younger mice not seen with the age-matched C3H animals. By the end of this experiment (72 days), all NZB and B/W F_1 mice previously tolerant now had antibody titers approaching those of control animals.

This escape from tolerance implies that the immune system of the NZB mouse, as it develops, loses its capacity to remain immunologically unresponsive. This could be due either to the generation of an entirely new cell line capable of responding to the antigen in question, or to the recovery of a previously tolerant or repressed population of antigen-reactive lymphocytes. An accelerated recruitment of immunologically competent stem cells, or the action of a thymic trophic factor, or both, might be responsible for either of these cellular events. Claman and McDonald have shown that the thymus plays a role in regulating recovery from immunologic unresponsiveness (*3*), and others have shown the importance of the thymus in establishing immunologic competence (*4*).

In the NZB and B/W strains, there is a remarkable correlation between relative resistance to tolerance induction in young adult mice and rapid loss of tolerance previously induced in weanlings. This correlation between abnormalities of tolerance induction and duration suggests a common pathologic mechanism.

The rapid loss of tolerance in weanling mice has certain implications for the pathogenesis of serologic autoreactivity. Our results with BGG provide an experimental instance whereby the NZB and B/W F_1 mice lose tolerance to an antigen which, in a nonautoimmune strain, leads to long-term and profound suppression of antibody formation. If the same relation pertains to autoantigens, then initial tolerance to the more immunogenic determinants of these antigens might likewise be lost over a period of time.

Finally, although in our study there was a significant difference between weanling NZB, B/W F_1, and comparable C3H mice, exposure of NZB or B/W F_1 animals to self or to foreign antigens at still earlier times of life might render them tolerant for longer periods of time. The timing and extent of exposure to antigen will obviously be very critical in any attempt to restore or maintain self-tolerance in these mice.

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References and Notes

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Thymus-Dependent Lymphocytes: Destruction by Lymphocytic Choriomeningitis Virus

Abstract. *Selective destruction of small lymphocytes in the thymus-dependent areas of lymph nodes and thymocytes was observed in mice infected with lymphocytic choriomeningitis virus. These changes were clearly evident in lymphoid and splenic tissue 3 days after infection and in the thymus by day 7. The destructive changes paralleled growth of the virus in these organs. The findings show that infection with lymphocytic choriomeningitis virus can temporarily cause the equivalent effect of neonatal thymectomy, that is, a "viral thymectomy," which appears to be related to the ability of this virus to cause persistent infection.*

The means whereby certain viruses sometimes cause disease, but at other times produce inapparent persistent infection of the adult animal, is not completely understood. It now appears from the work reported here that one such agent, lymphocytic choriomeningitis (LCM) virus, may do this by damaging, in the first few days after infection, those immune cells that normally exterminate the virus.

The pathogenesis of LCM virus infection of the mouse has long been known to involve a lymphocytic infiltration of the tissues (*1*). Tissue damage, illness, and recovery from the virus infection are believed to be due to a cellular response (*2*) involving a delayed hypersensitivity reaction with rejection of infected tissue similar in mechanism (*3*) to the homograft response. Agents known to reduce the cellular immune response ameliorate or abolish both acute clinical disease, mortality, and histopathology but not growth of the virus; these include cortisone (*4*), x-irradiation (*5*), amethopterin (*3, 6*), thymectomy (*7–9*), and antiserum to lymphocytes (*10, 11*). Inoculation of viscerotropic LCM virus close to or before the time of birth induces immunological tolerance to the virus and a persistent infection without the normal LCM disease (*4, 12*); inoculation of the adult animal with large doses of this virus induces a similar but less permanent state (*13*) which we refer to as high dose immune paralysis (HDIP). Conversely, grafting the tolerant animal with mature isologous lymphocytes has been shown to