Antibody-Mediated Immunodepression in New Zealand Black Mice[1] (36184)

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Young adult New Zealand Black (NZB) mice have been shown to be hyperresponsive to immunization with a variety of foreign antigens, including sheep erythrocytes (SRBC) (1, 2). In addition, elevated numbers of hematopoietic stem cells have been detected in this mouse strain as demonstrated by high rates of survival following acute exposures to ionizing radiation (3). These observations have suggested (1) that the possession of an expanded pool of antigen-sensitive cells might present increased opportunities for interaction with autologous as well as foreign antigens, leading to the autoimmune manifestations characteristic of NZB mice (4). It was additionally speculated (3) that these augmented cell numbers could arise as a consequence of deficient feedback control at some level of the immunologic process.

It is now widely established that administration of specific hyperimmune serum can depress or prevent primary antibody formation (5–9). In the present report, experiments were designed to investigate the potentialities of antisera derived from NZB mice to influence the initiation of immune responses to foreign and autologous antigens. Our findings indicated that NZB hyperimmune serum to SRBC was highly effective in limiting anti-SRBC antibody formation, while passive transfer of serum from antinuclear antibody (ANA)-positive and Coomb's positive NZB donors to preautoimmune recipients did not significantly alter the subsequent development of these autoantibodies.

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Materials and Methods. NZB mice raised in this laboratory were derived from breeding stock (generations 57 and 58) obtained from Otago University Medical School, Dunedin, New Zealand. Two-month-old BALB/c mice were obtained from The Jackson Laboratory, Bar Harbor, ME.

Hyperimmune anti-SRBC serum was prepared by ip injections of 10^9 saline-washed SRBC into 17, 5-month-old NZB mice on days 0, 14, 21, and 28. Mice were bled one week after the last injection, and a serum pool was prepared which had a SRBC agglutinin titer of 1:1536. Sixteen NZB mice were similarly injected with saline and bled as a source of control serum. Pools of immune serum were also obtained from six 5-month-old donors on each of days 4 and 7 post-primary immunization by single injection of 1.5×10^9 SRBC. For purposes of serum administration, selected dilutions in saline were injected ip in 0.20 ml volumes. Spleen plaque-forming cell assays (10) were performed on recipient 2-month-old mice 4 days after primary immunization with 5×10^8 SRBC.

A serum pool derived from 27 one-year-old Coomb's positive NZB mice served as the source of anti-erythrocyte autoantibody. Serum from these donors was injected ip as 0.10 ml into 15 NZB recipients at 16 days of age, and subsequently in 0.25 ml volumes at 2-month intervals until the age of 11 months. Thirteen untreated littermates served as controls. Equal numbers of each sex were used, and direct Coomb's tests were carried out as described by Norins and Holmes (11).

A serum pool obtained from 23 seven-month-old, ANA-positive donors served as

TABLE I. Plaque-forming Cells from Spleens of NZB Mice Receiving Serum from SRBC-immunized or Saline-injected Donors.

Expt. no.	Type of NZB immune serum	Number of plaque-forming cells per spleen[a]			
		Volume of control serum injected (ml)	Volume of antiserum injected (ml)		
		0.20	0.20	0.02	0.002
1	4th-day post-primary	83033	127800	—[b]	—
2	7th-day post-primary	54500	59833	—	—
3	hyperimmune	206600	200	1893	42000

[a] Each value represents the average for 3 mice assayed 4 days after ip immunization with 5×10^8 SRBC. Serum was injected 24 hr after (Expt. 1 and 2) or 24 hr before (Expt. 3) immunization.

[b] Not done.

antinuclear antiserum. This serum gave an ANA titer of 1:160 by immunofluorescence staining. For antinuclear antibody studies, 9 NZB mice received 5 injections each of 0.05 ml undiluted ANA-positive serum at 7, 14, 20, 30 and 45 days of age; 6 NZB mice received 1 injection of this serum at 7 days of age with injections of Medium 199 at the other times; 12 control mice received injections of Medium 199 only at these 5 age periods. Mice were tested periodically for ANA by an indirect immunofluorescence method using chicken erythrocyte nuclei as antigen (12–14).

Results. Anti-SRBC plaque responses. The passive administration of 0.20 ml of serum collected from NZB donors 4 or 7 days after primary SRBC immunization had little influence on subsequent spleen antibody plaque formation (Table I, Expt. 1 and 2). There was some stimulation by 4-day antiserum (Expt. 1), possibly due to the macroglobulin character of this antibody (6). In contrast, injection of hyperimmune anti-SRBC serum was found to be highly effective in depressing responses of recipient animals (Table I, Expt. 3). Here, 0.20 ml serum diminished the response to about 0.1%, 0.02 ml to 1%, and 0.002 ml to 20% of that obtained with control mice injected with 0.20 ml of serum obtained from saline-injected donors.

It was of interest to determine the duration of this inhibitory effect, and also to test whether NZB antisera would be equally effective in reducing the immune response of recipient BALB/c mice. The depressive effects of 0.002 ml antiserum administered 24 hr before immunization was observed (Table II) to be comparable in NZB and BALB/c strain animals. Also, injection of 0.02 ml one month before immunization was equally depressive, although the effect was no longer evident 2 months following serum injection.

Development of positive Coomb's tests. It also seemed relevant, with respect to possible aberrant control mechanisms, to determine the development of Coomb's positive hemolytic anemia in NZB mice receiving periodic injections of Coomb's positive sera from an early age. In this regard, the times of onset and incidence of positive Coomb's reactions were found not to be markedly different for experimental and control groups (Table III), although the incidence of positive tests from 10 to 14 months of age appeared to plateau at 50–60% in the treated animals as compared to a progressive increase in Coomb's reactivity in untreated control NZB mice. Upon sacrifice, increased spleen weights were observed to be almost identical for both groups (Table III) suggesting similar progression of hemolytic disease.

TABLE II. Duration of Depressive Effect of NZB Hyperimmune Serum on Antibody Plaque-formation in NZB and BALB/c Recipient Mice.

Recipient strain	Percentage of control antibody plaque response[a]		
	Time of immunization post-serum injection (ml serum)		
	1 day (0.002)	1 month (0.02)	2 months (0.02)
NZB	6	19	83
BALB/c	20	17	124

[a] Control NZB and BALB/c mice were treated with identical volumes of normal NZB serum. Each percentage value listed in the table represents the average for 3–4 mice assayed 4 days after immunization with 5×10^8 SRBC.

Antinuclear antibody formation. Indirect immunofluorescence assays have demonstrated that NZB mice develop a high incidence of antinuclear antibody at a very early age (13). Such an early response is delineated in Fig. 1, where 40% or more of the animals showed positive ANA responses by 30 days of age. It is also observed that a single injection of 0.05 ml ANA-positive serum at 7 days of age (B) or 5 injections of this serum between 7 and 45 days of age (C) had no notable effect on the appearance of antinuclear antibody in the 60-day age period studied.

Discussion. Previous observations of the heightened capacity of NZB mice to produce 19S antibody plaque-forming cells (PFC) (1) and to attain elevated serum agglutinin titers following primary SRBC immunization (2) have suggested a possible impairment of, or a higher threshold for, the activation of feedback controls limiting antibody formation. The present studies have indicated that administration of antiserum obtained from NZB mice during the early primary response to SRBC (4 and 7 days postimmunization) was not immunodepressive; also that treatment with serum obtained 4 days after immunization effected a slight enhancement, an observation consistent with previous findings (6) that specific IgM antibody may stimulate primary antibody formation.

In contrast, NZB hyperimmune serum to SRBC was strongly inhibitory of antibody formation in both NZB and BALB/c recipients, the extent of inhibition being related to antiserum dose. Additionally, the inhibitory effect of 0.02 ml of this serum was observed to persist for up to 2 months. Thus, a deficiency in 7S antibody-mediated feedback inhibition in NZB mice could not be demonstrated in the present experiments. More extensive studies along these lines would be necessary to establish any possible quantitative differences between NZB and other mouse strains.

TABLE III. Development of Positive Coomb's Tests and Terminal Spleen Weights for Controls and NZB Mice Inoculated with Coomb's Positive NZB Serum.

Treatment	Age in months					Avg spleen wt (g) at 14 mos (range)
	6	8	10	11	14	
Coomb's positive serum	0/15[a]	3/14	7/14	8/14	7/13	0.560 (0.119–1.882)
untreated	0/13	4/13	5/12	9/12	9/11	0.568 (0.144–1.529)

[a] Results are presented as number of mice showing positive Coomb's tests per total number of mice in the group.

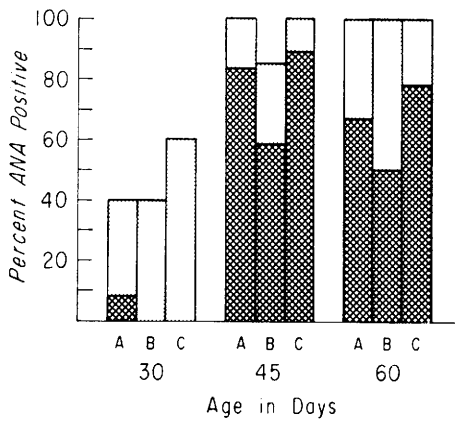

FIG. 1. Percent of mice demonstrating designated ANA immunofluorescence response: stippled area = weakly positive (titer of 1:20), cross-hatched area = strongly positive (titers of 1:40–1:160). Individual bars from left to right comprise groups of mice receiving (A) 5 injections of medium 199 only (controls), (B) one injection of ANA-positive serum and 4 of medium 199, and (C) 5 injections of ANA-positive serum, all between 7 and 45 days of age.

The inability to alter the course of autoantibody formation by passive administration of ANA-positive and Coomb's positive sera, noted here, might have resulted from a number of factors. It has been shown, for example, that a significant portion of the immunoglobulin coating NZB red cells is IgM and that NZB mice manifest marked elevation in serum macroglobulin levels from an early age (15). Since it has been observed that simultaneous adminstration of specific 19S antibody will counteract the suppressive effects of 7S antibody (6), it is possible that the ratios of immunoglobulin classes in the mouse sera employed here were not propitious for effective feedback inhibition. Similar considerations may apply in the case of antinuclear antibody positive sera, although Norins and Holmes (16) have identified these antibodies as 7S by sedimentation characteristics and mercaptoethanol resistance.

Another factor may be that the recipient NZB mice would possess autologous antigen, and administration of serum containing autoantibodies could bring about the formation of antigen–antibody complexes in this state of antigen excess, a situtation which at times has been demonstrated to enhance rather than depress antibody formation (17). Conceivably, feedback inhibition of autoantibody formation may be achievable through frequent injections of concentrated, high affinity 7S antibody prepared by active immunization with erythrocyte and nuclear antigens. This is possibly one approach to the manipulation of autoimmunity and as such might merit further investigation.

Summary. Passive administration of hyperimmune antiserum into syngenic NZB recipients produced a marked depression of spleen anti-SRBC plaque formation. This antiserum was equally effective in BALB/c mice, depression persisting in both strains for as long as 2 months following ip injection of 0.02 ml serum. These observations would suggest that the immunological hyperresponsiveness previously described for NZB strain mice does not derive from a deficiency in 7S antibody-mediated feedback inhibition of the immune response. Transfer of serum from 1-year-old Coomb's positive mice and from 7-month-old ANA-positive animals into preautoimmune baby NZB recipients was without effect on the subsequent course of development of antinuclear antibody and of Coomb's reactivity. The inability to alter the course of autoantibody formation by such passive transfer of sera may involve a number of factors which are discussed.

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