Antibody-Mediated Inhibition of Primary Antibody Formation *in Vivo* and *in Vitro* in NZB Mice[1] (37079)

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The spontaneous production of autoantibodies by New Zealand Black (NZB) mice is well documented (1–8), and is associated with the auto-immune hemolytic anemia and lupus type glomerulonephritis characteristic of these mice. Studies with several NZ hybrid strains have indicated that the production of these autoantibodies is under genetic control involving only a few genes (9–11).

In considering the possible mechanism of action of this control, it is imperative to determine whether this control is exerted only on the specific autoantibody production or on all types of antibody specificities. Several studies have indeed shown (12–18) that NZB mice are hyperresponsive in antibody formation to some antigens although a depression of cell-mediated immunity has also been found (19–20). Such hyperresponsiveness might be due to one or several of three broad causes: an increased number of specific precursor cells, a more rapid proliferation or differentiation of a normal sized precursor pool, or thirdly, a deficiency in the negative feedback control of antibody formation that is exerted by antibody itself (21–23).

This present report described studies *in vivo* and *in vitro* on this latter possibility, and indicate that antibody-mediated immunodepression is normal in NZB mice.

Materials and Methods. The NZB mice used in this study were derived from the colony of Dr. M. Bielschowsky at the 42nd inbred generation; the strain was then maintained in this Institute by brother–sister mating. Mice of the hybrid crosses (NZB × NZC)F_1, (CBA × C57Bl)F_1 and inbred CBA and BALB/c mice were obtained from colonies maintained in the Institute.

Anti-sheep red blood cell (SRBC) sera were prepared by intraperitoneal injection of 0.2 ml of a 10% SRBC suspension in 2 mo old NZB and CBA mice. Mice were bled 14 days later. Both serum pools had a SRBC agglutination titer of 1:12800.

In vitro cell cultures were set up as described by Marbrook (24) and modified by Diener and Armstrong (25). In brief, a mixture of 15×10^6 spleen cells and 8×10^6 SRBC in 1 ml minimal Eagle's medium supplemented with 5% fetal calf serum were placed in a tube sealed off by a dialysis membrane and suspended from the stopper of an Erlenmeyer-type flask containing 50 ml tissue culture medium. For each group, quadruplicate cultures were performed, using a pool of spleen cells derived from 5 to 8 individual mice. Groups of mice (4–6 per group) were immunized *in vivo* intravenously with 0.1 ml of 10% SRBC suspensions.

Direct antibody-forming cells (d-AFC) to SRBC were enumerated at various time intervals by Cunningham and Szenberg's modification (26) of the hemolysin plaque assay of Jerne and Nordin (27).

In *in vivo* suppression studies, SRBC were given intravenously and antiserum was given at the same time intraperitoneally.

Results. In vivo *anti-SRBC immune response.* The intravenous administration of 0.1 ml of a 10% SRBC suspension to NZB mice results in considerably higher numbers of direct antibody-forming cells in spleen than in BALB/c or (CBA × C57Bl)F_1 hybrid mice

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TABLE I. Generation of Direct Antibody-Forming Cells After *in Vivo* SRBC Immunization.

After Immunization[b] (days)	Direct antibody-forming cells/spleen[a]		
	NZB	BALB/c	(CBA × C57Bl)F_1
5	240,485 ± 13,587	87,914 ± 7961	53,883 ± 3404
10	121,875 ± 9650	11,875 ± 1895	19,125 ± 1050
15	36,000 ± 3340	3131 ± 234	4475 ± 233
21	8752 ± 4962	1222 ± 106	1817 ± 152

[a] Mean ± standard deviation of 4 mice/group (6–8 wk old).
[b] All mice 0.1 ml of 10% SRBC iv on Day 0.

(Table I). This was observed when plaque tests were performed at either 5, 10, 15 or 21 days postimmunization.

Intraperitoneal administration of 0.1 ml of syngeneic anti-SRBC serum was found to depress the *in vivo* generation of direct anti-SRBC antibody-forming cells in 8 wk old NZB mice to 29% of controls, and to 45% with 8 wk old CBA mice (Table II).

In vitro *anti-SRBC immune response.* The primary immune response to SRBC *in vitro* at Day 4 showed some differences between spleen cells from mice of NZB, CBA and (NZB × NZC)F_1 hybrid origin (Table III). CBA spleen cells gave a 3 times higher anti-SRBC response than NZB spleen cells, but the NZB cell response was slightly higher than that of (NZB × NZC)F_1 hybrid spleen cells. All data given in Table III refer to cell cultures containing normal NZB or normal CBA serum in a final dilution of 1/50. No significant differential effect of these two sera on the cultures was observed.

The inhibitory effect of anti-SRBC sera of NZB and CBA origin on the generation of anti-SRBC antibody forming cells *in vitro* is given in Table IV. There was no major difference detectable between the inhibition of NZB, CBA or (NZB × NZC)F_1 hybrid spleen cells, with either NZB or CBA anti-SRBC serum. Thus, with a final serum dilution of 2×10^{-5}, approximately a 50% depression of the response was observed in all instances, and a 95% depression of the response was observed with a 2×10^{-3} dilution for NZB and CBA spleen cells, although the (NZB × NZC)F_1 response could only be inhibited to 80% of the control value.

Discussion. The results presented clearly demonstrate that the initiation of primary antibody responses to SRBC by NZB spleen cells, either *in vitro* or *in vivo,* is susceptible to passive antibody-mediated immunodepression in a similar magnitude to that of other mouse strains. These studies therefore appear to negate the possibility that NZB mice have a defective feedback inhibition that could result in hyperresponsiveness to certain antigens. A similar observation with *in vivo* studies has also been reported by Morton and Siegal (28).

Clearly, other possible explanations for this hyperresponsiveness of NZB mice to SRBC must therefore be considered. The demonstration of increased responsiveness to SRBC has been made in several studies (12–17) with *in vivo* immunization. However it has been stressed (16) that this is not a general

TABLE II. Inhibition of the Generation of Direct Antibody-Forming Cells *in Vivo* by Means of Anti-SRBC Sera.

Mouse strain[a]	Serum[b]	Direct antibody-forming cells/spleen[c]
NZB	Normal NZB	9445 ± 627
NZB	NZB anti SRBC	2785 ± 227
CBA	Normal CBA	2110 ± 341
CBA	CBA anti SRBC	950 ± 114

[a] NZB and CBA mice were immunized with SRBC by iv injection of 0.1 ml of a 10% SRBC suspension.
[b] Equal numbers of NZB and CBA mice received at the day of immunization 0.1 ml of the designated serum intraperitoneally. Hemagglutination titer against SRBC of both antisera was 1/12,800. (Normal sera have no detectable activity.)
[c] The mice were killed 15 days after immunization, and the number of d-AFC per spleen was determined; mean ± standard deviation 4–6 animals/group.

TABLE III. Generation of Direct Antibody-Forming Cells *in Vitro* in the Presence of Normal Mouse Serum.

Normal mouse serum[a]	Direct antibody-forming cells/culture[b]		
	NZB	CBA	(NZB × NZC)F_1
NZB	855.2 ± 43.4	2418.75 ± 223.7	523.75 ± 77.5
CBA	805.0 ± 38.1	2550.0 ± 220.7	478.2 ± 37.2

[a] Final dilution, 1/50.

[b] Mean number of direct antibody-forming cells ± standard deviation at Day 4 of the culture (quadruplicate cultures).

hyperreactivity of NZB mice to all antigens, and for example, NZB mice are poorer responders to hemocyanin than A/J or BALB/c mice (16). It is therefore most unlikely that this "hyperresponsiveness" to certain antigens, has anything to do with the auto-immune responses of these mice. Rather, it is more likely that this is another example of specific immune response genes, that control either quantitatively or qualitatively the antibody production to defined antigens (29). On this thesis, it would be more relevant to know whether the T and/or B cell precursor pools with specific anti-SRBC reactivity were increased in number in NZB mice. An increased number of background plaque-forming cells in NZB mice compared to C3H mice has been reported (30).

One unexpected aspect of our studies was the observation that *in vitro* primary antibody production by NZB cells was not greater than that of CBA mice. In fact the *in vitro* NZB response was only 32% that of the CBA response, whereas *in vivo*, the CBA response was only about 22% that of the NZB response. These observations may suggest that there is a serum factor responsible for the *in vivo* hyperreactivity and this is in insufficient concentration in the *in vitro* studies.

Alternatively, as the *in vitro* response requires the participation of at least three cell types, B cells, T cells and macrophages (31), it may be that one of these populations is limiting in the *in vitro* studies. For example, although NZB mice might have more B cells reactive to SRBC than other mouse strains,

TABLE IV. Inhibition of the Generation of Direct Antibody-Forming Cells *in Vitro* by Means of Anti-SRBC Sera.

Serum	Final serum dilution	Direct antibody-forming cells/culture[b] (%); spleen cells:		
		NZB	CBA	(NZB × NZC)F_1
Normal NZB	2×10^{-2}	100 ± 5.06	100 ± 9.30	100 ± 14.28
	2×10^{-2}	2.2 ± 0.73	6.1 ± 0.77	15.7 ± 1.49
	2×10^{-3}	3.6 ± 0.97	4.2 ± 0.52	17.2 ± 1.55
NZB anti-SRBC[a]	2×10^{-4}	6.4 ± 2.06	14.6 ± 1.37	19.6 ± 3.82
	2×10^{-5}	54.1 ± 6.30	57.5 ± 3.01	38.2 ± 1.87
	2×10^{-6}	87.5 ± 2.40	111 ± 9.09	95 ± 6.41
Normal CBA	2×10^{-2}	100 ± 4.74	100 ± 8.6	100 ± 8.12
	2×10^{-2}	5.3 ± 1.29	3.8 ± 0.64	17.9 ± 2.28
	2×10^{-3}	4.5 ± 1.05	5.8 ± 0.71	17.7 ± 2.47
CBA anti-SRBC[a]	2×10^{-4}	5.3 ± 1.55	10.1 ± 1.34	21.8 ± 3.14
	2×10^{-5}	40.5 ± 5.52	42.3 ± 4.0	40.2 ± 7.10
	2×10^{-6}	74 ± 5.18	95.4 ± 5.8	89 ± 13.67

[a] Hemagglutinating titer of 1:12,800.

[b] d-AFC per culture ± standard deviation of at least 4 cultures, determined at Day 4. Results are expressed as the percentage of the respective control of spleen cells in normal serum. Absolute values of the controls were similar to those shown in Table III.

the NZB mice may have lower numbers of effective T cells. Thus *in vivo*, the number of T cells may be sufficient to trigger the excessive response of B cells, whereas *in vitro* with the number of cells used per culture, the T cells may be in a limiting concentration.

Summary. The immune response to SRBC was studied *in vivo* and *in vitro* with NZB mice and several other normal mouse strains. In both situations the presence of passive anti-SRBC antibody, had a marked immunodepressive effect. Equal depression was observed with NZB or CBA antisera. The results negate the possibility that NZB hyperresponsiveness *in vivo* to SRBC is due to a failure in feedback inhibition.

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