COMPARISON OF THE IMMUNE RESPONSIVENESS OF NZB AND NZB × NZW F_1 HYBRID MICE WITH THAT OF OTHER STRAINS OF MICE*

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The natural history of the F_1 hybrid of NZB and NZW mice (NZB/W) is characterized by the spontaneous appearance of serum antinuclear antibodies followed by the development of a fatal subacute glomerulonephritis very similar to the human lupus nephritis [see review by Howie and Helyer (1)]. A close temporal relationship between the presence of serum antinuclear antibodies and the manifestations of kidney damage has been demonstrated, and it is very likely that the kidney lesions result from the glomerular localization of nuclear antigen-antinuclear antibody complexes (2). However, the events leading to the formation of antinuclear antibodies are unknown. Since DNA-like antigens have been detected in sera from NZB/W mice before the occurrence of antinuclear antibodies (2), the possibility exists that DNA antigens from endogenous or exogenous sources appear in excessive amounts or unusual forms in these mice. In addition to an increased antigenic stimulus, a heightened genetically determined immune responsiveness could favor the synthesis of antinuclear antibodies. With regard to the genetic constitution of NZB/W mice, it should be stressed that no immunopathologic abnormalities have been reported in NZW mice, whereas NZB mice develop a high incidence of spontaneous autoimmune hemolytic anemia and a low incidence of glomerulonephritis (1).

Since the description of the immune response to sheep red blood cells in NZB mice by Diener (3), several studies of the immune responsiveness of NZB and/or NZB/W mice have been reported which claim various abnormalities of the immune system of these strains, such as hyporesponsiveness (4, 5), hyperresponsiveness (6–8), early maturation (9, 10), or relative inability to be made tolerant (6, 8). However, the validity of several of these conclusions could be questioned, since they were based upon the comparison of the antibody response of NZB or NZB/W mice against one strain of normal mice and/or one kind of antigen only.

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The present study was undertaken to compare various parameters of the immune response of NZB/W mice (and NZB mice in some experiments) with that of three other strains, A/J, BALB/c, and CBA/J, using four different antigens, namely SRBC,[1] keyhole limpet hemocyanin, bovine serum albumin, and human γ-globulin. It will be shown that important strain differences exist in the immune response of mice, but that the relative immune responsiveness depends very much upon the nature of antigen. As compared to the other strains tested, NZB/W mice were found to be relatively high responders to some antigens (SRBC and BSA) and low responders to others (KLH). Their ability to become unresponsive after treatment with ultracentrifuged HGG did not differ significantly from that of BALB/c and A/J mice.

Materials and Methods

Animals.—(NZB × NZW)F_1 hybrid mice (NZB/W) were used in all experiments. NZB and NZW strains were originally obtained from the Laboratory Animal Center, Medical Research Council, Surrey, England, and inbred in our laboratory for 3 years. The NZB female and NZW male cross and the reciprocal mating were both randomly performed. A/J and CBA/J mice were procured from Jackson Laboratory, Bar Harbor, Maine. BALB/c mice were obtained from our own breeding colony.

The immune responsiveness of all strains was studied in 6 and 20 wk old male mice. Each experimental group included 10 or more mice.

Immunization and Antibody Determinations.—

Sheep red blood cells: Immunization was obtained by one single intraperitoneal injection of 5×10^8 SRBC in 0.5 ml of 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.2 (phosphate-buffered saline). The animals were bled by puncture of the retroorbital sinus 5, 7, and 12 days later. All sera were heated at 56°C for 30 min. Hemagglutinins were titrated as described previously (11), using 2-fold dilutions of antiserum in PBS containing 1% heat-inactivated normal mouse serum. Agglutination patterns were read after incubation at room temperature for 18 hr. Agglutinin titer is recorded as the $\log_2$ of the reciprocal of the dilution giving a 1+ hemagglutination pattern. After reading, the tubes were centrifuged at 700 *g* for 10 min. The supernatant was discarded, the cells were resuspended in 0.7 ml of fresh guinea pig serum diluted 1:40 in Veronal buffer, and incubated at 37°C for 1 hr. Hemolysin titer is recorded as the $\log_2$ of the reciprocal of the dilution giving complete hemolysis.

Sera of each experimental group were pooled for the determination of 2-mercaptoethanol-resistant antibodies. 0.5 ml of 0.2 M 2-mercaptoethanol was added to 0.5 ml of serum diluted 1:10 in PBS. After incubation at 37°C for 1 hr, the sera were serially diluted and titrated as described above. End points were read in comparison with those displayed by sera similarly treated, but without addition of 2-mercaptoethanol.

Keyhole limpet hemocyanin: Mice received two intraperitoneal injections of 0.2 mg associated (12) KLH made 4 wk apart. Within each strain, groups of 7–12 mice were bled out at days 14 and 28 after the first injection, and at days 7 and 14 after the second injection. Sera from three or four animals were pooled and tested for anti-KLH activity, using a modified quantitative precipitin technique with ^{131}I-labeled dissociated KLH (13). The results

[1] *Abbreviations used in paper:* ANA, antinuclear antibody; BSA, bovine serum albumin; HGG, human γ-globulin; KLH, keyhole limpet hemocyanin; PBS, sodium phosphate (0.01 M)–NaCl (0.15 M), pH 7.2; SRBC, sheep red blood cells.

are expressed as micrograms of dissociated KLH nitrogen precipitated by 1 ml of antiserum at the point where 80% of the KLH added was precipitated (P^{80} units).

Immunoglobulin classes with anti-KLH activity were determined by radioimmunoelectrophoresis according to the method described by Yagi et al. (14). Immunoelectrophoresis was performed on microscope slides (15). Specific antisera to mouse immunoglobulin classes (kindly provided by Dr. H. M. Grey) were allowed to diffuse for 24 hr at room temperature. The slides were washed with 0.15 M borate buffer, pH 8.8, for 24 hr. ^{131}I-labeled dissociated KLH (10 μg/ml and approximately 0.1 μc ^{131}I/μg) was added to the center trough. After 24 hr at room temperature, the slides were extensively washed with borate buffer, air-dried, and overlaid with X-ray film (Kodak industrial type KK) for 3–5 days, after which the film was developed. Slides were subsequently stained with amido black.

Bovine serum albumin: Mice were injected intraperitoneally with 0.2 ml of complete Freund's adjuvant containing 0.2 mg of BSA (Armour Pharmaceutical Co., Kankakee, Ill.). The animals were bled by puncture of the retroorbital sinus 14 and 28 days later. Antibodies were measured by the antigen-binding capacity (ABC) test described by Farr (16), using two antigen concentrations of ^{131}I-labeled BSA (0.02 and 0.2 μg nitrogen/ml). The serum dilution at which 33% of ^{131}I-labeled BSA was specifically bound to globulin was used as the measure of relative antigen-binding capacity. The results are expressed as micrograms of antigen nitrogen bound to 1 ml of undiluted antiserum (ABC-33).

Human γ-globulin: Human γ-globulin, Cohn Fraction II, was obtained through the courtesy of the American Red Cross and prepared by E. R. Squibb, New York (batch 2087). Heat-aggregated (56°C, 30 min) sodium sulfate–precipitated (0.62 M) HGG (17) or HGG in incomplete Freund's adjuvant was used for immunization. 0.5 ml of aggregated HGG containing 0.5 mg of protein was injected intraperitoneally; 0.2 ml of adjuvant containing 0.1 mg of HGG was injected subcutaneously.

For the induction of unresponsiveness, HGG was purified by passage through a diethylaminoethyl (DEAE) cellulose column, using 0.01 M sodium phosphate buffer, pH 8.0, as eluent. 1 hr prior to use, this preparation was ultracentrifuged at 100,000 *g* for 2 hr (HGG_{100}) in a Spinco model L preparative ultracentrifuge. After ultracentrifugation, the upper third of the tubes was carefully removed and injected within ½ hr into mice. Similar experiments were carried out with HGG dissolved in PBS and centrifuged at 20,000 *g* for 30 min (HGG_{20}). With both preparations, 0.5 ml containing 0.5 mg protein was injected intraperitoneally.

The immune status was determined by following the whole body radioactivity of mice injected with 10 μg of ^{131}I-labeled ultracentrifuged HGG (18). Antibodies to HGG fragments were measured by the antigen-binding test described previously (19), using ^{131}I-labeled Fab and ^{125}-labeled Fc. The serum dilution at which 33% of the radiolabeled antigens were specifically bound to globulin was used as a measure of relative antigen-binding capacity.

Iodination.—Iodination of proteins was performed according to the method described by McConahey and Dixon (20).

RESULTS

Immune Response to SRBC.—The hemagglutinin response of five strains of mice is recorded in Table I. In all strains, hemagglutinin titers were higher on day 5 than on day 7. At day 5 NZB/W, NZB, and BALB/c mice, the best responders, had similar titers, whereas A/J mice had a slightly lower response. CBA/J mice were the least responsive, with a four 2-fold dilution end point difference when compared to the high responder mice. In all strains, most of day 5 antibodies were mercaptoethanol-sensitive (Fig. 1). On day 12, the

hemagglutinin titers were higher than on day 5 in NZB/W and NZB mice, while the reverse was true for the other strains (Table I). A maximal difference of five 2-fold dilution end points was found between NZB and CBA/J antisera. Between 1- and 2-fold reduction in titer was observed after mercaptoethanol treatment (Fig. 1).

Similar strain differences were found when day 5 hemolysin titers were compared, whereas only a maximum of three 2-fold dilution end points was observed on day 12 (Table I). Mercaptoethanol treatment produced reduction in titers similar to that observed with hemagglutinins.

Immune Response to KLH.—Previous studies (21) indicated that 0.2 mg KLH given intraperitoneally to A/J mice induced a poor primary precipitin response but prepared the animals for a strong secondary response. The present

TABLE I

*Primary Response of Various Mouse Strains to Sheep Red Blood Cells**

Strain	Mean hemagglutinin titer‡			Mean Hemolysin Titer‡		
	Day 5	Day 7	Day 12	Day 5	Day 7	Day 12
NZB/W	12.7	12.1	13.7	13.1	11.1	9.3
NZB	13.7	11.9	14.9	13.1	11.5	10.1
A/J	11.3	10.7	11.1	11.3	9.7	8.7
BALB/c	13.7	11.7	11.3	13.3	11.1	8.7
CBA/J	9.7	9.3	9.7	9.7	9.3	6.9

* 5×10^8 SRBC injected intraperitoneally into 6 wk old male mice (10 mice/group).

‡ Reciprocal of $\log_2$ dilution.

experiments confirmed the almost complete absence of a detectable primary precipitin response to KLH in mice. No precipitating antibody was detectable 14 days after primary immunization in sera of all strains studied. By the 28th day, P^{80} values of about 0.4–1.2 were found in A/J mice antisera, whereas the other strains had no detectable precipitating antibody.

The secondary antibody response was measured 7 and 14 days after reinjection of 0.2 mg KLH at day 28. In all strains, serum antibody levels were higher on day 7 than on day 14. As shown in Table II, the secondary response to KLH was profoundly influenced by the strain of mouse. Serum antibody levels 7 days after reinjection of KLH were 40 times higher in A/J mice than in CBA/J mice. NZB/W and NZB mice had about one-tenth to one-sixth of the antibody of A/J mice.

In order to determine the influence of maturation of the immune system, the secondary response to KLH was measured in animals immunized at 6 or 20 wk of age (Table II). No difference was found in NZB/W mice, whereas a 2-fold (or 4-fold) increase in serum antibody levels was demonstrated in older A/J and CBA/J mice (or BALB/c mice).

Classes of immunoglobulins possessing anti-KLH activity in day 7 secondary sera were determined by radioimmunoelectrophoresis, using specific antisera to mouse immunoglobulins. No antibody activity was associated with γM or γA immunoglobulins. In all strains, anti-KLH antibody activity was present in

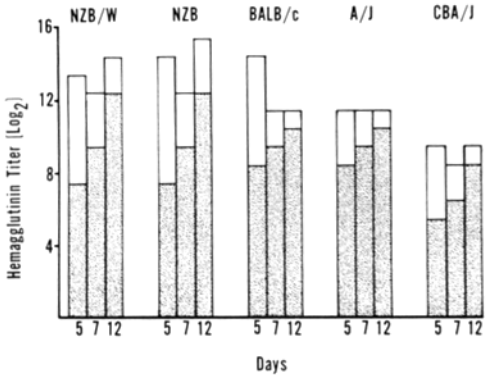

FIG. 1. Mercaptoethanol sensitivity of hemagglutinins in antisera of mice immunized with sheep red blood cells. Open bars, total antibody activity; stippled bars, mercaptoethanol-resistant antibody activity.

TABLE II

*Secondary Response of Various Mouse Strains to Keyhole Limpet Hemocyanin**

Strain	Anti-KLH antibody titer‡	
	6 wk old§	20 wk old§
NZB/W	12.0	15.3
NZB	N.D.‖	19.8
A/J	81.1	159.1
BALB/c	12.0	48.7
CBA/J	2.0	4.2

* Two intraperitoneal injections of 0.2 mg KLH made 28 days apart.

‡ P^{80} units determined 7 days after secondary injection (average of 7–12 mice).

§ Age at the time of the first injection.

‖ Not done.

the γG_1 immunoglobulins, as shown in Fig. 2. Strong binding of radioactive KLH by γG_2 antibody was also found in antisera from A/J mice and, to a much lesser extent, BALB/c and NZB/W mice.

It should be stressed that studies of antigen-binding capacities of anti-KLH sera from the four strains confirmed the strain variation in antibody response to KLH noted in the present study using a precipitin test.

Immune Response to BSA.—The antibody response to a single intraperitoneal

injection of BSA in complete Freund's adjuvant is illustrated in Table III. It can be seen that serum antibody levels were higher in NZB/W and NZB mice at day 14 after antigen injection. However, when measured at day 28, the anti-BSA response of A/J mice was greater than that of these two strains. No important difference in responsiveness was found between 6 and 20 wk old mice, except in the CBA/J strain, where older mice had antibody serum titers 8-fold higher than younger mice.

Since it is known that antigen-binding capacity values are influenced by the relative avidity of antibodies (15), the effect of antigen dilution upon the ABC values was determined using two ^{131}I-labeled BSA concentrations, 0.02

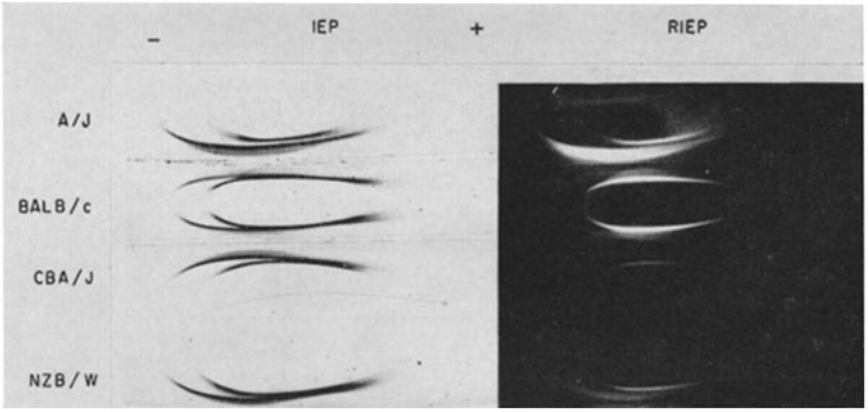

FIG. 2. Paired immunoelectrophoresis (IEP) and radioimmunoelectrophoresis (RIEP) preparations of anti-KLH antisera from various strains of mice. The antiserum added to the troughs was specific for mouse γG_1 and γG_2 immunoglobulins.

and 0.2 μg nitrogen/ml. The results, expressed as ratios between the ABC value obtained with the lower antigen concentration and that obtained with the higher antigen concentration, are reported in Table IV. It can be seen that ratios were usually similar in antisera obtained at the same time after antigen injection indicating that the results presented above reflected a difference in antibody amount rather than in antibody quality.

Immune Response to HGG.—Studies of the antibody response to HGG dealt with two antigen preparations, heat-aggregated, sodium sulfate-precipitated HGG, and HGG incorporated in incomplete Freund's adjuvant. As determined by following the whole-body radioactivity, all animals showed immune elimination of 10 μg ^{131}I-labeled HGG injected intraperitoneally 7–10 days after administration of each antigen preparation. Using an antigen-binding test employing ^{131}I-labeled Fab and ^{125}I-labeled Fc, binding capacities for HGG fragments were measured in antisera collected 14 or 21 days after antigen

injection. Since antisera displayed no or little binding of Fab, data dealing only with anti-Fc activity are presented in Table V. It was found that NZB/W mice responded to both antigen preparations better than the three other strains, although the maximal difference in antibody serum levels did not exceed 3-fold.

TABLE III

*Immune Response of Various Mouse Strains to Bovine Serum Albumin in Adjuvant**

Strain	Binding capacity‡			
	Day 14§		Day 28§	
	6 wk old‖	20 wk old‖	6 wk old	20 wk old
	ABC-33/0.01 μg N		*ABC-33/0.01 μg N*	
NZB/W	4.0	7.5	41.6	66.5
NZB	4.3	N.D.¶	21.1	N.D.
A/J	1.4	3.6	54.5	85.9
BALB/c	0.8	3.2	13.9	20.8
CBA/J	<0.1	0.8	2.0	15.9

* One intraperitoneal injection of 0.2 mg BSA in complete Freund's adjuvant (10 mice/group).

‡ Micrograms nitrogen BSA bound/ml of undiluted serum.

§ Time after immunization.

‖ Age at the time of injection.

¶ Not done.

TABLE IV

*Effect of Antigen Dilution upon the Binding Capacities of Anti-BSA Sera from Various Strains of Mice**

Strain	[ABC-33 (0.01 μg N)]/[ABC-33 (0.1 μg N)] × 100	
	Day 14‡	Day 28‡
NZB/W	28.4	40.0
A/J	36.4	45.9
BALB/c	35.0	42.0
CBA/J	29.0	41.5

* 20 wk old male mice immunized with 0.2 mg BSA in complete Freund's adjuvant (10 mice/group).

‡ Time after immunization.

Induction of Unresponsiveness to HGG.—In the first series of experiments, mice were given intraperitoneally 0.5 mg DEAE-isolated, ultracentrifuged (100,000 *g*, 2 hr) HGG 7 days prior to subcutaneous challenge with 0.1 mg HGG incorporated in incomplete Freund's adjuvant. 7 days after challenge the mice were tested for their ability to eliminate 10 μg ^{131}I-labeled HGG (I*HGG) injected intraperitoneally, whereas anti-Fc antibody levels were

determined in sera collected 3 wk after challenge. All control mice administered challenge antigen only showed immune elimination of I*HGG. In contrast,

TABLE V

*Immune Response of Various Mouse Strains to Human γ-Globulin**

Strain	Binding Capacity (ABC-33/0.01 μg N)‡		
	Aggregated HGG: day 14§	HGG in incomplete Freund's adjuvant	
		Day 14§	Day 21§
NZB/W	0.41	0.58	0.61
A/J	0.24	0.54	0.56
BALB/c	0.11	0.20	0.24
CBA/J	0.26	0.31	0.38

* 6 wk old male mice immunized with one intraperitoneal injection of 0.5 mg of aggregated HGG or one subcutaneous injection of 0.1 mg HGG in incomplete Freund's adjuvant (10 mice/group).

‡ Micrograms nitrogen Fc bound/ml of undiluted serum. Anti-Fab ABC values were negative.

§ Time after immunization.

TABLE VI

*Effect of Soluble HGG on the Induction of Unresponsiveness to HGG in Adjuvant in Various Strains of Mice**

Strain	HGG_{100}		HGG_{20}	
	Immune elimination‡	Day 21 control response§	Immune elimination‡	Day 21 control response
		%		%
NZB/W	2/10‖	3 (2)¶	8/10	11 (8)
A/J	2/10	2 (2)	10/10	19 (10)
BALB/c	3/10	11 (3)	10/10	14 (10)
CBA/J	1/10	3 (1)	4/10	5 (4)

* Mice were injected with 0.5 mg ultracentrifuged (100,000 *g*, 2 hr) HGG (HGG_{100}) or centrifuged (20,000 *g*, 30 min) HGG (HGG_{20}) 1 wk prior to challenge with 0.1 mg HGG in incomplete Freund's adjuvant.

‡ Determined by following the elimination of 10 μg I*HGG injected 7 days after challenge.

§ Percentage of anti-Fc binding values in antisera of mice injected with challenge antigen only.

‖ Positive/total.

¶ Number of mice with detectable antibody.

pretreatment with ultracentrifuged HGG induced unresponsiveness to HGG in adjuvant, as indicated by the absence of immune elimination in most of the mice and by the presence of little or no serum antibody (Table VI).

Since the four strains tested showed no apparent difference in their ability to become unresponsive after administration of HGG ultracentrifuged as

indicated above, the same type of experiments were performed using HGG centrifuged at 20,000 *g* for 30 min. The results (Table VI) indicate that all A/J and BALB/c mice and 8 out of 10 NZB/W mice eliminated I*HGG very rapidly, whereas only 4 out of 10 CBA/J mice showed an immune elimination. However, when anti-Fc binding values were compared in sera collected 21 days after challenge, all mice treated with centrifuged HGG had less than 20% of the amount of antibody present in mice injected with challenge antigen only. Again, NZB/W mice did not appear to behave differently from the other strains tested.

DISCUSSION

The present study confirms and extends previous reports dealing with strain differences in the immune response of mice to complex antigens (22–28). Our results demonstrate that a difference in the amount of antibody produced against one antigen does not necessarily indicate differences in the general immunological capacity of mouse strains, and illustrate the difficulty of drawing any general conclusion from experiments limited to one antigen and/or a few strains. From the study of the antibody response to KLH, one might conclude that NZB/W mice have an immune hyporeactivity, whereas studies of the antibody production to SRBC might suggest the contrary. Similarly, comparative studies between NZB/W and CBA/J mice only would suggest a general hyperresponsiveness of the former strain, since they produced more antibodies against the four antigens studied. No such conclusion would be reached if the immune response of NZB/W mice were compared with that of A/J mice, which responded better to KLH and BSA, similarly to HGG, and less to SRBC.

The same difficulties mentioned above apply to the interpretation of data concerning induction of tolerance in mice. In contrast with the report of Staples and Talal (8), the present study demonstrates that induction of unresponsiveness to HGG could be achieved in 6 wk old NZB/W mice by using ultracentrifuged (100,000 *g*) HGG. Simiar results were obtained by Weigle,[2] using sodium sulfate-fractionated HGG in 5–6 month old NZB/W mice. By comparison with the other strains tested, NZB/W mice did not behave differently from A/J and BALB/c mice as far as induction of tolerance to HGG was concerned. This similarity was particularly evident when HGG centrifuged at 20,000 *g* (HGG_{20}) was used for the induction of unresponsiveness. After challenge, most, if not all, NZB/W mice, and all A/J and BALB/c mice, showed a very rapid immune elimination of I*HGG, suggesting a priming effect of HGG_{20}. However, a state of hyporesponsiveness was also present in the same animals, as indicated by the serum antibody levels, which reached 10–20% of the values found in animals receiving the challenge antigen only.

[2] W. O. Weigle. Personal communication.

As mentioned by Golub and Weigle (29), the findings that the dose of ultracentrifuged HGG needed to induce an unresponsive state may vary from one strain of mice to the other indicate that caution is necessary in the interpretation of data concerned with the relative ease with which mice become unresponsive to a given antigen. This remark has to be kept in mind particularly with regard to the suggestion that the relative inability of NZB/W mice to become tolerant to some protein antigens could indicate a lack or loss of tolerance to self-antigens (30).

Another difficulty emphasized by our study concerns the variation with age of the immune response to a given antigen. Serum anti-BSA peak titers were 8 times higher in CBA/J mice immunized at 20 rather than 6 wks of age. However, no such difference was observed in the immune response of the same strain to KLH, although older BALB/c mice produced 4 times more anti-KLH antibodies than younger mice. Studies of the relationship between age and immune responsiveness have demonstrated that the antibody-forming potential of mice to foreign red cells greatly increases during the first months of extrauterine life (31). Moreover, the magnitude of the increase, as well as the age at which immune maturity was fully reached, differed between two strains immunized with rat red blood cells. In this respect, the immune responsiveness of NZB mice to SRBC was found to develop at a very early age (9, 10). However, the finding that early maturation was not observed with pig or chicken red blood cells ruled out the possibility of a general early development of immunocompetence in this strain (9).

It is apparent, from the results of the present work, that NZB/W mice do not possess a general immune hyperresponsiveness or a relative inability to be made tolerant to HGG. Of greater importance is the possibility that these mice have a genetically determined hyperreactivity to the antigens involved in their autoimmune disease, as suggested by the findings that young NZB/W mice produced much more antinuclear antibody than five other strains of mice after immunization with DNA bound to methylated bovine serum albumin.[3] It is of interest that, in the parental strains, the NZW mice, which have a very low incidence of spontaneously occurring ANA, made as much ANA upon immunization as did the hybrids, while the NZB mice, which develop naturally a moderate incidence of ANA, responded relatively poorly to immunization. These findings suggest that the very high incidence of ANA spontaneously occurring in NZB/W mice may be the result of their possessing lymphoid cells highly responsive to nuclear antigens as in their NZW parent and nuclear antigenic stimulation, perhaps similar to the situation in their NZB parent. The importance of a heightened antigenic stimulus for the development of the autoimmune disease is further suggested by the considerable enhancement of ANA production in NZB, NZW, and NZB/W mice after

[3] P. H. Lambert. Manuscript in preparation.

neonatal infection with either polyoma virus or lymphochoriomeningitis virus (32). Although the mechanisms underlying this enhancement are unknown, the possibility exists that the viral infections may increase the liberation and/or cause an abnormal degradation of endogenous nuclear material.

SUMMARY

The immune responsiveness of (NZB × NZW) F_1 hybrid mice (NZB/W) has been compared with that of three other strains of mice, A/J, BALB/c, and CBA/J. The antigens used included sheep red blood cells (SRBC), keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), and human γ-globulin (HGG). It was found that important strain differences existed in the amount of antibody produced, but the relative immune responsiveness depended very much upon the nature of antigen. By comparison with the other strains tested, NZB/W mice had a higher antibody production to some antigens (SRBC and BSA) but were low responders to others (KLH).

Induction of unresponsiveness to HGG by treatment with ultracentrifuged HGG was studied in the strains cited above. NZB/W mice became tolerant after injection of HGG ultracentrifuged at 100,000 *g* for 2 hr. Similar experiments carried out with another preparation of HGG (centrifuged at 20,000 *g* for 30 min) failed to reveal any abnormal behavior of NZB/W mice as compared to BALB/c or A/J mice.

These results do not support the concept that NZB/W mice possess a general immune hyperreactivity or a relative inability to be made tolerant to protein antigens. However, they do not rule out the possibility that these mice have a genetically determined hyperresponsiveness to some antigens, in particular to nuclear antigens.

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