

GENETIC STUDIES IN NZB MICE

I. Spontaneous Autoantibody Production

By E. S. RAVECHÉ, A. D. STEINBERG, L. W. KLASSEN, AND J. H. TJIO

(From the National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014)

NZB mice spontaneously produce a variety of autoantibodies and serve as a model of autoimmunity (1). As they age, NZB mice produce antibodies to erythrocytes, thymocytes and T cells, and nucleic acids, especially single-stranded DNA (ssDNA)¹ (2).

Naturally occurring thymocytotoxic autoantibodies (NTA) have been found early in life in NZB mice (3). These antibodies are present in almost all NZB mice by 3 mo of age, and are associated with an age-dependent loss of T cells in these mice (4, 5). Sera with high titer NTA activity were shown to affect a subpopulation of T cells by selectively decreasing the response of normal spleen cells to the mitogen Concanavalin A (6). NTA appear to be functionally important. These antibodies impair cellular immune responses (7) and cause a loss of suppressor T cells (8). Although other mouse strains may produce NTA later in life, the prevalence and titer of NTA are much lower than in NZB mice (3).

Because both NTA and antinucleic acid antibodies are characteristic of NZB mice and may be important in the pathogenesis of autoimmunity (9), we set out to investigate the mode of inheritance of the propensity for development of these autoantibodies.

Reciprocal F₁ hybrids between the DBA/2 and NZB strains were studied to determine whether or not the inheritance was dominant or regulated by a gene on the X chromosome. Further studies were performed in backcrosses. The DBA/2 strain was chosen as one of the parental strains because: (a) it is H-2^d like NZB mice (10), (b) like NZB mice it expresses GP70 viral protein antigens in large quantities on the surface of mononuclear cells (T. M. Chused and H. C. Morse, III, personal communication) and (c) it does not spontaneously produce large quantities of either NTA or anti-ssDNA (11, 12). Thus, the possibility of an H-2-linked gene controlling either NTA or anti-ssDNA production could be eliminated, and the contribution of GP70 expression minimized. We found that the hybrids were intermediate between high producing (NZB) and low producing (DBA/2) parents with regard to both NTA and anti-ssDNA production. In preliminary studies (13), NTA were produced by female but not male F₁ hybrids. Castration of the males abolished this sex difference; castrated males had the same incidence and titer of NTA as did their female littermates. This was consistent with the notion that male sex hormones can exert a negative immunoregulatory effect (14). We therefore concluded that further genetic studies of the regulation of autoantibody production must deal directly with the problem of sex effects. It appeared to us that the best way to perform a genetic analysis of a sex-influenced trait was to test gonadectomized progeny.

In this paper we report a study of the mode of inheritance of the propensity for production of thymocytotoxic autoantibodies. Because sex hormones influenced the expression of these antibodies, a genetic study was carried out utilizing go-

¹ *Abbreviations used in this paper:* C, complement; NTA, naturally occurring thymocytotoxic autoantibodies; ssDNA, single-stranded DNA.

nadectomized offspring to better determine the precise genetic mechanisms in the absence of the modulating influence of sex hormones. In addition, we studied the genetic control of antibodies to ssDNA in the same mice. We found that inheritance of the tendency to develop NTA is not linked to the tendency to develop ssDNA antibodies. However, each is primarily controlled by a single gene locus.

Materials and Methods

Mice.—All mice were derived from colonies at the National Institutes of Health Animal Production Unit. NZB/N and DBA/2N mice were reciprocally crossed in our laboratories to produce (NZB X DBA/2) F_1 and (DBA/2 X NZB) F_1 progeny. Male and female F_1 hybrids were backcrossed to both parental stains. Virgin mice of both sexes from parental strains, F_1 hybrids, and backcrosses were chosen for surgery at 4–5 wk of age. Animals were injected intraperitoneally with sodium pentobarbital before surgery. In female mice, the ovaries were removed through two bilateral flank incisions (15). In males, both testes were removed through a single scrotal incision. The vas deferens was tied, and each testis and epididymis was removed. Vasectomy of select males were performed by vasoligation above and below the transection.

Sera.—Mice were bled at 1 yr by orbital sinus puncture under light ether anesthesia. The blood was allowed to clot at room temperature for about 2 h, after which the sera were removed, centrifuged to remove cellular debris, and stored at -20°C until used. Because of a laboratory accident (freezer breakdown) sera from gonadectomized (NZB X DBA/2) F_1 females were not available for study with regard to ssDNA antibodies and serum IgM concentrations.

NTA Assay.—The presence of NTA was measured as described previously (16), with only minor modifications. Thymocytes from 4- to 6-wk-old C57BL/6N mice were incubated with 30 μCi of ^{51}Cr per 10^7 cells for 30 min at 37°C in RPMI-1640 medium. Labeled thymocytes (5×10^4) were added to 50 μl of serially diluted test serum and incubated for 30 min at room temperature followed by 30 min at 4°C . Cells were centrifuged, washed twice in medium, and incubated with 50 μl of a 1:5 dilution of rabbit complement (previously absorbed with mouse thymocytes) for 30 min at 37°C . The suspension was then centrifuged, and 50 μl of supernate removed and counted in a gamma spectrometer. The positive control was a pool of NZB sera with known NTA activity; the negative control consisted of sera from 1-yr-old female DBA/2 mice. Maximum ^{51}Cr release was obtained by freeze-thawing three times. Cytotoxicity of complement (C) alone was also determined. Percentage cytotoxicity was calculated as

$$\frac{(\text{cpm test serum} - \text{cpm C})}{(\text{cpm freeze-thaw} - \text{cpm C})} \times 100.$$

All sera were assayed in duplicate with good reproducibility. The cytotoxic titer was the last dilution giving $>50\%$ ^{51}Cr release. A positive titer in this assay is 1:4.

Measurement of IgM Levels.—Serum IgM concentrations were measured by radial immunodiffusion in Meloy immunodiffusion plates (Meloy Laboratories, Inc., Springfield, Va.). Sera were added to wells cut in agarose gel impregnated with monospecific IgM antibody. The plates were incubated for 18 h at room temperature in a humidified atmosphere, and the diameter of the precipitin ring measured and compared to known standards run on the same plate.

Measurement of Anti-ssDNA Antibodies.—A previously described modified Farr technique was employed (17). The reaction mixture (100 μl) contained 50 ng ^{14}C -labeled heat-denatured *Escherichia coli* DNA (50,000 dpm/ μg) and 25 μl of test serum (previously heated to 56°C for 30 min) in borate buffer, pH 8.0. The mixture was incubated for 1 hr at 37°C , then refrigerated overnight at 4°C , after which an equal volume (100 μl) of 70% saturated ammonium sulfate was added. The mixture was then incubated at 0°C for 1 h and centrifuged at 1,000 g for 20 min. 100 μl of supernate was removed, and the amount of radioactivity determined in a liquid scintillation counter. Positive and negative controls were run in duplicate or triplicate with test sera. A single batch of fetal calf serum (FCS) was always assayed as a negative control for nonspecific binding of the ligand. The percent binding by the FCS was subtracted from each test serum. A test was considered positive if the binding was greater than the highest binding ever recorded for FCS as well as 2 SD greater than the mean of replicate runs of normal mouse sera.

TABLE I
Study of NTA Production in Intact NZB, DBA/2, F₁, and Backcross Mice

Intact animal	Sex	Positive NTA test		Combined sex frequency
		No./total	Frequency	
NZB	F	14/16	0.88	0.91
NZB	M	16/17	0.94	
DBA/2	F	1/14	0.07	0.04
DBA/2	M	0/14	0.00	
(NZB × DBA) F ₁	F	13/28	0.46	0.24
(NZB × DBA) F ₁	M	0/26	0.00	
F ₁ × NZB	F	13/24	0.54	0.48
F ₁ × NZB	M	9/22	0.41	
F ₁ × DBA/2	F	1/30	0.03	0.09
F ₁ × DBA/2	M	4/25	0.16	

Results

Prevalence of NTA.—Table I shows the prevalence of NTA in the two parental strains, the F₁ hybrids, and the backcrosses to both parental strains. In the right column of Table I, the incidence of NTA is reported without regard to sex. The F₁ hybrids have a higher incidence of NTA than does the DBA/2 parental strain. Backcrosses to the NZB strain have an intermediate incidence of NTA between the NZB parental strain and the F₁ hybrids, whereas the backcrosses to the DBA/2 strain have as low an incidence as the DBA/2 parental strain. The data on the right side of Table I do not suggest a simple genetic mechanism. In the center of Table I the incidence of NTA is reported by sex. In the case of the F₁ hybrids, the males had a significantly lower incidence of NTA than did the females ($P < 0.001$). NTA production is not an X-linked trait because F₁ males of both reciprocal crosses did not produce NTA (13, and unpublished data). The sex difference in the incidence of NTA is attributed to sex hormones, and can be abolished by castration (13). No meaningful genetic analysis could be made from these findings of intact animals. Therefore, the genetic control of NTA production in castrated offspring was studied.

Effect of Gonadectomy on the Prevalence of NTA.—In Table II the incidence of NTA in gonadectomized male and female mice is shown. Castration of the males increased the incidence of NTA and thereby removed the sex differences observed in intact F₁ mice. The differences between males and females with regard to NTA incidence in gonadectomized animals are insignificant; therefore, the sexes were combined. In the right-hand column and at the bottom of Table II the expected incidence of NTA is shown; the calculations are based on the observed NTA incidence in gonadectomized parental strains. This calculation assumes that: (a) each parental strain has a distribution of NTA production (incomplete penetrance), (b) each parental contribution to offspring NTA production is equivalent to a random sample of the parental distribution, and

TABLE II
*Study of the Genetic Factors Influencing NTA Production by Analysis of
 Gonadectomized Offspring*

Gonadectomized animal	Sex	Positive NTA Test		Combined sex frequency	Expected frequency*	χ^2 (P)
		No./total	Frequency			
NZB	F	9/10	0.90	0.94	—	
NZB	M	8/8	1.00			
DBA/2	F	1/9	0.11	0.09	—	
DBA/2	M	1/13	0.08			
(NZB × DBA) F ₁	F	9/15	0.60	0.52	0.52‡	0.00 (P > 0.75)
(NZB × DBA) F ₁	M	13/27	0.48			
F ₁ × NZB	F	8/12	0.67	0.71	0.73§	0.03 (P > 0.50)
F ₁ × NZB	M	7/9	0.78			
F ₁ × DBA	F	4/14	0.29	0.21	0.31	1.44 (P > 0.50)
F ₁ × DBA	M	2/15	0.13			

* Expected incidence calculated on the basis of equal contribution by both parents.

‡ $\frac{1}{2}(0.94) + \frac{1}{2}(0.09) = 0.52$.

§ $\frac{1}{2}(0.94) + \frac{1}{2}(0.52) = 0.73$.

|| $\frac{1}{2}(0.52) + \frac{1}{2}(0.09) = 0.31$.

(c) each parent contributes equally to the genetic control of NTA production in the offspring (gene dosage).

Another genetic analysis is based upon the assumption that NZB mice carry a gene or genes that lead to 100% incidence of NTA (assuming modifying factors such as male sex hormones are eliminated). The model for this calculation (assuming complete penetrance) is shown in Fig. 1. The observed relative frequencies in gonadectomized animals are very close to, and not significantly different from, this theoretical model (Table III). However, in intact F₁ and backcross mice, the observed relative frequencies are significantly different from the expected theoretical relative frequencies. Thus, only the gonadectomized offspring (especially males) provided data compatible with the theoretical model.

IgM Concentration.—NTA are predominantly of the IgM class (3, 18). We therefore felt obliged to look for a possible correlation between NTA and IgM serum concentration in individual mice. In Fig. 2 the serum IgM concentrations for the two parental strains, the F₁ hybrids, and the backcrosses are shown. Overall, the amount of serum IgM increases as the contribution of the NZB genome increases. In the DBA/2 strain, the males have significantly less serum IgM than do the females; this sex difference wanes as the cross approaches the NZB genome. Thus, NZB males and females had similar IgM concentrations just as they had similar quantities of NTA. The gonadectomized males have higher IgM concentrations than intact males suggesting that male sex hormones nonspecifically decrease total serum IgM levels.

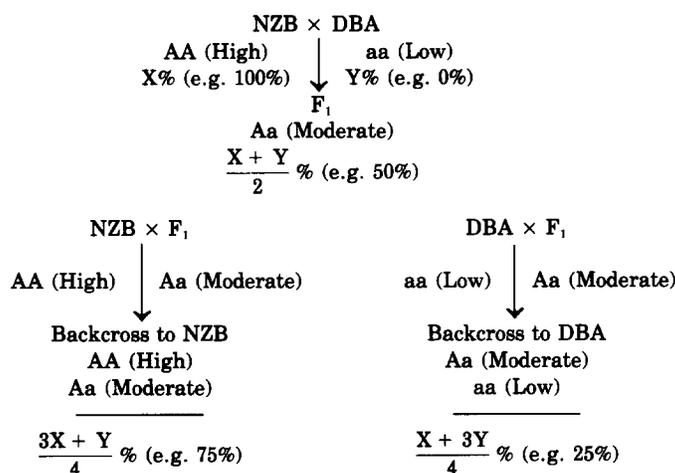


FIG. 1. Gene dosage model. Aa, moderate production; AA, high production.

TABLE III
 Comparison of NTA Frequencies* in Intact and Gonadectomized NZB, DBA/2, F₁, and Backcross Animals

Animal	Expected‡	Intact		Gonadectomized	
		Observed	χ^2 (P)	Observed	χ^2 (P)
NZB	1.00	0.91	0.27 (P > 0.50)	0.94	0.06 (P > 0.75)
DBA/2	0.00	0.04	0.04 (P > 0.75)	0.09	0.18 (P > 0.50)
(NZB × DBA/2) F ₁	0.50	0.24	14.52 (P < 0.005)	0.52	0.10 (P > 0.75)
F ₁ × NZB	0.75	0.48	18.12 (P < 0.005)	0.71	0.15 (P > 0.50)
F ₁ × DBA/2	0.25	0.09	7.33 (P < 0.01)	0.21	0.29 (P > 0.50)

* Frequencies based on calculations in Tables I and II.

‡ Based on theoretical gene model (Fig. 1).

The IgM levels followed a pattern similar to that of NTA: (a) both had decreased levels in males, (b) the DBA/2 strain was the lowest, (c) backcrosses to the DBA/2 were intermediate between the DBA/2 strain and the F₁ hybrids, and (d) the backcrosses to the NZB strain were intermediate between the F₁ hybrids and the NZB strain. In Fig. 3 A individual F₁ hybrid sera were analyzed for both the amount of serum IgM and the amount of thymocytotoxicity. Using Spearman rank correlation analysis, no significant correlation was observed. There was also no correlation between IgM concentrations and thymocytotoxicity in backcrosses. Although both IgM levels and NTA are increased in NZB mice and their offspring, and in castrated males as compared with intact males, the increase in NTA is not the result solely of the increased IgM levels.

Spontaneously Produced ssDNA Antibodies.— In Fig. 4 the amount of ssDNA antibodies is shown for both parental strains, F₁ hybrids, and backcrosses. In

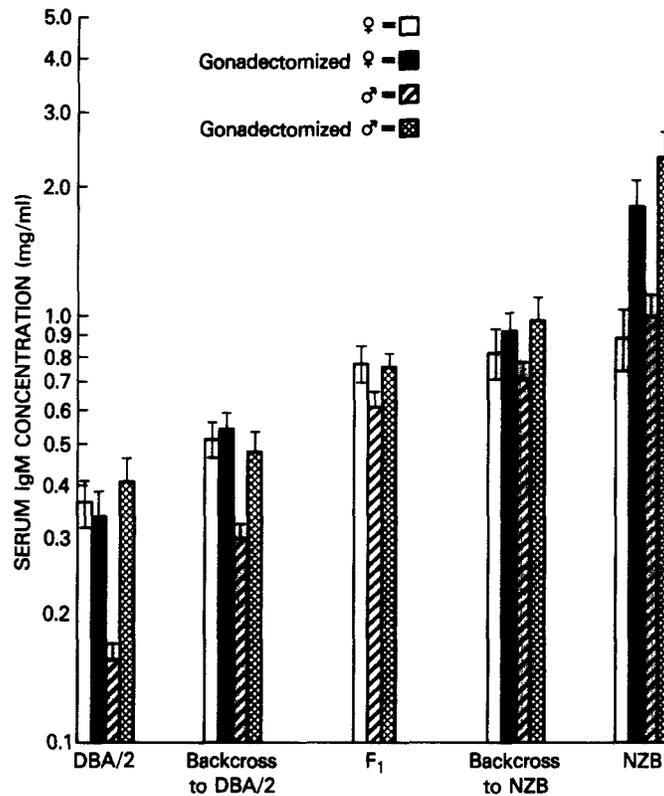


FIG. 2. Serum IgM concentrations were determined in intact and gonadectomized mice of both sexes. The means of 10 mice are shown in each column. Bars indicate the standard error of the mean. The difference between intact males and intact females was significant for DBA/2, and backcross to DBA/2 mice ($P < 0.05$). The difference between intact males and gonadectomized males was significant ($P < 0.05$) for all groups except for F₁ mice ($0.1 > P > 0.05$). Intact and gonadectomized females did not differ significantly except in the case of NZB mice ($P < 0.025$). Castrated males did not differ significantly from castrated females with regard to serum IgM concentrations.

all cases the intact males produced significantly less anti-ssDNA than did the gonadectomized males ($P \leq 0.05$, Student's t test). In all cases, except in the NZB strain, the females had more binding than the males. Gonadectomy of the males elevated their ssDNA binding to levels at least as high as the corresponding females. Analysis of the genetic contribution of the NZB parent to ssDNA antibodies is shown in Table IV. Unlike the case of NTA, groups of animals divided themselves very well into nonoverlapping positives and negatives. All NZB mice produced anti-ssDNA but none of the DBA/2 mice did, even when castrated (unpublished data). F₁ mice (both NZB and DBA/2 mothered) all produced anti-ssDNA providing males were castrated to reduce the "suppressive" influence of male sex hormones. Vasectomy and sham castration gave results similar to those found in intact males (unpublished data). Backcrosses to NZB mice gave a frequency close to 100%, and backcrosses to DBA/2 mice gave a frequency close to 50% (again providing males were castrated). These

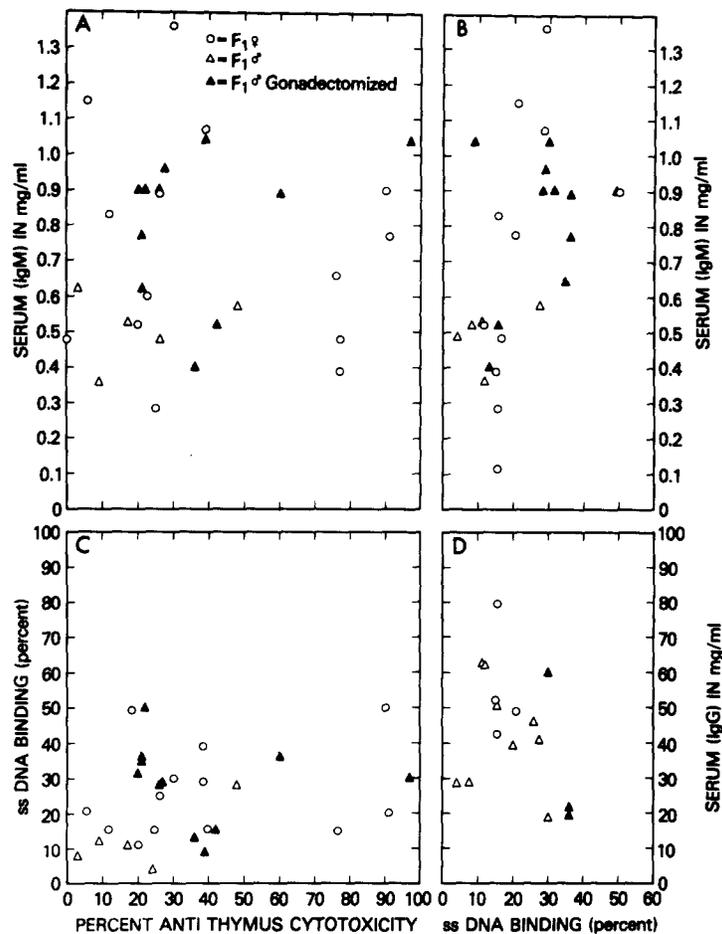


FIG. 3. (A) Serum IgM is plotted against NTA for individual sera. There was no significant correlation between the two measures ($r_s = -0.2$, $P > 0.06$). In addition, there was no significant correlation for subgroups (F₁ females, $r_s = 0.03$, $P > 0.05$; F₁ males, $r_s = -0.10$, $P > 0.05$; F₁ gonadectomized males $r_s = 0.21$, $P > 0.05$). (B) Serum IgM is plotted against antibodies to ssDNA. There was significant correlation between the two measures ($r_s = 0.56$, $P < 0.01$). (C) Antibodies to ssDNA are plotted against NTA in individual sera. No significant correlation was found between the quantities of the two antibodies for all sera ($r_s = -0.12$, $P > 0.05$). (D) Serum IgG is plotted against ssDNA binding. Overall there was no significant correlation ($r_s = -0.36$, $P > 0.05$).

data are best explained by a single dominant gene which predisposes to spontaneous anti-ssDNA production.

In anti-ssDNA-positive mice, the quantity of anti-ssDNA is not completely explained by a single dominant gene (Fig. 4). F₁, backcrosses to NZB, and NZB mice show a progressive increase in amount of anti-ssDNA, even though all have a 100% incidence of these antibodies. Quantitative analysis of the ssDNA binding suggests that the quantity of anti-ssDNA produced is controlled by a single regulatory gene. Complete data for castrated mice are available for males. Thus, DBA/2 male mice had close to 0%, F₁ mice 20%, and backcross to

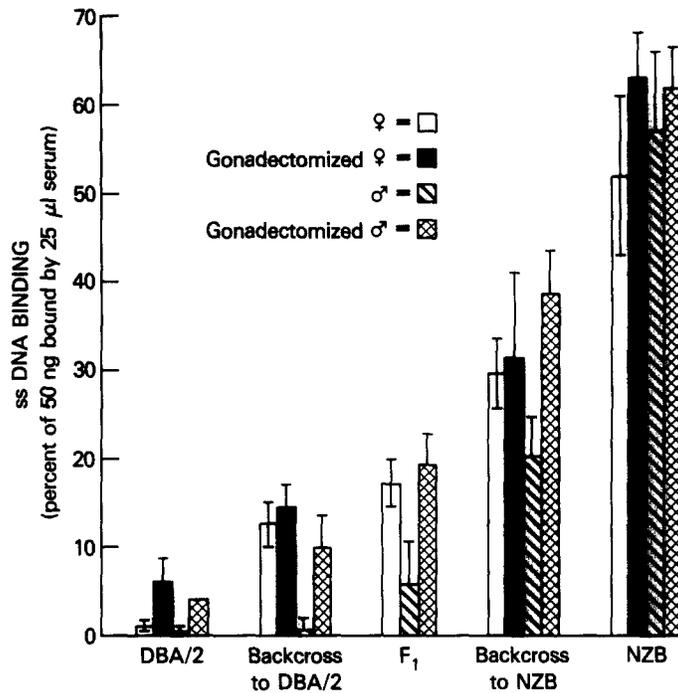


FIG. 4. Mean percent of 50 ng ¹⁴C-labeled ssDNA bound by individual sera is shown in the vertical columns. Groups consisted of 10-28 mice. Bars represent the standard error of the mean. Intact females had significantly more anti-ssDNA than intact males in the F₁ mice and backcrosses to DBA/2 ($P < 0.05$). Gonadectomized males were significantly higher ($P < 0.05$) than intact males in all groups except the NZB mice. In all groups, castrated females and castrated males did not differ with regard to anti-ssDNA antibodies ($P > 0.05$).

DBA mice 10% ssDNA binding. NZB had close to 60%, and backcrosses to NZB had 40% binding (Fig. 4). This strongly suggests either (a) a gene dosage type of inheritance by a single-gene locus for anti-ssDNA (in Fig. 1, X = 60%, Y = 0%) or (b) one dominant and one regulatory gene as shown in Fig. 5. These two models cannot be distinguished by the data in Fig. 4.

Correlations of ssDNA Antibodies with NTA and Ig.—The NZB strain produces both NTA and antibodies reactive with ssDNA. To investigate the possible relationship between these two autoantibodies, individual sera were analyzed for the quantities of both NTA and ssDNA antibodies. Overall, there was no correlation between the percent cytotoxicity (NTA) and ssDNA binding. This is illustrated for F₁ mice in Fig. 3C. Using Spearman rank order analysis, there was no correlation between NTA levels and ssDNA antibodies in NZB, DBA, F₁, or backcrosses. By disregarding the quantity of autoantibody and considering only positive or negative expression of the trait, analyses can be performed in backcross mice to determine whether or not NTA and ssDNA antibody traits are linked. If the two genes segregate independently, there should be 50% of the parental types and 50% of the recombinant types. On the other hand, if the two loci are linked, the frequency of the recombinant types should be significantly lower than that of parental types (that is <50%). In

TABLE V
*Distribution of NTA- and ssDNA-Positive and Negative Mice
 for All Backcrosses (to Both Parents)*

	NTA +	NTA -
ssDNA +	26	44
ssDNA -	13	45

$\chi^2 = 1.32, P > 0.20.$

these experiments, parental types would be either positive for both NTA and anti-ssDNA or negative for both. Recombinant types would be positive for one trait and negative for the other. The observed ratios of the parental and recombinant types (Table V) do not differ significantly from the ratios expected if the two traits were not linked ($\chi^2 = 1.32, P > 0.20$). Furthermore, in the 28 female backcrosses to DBA/2, one-half of the mice produced anti-ssDNA and one-half did not (Table IV). In the 14 animals with anti-ssDNA, the mean percent thymocytotoxicity (NTA) at a 1:4 serum dilution was 8.71 ± 2.3 and in the 14 animals without anti-ssDNA the mean percent thymocytotoxicity was 14.38 ± 5.7 . This further demonstrates that the genetic predisposition to the development of each of these two autoantibodies is inherited independently. Finally, because the amounts of these two autoantibodies are unrelated, it is likely that separate secondary (? regulatory) genes independently control the magnitude of the production of the two autoantibodies.

Serum IgG levels were not significantly correlated with the amount of ssDNA antibodies produced (Fig. 3 D). However, serum IgM levels were correlated with the amount of ssDNA antibodies (Fig. 3 B).

Discussion

An increasing body of evidence suggests that sex hormones influence spontaneous autoantibody production (19-21) and antibody response to immunization (22-25). Although female sex hormones may have some immunoregulatory effects, the predominant mechanism is an immunosuppressive effect of male sex hormones. The immunosuppressive effect is probably not related to direct suppression of B cells, but rather, indirectly, through an effect on thymocyte development and T cells (26). Because sex hormones may affect antibody production, it becomes important to consider the sex of study animals in genetic analyses of control of antibody production. This is especially relevant for situations in which males and females differ markedly. An extreme example of this phenomenon is the spontaneous production of NTA in NZB hybrids; male hybrids do not produce NTA, whereas many females do. Theoretically, one way to avoid this problem is to use only females. However, this is not possible when an X-linked immune response gene is under investigation. In such a situation, castration of progeny may allow for an adequate genetic analysis.

We found that only by looking at gonadectomized animals could a model be constructed for the genetic contribution of NZB parents to the appearance of NTA in offspring. A single locus in which there was a gene dosage effect could account for the observed results. If the NTA trait were dominant, all the F_1

hybrids would produce high titers of NTA, and if the trait were recessive none of the F_1 animals would produce NTA; neither was the case. In fact, 52% of the hybrids produced NTA. If a single gene for the phenotype is postulated, the F_1 hybrids would be heterozygous and could have only one gene predisposing to NTA, whereas the NZB strain has two genes. If there were a gene dosage effect, the F_1 hybrids would produce half as much NTA. Unfortunately, NTA production is variable in terms of time of onset and titer, even in the highly inbred NZB strain. As a result, the arbitrary selection of a positive test of 50% kill at a 1:4 serum dilution gave not a 100%, but approximately a 50% incidence level. It is possible that examination at a later time (e.g. 2 yr) would have allowed the F_1 mice to be closer to 100%. Unfortunately, at that age many NZB parents and backcrosses to NZB mice might already be dead of their autoimmune disease.

A model that explains these observations is shown in Fig. 1. The offspring receives an equal genetic contribution from each parental type for the phenotypic expression of the trait. However, each parental type has a distribution of the trait. Thus, an offspring has a probability of having the trait equal to the sum of each parent's probability divided by 2. The observed phenotypic expression in F_1 and backcross mice is very close to that expected whether calculated on the basis of observed expression (incomplete penetrance) in the parents (Table II) or an all-or-nothing expectancy (complete penetrance) in the parental strains (Table III). That both methods successfully approximate the observation shows the closeness of the phenotypic expression of the trait in NZB mice to 1.0 and in DBA/2 mice to 0.0.

Other alternative hypotheses are possible. The most plausible alternative is that NTA production is controlled by a single dominant gene but modified by an additional gene that controls the level of autoantibody production. One such model is shown in Fig. 5. Assuming that these two genes are not linked, an animal that is homozygous at both of these loci would produce maximal levels of NTA. If an animal were heterozygous at both loci, the recessive trait for excessive production of autoantibodies would not be expressed, but the dominant NTA trait would be expressed though not maximally. If an animal were heterozygous at the NTA locus but homozygous at the locus which controlled autoantibody levels, the production of NTA could be maximal. Either a gene dosage theory (Fig. 1) or a modifying gene theory (Fig. 5) would give the same expected results for NTA production. It is theoretically possible to distinguish a single gene (with a gene dosage effect) from a two-gene loci model (a dominant gene with a recessive modifying gene) by examining large numbers of F_2 mice. In the former, one would expect a 1:2:1 ratio, whereas in the latter one would expect a 4:9:3 ratio of low:moderate:high; however, in practice such an analysis might be extremely difficult because of the closeness of the two sets of ratios. Other genetic models cannot be excluded; however, the two presented here reasonably approximate the data.

The genetic analysis of the ssDNA antibody production suggested a single dominant gene that led to spontaneous anti-ssDNA production. The quantity of anti-ssDNA in positive mice is determined either by a gene dosage effect (Fig. 1) or a regulatory gene (Fig. 5). The genetic control of levels of ssDNA and NTA antibodies appear to be distinct.

In contrast, the level of ssDNA antibodies and quantity of IgM in individual mice were found to be correlated. This suggests that a regulatory gene model (Fig. 5) best explains the quantitative differences in anti-ssDNA levels among positive mice. In addition, these data suggest that the gene that regulates ssDNA antibody levels may be the same as (or linked to) the gene controlling the quantity of IgM.

It has been demonstrated in the present experiments that IgM levels of males are decreased relative to female littermates in intact DBA/2, backcrosses to DBA, and F₁ mice. This could not be strictly correlated with the decrease in NTA. Thus, the decrease in total IgM in intact males does not alone account for the decrease in this autoantibody. Male sex hormones may act to nonspecifically decrease total levels of IgM; they may work in another manner to prevent the formation of autoantibodies.

Large numbers of matings have been performed with NZB mice to determine the mode of inheritance of autoimmune traits. Two types of matings have been evaluated: (a) crosses with other New Zealand strains, and (b) crosses with non-New Zealand strains. In crosses of NZB with NZC mice, 100% of the F₁ animals were Coombs positive, and 74% of the backcrosses to NZC were Coombs positive, suggesting that a single gene was responsible for the abnormality (27). Additional studies of NZB X NZC mice have suggested both a dominant and recessive gene for antierythrocyte antibodies (28). Braverman (29) also studied Coombs positivity in crosses of NZB with NZW mice. He concluded that there is a dominant gene in NZB mice that determines Coombs positivity whereas the NZW carries "a modifying gene" that in the presence of the NZB gene allows for a positive antinuclear response. Thus, the non-NZB New Zealand strains contribute to the disease state observed in the backcrosses. It seems that the severity of the disorder in NZB crosses with other New Zealand strains depends on the particular New Zealand strain. With regard to antierythrocyte antibodies, the current view is that more than one gene, at least one of which may not be uniquely associated with the NZB strain, is involved in the phenotypic expression of these autoantibodies (30).

Additional studies of crosses with NZB and other New Zealand strains focused largely on questions of disease signs. Because disease may depend upon many factors, it may not be a good phenotype to study in genetic analysis. This is especially true of immune complex glomerulonephritis to which a variety of different antibodies appear to contribute. Despite these difficulties, the occurrence of lupus nephritis in NZB X NZW F₁ mice has been postulated to depend on the action of at least two dominant or codominant genes, at least one gene from each parent (31).

A variety of crosses of NZB with non-autoimmune, non-New Zealand strains produced F₁ hybrids with relatively mild autoimmune disease (32-35). Analysis of NZB X AKR F₁ mice and backcrosses to AKR parents suggested that three to five unlinked genes controlled the production of antierythrocyte autoantibodies and that one of the genes is on the X chromosome (32). In addition, autoimmune traits were found to be independent of xenotropic viral expression (36).

The main difficulty with all genetic analyses of inheritance of autoimmune traits is the complexity of the disease process. The production of disease signs and autoantibodies may be the end result of the action of several genes.

Autoantibody production, once considered manifestations of abnormal autoimmunocompetent lymphocytes, now are thought possibly to result from aberrant immunoregulation (37). Despite these problems, previous studies have suggested a limited number of genes controlling a variety of autoimmune phenomena in NZB offspring. We have confirmed and extended these suggestions in the present study. We have provided evidence for a single dominant gene controlling the expression of spontaneous anti-ssDNA antibodies. The quantity of anti-ssDNA produced is controlled either by gene dosage (i.e., two genes lead to more anti-ssDNA than one gene) or, more likely, by a second regulating gene. Spontaneous production of NTA appeared to be inherited as a single codominant trait. No linkage between the gene regulating NTA production and the genes regulating antibodies to ssDNA was found.

Inasmuch as both NZB and DBA/2 have the same H-2 type, the locus controlling production of either autoantibody appears not to be within the *H-2* complex. Other investigators have provided evidence for non-*H-2* immune response genes (15, 38-40). In addition to non-*H-2* control of antigen recognition, there is non-*H-2* genetic control of the amount of antibody synthesized (41, 42). For example, the control of anti-L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ is under H-2 control but the amount of antibody produced by responders is controlled by non-*H-2*-linked genes (43). At least one of the genes controlling the magnitude of the antibody response is linked to the immunoglobulin heavy chain allotype linkage group (43, 44). The possibility that the production of autoantibodies is linked to heavy chain allotype is under investigation.

Finally, the role of sex hormones in modifying disease has not been adequately considered in previous genetic analyses. In the present studies, it was of considerable interest that intact offspring did not provide clear-cut data with regard to the genetic contribution of NZB mice to autoantibody production in their offspring. However, when gonadectomized offspring were studied, the data suggested that a single codominant gene controlled the presence of NTA and that an independent dominant gene controlled the production of autoantibodies to ssDNA. The quantity of each autoantibody is determined either by gene dosage or an independent regulatory gene. In addition to these findings, this study provides evidence for the theoretical claim that genetic analyses of phenotypes modified by sex hormones are best analyzed in gonadectomized progeny.

Summary

The appearance of naturally occurring thymocytotoxic autoantibodies (NTA) and spontaneously produced antibodies to single-stranded DNA (ssDNA) was studied in NZB, and DBA/2 mice and their F₁ and backcross progeny. NTA production was markedly decreased in males; however, castrated males produced quantities of NTA similar to those of females. Because the amount of NTA was influenced by sex hormones, it was necessary to gonadectomize all progeny to determine the mode of inheritance. Such studies suggested that NTA production was determined by a single locus with a gene dosage (codominant) mode of expression.

The spontaneous production of antibodies to ssDNA appeared to be inherited as a single dominant genetic trait. The quantity of anti-ssDNA was also found

to be under additional regulation; either a gene dosage effect or more likely a regulatory gene. The genes controlling the presence and quantity of ssDNA antibodies were not linked to the gene controlling the appearance of NTA.

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