

GENETIC STUDIES IN NZB MICE

V. Recombinant Inbred Lines Demonstrate That Separate Genes Control Autoimmune Phenotype

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The genetic basis for autoimmunity in New Zealand mice has been investigated for two decades. These studies have suggested that several genes underlie the autoimmune process (1-10). The advent of new murine models of autoimmunity that display some of the traits of NZB mice while lacking others has led to a resurgence of interest in the genetic basis for autoimmunity (11-14). The major question is whether there is a single fundamental defect that gives rise to autoimmunity.

We have investigated this question by trying to determine whether the autoimmune traits in NZB mice are linked to or dependent upon another trait for their appearance. We have previously shown (15) by classical genetic analyses of F_1 and backcrosses of NZB \times DBA/2 mice that antibodies against T cells and antibodies directed against single-stranded DNA (ssDNA)¹ were genetically determined traits not linked to each other. Moreover, they were expressed in a gene dosage fashion. Other traits, splenic aneuploidy and splenomegaly, were inherited as recessive traits; they were found only in backcross mice (16). By analyzing several other crosses of NZB mice with non-autoimmune mice, we hoped to determine whether these were consistent findings.

In addition to classical genetic studies, we have also used a powerful genetic tool, recombinant inbred (RI) lines, to further analyze the inheritance of autoimmunity (17-20). RI lines were obtained by mating an NZB mouse with a non-autoimmune mouse. The F_2 mice were separately mated to initiate the RI lines and then brother-sister mated at each successive generation. Each RI line can have any combination of parental genes. After several generations of inbreeding, individual RI lines become largely homozygous at most loci. Therefore, recessive genes are observed as easily as dominant genes. Our studies indicate that multiple genes underlie NZB disease; no single gene for autoimmunity was discovered.

Materials and Methods

Mice. The progenitor strains that gave rise to the various RI lines were obtained from the Division of Research Services, National Institutes of Health, Bethesda, Md. The RI lines were derived from pairs of (ALN \times NZB) F_2 or (inbred NIH Swiss [NFS] \times NZB) F_2 mice. Each RI line was then inbred by brother-sister mating for at least 14 generations. B lines are RI lines produced from (NZB \times ALN) matings and C lines are produced from (NFS \times NZB) matings.

¹ *Abbreviations used in this paper:* NTA, naturally occurring thymocytotoxic autoantibodies; RI, recombinant inbred; SIp, sex-limited serum protein; ssDNA, single-stranded DNA.

To date, we have studied 12 B lines and 15 C lines. Individual mice from each RI line were then studied for various NZB traits. At least 10 males and 10 females from every line were analyzed. The measurements of various autoantibodies, splenomegaly, aneuploidy, and abnormal cell cycle kinetics were performed on mice at 1 yr of age. A line was considered to be positive for a trait if at least 60% of the mice from that line manifested the trait. A few RI lines were significantly different from both NZB parents and normal parents.

In addition to RI mice, various other mice were studied. NZB/N and DBA/2 mice were crossed in our laboratory to produce F₁ progeny. F₁ hybrids were backcrossed to both parental strains. Additional crosses involved (NZB × C3H/HeN) and (NZB × BALB/c); both of these F₁ hybrids were backcrossed to their respective parents.

Sera. Mice were bled by retro-orbital sinus puncture under light ether anesthesia. The blood was allowed to clot for 2 h at room temperature; the sera were removed and stored at -20°C until used.

Naturally Occurring Thymocytotoxic Autoantibodies (NTA). NTA was measured by a chromium release assay using selected rabbit complement as previously described (15). A positive test was >50% cytotoxicity at a serum dilution of 1:4.

Antibodies Directed against ssDNA. Spontaneously occurring antibodies reactive with ssDNA were measured by the Farr technique as previously described (15). Positive sera had >25% binding.

Antibodies Directed against Erythrocytes. Antibodies against erythrocytes were measured by a direct Coombs test (21). A test was considered strongly positive when it had a titer of >6 wells (1:640 final dilution of the anti-Ig reagent).

Serum IgM Concentration. IgM levels were determined by radial immunodiffusion with Meloy plates (Meloy Laboratories Inc., Springfield, Va.). Known positive and negative controls were included on each plate. An IgM concentration was considered abnormal if it was >0.6 mg/ml.

Splenomegaly. Immediately after a mouse was killed by cervical dislocation, the spleen was removed and weighed. Spleen weights >250 mg were considered abnormal.

Aneuploidy. Mitotic spleen cells were analyzed for chromosome number as previously described (16). If >10% of the spleen cells had at least 42 chromosomes, the mouse was considered abnormal.

Cell Cycle. Spleen cells were stained with propidium iodide (22) and analyzed on a TPS I cell sorter (Coulter Electronics, Inc., Hialeah, Fla.) for the amount of fluorescence due to DNA content. Percentages of cells in G₁, S, and G₂ + M phases of the cell cycle were determined using a mathematical analogram. Percentages of cells >10% in S or >2% in G₂ + M were considered abnormal.

Sex-limited Serum Protein (Slp). Slp was measured by radial immunodiffusion as previously described (23). Abnormal female production of Slp was >0.5 U/ml.

Results

Prevalence of Autoimmune Traits in RI Lines. Table I shows the prevalence of various traits in the RI lines derived from NZB matings. Parent B, which gave rise to the B lines, displayed none of the abnormal traits found in NZB mice. However, parent C, which is an inbred NFS, did produce high-titered anti-T cell antibodies. These anti-T cell autoantibodies were not associated with excessive IgM production, whereas the two traits are associated in NZB mice. Anti-T cell antibodies appear to be regulated by separate genes in the two strains because several NFS × NZB lines did not produce anti-T cell antibodies. In NZB mice, the control is by a single locus (15; and data from the B lines).

No RI line displayed all of the traits of the NZB parent. Lines C-10 and C-11 displayed six of the seven NZB traits studied. Line B-8 displayed none of the autoimmune traits of NZB and was therefore similar to the non-autoimmune ALN parent, with the exception of abnormal female production of Slp. The abnormal female production of Slp has not been found to be associated with any immunological

TABLE I
Prevalence of Various Traits Characteristic of NZB Mice in RI Lines*

RI line	NTA‡	↑ IgM	Anti-ssDNA	Anti-erythrocytes	Slp	Splenomegaly	Hyperdiploidy	↑ Cells in S phase
NZB	+ (90)	+ (90)	+ (80)	+ (100)	+ (100)	+ (90)	+ (70)	+ (90)
Parent B	- (10)	- (0)	- (10)	- (0)	- (0)	- (0)	- (0)	- (0)
Parent C	+ (100)	- (0)	- (0)	- (0)	- (0)	- (10)	- (0)	- (10)
B-1§	- (13)	+ (80)	- (9)	- (0)	- (0)	- (0)	- (0)	- (0)
B-2	+ (100)	- (12)	+ (75)	- (0)	+ (100)	- (0)	- (0)	- (0)
B-3	+ (67)	+ (67)	+ (80)	- (0)	+ (100)	- (0)	- (0)	- (0)
B-4	- (0)	- (0)	+ (100)	- (0)	+ (100)	- (0)	- (0)	- (0)
B-5	+ (67)	+ (100)	- (17)	- (0)	+ (100)	- (0)	- (0)	- (0)
B-6	ND	+ (73)	+ (83)	ND	- (0)	+ (100)	+ (66)	ND
B-7	+ (86)	+ (60)	+ (100)	- (0)	+ (100)	+ (100)	- (0)	ND
B-8	- (0)	- (0)	- (0)	- (0)	+ (100)	- (0)	- (0)	- (0)
B-9	- (0)	- (0)	+ (83)	ND	- (0)	- (0)	- (0)	+ (100)
B-10	- (0)	- (0)	+ (83)	ND	+ (100)	- (0)	- (0)	- (0)
B-11	- (0)	- (20)	- (0)	ND	+ (100)	+ (100)	+ (60)	ND
B-12	+ (100)	+ (100)	+ (100)	ND	+ (100)	ND	ND	ND
B-13	- (0)	- (0)	+ (100)	- (0)	+ (100)	- (0)	- (0)	- (0)
B-14	- (25)	+ (60)	- (0)	- (0)	- (0)	- (0)	- (0)	ND
B-15	+ (82)	+ (86)	- (13)	- (0)	- (0)	- (0)	- (0)	- (0)
C-1	+ (75)	+ (75)	+ (100)	- (0)	+ (100)	- (0)	+ (71)	+ (100)
C-2	- (27)	- (31)	+ (100)	+ (60)	- (0)	+ (100)	+ (80)	- (0)
C-3	- (14)	- (0)	+ (90)	+ (75)	- (0)	- (0)	- (0)	- (0)
C-4	+ (90)	+ (80)	- (21)	- (0)	- (0)	+ (75)	+ (100)	- (0)
C-5	+ (83)	- (0)	- (31)	- (17)	+ (100)	+ (100)	+ (100)	- (0)
C-6	- (0)	- (0)	- (10)	- (0)	+ (75)	- (0)	- (0)	- (0)
C-7	+ (63)	+ (100)	- (35)	- (15)	- (0)	- (0)	+ (60)	+ (60)
C-8	- (25)	- (0)	- (0)	- (0)	ND	+ (100)	+ (66)	+ (100)
C-9	+ (100)	+ (100)	+ (100)	- (0)	ND	+ (100)	- (0)	+ (100)
C-10	+ (71)	+ (71)	+ (100)	+ (67)	ND	+ (100)	+ (100)	- (0)
C-11	+ (100)	+ (100)	+ (100)	- (0)	ND	+ (100)	+ (100)	+ (100)
C-12	+ (83)	+ (100)	+ (100)	- (0)	ND	+ (100)	+ (100)	- (0)

↑ indicates increase over normal.

* Only female data are presented because there were sex differences in some lines. The percentage of 10-30 mice examined expressing the trait in an individual line, or parent strain, is shown in parentheses.

‡ Plus sign indicates presence of trait; minus sign indicates lack of trait.

§ B-1, B-2, B-3, etc., are each separate RI lines. There are 12 different B lines and 15 different C lines.

|| Not determined.

abnormalities in the present study and in separate backcross experiments (data not shown).

A summary of the prevalence of NZB traits in the two different RI lines is presented in Table II. NTA, anti-ssDNA, and increased IgM each occurred in ~50% of the lines, which suggests control by single genes. The results in B lines and C lines were similar. However, B lines differed significantly from C lines in the incidence of splenomegaly and hyperdiploidy. In addition, none of the B lines displayed anti-erythrocyte autoantibodies, whereas 25% of the C lines were positive for anti-erythrocyte autoantibodies (although this difference is not statistically significant, we believe that it might be biologically important).

Based on the incidence of autoimmune traits in RI lines, various genetic models

TABLE II
Summary of RI Lines

RI lines	NTA	↑ IgM	Anti-ssDNA	Anti-erythrocytes	Females with Slp	Splenomegaly	Hyper-diploidy	↑ Cells in S phase
B lines	6/14* (43)	8/15 (53)	9/15 (60)	0/10 (0)	10/15 (67)	3/14‡ (21)	2/14‡ (14)	1/10 (10)
C lines	8/12 (67)	7/12 (58)	7/12 (58)	3/12 (25)	3/7 (43)	8/12‡ (67)	9/12‡ (75)	5/12 (42)
All lines	14/26 (54)	15/27 (56)	16/27 (59)	3/22 (14)	13/22 (59)	11/26 (42)	11/26 (42)	6/22 (27)

* Fractions represent number over total. Percentages are in parentheses.

‡ B lines significantly different from C lines by Fisher exact test (two-tailed), $P < 0.05$.

TABLE III
Expected Outcome of Different Modes of Inheritance in RI Lines

Type	NZB genotype required for phenotype (+)	Genotype of normal parent without phenotype (-)	Genotypes of possible RI lines and phenotype (+ or -)	Frequency of expression of RI lines %
I One gene	AA(+)	aa(-)	AA(+) aa(-)	50
II Two genes	AABB(+)	aabb(-)	AAbb(-) AABB(+) aabb(-) aaBB(-)	25
III Three genes	AABBCC(+)	aabbcc(-)	AABBCC(+) AAbbcc(-) aaBBcc(-) aaBBCC(-) aabbCC(-) AABBcc(-) aabbcc(-) AAbbCC(-)	12.5
IV Two genes; normal parent with one gene but lacking expression of trait	AABB(+)	AAbb(-)	AABB(+) AAbb(-)	50
V Three genes; normal parent with one gene but lacking expression of trait	AABBCC(+)	AAbbcc(-)	AABBCC(+) AAbbcc(-) AABBcc(-) AAbbCC(-)	25
VI One trait; controlled in individual strains by different single genes and both parents expressing trait	AAbb(+)	aaBB(+)	AAbb(+) AABB(+) aaBB(+) aabb(-)	75
VII Three genes; normal parent with two genes but lacking expression of trait	AABBCC(+)	AABBcc(-)	AABBCC(+) AABBcc(-)	50

were analyzed for closeness of fit to the observed data for both B and C RI lines. In Table III, the expected incidence of traits in RI lines is shown for various modes of inheritance. As inbreeding proceeds, heterozygotes are diminished, and full homozygosity at all loci is approached. As a result, dominant and recessive genes are detectable with equal frequency. Theoretically, each RI line represents multiple replicates of an individual F_2 recombinant mouse. The presence of multiple copies

diminishes problems of penetrance in genetic analysis. For instance, in NZB mice, NTA are expressed with a 0.91 frequency by standard genetic analysis of F₁ and backcross mice (15). This lack of complete penetrance complicates F₂ analyses. However, in RI line analysis, all but one mouse in an RI line may display anti-T cell antibodies, and this line can easily be identified as positive for the presence of NTA. This advantage is even more obvious when penetrance is only in the 0.7–0.8 frequency range, as is the case for aneuploidy.

In Table IV, the most likely genetic model that was shown in Table III is assigned to each of the traits studied in the RI lines. Genetic mechanisms for the traits studied in the RI lines were assigned on the basis of (a) genetic models already determined on the basis of F₁ and backcross studies (15); (b) prevalence of a trait in B lines and linkage with other traits; and (c) comparison of the prevalence of the traits in C lines

TABLE IV
Most Probable Type of Inheritance of Traits in RI Lines Based on the Models Shown in Table III

RI lines	NTA	↑ IgM	Anti-ssDNA	Anti-erythrocytes	Slp	Spleno-megaly	Hyperdiploidy	↑ cells in S phase
B lines	I*	I	I	III	I	II	II	III
C lines	VI	I	I	V	I	IV	IV	VII
Minimum number of required genes	1	1	1	3	1	2	2	3
Minimum number of genes for NZB phenotype	1	+ 0‡	+ 1	+ 1§	+ 1	+ 1	+ 0¶	+ 1** = 6

* Roman numerals stand for models shown in Table III.
 ‡ Linked to NTA.
 § The other two could be (i) suppressor gene and (ii) either of the previous two genes.
 || One could be one of the previous genes.
 ¶ Could be any of the previous genes.
 ** The other two genes could be any of the previous genes.

TABLE V
Analysis of Linkage of Autoimmune Traits in RI Lines

	Hyperdiploidy (+)	Hyperdiploidy (-)
Spleno-megaly (+)	9	2
Spleno-megaly (-)	2	13*
	High IgM(+)	High IgM (-)
NTA (+)	12	2
NTA (-)	2	10*

The appearance of NTA is linked to the presence of hypergammaglobulinemia M, and the presence of splenic hyperdiploidy is linked to the presence of an enlarged spleen. No other associations among the various traits studied were found.
 * P < 0.001.

with that in B lines. Among the B lines, NTA and anti-ssDNA autoantibodies appear to be under the control of separate single genes. C lines confirm these findings.

The incidence of splenomegaly in B lines is 3/14. If two genes were involved, the expected incidence would be 3.5/14. This is close to the observed value. In C lines, the incidence is much higher, suggesting that the normal NFS parent, which does not display splenomegaly, has one of the two genes that leads to an enlarged spleen.

The number of RI lines in which the females produce SIp is similar to the number of lines that would be expected to have abnormal female SIp production if the trait were controlled by a single gene. A previous report with a limited number of RI lines has indicated that abnormal female production of SIp was controlled by one or two genes (24). In the RI lines, the presence of female SIp was not strongly associated with any of the autoimmune traits studied.

Association of Autoimmune Traits in RI Lines. One of the uses of RI lines is the location of genes on particular chromosomes through linkage analysis with known genetic markers. Additional studies will be required to determine the location of particular genes. Our initial investigations have been confined to the determination of the expression of autoimmune traits in RI lines and their linkage. If two traits segregated independently, the distribution should be random. On the other hand, if the two loci are linked, the frequency of recombinant types, which are positive for one trait and negative for the other, should be significantly lower. The observed distribution for splenomegaly and hyperdiploidy varies significantly from that expected if the two traits were not linked (Table V). Therefore, splenomegaly and the presence of chromosomal hyperdiploidy appear to be linked. This might occur if at least one of the genes for splenomegaly also is one of the genes for hyperdiploidy. Alternatively, one or two genes for the two traits might be closely linked, but not identical. In addition, NTA was also associated with increased IgM levels (Table V). These traits may also be closely linked, but not controlled by the same gene, thereby accounting for the four divergent lines. Alternatively, the same major gene may code for both, but minor genes could account for the divergent lines [IgM (+) and NTA (-), or

TABLE VI
Distribution of Hyperdiploidy- and Splenomegaly-positive and -negative Mice for All Backcrosses

	Splenomegaly (+)	Splenomegaly (-)
Hyperdiploidy (+)	15	2
Hyperdiploidy (-)	14	10*

* $P < 0.05$, Fisher exact test (one-tailed).

TABLE VII
Classical Genetic Analysis in Females of Anti-Erythrocyte Antibodies

Cross	Positive for Anti-erythrocytes
NZB	15/15* (100)
BALB/c	1/12 (8)
(BALB/c × NZB) F_1	1/12 (8)
F_1 × NZB	14/22 (64)
BALB/c × F_1	1/14 (7)

* Fractions represent number over total. Percentages are in parentheses.

NTA (+) and IgM (-)]. No other associations among the various traits were found besides splenomegaly-hyperdiploidy and IgM-NTA.

Comparison of Results Obtained with RI Lines with Those Obtained by Classical Backcross Analysis. To ascertain if the proposed genetic models for the inheritance of a trait in the RI mice is similar to that obtained by classical genetic analysis, the NZB strain was separately mated with non-autoimmune DBA/2, C3H/Hen, and BALB/c mice, and the F₁ and backcross progeny were analyzed for the presence of hyperdiploidy and splenomegaly, two traits found to be linked in the RI lines. Both hyperdiploidy and splenomegaly were inherited in a recessive manner (data not shown). Hyperdiploidy was controlled by at least one recessive gene and perhaps one or more additional genes, one of which might be present in BALB/c mice. In addition, hyperdiploidy and splenomegaly were studied for any association with each other. 88% of the time hyperploidy was observed, splenomegaly was also present (Table VI). This is significantly different from what would be expected if no association between the two traits were present, and is similar to the results obtained in RI lines.

In addition, F₁ and backcross animals from crosses of the NZB strain with the non-autoimmune strain BALB/c were studied for the presence of anti-erythrocyte antibodies (Table VII). The trait was inherited in a recessive manner and was controlled by at least one recessive gene and any number of dominant genes. Analysis in RI mice indicated that anti-erythrocyte antibodies are controlled by more than one gene. The combined results suggest that anti-erythrocyte antibodies are controlled by one recessive gene and one or two dominant genes.

Discussion

The use of RI lines, in addition to classical genetic studies, has provided insight into the genetic basis for autoimmunity in NZB mice. This analysis has indicated that multiple genes (at least six) are responsible for the autoimmune traits of NZB mice. That is, these genes are present in NZB mice, but not in several non-autoimmune strains studied. Anti-T cell antibodies and antibodies to ssDNA were each found to be inherited as single traits controlled by unlinked genes in RI mice. These conclusions have been supported by standard genetic analyses of F₁ and backcross mice. The results are consistent with our previous report of a single dominant gene for ssDNA expression and a separate gene for anti-T cell antibodies (15). Our results are also consistent with the suggestions of others (25) that different traits of NZB mice are unlinked.

Studies of NZB × NZC crosses suggested that a single dominant gene was responsible for anti-erythrocyte autoantibody production (5). Additional analyses indicated the involvement of a dominant gene and also a recessive gene (10) or, as Burnet and Holmes postulated (1), the involvement of at least three genes. Our studies, both in backcross mice and in RI mice, support the idea that at least two and possibly three genes (one of which is recessive) are responsible for anti-erythrocyte autoantibodies.

Splenic B cells of NZB mice spontaneously secreted many times more IgM than those of normal strains (26). This spontaneous hypersecretion of IgM is due to at least two independently segregating mechanisms (27). One was a single dominant gene that determined the number of IgM-containing cells; a second recessive gene controlled the amount of IgM secreted per cell. However, the amount of IgM spontane-

ously secreted per cell was regulated by the number of IgM-containing cells in the population (27). In the experiments reported herein, IgM levels seem to be under single gene control, similar to the control of the number of IgM-containing cells (27). It is possible that other genes affect serum IgM levels, but the major genetic control appears to be a single gene.

The RI analysis and the classical F_1 and backcross analyses actually provide somewhat different and complementary information. The F_1 studies show whether a trait is expressed in a dominant fashion. If it is dominant, backcrosses to the parent not expressing the trait (in this case, the non-NZB parent) will allow for an analysis of the number of dominant genes required for the trait. If the trait is not expressed in F_1 mice, backcrosses to the parent expressing the trait (in this case, NZB) will allow an assessment of the number of recessive genes necessary for expression of the trait. However, such an analysis will not give an estimate of the number of genes necessary for the trait if there are both recessive and dominant genes necessary for the trait. This difficulty is overcome in the RI analysis. In RI mice, dominant and recessive genes are expressed equally; therefore, the frequency of a given trait is determined by the number of genes necessary for its expression; that is, the number of genes in which the "normal" and "abnormal" parents differ with regard to expression of that trait. Thus, if 50% of the lines express a trait, the two parental strains differ with respect to only one gene; a single gene from the abnormal parent is most likely responsible. A trait that depends upon the presence of two abnormal genes would lead to a 25% frequency in the RI lines. The use of RI lines has many advantages over F_2 analyses. One advantage is minimization of the problem of incomplete penetrance of a trait by virtue of multiple animals with the same genetic make-up. As a result, a trait with only 70 or 75% penetrance can easily be identified in an individual RI line because several animals from the line are studied. In contrast, incomplete penetrance of that degree completely mars F_2 analyses. In our RI analysis, major genes determining a trait have been analyzed; however, minor genes that might influence the magnitude of the expression of a trait have, for simplicity, not been considered. It should be recognized that each RI line in its own right represents an inbred line with various manifestations of autoimmunity. Further studies of individual RI lines might help to elucidate the contributions of minor genes to the expression of individual autoimmune traits.

In the present study, we benefitted from the availability of RI lines from NZB matings with more than one non-NZB parent. This allowed us to estimate the genetic contribution to various traits of the non-NZB parent, and, thereby, to assign more correctly the contribution to the trait of genes unique to NZB mice. For example, B lines and C lines differed significantly in the expression of splenomegaly and hyperdiploidy. If only C lines were studied, one would propose that splenomegaly might be controlled by one gene on the basis of a 67% frequency. However, the 21% frequency of splenomegaly in B lines points to two genes, with the C parent possessing one of the two genes necessary for expression of the trait. Studies of additional RI lines with other non-NZB parents will allow gene assignments to be made with even greater confidence.

Some of the NZB traits were found to be linked in RI lines. Splenomegaly was found to correlate with the presence of splenic hyperdiploidy. 82% of the RI lines that displayed hyperdiploidy also had splenomegaly. A similar significant association

(88%) was found in classical backcross analyses. We have previously found (28) that hyperdiploid spleen cells are derived from NZB bone marrow stem cells. We presume that the splenomegaly and the hyperdiploidy have related cellular bases, thereby explaining their strong concordance. These abnormal B cells have been found to be limited to the Lyb-5⁺ subset (28, 29). The same may be true of the abnormal increase in cycling cells in the S phase of DNA synthesis. It is likely that rapidly replicating B cells contribute to the enlarged spleen, to hyperdiploidy, and to the abnormal cell cycle observed in NZB mice.

The present study has helped to resolve the question of a gene necessary for the expression of generalized autoimmunity in NZB mice. If such an "autoimmunity gene" existed, only half of the RI lines would be expected to possess this prerequisite gene, and only these lines could express autoimmune traits. Lines that did not receive the autoimmunity gene could possess genes for individual autoimmune traits, but could not express them in the absence of a postulated autoimmunity gene. Because each RI line has a 50% chance of inheriting each NZB gene, the requirement for both a gene for an individual trait, and an autoimmunity gene for its expression, would mean that only 25% of the lines could express the trait. As many as 50% of the lines could express the trait if the gene for the trait and the autoimmunity gene were closely linked. However, both anti-T cell antibodies and anti-ssDNA antibodies were found to be unlinked and present in 50% of the RI lines. This observation argues strongly against an autoimmunity gene necessary for the expression of autoimmune traits. Rather, it appears that NZB disease is based upon several genes, which, in the aggregate, allow the expression of the autoimmune syndrome of NZB mice.

Summary

The genetic basis for autoimmunity in NZB mice has been investigated through analysis of recombinant inbred lines produced by mating NZB mice with two different non-autoimmune strains. Several genes (at least six) were found to be necessary for the production of eight traits characteristic of the NZB mice that were studied. No fundamental genetic defect (an "autoimmunity gene") was identified that could give rise to the various autoimmune traits studied. This study strongly suggests that NZB disease results from the actions of several separate genes that together result in the characteristic manifestations of autoimmunity.

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