The Journal of Immunology

RESEARCH ARTICLE | FEBRUARY 01 1980

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*J Immunol* (1980) 124 (2): 787–789.
https://doi.org/10.4049/jimmunol.124.2.787

0022-1767/80/1242-0787$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 124, No. 2, February 1980
*Printed in U.S.A.*

# ANTIBODY GENE LINKAGE STUDIES IN (NZB × C58) RECOMBINANT-INBRED LINES[1,2]

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**A set of recombinant inbred mouse strains has been constructed from the cross of NZB by C58. These strains provide new tools for the analysis of antibody heavy and light chain genes and NZB autoimmunity. Typing of heavy chain genes confirmed the allotype linkage of the dextran response locus *Igh-Dex*. One strain has fixed a recombinant chromosome carrying NZB allotype genes and the *Dex*$^+$ variable region gene from C58. Two κ-light chain variable region genes, *Igk-Pc* and *Igk-Ef1*, co-segregated with the *Lyt-2* lymphocyte alloantigen locus. This confirms the tight linkage of these loci and decreases the maximum estimate of the recombination frequency between them to 1.7%.**

Donald Bailey's introduction of recombinant inbred (RI)[3] strains of mice has provided an important tool for genetic analysis. His original CXB lines (1) and RI lines subsequently developed by Taylor *et al.* (2), such as the AKXL lines, have been extensively used for the linkage analysis of a variety of markers. We describe here preliminary studies on a new set of RI lines. These originated from the F2 generation of crosses between NZB and C58 and are designated NX8 lines. At present, 12 lines that originated from independent matings of (NZB × C58)$F_2$ mice have been inbred for more than 20 generations.

This parental combination was chosen because NZB and C58 differ at a number of loci relevant to the immune system. In this paper the strain distribution patterns (SDP) of the alleles at certain of these loci are described, and resultant linkage information is analyzed. One of the strains, NX8-3, has a recombinant genotype at the heavy chain locus. It is hoped that the NX8 RI lines will be of use in studying the genetic control of many immunologic traits and especially certain traits unique to either the NZB or C58 strains.

Received for publication October 2, 1978.
Accepted for publication November 1, 1979.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This investigation was supported in part by United States Public Health Service Grants GM20964, AI12533, AI13797, CA06927, RR05539, American Cancer Society Grant IM157, and an appropriation from the Commonwealth of Pennsylvania, and by the Medical Research Council of Canada, Grant MA-4317.
[2] These recombinant inbred (RI) lines are designated NX8 followed by a numeral denoting the particular line, e.g., NX8-1, etc.
[3] Abbreviations used in this paper: RI, recombinant inbred; SDP, strain distribution pattern; PC-KLH, phosphorylcholine-derivatized keyhole limpet hemocyanin.

## MATERIALS AND METHODS

The progenitor strains that were crossed to give rise to the NX8 RI strains were NZB/NIcr, an NZB substrain obtained from the National Institutes of Health and maintained at The Institute for Cancer Research since 1970, and C58/J, purchased from The Jackson Laboratory, Bar Harbor, Maine. A panel of 20 NX8 RI lines was initiated from 20 pair of (NZB × C58)$F_2$ mice. To ensure equal contributions of the two progenitor strain X and Y chromosomes into the RI panel, reciprocal crosses were done. Thus, strains 1 through 5 were derived from $F_2$ mice from the cross (NZB × C58)$F_1$ × (NZB × C58)$F_1$, strains 6 through 10 were initiated from [(NZB × C58)$F_1$ × (C58 × NZB)$F_1$] mice, strains 11 through 15 from [(C58 × NZB)$F_1$ × (C58 × NZB)$F_1$], and strains 16 through 20 from [(C58 × NZB)$F_1$ × (NZB × C58)$F_1$] (the female parent is listed first). Each strain was then inbred by brother-sister mating. Twelve strains are now extant and have been inbred for more than 20 generations. In selecting mice for testing in this study, care was taken to stay as close as possible to the line of descent of each strain. All retired mating pairs of each strain were tested for *Igh-1* and *Igh-Dex* alleles, and many were tested for *H-2* types. Generally sera from four mice for each strain were tested as a pool for *Igk-Pc*, and two to four of these same mice were individually typed for *Lyt-2*. Sera pooled from two to four different but closely related mice were typed for *Igk-Ef1*.

*Igh-1.* The allotypes of the RI strains were determined by Ouchterlony techniques and by inhibition of hemagglutination of myeloma protein-sensitized sheep erythrocytes (3).

*Igh-Dex.* As previously described (4), the nature of the antibody response of mice to α-1,3-linked dextrans is controlled by a single gene that affects the titer, light chain content, and variable region idiotype of anti-dextran antibodies. C58, like BALB/c carries the *Dex*$^+$ or *Dex*$^a$ allele and responds with a high titer of λ-containing antidextran antibodies that have the idiotype of the J558 myeloma protein. NZB, like C57BL/6, has the *Dex*$^-$ or *Dex*$^o$ allele and responds poorly to dextran, with κ-containing, idiotype-negative antibodies. NX8 RI mice were immunized i.p. with 50 μg dextran B1355S in saline and bled 1 week later. The presence of the J558 idiotype in anti-dextran antibodies was determined by anti-idiotype inhibition of hemagglutination of dextran-sensitized sheep erythrocytes (4).

*H-2.* H-2 typing was performed by a modified hemagglutination technique (5) by using anti-*H-2K*$^k$ (D-23) and anti-*H-2D*$^d$ (D-4) alloantisera obtained from the Transplantation Immunology Branch, National Institutes of Health.

*Lyt-2.* Lyt-2 antigen was detected by indirect immunofluorescence on thymocyte suspensions under conditions described elsewhere (6). The positive pattern of dots on thymocyte surfaces was consistent with previously reported immunofluores-

cence for Lyt antigens. (NZB × C58)$F_1$ heterozygote as well as parental controls were tested at the same time as the RI lines. This assured that the reagents were used at sufficient levels to detect heterozygous as well as homozygous positive and negative animals for both Ly 2 specificities.

Anti-Lyt-2.1, C57BL/6/*H-2*$^k$ (B6/H-2$^k$) anti-CE normal thymocytes, was a generous gift of Doctors F-W. Shen and E. A. Boyse. This reagent detects Lyt-2.1 antigen expressed by the *Lyt-2*$^a$ allele of C58 mice. The serum was preabsorbed with NZB lymphoid cells to avoid contaminating specificities (Mathieson, Sharrow, and Bottomly, unpublished results). After absorption it was shown to be positive with the appropriate fluorescent pattern on DBA/2N, C58 and B6/Ly 2.1 (an Ly congenic strain of mice) and negative on thymocytes of NZB, B6 and B6/$G_{1X}$+ mice. For standardization, 25 μl of 1:20 diluted, absorbed antiserum was used on $10^6$ cells.

Anti-Lyt-2.2, (C3H/HeN × B6/Ly 2.1)$F_1$ anti-B6 normal thymocytes, was prepared by B. J. M. and detects the Lyt-2.2 antigen expressed by the *Lyt-2*$^b$ allele of NZB. This serum was absorbed as a precautionary measure with AKR/N lymphomas and B6/Ly-2.1, 3.1 normal lymphoid cells. This serum was positive in immunofluorescence tests with NZB and B6 thymocytes and negative on C58, DBA/2N and B6/Ly-2.1 thymocytes. Standard tests with this antiserum were performed with 50 μl of 1:15 diluted, absorbed antiserum on $10^6$ cells.

The fluorescent reagent in these tests were affinity purified, goat anti-mouse Ig prepared by P. S. Campbell and kindly provided by Dr. R. Asofsky, NIAID, Bethesda, Maryland (7).

*Igk-Pc.* Mice were immunized with two i.p. injections of 100 to 200 μg of phosphorylcholine-derivatized keyhold limpet hemocyanin (PC-KLH, five groups per 100,000 daltons) in complete Freund's adjuvant. Sera were collected 4 to 8 days after the second injection and antibodies to PC purified by affinity chromatography as previously described (8). The presence of the HOPC8 L chain in isoelectric focusing gels was determined by comparison with purified HOPC8 L chains. The phenotypic variants, Pc-A and Pc-B which are controlled by *Igk-Pc*$^a$ and *Igk-Pc*$^b$, were identified by comparison with AKR/J and C57L/J, respectively (9). Both phenotypes can be detected when one is present in as little as one-twentieth the concentration of the other.

*Igk-Efl.* Normal light chain isoelectric-focusing profiles were analyzed as previously described (10, 11). This procedure involved purification of normal immunoglobulin by using protein A-Sepharose, complete reduction and alkylation with $^{14}$C-iodoacetamide, separation of heavy and light chains by urea-formate gel electrophoresis and isoelectric focusing of the light chain fraction. This procedure permits the resolution of normal mouse light chains into approximately 75 to 80 recognizable bands (11). Differences involving at least four bands permitted distinction of the C58 and NZB light chain patterns. The C58 characteristics identified were 26–, 58+, 61+, 66+, indicating an elevation of bands 58, 61, and 66 and a reduction in band 26, compared with NZB. These differences were common to the inbred strains AKR/J, RF/J, PL/J and C58/J. The locus defined by this marker has been designated *Igk-Efl* and the allele carried by the above four strains is designated *Igk-Efl*$^a$ (12). All other inbred strains so far analyzed (including NZB) are designated *Igk-Efl*$^b$.

## RESULTS AND DISCUSSION

At the time of this study, the 14 NX8 lines described in this report had each been bred for at least 13 generations of brother-sister mating (Table I). It is probable ($p>0.9$) that each line was homozygous for any given locus. For the co-dominant markers *H-2* and *Igh-1* this was the case. *Igh-Dex* is a dominant marker, and heterozygosity could not be assessed. All 14 lines were tested for *Lyt-2* and the L-chain markers, *Igk-Pc* and *Igk-Efl*. Both alleles of *Lyt-2* were present in the NX8-9 and -20 strains. Some of the tested mice of strains 9 and 20 were heterozygous at the *Pc* locus, and heterozygosity at *Efl* was observed in strain NX8-9. That this degree of heterozygosity was present at F13 and beyond is not unlikely. The probability that two or more of 14 lines are not yet fixed at a specific locus at F13 is approximately 0.42. This heterozygosity may, however, result from a situation in which polymorphism at these loci or a closely linked locus is of selective advantage. These strains will be tested at later generations to examine this issue.

SDP for six loci have been obtained in most or all of the NX8 lines (Table I). As expected, *H-2*, *Lyt-2* and allotype (*Igh-1*) segregate as single, independent loci. Concordance between linked loci was observed. Thirteen out of the 14 lines are concordant for the *Igh-1* locus and *Igh-Dex*, the locus controlling the Vh region structure of anti-α1,3 dextran antibodies. Since in the derivation of these lines there are multiple opportunities for recombination, it is not unexpected that cross-overs between Vh and *Igh-1* could happen. Such a cross-over has occurred during the derivation of the NX8-3 line, which is *Igh-1*$^e$ and *Igh-Dex*$^a$. Based on these lines the recombination fre-

TABLE I

*Strain distribution patterns in the NZB × C58 recombinant inbred (NX8 RI) strains*[a]

| Inbreeding[b] | NZB | C58 | NX8 RI Strains[c] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | — | — | 1 F14 | 3 F13 | 4 F15 | 5 F16 | 6 F15 | 8 F13 | 9 F17 | 13 F16 | 15 F14 | 16 F15 | 17 F13 | 18 F15 | 19 F15 | 20 F15 |
| H-2 | d | k | N | 8 | 8 | N | N | nt | N | 8 | N | 8 | 8 | N | N | N |
| Igh-1 | e | a | N | N | 8 | 8 | N | N | 8 | 8 | N | 8 | 8 | 8 | N | 8 |
| Igh-Dex | 0 | a | N | 8 | 8 | 8 | N | N | 8 | 8 | N | 8 | 8 | 8 | N | 8 |
| Lyt-2 | b | a | 8 | 8 | 8 | 8 | N | N | ? | 8 | N | 8 | 8 | N | 8 | ? |
| Igk-Pc | b | a | 8 | 8 | 8 | 8 | N | N | ? | 8 | N | 8 | 8 | N | 8 | ? |
| Igk-Efl | b | a | 8 | 8 | 8 | 8 | N | N | ? | 8 | N | 8 | 8 | N | 8 | 8 |

[a] The table entries have the following meanings: 8 indicates that the particular RI strain has inherited the C58 allele at the designated locus; N indicates inheritance of the NZB allele; ? indicates heterozygosity, i.e., both alleles are present in the strain; nt means not tested. The same animals were typed for Lyt-2 and Igk-Pc; Igk-Efl typing was performed on closely related mice.

[b] The degree of inbreeding of the tested mice. The inbreeding process commenced with the F3 generation.

[c] The NX8-1 and -8 strains are now extinct.

quency between *Dex* and *Igh-1* is 0.018 (95% confidence interval: 0.001 to 0.05), a value not inconsistent with the value of 0.004 (0.002 to 0.008) found in tests on larger numbers of mice (13).

Concordance was found between *Lyt-2* and the two κ-light chain variable region (Vk) markers, *Pc* and *Ef1*, in the 12 lines that had fixed genotypes. This is consistent with earlier observations of linkage of these loci, including studies of congenic strains and the AKXL RI lines (12, 14). Two NX8 RI strains were still segregating both parental alleles at *Lyt-2* and the *Igk* loci. Of four NX8-20 mice typed, three were $Lyt\text{-}2^a$ homozygotes (C58-like), while one was heterozygous. In the *Pc* assay on serum pooled from these four mice, this heterogeneity was evident as a minor representation of NZB light chains. *Ef1* typing was done on different, but closely related, mice. These four mice expressed only the C58 allele. Apparently in this strain fixation of the C58 alleles at the *Lyt-2-Igk* complex is nearly complete. In the NX8-9 strain, however, both *Pc* and *Ef1* alleles were strongly expressed in the serum pools, and one of two mice tested was heterozygous at *Lyt-2*. The concordance of *Lyt-2* and the *Igk* loci in heterozygous mice in the unfixed lines is in agreement with the observation of close linkage of these loci in the fixed NX8 RI lines and in other RI and congenic strains. There is no suggestion of recombination between these loci in strains 9 and 20, but this could occur in the further inbreeding of these strains. Later generations will be examined for such an outcome.

In this study 12 RI lines or the equivalent of 48 backcross segregants have been tested for recombination between *Lyt-2* and *Igk* markers. This brings to 174 the number of chromosomes tested for recombination between *Lyt-2* and *Ef1* and to 157 the number between *Lyt-2* and *Pc* (12, 14). The probability of obtaining no recombinants in the former instance is equal to $(1 - r)^{174}$, where r is the probability of recombination in a single meiosis. From this, the upper 95% confidence limit for the recombination frequency between the *Lyt-2* and *Igk* loci can be calculated to be 0.017. This result indicates either that *Igk* genes are closely linked with *Lyt-2*, or that *Lyt-2* itself or a nearby locus selects the kinds of κ-chains a strain expresses.

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