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J Immunol 1999; 162:2623-2630; http://www.jimmunol.org/content/162/5/2623

References

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Analysis of MHC Class II Genes in the Susceptibility to Lupus in New Zealand Mice

Stephen J. Rozzo, Timothy J. Vyse, Chella S. David, Ed Palmer, Shozo Izui and Brian L. Kotzin

Hybrids of New Zealand Black (NZB) and New Zealand White (NZW) mice spontaneously develop a disease similar to human systemic lupus erythematosus. MHC and non-MHC genes contribute to disease susceptibility in this murine model. Multiple studies have shown that the NZW H2\textsuperscript{e} locus is strongly associated with the development of lupus-like disease in these mice. The susceptibility gene(s) within H2\textsuperscript{e} is not known, but different lines of evidence have pointed to class II MHC genes, either H2-E or H2-A (E\textsuperscript{e} or A\textsuperscript{e} in NZW). Recent studies from our laboratory showed that E\textsuperscript{e} does not supplant H2\textsuperscript{e} in the contribution to lupus-like disease. In the present work we generated C57BL/10 (B10) mice transgenic for A\textsuperscript{a} and Ab\textsuperscript{b} genes (designated B10.A\textsuperscript{a} mice) and used a (B10.A\textsuperscript{a} × NZB)F\textsubscript{1} × NZB backcross to assess the contributions of A\textsuperscript{a} genes to disease. A subset of backcross mice produced high levels of IgG autoantibodies and developed severe nephritis. However, no autoimmune phenotype was linked to the A\textsuperscript{a} transgenes. Surprisingly, in the same backcross mice, inheritance of H2\textsuperscript{b} from the nonautoimmune B10 strain was strongly linked with both autoantibody production and nephritis. Taken together with our previous E\textsuperscript{e} studies, the present work calls into question the importance of class II MHC genes for lupus susceptibility in this model and provides new insight into the role of MHC in lupus-like autoimmunity. The Journal of Immunology, 1999, 162: 2623–2630.

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Considerable evidence indicates that the development of systemic lupus erythematosus has a strong genetic basis, with contributions from MHC and multiple non-MHC genes (1). New Zealand hybrid mice are considered to be an excellent model of this human systemic autoimmune disease, and F\textsubscript{1} progeny of New Zealand black (NZB)\textsuperscript{4} and New Zealand white (NZW) mice spontaneously develop a severe lupus-like glomerulonephritis associated with the production of antinuclear autoantibodies. In studies of genetic susceptibility, genes encoded within or closely linked to the MHC have been shown to be important for the development of disease in these F\textsubscript{1} mice (1–11). Evidence supporting an important role for genes encoded within the H2\textsuperscript{e} locus of NZW mice has come from studies of (NZB × NZW)F\textsubscript{1} × NZW backcross mice and (NZB × NZW)F\textsubscript{2} intercross mice (2–6), crosses of NZB and NZW mice congenic for H2\textsuperscript{e} and H2\textsuperscript{b}, respectively (7), and backcross analyses of normal strains of mice (e.g., C57BL/6) congenic for H2\textsuperscript{b} (8, 9).

The genes encoded within H2\textsuperscript{e} that account for the genetic contribution to lupus susceptibility are not known. However, a number of studies have suggested that MHC class II genes, either H2-A\textsuperscript{a} (A\textsuperscript{a} or H2-E\textsuperscript{e}) (E\textsuperscript{e}); are likely candidates. For example, studies with mAbs to CD4 (12) and I-A\textsuperscript{a} (13) have shown that (NZB × NZW)F\textsubscript{1} disease is dependent on CD4\textsuperscript{+} T cells and class II MHC-bearing cells, respectively. In addition, studies of NZB mice congenic for H2\textsuperscript{e} vs H2\textsuperscript{b} indicated a role for the bm12 mutation of the I-A\textsubscript{b} chain in disease susceptibility (14). Studies also have shown that increased expression of I-E molecules can suppress lupus-like disease in New Zealand hybrid mice (15). Finally, the MHC class II hypothesis has been supported by studies of other murine models of autoimmunity, such as NOD mice with spontaneous diabetes or strains induced to develop experimental encephalomyelitis, experimental myasthenia gravis, or collagen-induced arthritis (16–22). In all these models of organ-specific autoimmunity, class II MHC genes have been shown to be important for disease susceptibility. However, some investigators have questioned the paramount importance of MHC class II genes in the development of murine lupus and have suggested that MHC class I or class III genes (e.g., Tnf\textsuperscript{a}) may at least partially account for the MHC contribution to disease (23, 24).

We recently studied the potential role of E\textsuperscript{e} genes in New Zealand murine lupus by comparing C57BL/6 (B6) mice transgenic for E\textsuperscript{e} genes (designated B6.E\textsuperscript{e} mice) and B6 mice congenic for the entire H2\textsuperscript{e} interval (B6.H2\textsuperscript{e} mice) in a backcross analysis (25). The development of nephritis in approximately 30% of (B6.H2\textsuperscript{e} × NZB)F\textsubscript{1} × NZB backcross mice was strongly linked with inheritance of H2\textsuperscript{e} (8, 25). In contrast, none of the similarly backcrossed B6.E\textsuperscript{e} mice with the E\textsuperscript{e} transgene developed nephritis. Although a subset of the (B6.E\textsuperscript{e} × NZB)F\textsubscript{1} × NZB backcross mice produced moderate levels of autoantibodies, this production was not linked with inheritance of the E\textsuperscript{e} transgenes (25). IgG autoantibody production was, however, linked with MHC heterozygosity determined by inheritance of H2\textsuperscript{b} from the B6 background of the B6.E\textsuperscript{e} mice.
Materials and Methods

Mice

Parental NZB/BIJN, C57BL/10 (B10), and C57BL/6J (B6) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and were maintained in the animal care facility at the National Jewish Medical and Research Center (Denver, CO). All congenic, transgenic, F₁, and backcross mice were bred and maintained at the National Jewish Medical and Research Center. Only female mice were studied for expression of disease.

B6 mice were made congenic for H₂ by mating these mice with NZW mice and backcrossing the progeny to B6 (8, 25). Inheritance of H₂ was monitored by immunofluorescence analysis of I-A expression and by screening for a simple sequence length polymorphism in the TNF-α gene (Tnfa). The congenic strain (designated B6.H2z) was made homozygous for H₂ after 12 generations. Congenic mice were analyzed for the length of the NZW chromosome 17 interval bred onto the recipient B6 strain. In relation to the MHC, analysis of markers approximately 1 cM proximal to MHC on chromosome 17 (D17Mit16; −18.1 cM from the centromere), within the MHC (Tnfa or H2α Alu repeat; −19 cM from the centromere), and about 4 cM distal to MHC (D17Mit49 or D17Mit50; −23.2 cM from the centromere) showed alleles inherited from NZW in the congenic mice.

In preparation for the generation of transgenic mice, genomic fragments encoding the Aa and Ab genes were isolated from an NZW splenic DNA cosmId library (see below). Transgenic mice were generated in the laboratories of Chella S. David using methods previously described (26, 27). (CBA/J × B10.M)F₁ or (SWR × B10.M)F₁ eggs were coinjected with 10⁵ total colonies were analyzed by Southern blotting for integration of the injected DNA. Three founders were bred, of which two were found to have both Aa and Ab genes. These lines were perpetuated by repeated backcrossing with B10 mice for seven generations. Inheritance of the transgenes was determined by PCR analysis of genomic DNA. Primer sequences (5'-3') to detect the Aa transgene were GGT ATA GT (forward) and GTC AAA GCT TCT CAG TTG AG (reverse), and primer sequences to detect the Ab transgene were GGG CCA CGG TTG TC (forward) and TAA GAG GCT CTG GGG GTA (reverse). Occasional offspring were also analyzed by immunofluorescence staining for expression of I-A after transfection into A20 cells. One of the lines with both Aa and Ab transgenes was subsequently designated B10.Aa. The other had lower levels of surface I-A expression and was designated B10.Aa lo. Integration sites for each of these lines were shown to mediate expression of I-A after transfection into A20 cells. The study mice were also bled (from the tail) at monthly intervals from the age of 5 mo. The blood was allowed to clot at room temperature, and the serum was stored at −20°C until analyzed for autoantibody levels.

Generation of NZW splenic DNA cosmId library

DNA extracted from NZW spleen cells was used to generate a cosmId library as previously described (29). Splenic DNA was partially digested with MboI to generate 35- to 45-kb fragments and ligated into BstHI-digested pCV 107 cosmId vector, packaged (Gigapack Gold, Stratagene, La Jolla, CA), and grown in Escherichia coli. The library was plated at approximately 10,000 colonies/filter, and 4.1 × 10⁸ total colonies were screened. Probes were generated from mRNA expressed by LPS-stimulated B cell blasts from NZW mice. PCR amplification of segments of the Aa and Ab genes, and cloning of PCR fragments into pEMBL. Before generation of the transgenic mice, the selected cosmId clones (see Fig. 1) were shown to mediate expression of I-A after transfection into A20 cells.

Analysis of B cell surface I-A expression

Spleen cells from the different parental strains and backcross mice were prepared and stained as previously described (30). The fluoresceinated mAbs used included 3F12 (anti-I-Aa), obtained from Dr. John Freed, National Jewish Medical and Research Center, 10-2.16 (anti-I-Ab), hybridoma cells obtained from American Type Culture Collection, Manassas, VA), and HB35 (anti-I-Aa,b), hybridoma cells obtained from American Type Culture Collection). Splenic B cells were also double stained using a biotinylated mAb to B220 (RA3-6B2, PharMingen, San Diego, CA) followed by avidin-phycocerythrin (PharMingen). In some experiments PBL were double stained with fluoresceinated 3F12 and biotinylated 10-2.16 followed by avidin-phycocerythrin. Fluorescence intensity was analyzed on an EPICS C flow cytometer (Coulter, Hialeah, FL). Viable mono-nuclear cells were gated by scatter analysis, and 1 × 10⁶ cells were collected for each Ab combination.
Analysis of thymus expression of I-A<sup>z</sup>

Thymus cells were also analyzed for the expression of I-A<sup>z</sup> by direct immunohistochemistry. Briefly, thymus tissue was frozen in embedding medium (O.C.T. compound, Miles, Elkhart, IN). Four-micron sections were cut and fixed, and endogenous peroxidase activity was blocked using 0.5% H<sub>2</sub>O<sub>2</sub>. The sections were incubated at 4°C overnight with biotinylated primary Ab followed by incubation with streptavidin-conjugated horseradish peroxidase for 1 h at room temperature. Finally, the sections were incubated with a solution of 3,3′-diaminobenzidine tetrahydrochloride and peroxide and counterstained with hematoxylin for 1 min. Sections were studied using light microscopy.

Typing for inheritance of H2 haplotype

Inheritance of H2<sup>H2</sup> vs H2<sup>H2a</sup> haplotypes in backcross mice was determined by analysis of genomic DNA for a simple sequence length polymorphism in Tnf (33). Oligonucleotide primers flanking the Tnf microsatellite were synthesized in the Molecular Resource Center at the National Jewish Medical and Research Center using an Applied Biosystems model 392 DNA synthesizer (Foster City, CA). Primer nucleotide sequences and the methods for SSLP mapping have been previously described (8).

Serological assays

Abs to chromatin were determined by ELISA as previously described (9, 11). Briefly, wells of microtiter plates were coated with calf thymus chromatin, 0.5 μg/ml and postcoated with gelatin. Serum samples were diluted 1/300 before adding them to Ag-coated wells for 90 min. After wells were incubated with peroxidase-conjugated Ab for mouse IgG, substrate was added, and OD was determined with an automated spectrophotometer. The results were plotted against a standard curve obtained using control (NZB × NZW)F<sub>1</sub> sera as previously described (9). IgG subclass anti-chromatin autoantibody levels were assayed using the same anti-chromatin ELISA, but IgG subclass-specific second step Abs were used as detecting reagents as previously described (9).

The production of autoantibodies to gp70 was quantitated as serum levels of gp70-anti-gp70 immune complexes (gp70 IC), since the relative excess of gp70 in sera makes free anti-gp70 Abs difficult to detect (34). These complexes were measured by ELISA after precipitation of the serum with polyethylene glycol (average m.w., 6000) as previously described (35). The results are expressed as micrograms per milliliter of gp70 complexed with anti-gp70 Abs. Although gp70 is detectable in the serum of nearly all murine strains, only lupus-prone strains produce autoantibodies to gp70 and form gp70 IC (36).

For certain comparisons, mice were separated into groups based on their serum levels of a particular autoantibody. The cut-offs used to group mice in the current study were originally determined in (NZB × NZW)F<sub>1</sub> × NZW backcross mice by dividing the frequency distribution of autoantibody levels on the basis of tertiles. This separation into autoantibody phenotypes identified one-third of mice with low/negative levels and one-third of mice with high levels for each autoantibody measured. Backcross mice with intermediate levels were defined as the middle third. The cut-offs for anti-chromatin and gp70 IC autoantibodies correlated well with low levels of production in NZW and nonautoimmune strains and high levels of production in (NZB × NZW)F<sub>1</sub>, mice (9, 25).

Statistical analysis

The linkage of the A<sup>a</sup> transgene or MHC type with nephritis was quantified by χ<sup>2</sup> analysis, using a standard (2 × 2) contingency matrix. Evidence that these genes are linked with autoantibody levels as quantitative trait loci (QTL) was determined by using the linkage program, MAPMAKER/QTL (37, 38). The autoantibody levels were log<sub>10</sub> transformed before analysis with MAPMAKER/QTL because this tended to normalize their frequency distribution and improve the accuracy of MAPMAKER/QTL (37). It is emphasized that these analyses were directed at MHC genes or transgenes and were not part of a genome-wide screen for linked loci. The statistical threshold used for significant linkage was p < 0.01, based on recommendations that this cut-off be used to confirm linkage in a new dataset (39).

In separate analyses the frequency of nephritis was compared in H2<sup>H2</sup>-congenic B6 mice and A<sup>a</sup>-transgenic B10 mice by Fisher’s exact test. The mean values for particular autoantibodies in different backcrosses were compared using the nonparametric Dunn procedure of the Kruskal-Wallis test (two-tailed).

Analysis of I-A<sup>z</sup> expression in transgenic mice

To study the roles of A<sup>a</sup> genes in the lupus-like disease of (NZB × NZW)F<sub>1</sub> mice, cosmids clones of A<sup>a</sup> and A<sup>b</sup> were isolated from an NZW genomic library. Restriction maps of both clones are shown in Fig. 1. Double-transgenic mice were then prepared by coinjecting both clones into (CBA × B10.MF<sub>2</sub>) or (SWR × B10.MF<sub>2</sub>), eggs and selecting for founder mice that expressed both A<sup>a</sup> and A<sup>b</sup> transgenes. After backcrossing the transgenes onto a B10 background, two lines that differed in I-A<sup>z</sup> expression levels were selected for use in the present studies. These were named B10.A<sup>a</sup> and B10.A<sup>a</sup>lo based on relative levels of both A<sup>a</sup> and A<sup>b</sup> mRNA expression and relative levels of splenic B cell surface expression of I-A<sup>β</sup>. As shown in Fig. 2, I-A<sup>β</sup> expression on splenic B cells of the B10.A<sup>a</sup> and B10.A<sup>a</sup>lo lines was approximately twofold and 10%, respectively, compared with H2<sup>H2a</sup>-congenic B6 mice. As expected, both transgenic lines expressed levels of endogenous I-A<sup>b</sup> similar to that expressed in normal B10 mice, whereas MHC-congenic B6.H2<sup>z</sup> mice did not express I-A<sup>a</sup>.

We verified that expression of A<sup>a</sup> and A<sup>b</sup> mRNA in transgenic mice was also associated with expression of I-A<sup>a</sup> and I-A<sup>b</sup> surface proteins. The mAb recognizing I-A<sup>a</sup> cross-reacts with I-A<sup>b</sup> (present in the B10 transgenic lines) but not with I-A<sup>a</sup>. We therefore studied (B10.A<sup>a</sup> × NZB)F<sub>1</sub> × NZB backcross mice and selected progeny that were H2<sup>H2d</sup> by genotyping. Peripheral blood cells from transgene-positive and transgene-negative mice were then double stained with mAbs directed to I-A<sup>a</sup> and I-A<sup>b</sup>. Fig. 3 shows that both proteins were expressed on the surface of the same cells in transgenic-positive mice, as in B6.H2<sup>z</sup> congenic controls. Cells from backcross animals genotyped as transgene-negative failed to stain positive with either I-A<sup>a</sup> reagent.

We also stained thymus tissue sections and analyzed the expression pattern of I-A<sup>a</sup> or I-A<sup>a</sup>-<sup>b</sup> by immunohistochemistry. Sections from B10.A<sup>a</sup> transgenic mice stained positively for expression of I-A<sup>a</sup> in a pattern indistinguishable from NZW and B6.H2<sup>z</sup> mice (data not shown). B6, B10, and B6.E<sup>a</sup> mice were negative for thymic A<sup>a</sup> expression. B10.A<sup>a</sup> mice also showed appropriate staining for I-A<sup>a</sup>-<sup>b</sup> similar to B6, B10, and B6.E<sup>e</sup> controls. In contrast, NZW and B6.H2<sup>z</sup> mice were negative for expression of thymic I-A<sup>a</sup>-<sup>b</sup>. (B10.A<sup>a</sup> × NZB)F<sub>1</sub> × NZB and (B10.A<sup>a</sup>lo × NZB)F<sub>1</sub> × NZB backcross mice (collectively referred to as A<sup>a</sup>-backcross mice) were bred to examine the contribution of A<sup>a</sup> to lupus-like disease. PCR amplification of genomic DNA using primers capable of distinguishing A<sup>a</sup> and A<sup>b</sup> products was used to analyze transgenic lines and backcross mice for inheritance of the transgenes. Fig. 4 shows representative results for H2<sup>H2a</sup>-positive and -negative control strains, transgene-positive and -negative strains, and backcross mice. As expected, neither A<sup>a</sup> nor A<sup>b</sup> PCR products were observed for DNA from NZW (H2<sup>H2a</sup>) or B10 mice (H2<sup>H2b</sup>). Alternatively, both NZW (H2<sup>H2a</sup>) and H2<sup>H2c</sup>-congenic B6 mice showed the presence of both genes, as did mice from transgenic B10.A<sup>a</sup> and B10.A<sup>a</sup>lo lines. In backcross mice constructed using either the B10.A<sup>a</sup> or B10.A<sup>a</sup>lo strains, inheritance of both transgenes or lack thereof was always concordant, consistent with integration of the transgenes into the same chromosomal position. Backcross mice were also screened for expression of A<sup>b</sup> mRNA and splenic B cell surface expression of I-A<sup>b</sup> using RT-PCR analysis and flow cytometry, respectively. Agreement among all these analyses for transgene inheritance and expression was consistently observed.
Analysis of backcross mice for contribution of MHC genes to nephritis

Previous studies have shown that (B6.H2\(^s\) × NZB)\(F_1\) mice do not develop severe lupus-like disease, but that a subset of (B6.H2\(^s\) × NZB)\(F_1\) × NZB backcross mice produces high levels of autoantibody production and die from lupus nephritis within 12 mo (8, 40). Disease development was strongly linked to inheritance of the H2\(^s\) congenic interval (8). For the current study we bred two similar backcrosses using the A\(^z\) transgenic B10 lines to study the influence of A\(^z\) on disease. Female mice with severe nephritis or no nephritis after staining and analyzing by two-color immunofluorescence. Using the gates shown at the left, the percentages of I-A\(^b\) positive cells (mean channel of fluorescence) were: B6.H2\(^z\), 2.2% (2.9); B10.A\(^{z\ lo}\), 84% (63); B10.A\(^z\), 96% (95); and B6, 99% (100). At the right (I-A\(^b\)), the percentages of positive cells (mean channel of fluorescence) were: B6, 2.3% (1.7); B10.A\(^{z\ lo}\), 7.4% (3.5); B6.H2\(^z\), 95% (46); and B10.A\(^z\), 97% (109).

Analysis of backcross mice for the contribution of MHC genes to autoantibody production

To further study whether the A\(^z\) transgenes contributed to autoimmunity in New Zealand mice, we quantitated serum levels of IgG autoantibodies to chromatin and gp70 immune complexes. Previous studies have shown that IgG antinuclear autoantibody production is coordinately controlled and that serum levels of anti-chromatin autoantibodies are highly correlated with levels of IgG autoantibodies to dsDNA and to histones (11). The linkages of these serological traits with the different MHC genotypes were analyzed as quantitative trait loci. The results in Table II are strongly concordant with the results shown above for nephritis. The A\(^z\) genotype showed no linkage or trend for linkage with any serological trait. In contrast, in the same mice, H2\(^{bd}\) vs H2\(^{bd}\) was strongly linked with total IgG anti-chromatin levels and gp70 IC. Linkage with each of the IgG subclass anti-chromatin autoantibodies was also apparent, with the strongest linkage for IgG2a anti-chromatin Abs. No significant difference was observed between (B10.A\(^{z\ lo}\) × NZB)\(F_1\) × NZB backcross mice. Whereas inheritance of the entire H2\(^z\) locus significantly increased disease susceptibility in (B6.H2\(^s\) × NZB)\(F_1\) × NZB backcross mice, inheritance of the A\(^z\) transgenes had no influence on disease incidence in either A\(^z\) backcross (Fig. 5B). Furthermore, differences in I-A\(^b\) expression between the two backcrosses were not important for development of lupus-like disease, and results for the A\(^z\) backcrosses are pooled for the remainder of this report.

Interestingly, B10.A\(^z\) backcross mice (transgene-positive or transgene-negative) demonstrated a significantly increased frequency of lupus nephritis compared with H2\(^z\)-positive and H2\(^z\)-negative (B6.H2\(^s\) × NZB)\(F_1\) × NZB backcross mice (p < 2.5 × 10\(^{-13}\)) or B6.E\(^z\) (p < 7.0 × 10\(^{-19}\)) mice (Fig. 5A) (8, 25).

In the (B10.A\(^z\) × NZB)\(F_1\) × NZB backcrosses, mice differed based on their A\(^z\) genotype as well as inheritance of H2\(^z\) from the B10 strain. Thus, backcross mice were either homozygous for H2\(^z\) or were heterozygous for H2\(^{bd}\). Table I shows a linkage analysis of nephritis with all the different MHC genotypes. As predicted from the data shown above, there was no trend for linkage with the A\(^z\) genotypes. This is irrespective of whether mice were H2\(^{bd}\) or H2\(^{bd}\) (data not shown). In contrast, inheritance of H2\(^s\) showed significant linkage with nephritis (p < 1 × 10\(^{-4}\)), with an odds ratio of 4.45.

The relevance of these autoantibody studies for development of disease is supported by the association of gp70 IC and anti-chromatin levels with severe proteinuria in the A\(^z\) backcross mice. Mice were segregated according to their levels of gp70 IC, defined as high (>3.5 μg/ml), intermediate (0.5–3.5 μg/ml), or low (<0.5 μg/ml), and whether they had severe proteinuria (9, 25). Similarly, mice were categorized by their IgG anti-chromatin level, defined as high (>4.6 U/ml), intermediate (1.0–4.6 U/ml), or low (<1.0 U/ml). Using a 3 × 2 contingency table and \(\chi^2\) analysis, a strong association with nephritis was found for gp70 IC (\(\chi^2 = 16.6; p < 2.5 \times 10^{-4}\)) and less so for IgG anti-chromatin Abs (\(\chi^2 = 7.9; p < 0.02\)). Compared with healthy (B10.A\(^z\) × NZB)\(F_1\) × NZB mice,
age-matched (B10.A' × NZB)F1 × NZB mice with severe proteinuria produced significantly greater amounts of autoantibodies ($p < 6.0 \times 10^{-4}$ for total IgG anti-chromatin, $p < 2.0 \times 10^{-4}$ for IgG2a anti-chromatin, $p < 5 \times 10^{-4}$ for IgG3 anti-chromatin, and $p < 1.0 \times 10^{-4}$ for gp70 IC).

**Discussion**

The present studies were based on the hypothesis that class II MHC genes underlie the contributions of particular MHC haplotypes to increased lupus susceptibility in New Zealand hybrid mice. This hypothesis appeared to be supported by different lines of reasoning and by multiple studies, including the required role for both CD4+ T cells and class II-bearing cells in (NZB × NZW)F1 mice (12, 13). The present work focused on the strong contribution of $H2^b$ from the NZW parent to IgG autoantibody production and nephritis in (NZB × NZW)F1 mice (1–9). Recent experiments from our laboratory showed that $Ea^b$ and $Eb^b$ genes did not influence disease development. The present studies strongly suggest that $A^c$ genes are also not the sole basis for this effect of MHC.

Our experimental design was based on transgenic expression of genomic $A^c$ clones with wild-type promoter and enhancer elements. We developed two different lines of $A^c$ transgenic mice, one with twofold higher and another with considerably lower expression of surface I-A$^c$ on B cells compared with $H2^d$-positive mice. Neither transgene showed a trend for influencing disease expression in the respective backcrosses. For peptide presentation in the peripheral lymphoid tissues, cells in transgenic mice with higher expression should have functioned similarly to those in wild-type animals, especially considering that autoimmunity in older (NZB × NZW)F1 mice correlates with higher expression levels of class II MHC molecules (41). We also compared $A^c$ transgenic mice with $H2^d$-positive or -negative control strains for expression of I-A$^c$ in the thymus, since this is the major site where class II MHC expression affects T cell development. The results indicated normal patterns of thymic I-A$^c$ expression in the transgenic mice.

The lack of effect of $A^c$ genes on autoimmunity also indicates that mixed haplotype I-A$\alpha$/$\beta$- and mixed isotype I-E$\alpha$/$\beta$ molecules do not explain the effect of $H2^d$ on disease in (NZB × NZW)F1 mice as previously suggested (42). Since $H2^d$-encoded molecules were present in the backcross animals, I-Ab$^d$ should have been equally likely to pair with I-Aa$^b$ or I-E$\alpha$ as in wild-type $H2^d$ backcross mice. This is consistent with a previous report showing that an Ab$^b$ transgene alone expressed in $H2^d$ homozygous

![FIGURE 3. Analysis of PBL from transgenic and control mice for expression of surface I-A$^c$ and I-A$\beta$. mAb 3F12 (anti-I-A$\alpha$) cross-reacts with I-A$^c$ and I-A$\alpha$, but not I-A$\alpha^d$ (31). Therefore, (B10.A' × NZB)F1 × NZB backcross mice were first selected for the absence of $H2^d$ by genotyping. PBL from transgene-positive and transgene-negative mice, determined by genotyping, were then double stained with anti-I-A$^c$ and anti-I-A$\beta$. The percentage of positive cells in each quadrant is indicated.](http://www.jimmunol.org/)
NZB × NZW.H2d)F1 mice resulted in no greater autoantibody production and nephritis than was found in (NZB × NZW)F1 mice (43). In our studies, the lack of effect of the transgene on lupus-like disease was also not influenced by inheritance of H2b/d vs H2d/d and therefore was not related to competition from I-Aαb or I-Aβb for pairing.

Although the Aα transgenes had no effect on autoimmune disease, inheritance of H2d in the normal B10 background greatly enhanced IgG autoantibody production and nephritis in the same backcross. These results are consistent with a large body of evidence indicating that MHC heterozygosity is important for full expression of disease in New Zealand hybrid mice (2–11; reviewed in Refs. 1 and 25). Previous studies have consistently shown that New Zealand hybrid or backcross mice that are H2d/z have increased IgG autoantibody production and increased incidence of nephritis compared with genetically similar mice with a double dose of either H2d or H2z genes. The mechanism by which this H2 heterozygosity confers greater disease susceptibility than H2 homozygosity is unknown. Consistent with our current results, recent studies have shown that inheritance of H2b, in the context of H2d or H2z, also enhances IgG autoantibody production and nephritis (10, 25). It may be important that heterozygous H2b/d mice have only one copy of H2d genes, like H2d/z mice, which is not recapitulated by transgenic expression of individual class II genes.

Table 1. Linkage of nephritis with MHC and transgene genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% with Nephritis</th>
<th>Linkage</th>
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<tr>
<td>H2d/d (vs H2d/d)</td>
<td>80 (vs 47)</td>
<td>15.2</td>
</tr>
<tr>
<td>Aα Tg+ (vs Tg−)</td>
<td>63 (vs 61)</td>
<td>0.6</td>
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</tbody>
</table>

*O.R., odds ratio, for H2d/d vs H2d/d or Tg+ vs Tg− calculated as: [(No. diseased mice with gene) × (no. healthy mice without gene)] / [(no. diseased mice without gene) × (no. healthy mice with gene)].
Inheritance of H2b in our previous B6.Eb backcrosses (25) and in the current B10.Ab backcrosses was strongly linked with the production of IgG autoantibodies, especially IgG2a anti-chromatin Abs. IgG2a subclass anuclear Abs have been regarded as strongly nephritogenic. However, nephritis was not observed in the previous B6.Eb backcrosses. Interestingly, H2b in the current B10.Ab backcrosses showed much stronger linkage with gp70 IC compared with B6.Eb backcross mice. In some genetic analyses, gp70 IC vs aninuclear Abs have been implicated as the major pathogenic autoantibody in this model of lupus (11, 36). Consistent with this hypothesis, levels of gp70 IC showed a stronger association with severe nephritis in the current study than did IgG anti-chromatin Abs.

The linkage of H2b with IgG autoantibody production in the current backcross analysis raises questions similar to those that prompted the current studies. For example, is this effect mediated by class II genes or by other genes encoded with the MHC? Although the answer is unknown at this time, it is of interest that the answer is unknown at this time.

We thank Virginia Appel and Ellen Roper for technical assistance.

References
