Effects of MHC and Gender on Lupus-Like Autoimmunity in Nba2 Congenic Mice

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The lupus-like disease that develops in hybrids of NZB and NZW mice is genetically complex, involving both MHC- and non-MHC-encoded genes. Studies in this model have indicated that the $H2^{d/z}$ MHC type, compared with $H2^{d/d}$ or $H2^{z/z}$, is critical for disease development. C57BL/6 (B6) mice ($H2^{b/b}$) congenic for NZB autoimmunity 2 ($Nba2$), a NZB-derived susceptibility locus on distal chromosome 1, produce autoantibodies to nuclear Ags, but do not develop kidney disease. Crossing B6.Nba2 to NZW results in $H2^{b/c}$ F1 offspring that develop severe lupus nephritis. Despite the importance of $H2^c$ in past studies, we found no enhancement of autoantibody production or nephritis in $H2^{b/c}$ vs $H2^{b/b}$ B6.Nba2 mice, and inheritance of $H2^{z/c}$ markedly suppressed autoantibody production. (B6.Nba2 × NZW)F1 mice, compared with MHC-matched B6.Nba2 mice, produced higher levels of IgG autoantibodies to chromatin, but not to dsDNA. Although progressive renal damage with proteinuria only occurred in F1 mice, kidneys of some B6.Nba2 mice showed similar extensive IgG and C3 deposition. We also studied male and female B6.Nba2 and F1 mice with different MHC combinations to determine whether increased susceptibility to lupus among females was also expressed within the context of the $Nba2$ locus. Regardless of MHC or the presence of NZW genes, females produced higher levels of anti-chromatin autoantibodies, and female F1 mice developed severe proteinuria with higher frequencies. Together, these studies help to clarify particular genetic and sex-specific influences on the pathogenesis of lupus nephritis. The Journal of Immunology, 2005, 175: 6190–6196.

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5 Abbreviations used in this paper: SLE, systemic lupus erythematosus; IC, immune complex.

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mice in each genetic system. These studies provide further insight into the impact of the MHC on the disease process and also provide a simplified genetic system for understanding sex-related differences in lupus susceptibility and lupus pathogenesis.

Materials and Methods

Mice and detection of proteinuria

NZW (H2\(z^z\)), NZB (H2\(d^d\)), and B6 (H2\(b^b\)) mice were obtained from The Jackson Laboratory and were maintained in the animal care facility at University of Colorado Health Sciences Center (Denver, CO) in accordance with guidelines approved by the animal care and use committee. B6.Nba2 congenic mice were generated by backcrossing an NZB interval on distal chromosome 1 to the B6 strain as previously described (21). B6.H2\(d^d\)/H2\(d^d\) mice were obtained from The Jackson Laboratory, and B6 mice were made congenic for the NZW H2\(z^z\) (B6.H2\(z^z\)) as previously described (22).

Study mice were followed for the development of lupus nephritis by measuring proteinuria at monthly intervals as previously described (9, 11, 14). Mice with \(\geq 2\) + (100 mg/dl) on at least two consecutive occasions before 12 mo of age were designated as positive for high grade proteinuria and severe renal disease and predict mortality from renal failure in New Zealand hybrid mice has been previously demonstrated (9, 11, 14).

Measurement of IgG autoantibodies and total IgG Levels

Serum autoantibody levels to chromatin, dsDNA, ssDNA, and total histones were determined by ELISA as previously described (13, 14). Serum samples were tested at a dilution of 1/300. All assays were performed in duplicate, and OD determinations were converted to units per milliliter by comparison with a standard curve obtained with mAbs to the appropriate nuclear Ag as previously described (13, 14). Animals were considered positive for antinuclear Abs if levels were 2 SD above the median levels in age-matched B6 animals. Serum levels of total IgG were also determined by ELISA as previously described (13) using sera diluted 1/100,000. OD determinations were converted to units per milliliter by comparison with a standard curve obtained with known concentrations of polyclonal IgG (MP Biomedical).

Immunohistochemistry

Kidneys were snap-frozen in Tissue-Tek (OCT) and were kept at \(-70^\circ\)C until sectioning. Sections were blocked with 10% nonimmune goat serum before staining. Five-micron sections were cut, fixed in acetone, and stained for total IgG deposition using FITC-conjugated rabbit anti-mouse IgG Abs (MP Biomedical). Staining intensity was evaluated using ImagePro software (version 4.5; Media Cybernetics) on digitalized images acquired through a Pixera CL600 color CCD camera and an Olympus BX51 microscope. All pictures were taken at identical conditions. Blinded scores were determined for IgG and complement C3 staining using the following 0–4 scale: 0, no detectable staining; 0.5, trace staining in mesangium only; 1, staining in mesangium only (<50% of glomeruli); 2, staining in mesangium only (>50% of glomeruli); 3, strong staining in mesangium (>50% of glomeruli) with occasional staining of capillary loops; and 4, strong staining in mesangium (>50% of glomeruli) with widespread staining of capillary loops.

Statistical analysis

The statistical significance of differences in autoantibody levels and degree of IgG deposition between groups of mice was determined using the non-parametric Mann-Whitney U test. A survival curve comparison analysis was used to determine the statistical significance of proteinuria rates between different groups of mice.

Results

Effect of MHC haplotype on autoantibody production and nephritis in B6.Nba2 mice

Many studies have demonstrated the importance of MHC haplotype in mouse lupus, especially in the BWF1 model (7, 9–20). Therefore, we assessed autoantibody production in B6.Nba2 congenic mice bearing different relevant MHC haplotypes. Like H2\(b^b\)/H2\(b^d\) B6.Nba2 controls, H2\(b^d\)/H2\(b^d\) B6.Nba2 mice produced high levels of autoantibodies, with 100, 73, 50, and 45% of the mice positive for autoantibodies to chromatin, histone, ssDNA, and dsDNA, respectively. Importantly, heterozygosity at the MHC with H2\(z\) neither enhanced nor decreased autoantibody production, because there was no difference in autoantibody production between H2\(b^b\)/H2\(z\) and H2\(b^d\)/H2\(z\). B6.Nba2 mice (with the exception of anti-histone Abs) or between H2\(d^d\)/H2\(b^d\) B6.Nba2 mice (Fig. 1). A much smaller percentage of H2\(d^d\)/H2\(z\) and H2\(d^d\)/H2\(z\) mice were positive for autoantibodies, with overall levels similar to those in Nba2-negative mice. Serum levels of total IgG were slightly greater in B6.Nba2 mice (3.9 ± 0.37 mg/dl) on at least two consecutive occasions before 12 mo of age were designated as positive for high grade proteinuria.
Autoantibody levels and immune complex deposition in the kidneys of MHC-matched B6.Nba2 and (B6.Nba2 × NZW)F1 mice

Potential mechanisms for how NZW-encoded non-MHC genes might enhance the development of glomerulonephritis in (B6.Nba2 × NZW)F1 mice include 1) enhanced autoantibody production leading to increased IC deposition; 2) production of additional pathogenic autoantibody specificities with increased IC deposition; 3) increased susceptibility to IC deposition in the kidney; and 4) enhanced damage-inducing inflammatory responses to deposited ICs (23). To help differentiate among these possibilities, we first compared the levels of IgG antinuclear autoantibodies in H2b/z B6.Nba2 and (B6.Nba2 × NZW)F1 mice. (B6.Nba2 × NZW)F1 mice produced more anti-chromatin (H2b/b) antibody than did MHC-matched B6 mice (1.6 ± 0.21) and compared with B6.Nba2-negative B6 mice (2.2 ± 0.17) and compared with B6.Nba2 (H2b/b) mice produced high levels of IgG antinuclear autoantibodies, yet did not develop lupus nephritis. However, when these congenic mice were crossed to NZW (H2z/z), severe lupus nephritis ensued. To determine whether the NZW contribution to lupus nephritis could be explained by inheritance of the H2c allele alone, we compared the incidence of severe proteinuria between H2b/b and H2b/z B6.Nba2 mice and (B6.Nba2 × NZW)F1 mice. Beginning at 5–6 mo, the percentage of (B6.Nba2 × NZW)F1 (H2b/z) mice with severe proteinuria progressively increased, and at 12 mo of age, 80% of these mice had developed severe proteinuria (Fig. 2). In contrast, by 12 mo of age, <10% of the H2b/b and H2b/z B6.Nba2 mice had developed severe proteinuria. These data indicate that inheritance of the H2b/z MHC haplotype without additional NZW-encoded genes is not sufficient for the development of lupus nephritis in this model.

Autoantibody levels and immune complex deposition in the kidneys of MHC-matched B6.Nba2 and (B6.Nba2 × NZW)F1 mice

Potential mechanisms for how NZW-encoded non-MHC genes might enhance the development of glomerulonephritis in (B6.Nba2 × NZW)F1 mice include 1) enhanced autoantibody production leading to increased IC deposition; 2) production of additional pathogenic autoantibody specificities with increased IC deposition; 3) increased susceptibility to IC deposition in the kidney; and 4) enhanced damage-inducing inflammatory responses to deposited ICs (23). To help differentiate among these possibilities, we first compared the levels of IgG antinuclear autoantibodies in H2b/z B6.Nba2 and (B6.Nba2 × NZW)F1 mice. (B6.Nba2 × NZW)F1 mice produced more anti-chromatin (p = 0.003), anti-histone (p = 0.003), and anti-ssDNA (p = 0.05) Abs, but not to dsDNA. We next assessed the extent of IgG and C3 deposition in the kidneys of B6.Nba2 (H2b/b and H2b/z) mice compared with (B6.Nba2 × NZW)F1 mice. Surprisingly, both 12-mo-old proteinuria-free B6.Nba2 mice and 7-mo-old proteinuria-positive F1 mice showed extensive IgG and C3 deposition in glomeruli in both mesangial and capillary loop areas (Fig. 3B). As expected, B6 (negative control) glomeruli exhibited only trace amounts of IgG or C3 deposition (Fig. 3B). The extent of kidney IgG deposition was scored on a 0–4 scale. Overall, scores were greater for F1 mice (Fig. 3C). However, scores overlapped between the two groups of mice, despite the nonoverlapping propensities to develop severe proteinuria.

MHC contribution to nephritis and autoantibody production in (B6.Nba2 × NZW)F1 and (B6 × NZW)F1 mice: prevention of disease in H2b/z mice

The above-described studies indicated that NZW-encoded non-MHC genes are required for renal disease in the context of Nba2. We therefore studied the influence of H2b/b, H2b/z, and H2z/z MHC haplotypes on autoantibody production and kidney disease in (B6.Nba2 × NZW)F1 mice. As shown in Fig. 4A, the frequency of severe proteinuria in H2b/z (B6.Nba2 × NZW)F1 mice progressed...
FIGURE 4. Effect of MHC haplotype on the development of lupus nephritis and autoantibody production. A. (B6.Nba2 × NZW)F1 mice homozygous for the H2b MHC haplotype (n = 10) did not develop severe proteinuria compared with mice inheriting H2d/c (n = 8) and H2d/c (n = 9). Nba2-negative mice also did not develop lupus nephritis (H2b/c, n = 10; H2d/c, n = 12; H2d/c, n = 9). The difference in proteinuria between H2b/c and H2d/c (B6.Nba2 × NZW)F1 mice was not statistically significant (p = 0.13). Serum samples from 7-mo-old (B6.Nba2 × NZW)F1 and (B6 × NZW)F1 mice with the indicated MHC haplotypes were analyzed for IgG autoantibodies to dsDNA (B), ssDNA (C), chromatin (D), and histone (E). (B6.Nba2 × NZW)F1 mice homozygous for the H2b MHC haplotype did not produce significant levels of autoantibodies compared with heterozygous mice. (B6.Nba2 × NZW)F1 mice with the MHC haplotypes of H2b/c and H2d/c produced more anti-ssDNA (p = 0.001 and p = 0.0002), anti-chromatin (p = 0.0004 and p = 0.0001), and anti-histone (p = 0.0001 and p = 0.0001) autoantibodies than (B6.Nba2 × NZW)F1 H2b/c mice.


Past studies have shown that female BWF1 mice exhibit a greater susceptibility to autoantibody production and lupus nephritis than male mice (4–6). To determine whether the lupus-like disease associated with the Nba2 locus (derived from the NZB parent) still maintains this influence of gender, autoantibody levels and the incidence of proteinuria in both male and female animals were assessed. The current studies showed that female B6.Nba2 mice produced higher levels of anti-dsDNA (p = 0.03), anti-ssDNA (p = 0.02), anti-chromatin (p = 0.03), and anti-histone autoantibodies (p = 0.05) than male B6.Nba2 mice (Fig. 5).

Enhancement of autoantibody production in females was also apparent in (B6.Nba2 × NZW)F1 mice (Fig. 6). Female (B6.Nba2 × NZW)F1 mice produced more anti-ssDNA (p = 0.0003), anti-chromatin (p < 0.0001), anti-dsDNA (p = 0.008), and anti-histone (p = 0.0003) autoantibodies than male (B6.Nba2 × NZW)F1 mice in the context of the H2b/c MHC haplotype (Fig. 6A). Total IgG levels were also 2-fold higher in H2b/c female compared with male (B6.Nba2 × NZW)F1 mice (data not shown). In the context of the H2d/c MHC haplotype, female mice produced more anti-chromatin (p = 0.001), anti-dsDNA (p = 0.002), and anti-ssDNA (p = 0.03) autoantibodies than male mice (Fig. 6B).

Female (B6.Nba2 × NZW)F1 mice also developed more severe renal disease (Fig. 6C). About 20% of female H2b/c (B6.Nba2 × NZW)F1 mice developed severe proteinuria by 6 mo, and by 12 mo, 80% were positive. In contrast, only 20% of male (B6.Nba2 ×
The current studies did not support an important role of H2\(^c\) from NZW in the B6.Nba2 or (B6.Nba2 × NZW)F\(_1\) model of disease. Mice heterozygous for H2\(^c\) did not demonstrate enhanced IgG antinuclear Ab production or nephritis. In B6.Nba2 mice, H2\(^b/b\) appeared to be the most disease-enhancing MHC haplotype, especially compared with H2\(^d/d\) and H2\(^d/c\), whereas in the F1 model, H2\(^c/c\) was completely suppressive of both autoantibody production and disease. The relative disease-promoting effect of H2\(^c\) compared with other haplotypes has been noted in other lupus-prone mouse strains, such as B6-Faslpr mice (24, 25), BXSB mice (26, 27), certain backcross studies of NZB and NZW mice (16, 22, 28, 29), and even some knockout models of lupus (30). In contrast to H2\(^c\) and H2\(^d\), the H2\(^b\) haplotype does not allow expression of an I-E\(\alpha\) protein and does not express an I-E molecule (31).

With regard to this difference, other investigators have observed partial prevention of mouse lupus in mice with high levels of I-E expression (24, 32, 33). Studies have suggested that peptides derived from I-E\(\delta\) and I-E\(\epsilon\) can prevent autoantibody production, perhaps through blocking of I-A presentation of other self peptides (24, 32–34).

The marked prevention of autoantibody production and disease by homozygosity for H2\(^c\) probably relates to mechanisms additional to I-E expression. For example, in the current studies, increased autoantibody production in H2\(^d/c\) vs H2\(^c/c\) F1 mice cannot easily be explained by levels of I-E, because both haplotypes encode functional I-E molecules about equally (22). Previous studies from our laboratory suggested that H2\(^c\) alone may not be efficient at presenting certain types of self Ags compared with other haplotypes (18). Furthermore, in separate studies, B6.Nba2 mice heterozygous for deficiency of Ab\(^b\) (i.e., Ab\(^{+/-}\)) mice produced higher amounts of autoantibodies than wild-type B6.Nba2\(^{+/-}\) (Ab\(^{+/-}\)) mice, consistent with the idea that lower levels of class II molecules (as would be the case for each class II molecule in a mouse heterozygous for MHC) reduces the numbers of T cells that are negatively selected in the thymus (35).

The lack of antinuclear Abs and glomerulonephritis in our studies of H2\(^c/c\) B6.Nba2 mice almost certainly relates to studies by...
Wakeland and colleagues (36) showing that the strongest disease-suppressor locus (Sles1) mapped to the H2z MHC region. In their studies, Sles1 also inhibited disease at the level of autoantibody production by congenic mice. Nevertheless, Sles1 is not sufficient to inhibit disease in every genomic context, because NZM2410 (H2z/z) mice produce autoantibodies and develop glomerulonephritis. Our study design also localizes the position of Sles1, because the congenic interval includes only 1 cM of NZW genomic DNA proximal and 4 cM distal to the H2z locus (22). Novel aspects of the H2z locus have been identified. For example, studies have described a novel allele of the factor B gene (class III region) in H2z vs other haplotypes that affects activation of the alternative complement pathway in the kidney (37).

Several explanations are possible for why B6.Nba2 mice do not develop glomerulonephritis. The current studies show that the levels of autoantibodies to chromatin and its constituents are lower in B6.Nba2 than in (B6.Nba2 × NZW)F1 mice, and it is possible that the B6.Nba2 levels do not achieve a threshold sufficient for disease. A second possibility is that different autoantibody specificities, critical for nephritis, are produced in F1, but not B6.Nba2 or H2zH2b. B6.Nba2, mice. Studies involving NZM.C57Lc4 mice, which develop a fatal glomerulonephritis in the absence of autoantibodies to dsDNA and other antigens, support this possibility (38). Nevertheless, we observed prominent IgG and C3 deposition in the glomeruli of B6.Nba2 mice, suggesting that the block of fatal glomerulonephritis was at a level downstream of autoantibody production and IC deposition. Although the extent of deposition was not as diffuse as that observed in the F1 mice, and levels in F1 mice might continue to increase well beyond B6.Nba2 levels, our results suggest that at least one component of the NZW non-MHC contribution to disease in the F1 is related to mechanisms of inflammation or destruction after IC deposition and complement activation. The data are consistent with recent studies suggesting that NZW mice are more susceptible than other strains to nephrotic serum-mediated glomerulonephritis and glomerular damage (39). It has also been well demonstrated that genetic defects (and probably polymorphisms) can affect glomerulonephritis after the stage of IC deposition and complement activation (40).

These studies also showed that female mice, whether B6.Nba2 or (B6.Nba2 × NZW)F1, develop increased levels of autoantibodies and nephritis compared with male counterparts. Previous studies in humans and BWF1 mice suggested that the effects of gender are related to sex hormones, both disease-suppressive effects of androgens and disease-enhancing effects of estrogens (1, 4–6, 41, 42). One major candidate gene for Nba2 is IFN-inducible gene 202 (Ifi202), which encodes a protein that inhibits apoptosis (21, 43). Preliminary gene expression studies indicated an enhancing effect of estrogens on gene expression (D. Choubey, M. R. Gubbel, and B. L. Kotzin, unpublished observations), and this may be involved in their enhancing effect on disease. However, the consistent effects of sex hormones in different lupus models, including single-focus models such as Sle1 mice (36), suggest that the effects of sex hormones on gene expression involve separate pathways that can consistently interact with nearly any group of lupus susceptibility genes.

In summary, the current studies showed that the development of disease in (B6.Nba2 × NZW)F1 compared with B6.Nba2 mice depends on NZW-encoded non-MHC genes. Additionally, H2z homozygosity suppressed autoantibody production in these models, which may be dependent upon multiple genes within the MHC region. In these studies, the lack of nephritis in B6.Nba2 mice appeared to be partly independent of IC deposition in the kidney,
suggesting that a component of the NZW genetic contribution required for kidney pathogenesis acts downstream of IC deposition. Whether this effect is dependent on sex hormones is unknown, because female mice exhibited both higher levels of autoantibodies and increased incidence of fatal lupus nephritis than male mice regardless of genetic background or MHC haplotype.

Disclosures

The authors have no financial conflict of interest.

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