HLA-DR Modulates Autoantibody Repertoire, But Not Mortality, in a Humanized Mouse Model of Systemic Lupus Erythematosus

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HLA-DR Modulates Autoantibody Repertoire, But Not Mortality, in a Humanized Mouse Model of Systemic Lupus Erythematosus

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To evaluate the disease-modulating role of HLA-DR2 and DR3 molecules, which have been associated with systemic lupus erythematosus, a humanized mouse model was examined. HLA-DR2 (DRB1*1502)- and DR3 (DRB1*0301)-transgenic mice were backcrossed to the New Zealand Mixed 2410 (NZM2410, H2z) strain. Seventh generation DR2 and DR3 transgene-positive animals along with their transgene-negative littermates and the parental strain NZM2410 were monitored for proteinuria, azotemia, death, and mortality between the backcrosses with and without HLA-DR2 or HLA-DR3. However, the genetic analysis of different backcrosses showed that heterozygosity at the endogenous H2-E locus (Ez/Eb) was strongly linked with acceleration of lupus nephritis in both HLA-DR2 and HLA-DR3 transgenics. More importantly, the presence of the HLA-DR2, but not the HLA-DR3, transgene significantly enhanced the production of anti-dsDNA, but not anti-ssDNA, anti-histone-dsDNA complex, or anti-histone Abs. In contrast, neither HLA-DR2 nor HLA-DR3 influenced the development of glomerulonephritis or the degree of immune complex deposition. Moreover, nephritic kidneys from mice with and without HLA-DR2 or HLA-DR3 transgenes showed similar patterns of cytokine expression. Collectively, these findings provide molecular evidence that the association of HLA-DR2 or HLA-DR3 with lupus susceptibility is related to the type of autoantibody rather than to disease mortality. The use of a humanized mouse model provides a way of dissecting the roles of human MHC genes in systemic lupus erythematosus pathogenesis.


Studies in both animal models and humans indicate that the development of systemic lupus erythematosus (SLE) has a strong genetic basis, with contributions from both MHC and multiple non-MHC genes (1–3). Lupus susceptibility genes most likely interact with one another at different levels of lupus pathogenesis, which, along with the appropriate environmental triggers, lead to the development of disease (4). Studies of genetic susceptibility indicate that genes encoded within or closely linked to the MHC are important for the development of disease. This is strongly supported by the linked association of genes encoded within the H-2z locus with lupus in several mouse models, including New Zealand hybrid mice and their derivatives (5, 6). The disease association with certain HLA haplotypes, including HLA-DR2 and HLA-DR3 (7–11) and HLA-B8 (11), and certain complement (12) and TNF polymorphisms (13) has been reported in human lupus.

The genes encoded within the MHC that contribute to lupus are not known. However, disease association studies in (NZB × NZW) F1 progeny indicate the requirement of heterozygosity at MHC class II (H2b3) for lupus susceptibility (6). Studies using transgenic mice reveal that MHC class II molecules may not totally account for the MHC contribution to disease susceptibility, nor do they provide protection from disease in certain lupus models (14, 15). Whether this is true for disease-associated HLA genes is unknown. In humans, disease association studies are further complicated by linkage disequilibrium and possible complimentary interactions among the genes within the HLA region. In particular, the association of HLA class II molecules with disease is stronger for subsets of autoantibodies and autoantibody-related clinical manifestations of SLE (16).

In the present study, we used HLA class II-transgenic mice to individually dissect the disease-modulating capabilities of these HLA genes, particularly HLA-DR2 and DR3, in the development of lupus. These mice were bred into the New Zealand Mixed/Aeg 2410 (NZM2410) strain, which is an inbred lupus murine line derived from the NZB and NZW mice, to generate the humanized strains carrying HLA-DR2 (DR2t.NZM2410) and HLA-DR3 (DR3t.NZM2410). These animals provided an opportunity to assess the role of HLA-DR2 or HLA-DR3 in the development of lupus nephritis and mortality. These HLA-DR transgenics also allowed for a differential evaluation of the roles of these human genes in the clinical manifestations of the mouse disease, suggesting a similar role(s) in human disease.

Materials and Methods

The mAbs

All mAb cell lines used in this study were obtained from American Type Culture Collection (Manassas, VA). These included anti-DR mAb L227 (17), anti-Ad mAb 10.2.16 (18, 19), and anti-E4 mAb Y-17 (20).

Transgenic mice

All mice were bred and maintained concurrently in the pathogen-free immunogenetic mouse colony at Mayo Clinic (Rochester, MN). The protocols of these studies were approved by the institutional animal care and use committee.
DISEASE-MODULATING ROLES OF HLA-DR IN A MOUSE MODEL OF SLE

New Zealand Mixed/Aeg 2410 (NZM2410, H2* mice have been previously described (21) and were purchased from The Jackson Laboratory (Bar Harbor, ME). The AβDR2 (DRB1*1502)- and AβDR3 (DRB1*0401)- transgenic mice have also been described previously (22–25). These HLA class II-transgenic mice have normal phenotypes without evidence of autoantibody production up to 12 mo of age, which covered the period of study. The HLA-DR2 and HLA-DR3 transgene-positive NZM2410 (DR2*NZM2410 or DR3*NZM2410) mice were generated by selective backcrossing of the progeny to NZM2410 for seven generations. The offspring from these NZM2410 and DR3*NZM2410 were confirmed by PCR analysis of tail DNA and by flow cytometry for cell surface expression using anti-HLA-DR (L227 mAb) on PBMCs (23, 24). All mice examined retained their endogenous H2*, as demonstrated by flow cytometric analysis for anti-HLA-DR (L227 mAb) and I-E* (Y-17 mAb). HLA-DR2 and HLA-DR3 expression in spleen and kidney was confirmed periodically by immunoperoxidase staining.

Evaluation of renal disease and mortality

Mice were studied from 2–12 mo of age or until they were moribund. They were monitored weekly for mortality and monthly for the presence of significant proteinuria, defined as albuminuria ≥300 mg/dl (26), at Balb/cix (Bayer, Elkhart, IN) for and significant azotemia, defined as whole blood urea nitrogen ≥26 mg/dl (26), at Azostix (Bayer).

Serological assays

Autoantibodies to ssDNA, dsDNA, histone-DNA complex, and histone were determined by ELISA as previously described (27). Briefly, microtiter plates (Maxisorb, Fisher Scientific, Pittsburgh, PA) were coated overnight with calf thymic histone (10 µg/ml in carbonate buffer (pH 9.4); Worthington Biochemical, Lakewood, NJ), and plasmid DNA-300 (1 µg/ml) in PBS (pH 7.2); Immunovision, Springfield, AZ) precoated with methylated BSA (mBSA) complex (Sigma, St. Louis, MO). To detect Abs to histone-DNA complex, mBSA-precoated plates were subsequently incubated overnight with 50 µg/ml sonicated and S1 nuclease-digested calf thymic histone, followed by 10 µg/ml calf thymic histones. This procedure has been previously demonstrated to give more specific results than nucleosome-coated substrates (27).

For anti-ssDNA Ab detection, plates were precoated overnight with mBSA, followed by calf thymic DNA that had been boiled for 15 min and chilled on ice. The wells were subsequently blocked with 3% BSA in PBS for 2 h at room temperature. Serum samples were diluted (1/300) in 3% BSA in PBS, added to Ag-coated wells, incubated at 37°C for 1 h, and washed five times with PBS containing 0.05% Tween 20. Subsequently, wells were incubated with peroxidase-conjugated goat anti-mouse Ig (BD PharMingen, San Diego, CA) for 1 h and washed. 3,3',5,5'-Tetramethylbenzidine (TMB) reagent (Sigma) was added, and the absorbance spectrum was determined with an automated spectrophotometer (Bio-Rad, Hercules, CA). Ig isotype and subclass anti-dsDNA were assayed in the same manner using IgM, IgG1, IgG2a, IgG2b. IgG3 specific second-step conjugates, and p-nitrophenyl phosphate substrate (Southern Biotechnology Associates, Birmingham, AL). All assays were performed in duplicate and were quantified against a standard curve obtained with the known positive control serum from NZM2410 as previously described (14, 15).

Histopathology

Moribund mice or mice with significant proteinuria and/or azotemia were sacrificed, and their kidneys were extracted and snap-frozen in OCT tissue embedding medium (Sakura Finetek, Torrance, CA). Cryostat sections were prepared, fixed, and stained with H&E as previously described (21).

For immunoperoxidase staining for HLA-DR2 and -DR3 (24), cryostat sections of OCT-embedded kidney tissues were acetone-fixed for 10 min at 4°C, quenched for endogenous peroxidase, and incubated for 30 min in phosphate buffer containing 10% normal mouse serum. Sections were washed briefly in phosphate buffer, incubated for 60 min with the optimal dilution of biotinylated-L227 (anti-DR mAb) in phosphate buffer containing 5% normal mouse serum, wash in phosphate buffer for 15 min, and incubated for another 30 min with avidin-biotin-peroxidase complex (ABC kit, Vector Laboratories, Burlingame, CA). Peroxidase reactivity was detected by incubation with 3,3'-diaminobenzidine (DAB substrate kit, Vector Laboratories). Kidneys from C57BL/6 and DRB1*NZM2410 littersates were used as negative controls.

Immunocomplex deposition was examined by direct immunofluorescence staining of kidney sections. Unfixed cryostat sections were blocked for 30 min with 5% BSA in PBS and incubated for 60 min with FITC-conjugated goat IgG anti-mouse C3 (ICN/Cappel, Aurora, OH) in PBS (pH 7.2) containing 5% BSA. Stained sections were washed three times in PBS for 15 min each time and examined by fluorescence microscopy.

Tissue cytokine expression

RNase protection assays were conducted to examine the expression of cytokines in the nephritic kidneys as previously described (28). Briefly, tissue RNA was extracted by Tripure (Roche, Indianapolis, IN) from cryostat sections. The RNA samples were electrophoresed on a 1% agarose gel to check for integrity and to rule out DNA contamination. RNAs were used for hybridization with 32P-labeled probes (TNF-α, leukotriene-β, TGF-α, IL-6, IFN-γ, IFN-β, TGF-β1, TGF-β2, TGF-β3, and macrophage migration inhibitory factor (MIF)) of a multiprobe RNase protection assay kit (BD PharMingen). Subsequent to hybridization, samples were subjected to RNase digestion, electrophoresed in 5% acrylamide/5 M urea gel, and analyzed by autoradiography. Quantification of autoradiograms was accomplished by phosphorimaging using the Storm System (Molecular Dynamics, Sunnyvale, CA). For these studies the cytokines examined included those reported to be important in the mediation and/or progression of lupus nephritis (29, 30).

Statistical analysis

Survival data were analyzed using the Kaplan-Meier method and the log-rank test (31). The median difference for particular autoantibodies in different backcrosses were tested prior-hoc using Kruskal-Wallis test (two-tailed) followed by post-hoc pair comparisons using Mann-Whitney rank sum test with the p value adjusted for the number of paired comparisons (32). The incidence of proteinuria was analyzed by χ2 test. Values of p < 0.05 were used for statistical significance.

Results

Genetic analyses of transgenic mice

To investigate whether HLA-DR molecules have a disease-modulating role in SLE, we introduced HLA-DR2 and -DR3 genes into the lupus mouse model NZM2410 (21) to simulate the situation in human disease, where these MHC class II genes are among other lupus susceptibility genes (16). It was our intention to develop NZM2410 mice congenic for HLA-DR2 and HLA-DR3. However, the progenies of these mice bred poorly, developed obesity, and had an increase in mortality, such that studies were limited to the seventh generation offspring. At this level, the animals examined theoretically carry ~99.22% of the parental NZM2410, with 0.88% of background genes introduced by the HLA class II (AβDR2* and AβDR3*) transgenic strains.

Four groups of seventh generation progenies were ascertained for the presence/absence of HLA-DR2 or HLA-DR3. HLA class II-positive mice were initially screened by PCR and confirmed for cell surface expression on PBMCs by immunofluorescence staining and flow cytometry (data not shown). The HLA-DR transgens have 25–30% of PBMCs expressing HLA class II molecules by flow cytometry, whereas the HLA class II-negative littersates have no detectable surface expression. The levels of HLA-DR expression on PBMCs were comparable to the levels of endogenous H2* among the transgene-positive mice (DR to A ratio, 0.85–0.90; DR to E ratio, 0.88–0.92; p > 0.1). The levels of endogenous H2* expression on PBMCs were unaltered by the presence of HLA transgenes (for DR*: A*, 30.07 ± 5.11%; E*, 27.8 ± 6.9%; for DR**: A*, 31.68 ± 5.68%; E*, 27.5 ± 5.7%; p > 0.1). All transgenic mice and their littermates retained the endogenous H2* from parental NZM2410.

Cell population analyses were also conducted for all the backcrosses. Results showed that there were no significant differences in the numbers of CD3+, CD4+, CD8+, and double-negative CD3− T cells, B cells, and monocytes among the transgenic mice, their negative littersates, and the parental strain NZM2410 (data not shown).

Role of HLA-DR molecules in lupus nephritis and mortality

Depicted in Figs. 1 and 2 is a comparative analyses of proteinuria and mortality among the five cohorts of mice examined. The results showed that neither HLA-DR2 nor HLA-DR3 influenced the
incidence of proteinuria. There were no differences in the cumulative incidence of proteinuria between HLA-DR2 transgenics and their negative littermates or between the HLA-DR3 transgenics and their respective transgene-negative littermates (Fig. 1A; \( p < 0.1 \) in both HLA-DR2 and HLA-DR3 cohorts). Similarly, the incidence of mortality was unaffected by the presence or the absence of the DR transgenes (Fig. 1B; \( p = 0.9748 \) in the HLA-DR2 cohorts and \( p = 0.7865 \) in the HLA-DR3 cohorts).

However, there was a curious subgroup difference between the HLA-DR2- and the HLA-DR3-transgenic cohorts. The HLA-DR2 transgenics and their negative littermates had a much higher incidence of proteinuria compared with the HLA-DR3 transgenics and their negative littermates (Fig. 1A; \( p < 0.001 \)). Moreover, the HLA-DR2 transgenics and their negative littermates died much earlier than the HLA-DR3 transgensics, their negative littermates, and the parental NZM2410 (Fig. 1B; \( p < 0.001 \)). We hypothesized that such difference in the Kaplan-Meier curves between the cohorts, i.e., DR2\(^+\) and DR2\(^-\) littermates as one group, and DR3\(^+\) and DR3\(^-\) littermates as another group, might be attributed to slight differences in genetic backgrounds of these seventh generation offspring.

To address this issue, mice were screened for the expression of endogenous H2-E\(^z\) by PCR and flow cytometry (data not shown). Analysis of the subgroups revealed that the mice were either homozygous or heterozygous at the H2-E locus (Fig. 2A; \( p = 0.001 \)). We hypothesized that such difference in the Kaplan-Meier curves between the cohorts, i.e., DR2\(^-\) and DR2\(^+\) littermates as one group, and DR3\(^-\) and DR3\(^+\) littermates as another group, might be attributed to slight differences in genetic backgrounds of these seventh generation offspring.

FIGURE 1. Incidence of proteinuria and survival curves in different backcrosses of mice. A, Incidence of proteinuria. Mice were monitored monthly for the presence of proteinuria starting at 2 mo of age. Proteinuria was defined as the presence of albumin \( \geq 300 \) mg/dl in the urine (26). Because there was no statistical difference between DR2\(^-\).NZM2410 and DR2\(^+\).NZM2410 and between DR3\(^-\).NZM2410 and DR3\(^+\).NZM2410, they were grouped as DR2 and DR3 cohorts, respectively, for the analysis; \( * \), \( p < 0.05 \), by one-tailed chi square test. B, Survival curves. Mortality was monitored weekly up to 12 mo. There was an increased mortality of DR2\(^-\).NZM2410 and DR2\(^+\).NZM2410 compared with either the parental strain NZM2410 (\( p < 0.001 \)) or DR3\(^-\).NZM2410 and DR3\(^+\).NZM2410 (\( p < 0.001 \)). The mortality was not different among the following groups: DR2\(^-\).NZM2410 and DR2\(^+\).NZM2410 (\( p = 0.9748 \)), DR3\(^-\).NZM2410 and DR3\(^+\).NZM2410 (\( p = 0.7865 \)), NZM2410 and DR3\(^-\).NZM2410 or DR3\(^+\).NZM2410 (\( p = 0.9433 \)).
We proceeded to examine the survival age among the groups and found similar patterns. Results showed that mice heterozygous at the H2-E locus (Ez/Eb) died earlier than the parental strain NZM2410. All mice that were heterozygous at the H2-E locus (Ez/Eb), regardless of the presence of HLA-DR transgenes, died by a mean age of 25–28 wk. In contrast, mice homozygous at the H2-E locus (Ez/Ez) died at an older age of 35–41 wk, much like the parental strain NZM2410 (39.77 ± 8.22 wk). The age at mortality of the homozygotes was unaltered by the presence of HLA-DR transgenes. It is evident from these studies that the high rate of mortality in these animals correlates strongly with proteinuria. The present data are consistent with previous studies indicating that proteinuria is a strong predictor of mortality in lupus (14, 15). We found that the high rates of mortality and proteinuria were highly correlated with the presence of azotemia (data not shown) and glomerulonephritis (see below).

**HLA-DR expression and renal pathology**

All mice found dead or moribund had histological evidence of severe nephritis. As in previous studies the kidney lesions were indistinguishable among different backcrosses and from the parental NZM2410 (21). As depicted in Fig. 3, the mice in different backcrosses had typical diffuse lupus proliferative glomerulonephritis, showing inflammatory infiltrates, thickened capillary wall, wire loops, and sclerosis indistinguishable from those in the parental NZM2410 (data not shown).

Immune complex deposition along the capillary walls was also present in diseased kidneys of all backcrosses (Fig. 4, A, C, E, and G). There was no difference in the histopathologic manifestations of the nephritic kidneys between homozygous (Ez/Ez) and heterozygous (Ez/Eb) transgenic mice (data not shown).

To determine whether the presence of HLA-DR2 or DR3 influenced disease severity, the expression patterns of these molecules were examined in the transgenics. Results showed that just as HLA-DR2 and HLA-DR3 molecules were found in PBMCs, as detected by flow cytometry, they were also abundantly expressed in the nephritic kidney (Fig. 4, B and F) and on splenocytes (data not shown). HLA-DR molecules were expressed on the mesangial cells and the infiltrating cells in the glomeruli as well as on tubular cells and inflammatory cells in the interstitium. As expected, these molecules were absent in the kidneys of the DRneg littermates (Fig. 4, D and H). Interestingly, there were no significant differences in kidney pathology between the HLA-DR transgenics and their negative littermates. All kidney tissues examined showed severe infiltration and immune complex deposition.

![Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** Histopathology of kidneys. H&E staining of kidney sections from different groups of backcrosses. A, DR2*.NZM2410; B, DR2neg.NZM2410; C, DR3*.NZM2410; D, DR3neg.NZM2410. Severe glomerulonephritis was found in all groups of mice. Magnification, ×400.
Results suggest that the quantitative difference in HLA-DR expression is about 5% higher than that of HLA-DR2 (data not shown). These findings indicate that HLA-DR expression in PBMC showed that HLA-DR3 expression is about 5% higher than that of HLA-DR2 (data not shown). Interestingly, there were no statistical differences in autoantibody levels among mice heterozygous (Ez/Eb) or homozygous (Ez/Ez) at the H2-E locus (data not shown). In contrast to the apparent lack of contribution of HLA-DR2 and HLA-DR3 to glomerulonephritis and mortality, DR2+NZM2410 mice produced significantly higher levels of anti-dsDNA compared with their DR2neg.NZM2410 (p = 0.013) littersmates and the parental NZM2410 (p = 0.003; Fig. 5). Isotype and subclass analysis of the anti-dsDNA Abs revealed that the predominant isotype was IgG2a, but not IgM, IgG1, IgG2b, or IgG3 (data not shown). A similar pattern was observed in anti-dsDNA IgG and anti-dsDNA IgG2a levels among mice heterozygous (Ez/Eb) or homozygous (Ez/Ez) at the H2-E locus (data not shown). However, there was a tendency toward a higher level of anti-ssDNA, anti-histone-dsDNA complex, and anti-histone Ab production among the DR2+ mice, albeit the levels were not statistically different from those in their negative littersmates or the other backcrosses. In contrast, there were no differences in anti-dsDNA Ab production between DR3+NZM2410 mice and their negative littersmates (p = 0.982). Subgroup analyses also showed no statistical differences in autoantibody levels among mice heterozygous (Ez/Eb) or homozygous (Ez/Ez) at the H2-E locus (data not shown). Interestingly, flow cytometry measurements of HLA-DR expression in PBMC showed that HLA-DR3 expression is about 5% higher than that of HLA-DR2 (data not shown). These results suggest that the quantitative difference in HLA-DR expression does not account for the preferential elevation of anti-dsDNA Ab production in DR2+, but not in DR3+, mice.

Cytokine expression in the nephritic kidneys

Proinflammatory cytokines and growth factors are important in mediating lupus nephritis (29, 30). Although the histopathologies of the nephritic kidneys among mice with and without HLA transgenes were very similar, it is possible that the presence of HLA-DR transgenes might bring about differences in the local cytokine environments in the diseased kidneys. Therefore, RNase protection assays were conducted to examine whether there were differences in the molecular composition of inflammatory mediators in the nephritic kidneys of the different backcrosses. The results showed that MIF was constitutively expressed in the prenephritic kidneys (Fig. 6). Other cytokines, such as TNF-α, TNF-β, leukotriene-β, and IFN-γ, were variably expressed in all nephritic kidneys examined. None of these cytokines was found in the prenephritic kidneys from all backcrosses (data not shown). In contrast, nephritic kidneys predominantly expressed TGF-β1 and TGF-β3 (Fig. 6). Other cytokines, such as TNF-α, TNF-β, leukotriene-β, and IFN-γ, were variably expressed in all nephritic kidneys examined. None of these cytokines was found in the prenephritic kidneys (data not shown). Regardless of the presence of the HLA-DR transgenes, nephritic kidneys from all backcrosses and the parental strain NZM2410 showed similar patterns and levels of cytokine genes after adjusting for the levels of housekeeping genes (L32 and GADPH).

Discussion

Although MHC class II genes have been associated with lupus susceptibility in several mouse models and in human lupus (5–11), the exact role of particular MHC genes or haplotypes in disease...
pathogenesis is unknown. The underlying basis of disease is undoubtedly complex. Assessing the role of specific HLA class II genes in human lupus is difficult due to the coexpression of several other class II molecules and the strong linkage disequilibrium among the HLA region genes (33). To circumvent this difficulty, we created transgenic mice to specifically determine whether the lupus-associated HLA class II alleles, HLA-DR2 (DRB1*1502) and HLA-DR3 (DRB1*0301), have any influence on the pathogenesis of lupus. Inasmuch as these DR alleles are considered to be the most relevant SLE-predisposing genes in multiple ethnic groups (7–10), we introduced DR2 and DR3 transgenes into a well-described lupus mouse model, NZM2410 (21, 34–36). Because this mouse strain spontaneously develops lupus-like disease, the introduction of either HLA-DR2 or HLA-DR3 genes permitted differential assessment of the roles of these genes in the disease process.

The present data clearly show that neither HLA-DR2 nor HLA-DR3 influences the mortality associated with lupus in this animal model (Fig. 1). Regardless of the presence of HLA-DR, virtually all mice died from glomerulonephritis (Figs. 3 and 4). Moribund mice developed severe proteinuria (Fig. 1A), ascites, splenomegaly, and pleural effusion in both sexes that were indistinguishable among the different backcrosses examined (data not shown).

However, the HLA-DR2 cohorts, i.e., DR2+ transgensics and their negative littermates, had earlier mortality (Fig. 1B) accompanied by high levels of proteinuria (Fig. 1A) compared with the HLA-DR3 cohorts (i.e., DR3+ transgensics and negative littermates) and the parental strain NZM2410. This suggests that there is a disease-accelerating role of non-HLA class II genes. As has been suggested by previous studies with mouse lupus models (5, 6), we postulate that such early mortalities in the HLA-DR2 cohorts may be related to heterozygosity at the H2-E locus, because the animals used in these studies were seventh generation offspring. This hypothesis was borne out by the genetic analysis of the NZM2410 DR lines, which revealed that most of the mice (74%) in the HLA-DR2 cohorts (i.e., DR2+ and DR2neg littermates) were heterozygous at the H2-E locus (E/E′), whereas the majority of mice (76%) in the HLA-DR3 cohorts (i.e., DR3+ and DR3neg littermates) were homozygous at the H2-E locus (E/E′).

The H2-E heterozygous mice would inherit the Eβb from the parental AjHHLA class II transgensics. It is quite possible that Eβb molecules could dimerize with Eαa derived from parental strain NZM2410, forming mixed H2-E heterodimers that have Ag-presenting capability relevant to the disease process. The formation of such heterodimers has been demonstrated in other lupus models (19). Indeed, our data show that H2-E heterozygous mice, regardless of the presence of HLA class II transgensics, died earlier at 25–28 wk, whereas H2-E homozygous mice died at the mean age of 35–41 wk, in a manner similar to the parental strain NZM2410 (Fig. 2). These findings are consistent with the studies that suggested the requirement of H-2 heterozygosity for the development of autoimmunity in New Zealand hybrid mice, the predecessor of NZM2410 mice (1, 6). The exact mechanism by which H2 heterozygosity confers greater disease susceptibility than H2 homozygosity is unknown. However, the enhancing effect on disease from mixed haplotype or possibly mixed isotype class II molecules (36) and the possible loss of homozygosity of candidate recessive SLE-suppressing genes linked to this region (37) have been suggested as possible mechanisms.

Because the mice used in this study are seventh generation offspring, there is a theoretical background genetic contamination of 0.88% from the parental HLA class II-transgenic mice. If this small genetic contamination contributes at all to the disease process, then its segregation to the offspring is expected to result in the random disease-accelerating phenotype associated with the acquisition of these “contaminating” background genes. However, our data clearly show the consistent finding of early mortality among heterozygous E/E′ mice compared with E/E′ homozygous mice. Thus, lupus-related mortality among these animals is more strongly correlated with the loss of homozygosity at E′, rather than the contaminating parental genes.

The most interesting finding of this study is the specific association of anti-dsDNA Ab production with HLA-DR2, but not HLA-DR3 (Fig. 5). HLA-DR2+ transgensics (DR2+.NZM2410), but not their negative littermates (DR2neg.NZM2410) or DR3+.NZM2410 mice, had higher levels of anti-dsDNA Ab. This was in marked contrast to the anti-ssDNA, anti-histone-dsDNA.
complex, and anti-histone Ab levels, which were not statistically different among the different groups of mice. The basis for the DR2 transgene-specific enhancement of anti-dsDNA Ab production is unclear, although previous studies have implicated H2-E heterozygosity as an important determinant in lupus mice (14, 15). The DR2-specific bias of anti-dsDNA Ab production was found in approximately equal numbers of H2-E heterozygous and homozygous DR2+NZM2410 mice. There were also no significant differences in anti-dsDNA Ab levels between heterozygous and homozygous mice in the HLA-DR3 cohorts and the parental NZM2410 (data not shown). The DR2-specific bias of anti-dsDNA Ab production may also not be due to transgene-related defects in hemopoiesis, because T and B cell maturation and monocyte numbers are comparable among all backcrosses (data not shown). Whatever the basis for the DR2 bias, our data provide the first molecular evidence supporting the epidemiological association of anti-dsDNA autoantibody production and HLA-DR2 in human lupus (9, 38).

Autoantibody production in lupus is generally thought to be an important determinant of nephritis. In mice, anti-dsDNA Abs are considered to be pathogenic and to induce lupus nephritis when introduced into normal recipients (39, 40). However, in the present study, there was no apparent correlation between the serum levels of anti-dsDNA with either mortality or severity of nephritis in DR2+NZM2410 (Figs. 1–5). Despite the higher levels of anti-dsDNA Abs in DR2+ transgenics, their kidney pathologies were indistinguishable from those of their DR2neg littermates, the DR3 cohorts, and the parental strain NZM2410. Although the pathogenicity of DR2-induced anti-dsDNA Abs, or its specific subtypes, remains to be elucidated, it is conceivable that nephritis itself may be initiated with lesser amounts of anti-dsDNA. It is also possible that nephritis may be caused by other anti-nuclear Abs (41), which would explain the equivalent levels of immune complex deposition among all backcrosses (Fig. 4). The molecular basis for the restriction of anti-dsDNA Ab to HLA-DR2, but not HLA-DR3, remains to be examined.

The expression of HLA-DR2 or DR3 molecules in the kidney was also found to have no effect on the histopathology of the lesions among the different backcrosses (Fig. 4). HLA class II molecules were expressed on various cells in the kidneys that are largely nonprofessional Ag-presenting cells (e.g. mesangial cells) and/or normal nonhemopoietic cells of the kidney (e.g. tubular cells). The HLA-DR+ cellular infiltrates are most likely lymphocytes and macrophages, but their presence did not induce any more kidney damage than that seen in the DRneg littermates. Our data corroborate a study in the MRL/lpr MHC class II knockout transplanted model, which demonstrates that expression of class II molecules in the kidney is not required for the development of lupus nephritis (42). Consistent with this hypothesis is the finding that cytokine expression profiles of the nephritic kidneys of all backcrosses are very similar (Fig. 6). All animals showing nephritis predominantly expressed TGF-β1 and TGF-β3, and variable levels of TNF-α, TNF-β, leukotriene-β and IFN-γ. Dysregulation of TGF-β has been previously reported to be associated with progressive renal disease in human lupus (43). In the present study MIF is also expressed at high levels in the nephritic kidneys and, curiously, at equivalent levels to those in prenephritic kidneys (data not shown). Although elevated levels of MIF in the sera of lupus patients have been reported (44), the pathogenic role of this cytokine in lupus nephritis is unknown.

In summary, our studies using the humanized mouse lupus models carrying HLA-DR2 and HLA-DR3 transgenes unequivocally demonstrate that the development of lupus nephritis and mortality are HLA-DR-independent phenomena. However, HLA-DR2, but not HLA-DR3, preferentially enhances the production of anti-dsDNA Abs. The pathogenicity, if any, of DR2-dependent anti-dsDNA Ab production is unclear, because the severity of lupus nephritis is indistinguishable among all mice cohorts examined. Whether anti-dsDNA Abs incite pathologic effects other than in the kidneys remains to be examined. Whatever the pathogenic role of these Abs may be, our data provide the experimental evidence in support of previous studies reporting the association of HLA-class II genes to autoantibody production (3, 16). We are in the process of developing HLA-DR2 and HLA-DR3-transgenic mice in the context of the NZM2410 background genes but lacking endogenous class II molecules. Studies of these mice may provide a better understanding of the role of HLA class II genes in the etiopathogenesis of lupus.

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