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

Tawatchai Paisansinsup, Abbe N. Vallejo, Harvinder Luthra and Chella S. David

*J Immunol* 2001; 167:4083-4090; doi: 10.4049/jimmunol.167.7.4083
http://www.jimmunol.org/content/167/7/4083

### Why The JI?
- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

**average**

### References
This article cites 39 articles, 8 of which you can access for free at:
http://www.jimmunol.org/content/167/7/4083.full#ref-list-1

### Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

### Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

### Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
HLA-DR Modulates Autoantibody Repertoire, But Not Mortality, in a Humanized Mouse Model of Systemic Lupus Erythematosus

Tawatchai Paisansinsup, Abbe N. Vallejo, Harvinder Luthra, and Chella S. David

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, autoantibody production, development of nephritis, and mortality. The results showed no significant differences in proteinuria, azotemia, or 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 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. The Journal of Immunology, 2001, 167: 4083–4090.
New Zealand Mixed/Aeg 2410 (NZM2410, H2β) mice have been previously described (21) and were purchased from The Jackson Laboratory (Bar Harbor, ME). The Aβ′DR2 (DRB1*1502)- and Aβ′DR3 (DRB1*0301)-transgenic mice have also been described previously (22–25). These HLA-DR2- and HLA-DR3-transgenic mice have normal phenotypes without evidence of autoantibody production up to 12 mo of age, which covered the period of the study. The HLA-DR2 and HLA-DR3 transgenic-positive NZM2410 (DR2′-NZM2410 or DR3′-NZM2410) mice were generated by selective backcrossing of the progeny to NZM2410 for seven generations. The offspring with DR2′-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 H2β, as demonstrated by flow cytometric analysis for I-A′ (10.2.16 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), as well as blood urea nitrogen ≥26 mg/dl (26), using Azostix (Bayer). Serologic assays

Autoantibodies to dsDNA, 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, Springdale, AZ) precoupled with methylated BSA (mBSA) complex (Sigma, St. Louis, MO). To detect Abs to histone-DNA complex, mBSA-precoupled 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-sDNA 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 (1/500) 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 substrate (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 according to 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, washed 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 DR2′-NZM2410 and DR3′-NZM2410 littersmates were used as negative controls.

Immune complex 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-β, leucotriene-β, TNF-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 (Aβ′DR2 + and Aβ′DR3 ′)-transgenic strains.

Four groups of seventh generation progeny 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 transgenics have 25–30% of PBMCs expressing HLA class II molecules by flow cytometry, whereas the HLA class II-negative littersmates have no detectable surface expression. The levels of HLA-DR expression on PBMCs were comparable to the levels of endogenous H2β among the transgenic-positive mice (DR to A′e ratio, 0.85–0.90; DR to E′ ratio, 0.88–0.92; p < 0.1). The levels of endogenous H2β expression on PBMCs were unaltered by the presence of HLA transgenes (for DR to A′e, 30.07 ± 5.11%; E′, 27.8 ± 6.9%; for DR to A′e, 31.68 ± 5.68%; E′, 27.5 ± 5.7%; p < 0.1). All transgenic mice and their littersmates retained the endogenous H2β 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 littersmates, 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
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 < 0.05, by one-tailed $\chi^2$ test). Survival curves. Mortality was monitored weekly up to 12 mo. There was an increased mortality of DR2+NZM2410 and DR2negNZM2410 compared with either the parental strain NZM2410 (p < 0.001) or DR3+NZM2410 and DR3negNZM2410 (p < 0.001). The mortality was not different among the following groups: DR2+NZM2410 and DR2negNZM2410 (p = 0.9748), DR3+NZM2410 and DR3negNZM2410 (p = 0.7865), NZM2410 and DR3+NZM2410 or DR3negNZM2410 (p = 0.9433).

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 DR2negNZM2410 and between DR3+NZM2410 and DR3negNZM2410, they were grouped as DR2 and DR3 cohorts, respectively, for the analysis; *p < 0.05, by one-tailed $\chi^2$ test. B, Survival curves. Mortality was monitored weekly up to 12 mo. Survival curves of mice heterozygous (E/Eh) or homozygous (E/Ez) at the H2-E locus were compared with the parental strain NZM2410 (p < 0.001).

FIGURE 2. Survival analysis of backcrosses according to the genotypic difference at the H2-E locus. Mice were examined for the expression of endogenous H2-E by PCR and flow cytometry. Mortality was monitored weekly up to 12 mo. Survival curves of mice heterozygous (E/Eh, A) or homozygous (E/Ez, B) at the H2-E locus were compared with the parental strain NZM2410. H2-E heterozygotes, regardless of the presence of HLA class II transgenes, died earlier than the parental strain NZM2410 (p < 0.001). In contrast, there was no difference in the survival of the parental strain and progenies homozygous at the H2-E locus (E/Ez) regardless of the presence of HLA class II transgenes (p = 0.1703).

earlier than the HLA-DR3 transgenics, 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 DR2neg littermates as one group, and DR3+ and DR3neg 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 by PCR and flow cytometry of PBMC (data not shown). Analysis of the subgroups revealed that the mice were either homozygous or heterozygous at the H2-E locus. Because the parental NZM2410 was Eh, and the parental Aβ2/DR2 or Aβ2/DR3 transgenics were Eo, these seventh generation NZM2410 DR transgenics and their littermates were either E/Eh or E/Eo. As shown in Fig. 2, H2-E homozygotes (E/Eh), regardless of the presence of HLA-DR transgenics, have similar survival curves as the parental strain (Fig. 2B; p = 0.1703). The H2-E heterozygotes (E/Eo), in contrast, died much earlier than the parental strain (Fig. 2A; p < 0.001). HLA-DR transgenics also did not alter the survival curves of the heterozygotes.
We proceeded to examine the survival age among the groups and found similar patterns. Results showed that mice heterozygous at the H2-E locus (E<sup>z</sup>/E<sup>b</sup>) died earlier than the parental strain NZM2410. All mice that were heterozygous at the H2-E locus (E<sup>z</sup>/E<sup>b</sup>) 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 (E<sup>z</sup>/E<sup>z</sup>) 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 (E<sup>z</sup>/E<sup>z</sup>) and heterozygous (E<sup>z</sup>/E<sup>b</sup>) 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 DR<sup>neg</sup> 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.** Histopathology of kidneys. H&E staining of kidney sections from different groups of backcrosses. A, DR2<sup>+</sup>.NZM2410; B, DR2<sup>neg</sup>.NZM2410; C, DR3<sup>+</sup>.NZM2410; D, DR3<sup>neg</sup>.NZM2410. Severe glomerulonephritis was found in all groups of mice. Magnification, ×400.
RESULTS show that the quantitative difference in HLA-DR expres-
sion about 5% higher than that of HLA-DR2 (data not shown). These
results suggest that the quantitative difference in HLA-DR expres-
sion 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 media-
tors in the nephritic kidneys of the different backcrosses. The re-
sults showed that MIF was constitutively expressed in the prene-
hritic kidneys from all backcrosses (data not shown). In contrast,
none of these cytokines was found in the prenephritic kidneys.

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

FIGURE 4. Immune complex deposition and HLA-DR expression in the kidneys of different backcrosses of mice. Frozen kidney tissues were stained
for immune complex (A, C, E, and G) and HLA-DR2 and HLA-DR3 molecules (B, D, F, and H). Immune complex deposition along the capillary loops
was found indistinguishable in all backcrosses, namely, DR2⁺,NZM2410 (A), DR2⁻, NZM2410 (C), DR3⁺,NZM2410 (E), and DR3⁻,NZM2410 (G). HLA-DR2 (B) and HLA-DR3 (F) were expressed in the mesangial cells and infiltrating cells in the glomeruli (arrows), tubular cells (filled arrowheads), and inflammatory cells in the interstitium (open arrowheads) in HLA class II-positive mice, but not in HLA class II-negative littermates (D and H for DR2⁻, and DR3⁻, respectively). Magnification, ×400.
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 ethic 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* transgenics 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* transgenics 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 Eb/e from the parental AβHLA class II transgenics. It is quite possible that Eb molecules could dimerize with Eα 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 transgenes, 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* transgenics (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

FIGURE 5. Autoantibody levels in different backcrosses of mice. Sera from mice at 6 mo of age were analyzed for anti-ssDNA, anti-dsDNA, anti-histone-dsDNA complex, and anti-histone by ELISA. All assays were performed in duplicate and were quantified against a standard curve obtained with known positive control sera from NZM 2410. Data are expressed as arbitrary units per milliliter. A unit is the serum dilution, which gives an OD590 of 0.5 that is equivalent to the positive control serum at 1/450 dilution. An adjusted value of $p < 0.016$ was considered statistically significant (adjusted $p$ value for 3 post-hoc paired comparison).
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 heterozy-
gosity as an important determinant in lupus mice (14, 15). The
DR2-specific bias of anti-dsDNA Ab production was found in ap-
proximately equal numbers of H2-E heterozygous and homozy-
gous DR2\(^+\),NZM2410 mice. There were also no significant dif-
fferences 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 num-
bers 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 lu-
pus (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

Homozygous DR2

NZM2410 (Figs. 1–5). Despite the higher levels of anti-
dsDNA Abs in DR2\(^+\) transgensics, their kidney pathologies were
indistinguishable from those of their DR2\(^{-}\) littermates, the DR3
cohorts, and the parental strain NZM2410. Although the pathoge-
nicity 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 re-
striction of anti-dsDNA Ab to HLA-DR2, but not HLA-DR3, re-
 mains 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 lympho-
cytes and macrophages, but their presence did not induce any more
kidney damage than that seen in the DR2\(^{-}\) littermates. Our data
corroborate a study in the MRL/lpr MHC class II knockout trans-
planted model, which demonstrates that expression of class II mol-
ecules 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 back-
crosses are very similar (Fig. 6). All animals showing nephritis
predominantly expressed TGF-\(\beta\) and TGF-\(\beta3\), and variable levels
of TNF-\(\alpha\), TNF-\(\beta\), leukotriene-\(\beta\) and IFN-\(\gamma\). Dysregulation of
TGF-\(\beta\) has been previously reported to be associated with pro-
gressive 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 mod-
els 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 en-
dogenous class II molecules. Studies of these mice may provide a
better understanding of the role of HLA class II genes in the etio-
pathogenesis of lupus.

Acknowledgments
We thank Michele Smart and Julie Hanson for technical assistance, Dr.
Joseph P. Grande (Department of Pathology, Mayo Clinic) for assistance
with histopathology, the Mayo Center for Patient-Oriented Research for
assistance with statistical analysis, and Mary Brandt for preparation of the
manuscript.

References