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Spontaneous lupus-like syndrome in HLA-DQ2 Transgenic Mice with a Mixed Genetic Background

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Abstract

To investigate the role of HLA-DQ2 in the pathogenesis of associated immune disorders, we generated transgenic mice that expressed HLA-DQ2 in the absence of endogenous murine class II molecules (AE^oDQ2). These AE^oDQ2 mice with a mixed genetic background spontaneously developed skin lesions on their ears, whereas control AE^oDQ6 genotype control mice (also with a mixed genetic background) did not. The skin lesions were characterized by deep subepidermal blistering with hydropic degeneration and lymphoid infiltration in the subepidermal area as determined by histopathology. Immunofluorescence analysis revealed thick band-like granular deposition of IgG, IgM, and a thin band of IgA deposition along the basement membrane. AE^oDQ2 mice also developed significant and progressive hematuria and proteinuria as compared to the AE^oDQ6 mice ($P < 0.05$). Histopathology showed immune complex deposits in the glomeruli of AE^oDQ2 mice. Immunofluorescence analysis showed progressive mesangial and capillary wall deposition of IgA, IgM, IgG and C1q in the kidney. With electron microscopy, the deposits showed a “fingerprint” substructure; and tubuloreticular structures were identified within endothelial cells. Conversely, these changes were not observed in AE^oDQ6 mice. Serum anti-dsDNA IgM and IgG levels were also significantly elevated among AE^oDQ2 mice compared to AE^oDQ6 mice ($P < 0.001$). In conclusion, AE^oDQ2 mice spontaneously develop an autoimmune

lupus-like syndrome and are useful model for this disease. It remains to be determined whether genetic admixture played a role in the development of this SLE-like syndrome in HLA-DQ2 transgenic mice.

Keywords

Autoimmune; DQ2; Glomerulonephritis; Lupus; MHC class II; Mice; Transgenic

Introduction

The etiopathogenesis of autoimmunity is based on a complex interplay of genetic background, environmental factors, and immune system regulation. Although multiple genes and loci contribute to the development of autoimmune diseases, the best known association is with the major histocompatibility complex (MHC) class II molecules. The HLA haplotype DQ2/DR3 (DQA1*0501-DQB1*0201; DRB1*0301) is associated with a number of autoimmune diseases including systemic lupus erythematosus (SLE)^{1–3}, autoimmune type 1 diabetes,⁴ Graves' disease,^{5–7} celiac disease,⁸ dermatitis herpetiformis,⁹ and autoimmune hepatitis.¹⁰ In contrast, other HLA molecules such as HLA-DQ6 protect against autoimmune diseases.^{7, 11, 12}

As a consequence of linkage disequilibrium between DQ2 and DR3 alleles, it is difficult to address the independent role of these molecules in disease susceptibility in humans. Hence, transgenic mice that express distinct HLA genes provide a valuable means: 1) to develop genetic models of associated disorders 2) to study the role of individual MHC class II molecules in the onset, severity, and manifestations of the disease and 3) to investigate how these alleles contribute to the pathogenesis of the disease. To study the exclusive role of DQ2 in the etiopathogenesis of associated autoimmune diseases, we have generated AE[°]DQ2 transgenic mice that lack both H-2A and H-2E genes. Thus, the CD4⁺ T cell repertoire in these mice is shaped by the human DQ2 molecule.

AE[°]DQ2 mice spontaneously developed an autoimmune syndrome similar to systemic lupus, which is in contrast to AE[°]DQ6 mice. This study reports the phenotypic characterization and pathologic features of the autoimmune lupus-like disease that developed in the AE[°]DQ2 transgenic mice.

Animals, Materials, and Methods

Generation of Transgenic mice

Dr. Mauro Rossi (Istituto di Scienze dell'Alimentazione, Roma, Italy) provided the cDNA construct for HLA-DQ2, which was placed downstream of an H-2E α promoter. The cDNA constructs of DQ2 (DQA1 05, DQB1 02) were then injected into FVB embryos to generate DQ2 transgenic mice (FVB.DQ2).¹³ Only one out of three original DQ2 founder mice was used for subsequent crosses. Drs. Diane Mathis and Christophe Benoist provided MHCII Δ/Δ (AE[°]) mice, which had a mixed background (B6 $\times$ 129/J).¹⁴ These AE[°] mice were then backcrossed once with FVB.DQ2 mice to generate AE[°]DQ2 mice (heterozygote).¹⁵ The AE[°]DQ2 mice were then intercrossed and the offspring screened by PCR to select AE[°]DQ2 mice. Therefore the background genes for the AE[°]DQ2 mice were a mix of FVB, B6, and 129/J.

AE[°]DQ6 mouse generation was a result of numerous crosses. DQ6 alpha chain expressing mice were originally generated separately from the DQ6 beta chain expressing mice. The DQA1*0103 (DQ6 α) mice were produced using (B6 $\times$ B10.M) F1 embryos. One of two

founder mice was used for subsequent crosses. These DQ6 α mice were then backcrossed to B10 for 3 generations (N3). The DQ β 1*0601 (DQ6 β) mice were produced using (CBA $\times$ B10.M) F1 embryos. One of eight original founder mice was used for subsequent crosses. These DQ6 β mice were then backcrossed to B10.M, for 10 generations (N10). Next, each individual line was backcrossed to the AB $^{\circ}$ line (also a gift of Drs. Diane Mathis and Christophe Benoist), which is a mix of (B6 $\times$ 129/J). The subsequent lines (AB $^{\circ}$ DQ α 1*0103 and AB $^{\circ}$ DQ β 1*0601) were then intercrossed to generate AB $^{\circ}$ DQ6 $\alpha\beta$ mice. AB $^{\circ}$ DQ6 $\alpha\beta$ mice were then backcrossed once to AE $^{\circ}$ mice to generate AE $^{\circ}$ DQ6 mice.¹⁶ Therefore, the background genes for the AE $^{\circ}$ DQ6 lines were a mix of B10, B6, and 129/J backgrounds.

Both AE $^{\circ}$ DQ2 and AE $^{\circ}$ DQ6 mice were bred within the pathogen-free barrier facility and were provided with regular mouse chow (LabDiet; PMI Nutrition International) and water while maintained in the conventional area of the Mayo Immunogenetics Mouse Colony (Rochester, MN). The experimental protocol was approved by the Mayo Foundation Institutional Animal Care and Use Committee.

Fluorescent *in situ* Hybridization (FISH) and Spectral Karyotyping (SKY) Analysis

Fluorescent *in situ* Hybridization (FISH) and spectral karyotyping (SKY) analysis were performed by the Cytogenetics Shared Resource (CSR) and Tissue and Cell Molecular Analysis (TACMA) core facilities at Mayo Clinic, Rochester. Mouse spleens from DQ2 and DQ6 transgenic mice were cultured and harvested; and metaphase chromosome slides were prepared for analysis. A FISH probe spanning the entire human HLA class II region was produced and was directly labeled with Vysis Spectrum OrangeTM. Specimen slides were hybridized with the FISH probe and were scanned for integration of the probe in each specimen. Metaphase coordinates for each specimen were documented for further analysis by SKY which was used to determine the chromosomal location of the insert.¹⁷

Flow Cytometry

Fluorescence-activated cell sorting (FACS) analysis was conducted by the Flow Cytometry/Optical Morphology Resource at Mayo Clinic, Rochester. For DQ2 and DQ6 cell surface expression, naïve mice were sacrificed and splenocytes extracted. Activation of splenocytes was conducted *in vitro* via LPS (10 μ g/ml for 24 hours). FACS analysis consisted of a pan DQ antibody (TU39), anti-B220 and anti CD11b antibodies. Isotype control for TU39 was mouse IgG2a. All antibodies were purchased from Becton Dickinson (San Jose, CA).

Urine Collection and Urine Analysis

Urine samples were either collected in metabolic cages or by bladder massage and were subjected to strip analysis (Multistix 10 SG; Bayer Corp., Elkhart, IN). Urine protein levels of 100 mg/dl or greater and urine blood levels of 1+ or greater were considered as being positive. Urine protein levels of 300 mg/dl or greater and urine blood levels of 3+ or greater were considered as severe proteinuria and severe hematuria, respectively.

Biopsy

Mice were sacrificed humanely at different ages ranging from 2 to 17 months. Ears and Kidneys were removed and immediately frozen in liquid nitrogen for immunofluorescent analysis or fixed in formalin for Hematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS) staining.

Histopathology

The formalin-fixed skin and kidney specimens were paraffin-embedded, sectioned and processed. Five-micron-thick skin and kidney specimens were stained with H&E for

histopathologic examination. Kidney specimens were also stained with the Periodic Acid Schiff (PAS) stain. All sections were viewed and images were taken using an optical light microscope (Leica DM IRB; Leica Microsystems, Wetzlar, Germany) and an Olympus AX70 Research Microscope (Olympus Corp., Tokyo, Japan). Scoring of the PAS stained kidney sections for the intensity and extent of renal lesions was done using the 0–4 scale described in Wang et al.¹⁸ in which 0 was given to a kidney with no histopathological changes and 4 was given to a kidney wherein obliteration of the glomerular architecture included >70% of glomeruli. The mean scores were determined for mice under three months of age and those greater than three months of age.

Immunofluorescence analysis

Five-micron-thick cryostat sections from each specimen were placed on frosted glass slides (Superfrost/Plus; Fisher Scientific, Pittsburgh, PA). FITC-conjugated goat anti-mouse IgA (1:20 dilution; Sigma-Aldrich, Saint Louis, MO), FITC-conjugated goat anti-mouse IgM (1/200 dilution; Sigma-Aldrich) or FITC-conjugated rabbit anti-mouse IgG (1:500 dilution; Sigma-Aldrich) were applied to detect mouse IgA, IgM or IgG deposits within the skin and kidney sections. Purified rat anti-mouse complement component C1q monoclonal antibody (1/50 dilution; Cedarlane Laboratories Limited, Hornby, Ontario, Canada), rabbit anti-mouse C3a polyclonal IgG (1/50 dilution; Santa Cruz Biotechnology Inc., Santa Cruz, CA) and rat anti-mouse C3b/iC3b/C3c (1/50 dilution; HyCult biotechnology b.v., Uden, The Netherlands) were used to detect C1q and C3. These antibodies were detected using Rhodamine Red-X-conjugated anti-rabbit or anti-rat IgG (1/200, Jackson ImmunoResearch Laboratories Inc., West Grove, PA). Slides were viewed and images were taken using an Olympus AX70 Research Microscope (Olympus Corp., Tokyo, Japan).

Electron Microscopy

Kidney specimens from two AE^oDQ2 mice (11 and 14 months of age) were prepared for electron microscopy by fixation for 24 hours in Trump's fixative¹⁹ (1% glutaraldehyde and 4% formaldehyde in 0.1 M phosphate buffer, pH 7.2), followed by rinsing for 30 minutes in three changes of 0.1 M phosphate buffer, pH 7.2. One micron sections were cut and stained with toluidine blue. The tissue was post-fixed in 1% OsO₄ for 1 hour and stained with 2% uranyl acetate for 30 minutes at 60°C. The tissue was dehydrated in serial concentrations of ethanol and propylene oxide and embedded in Spurr's resin.²⁰ Thin 90 nm sections were cut on a Reichert Ultracut E microtome, placed on 200-mesh copper grids, and stained with lead citrate. Micrographs were taken on a JEOL 1200 EXII running at 60 KV.²¹

Serum Samples

Blood samples were collected and kept for 1 to 2 hours at room temperature. After removing the clot, samples were centrifuged in sera collection tubes (BD Biosciences, Franklin Lakes, NJ) at 4000 RPM for 5 minutes. Sera were then extracted and stored at −70°C.

Anti-dsDNA Antibodies

Immulon 2 HD plates (Fisher Scientific) were coated with 5ug/ml calf thymus DNA (Sigma-Aldrich) overnight at 4°C. Based on the manufacturer's specification, this was a highly polymerized DNA which was predominantly double-stranded DNA (dsDNA). Plates were then blocked with 4% BSA/PBS (Sigma-Aldrich) for 1 hour. Serum samples were serially diluted (1/200 and 1/400) and incubated for 1 hour at room temperature. Biotinylated secondary antibodies (anti-IgG, and anti IgM: Jackson ImmunoResearch Laboratories) were diluted 1:200 (0.1% BSA/PBS) and incubated for 1 hour. HRP/Streptavidin (Jackson ImmunoResearch Laboratories) was diluted 1/2000 (0.1% BSA/PBS) and incubated for 30

minutes. 3,3', 5,5'-tetramethylbenzidine (TMB) (Sigma-Aldrich) was the substrate and H₂SO₄ was used as the stop solution.

Statistics

Statistical analysis was conducted using JMP™ version 6.0.0 software (SAS Institute Inc.; Cary, North Carolina). Nonparametric Wilcoxon rank sum test and Fisher's exact test were employed to assess statistical significance for continuous variables (weight, anti-DNA antibodies) and binary variables (urine protein and blood), respectively. Significance was accepted at $P < 0.05$.

Results

Characterization of AE^oDQ2 and AE^oDQ6 mice

The AE^oDQ2 mice and AE^oDQ6 mice were evaluated by FISH analysis to determine the number of insertion sites for the DQ2 and DQ6 transgenes. DQ2 mice had one insertion site in chromosome 8 (Figure 1A and 1C) and DQ6 transgenic mice had three insertion sites on chromosomes 2 and 8 (Figure 1B and 1D). Over five DQ2 and five DQ6 mice were evaluated by FACS analysis for the expression of the DQ2 and DQ6 proteins at the cell surface (figure 2). DQ2 is minimally expressed on the surface of unactivated B cells (B220+) and monocytes (CD11b+ cells) (2A–B), whilst DQ6 is more strongly expressed on unactivated B cells, T cells, and monocytes (2E–F). Activation with LPS treatment for 24 hours marginally increased the level of DQ2 on B cells and monocytes (2C–D), but significantly increased the level of DQ6 expression (2G–H).

Mice General Characteristics and Weight

Older adult AE^oDQ2 mice were observed to be generally ill and had low activity levels whereas aged matched AE^oDQ6 mice were healthy and active. Before 6 months of age, there was no significant difference in weight between the two genotype groups (mean weight $\pm$ SD was 27.1 g $\pm$ 4.5 versus 27.5 g $\pm$ 5.0 for AE^oDQ2 mice (N=13) versus AE^oDQ6 mice (N=17), $P=0.85$). Weight assessment at six months to one year of age revealed that body weight for AE^oDQ2 mice was significantly lower than the controls, AE^oDQ6 mice. The mean weight $\pm$ SD for AE^oDQ2 mice (N=8) was 23.5 g $\pm$ 4.1 versus 35.4 g $\pm$ 9.6 for AE^oDQ6 mice (N=17) at this age period ($P=0.001$). This difference in weight persisted into older (>1 year) age (21.9 g $\pm$ 3.5 versus 42.7 g $\pm$ 10.7 for AE^oDQ2 mice (N=4) versus AE^oDQ6 mice (N=18), $P=0.007$).

Spontaneous Development of Skin Lesions

Beginning at 6 months of age AE^oDQ2 mice spontaneously developed skin lesions primarily on the ears. The muzzle, tail and hind paws were the secondary sites. The ear lesions demonstrated erosions, ulcerations and crust formation leading to loss of normal architecture of ears upon progression (Figure 3A). The histopathology of the lesions revealed focal separation of the epidermis from the dermis with hydropic degeneration and lymphoid infiltration in the dermis. In most of the lesional areas there was a complete loss of epidermis and stratum corneum. Epidermal thickening and hyperkeratosis were observed in the areas where the epidermis was intact (Figure 3B). Minimal basal cell vacuolar changes were observed (Figure 3C).

Direct immunofluorescence examination was performed on the ear skin of the mice to determine if immunoglobulin deposits were present in lesional skin. IgG and IgM staining of ear skin in AE^oDQ2 mice revealed a diffuse, thick, band-like deposition with granular pattern in the basement membrane. IgA deposition was also detected in the basement membrane but the intensity was less than IgG and IgM. These features were similar to

cutaneous lesions of lupus. In contrast to AE^oDQ2 mice, AE^oDQ6 mice did not show any IgG, IgM or IgA deposition in the skin. There was no deposition of complement proteins C3 and C1q in the skin of either AE^oDQ2 or AE^oDQ6 mice (Figure 4).

Survival and Urine Analysis

Mice were monitored long term and a Kaplan Meier Curve generated. AE^oDQ2 mice began to die at 6 months of age and nearly 100% were dead after 12 months, whereas 100% of the AE^oDQ6 mice were still alive at 12 months of age. To assess mice for renal imaging a urine analysis test was performed in young (<6 months), middle-aged (6–12 months) and older (>12 months) mice. Of the young AE^oDQ2 mice, 8% developed proteinuria which increased to 57% for middle-aged mice. In contrast, only 6% control AE^oDQ6 mice had developed mild proteinuria by one year of age ($P=0.02$). Severe proteinuria (urine protein levels of 300 mg/dl or greater) was present in 14% of AE^oDQ2 mice by 12 months of age increasing to 67% for older mice. This significantly differed from the control AE^oDQ6 mice which never developed severe proteinuria even in the old age ($P=0.001$) (Figure 5A). Hematuria occurred in 43% of AE^oDQ2 mice by one year of age. The frequency increased to 83% for older mice. In contrast, none of the AE^oDQ6 mice developed hematuria ($P=0.02$ for middle-aged and $P<0.0001$ for older mice) (Figure 5B). Both genders were equally affected by hematuria and proteinuria (analysis not shown).

Renal Histopathology

Examination of the H&E and PAS stains of the kidneys revealed a progressive immune complex mediated glomerulonephritis in AE^oDQ2 mice (Figure 6). At 2 months and 3 months of age, the glomeruli of AE^oDQ2 mice appeared completely normal. By 8 months of age, glomeruli of AE^oDQ2 mice had developed minimal mesangial hypercellularity, with no discernible immune complex deposits. The tubules were back-to-back, with minimal interstitial fibrosis or tubular atrophy. No significant interstitial inflammatory infiltrates were identified and vessels were unremarkable. By 11 months of age, the glomeruli were hypocellular and contained extensive immune complex deposits within mesangial regions and along peripheral capillary loops. The capillary loop deposits markedly compromised peripheral capillary loop lumens. There was minimal interstitial fibrosis or tubular atrophy. By 14 months, glomeruli contained large subendothelial deposits, reminiscent of the “wire loop” lesions characteristically observed in lupus glomerulonephritis. Patchy lymphocytic interstitial infiltrates were identified at this time point. Conversely, the AE^oDQ6 mice did not show any of these glomerular, tubular, interstitial, or vascular abnormalities. Glomerular nephritis was evaluated for individual mice using Periodic Acid Schiff staining and graded according to severity ranging from 0 (no glomerular lesions) to 4 (>70% of glomeruli had obliteration of the glomerular architecture).¹⁸ AE^oDQ2 mice had a significant increase in severity over time, such that older mice (>3 months of age, $N=11$) had a mean of 2.5 ± 0.37 SEM and the young mice (<3 months of age, $N=9$) had a mean of 0 ± 0 SEM ($P<0.001$). AE^oDQ6 mice did not have a significant increase with age {old mice- (1.333 ± 0.667 SEM, $N=3$) vs young mice- (0.0 ± 0.0 SEM, $N=3$) ($P=0.06$)

Immunofluorescence

Direct immunofluorescent analysis of kidneys was performed for IgM, IgG and IgA in 10 AE^oDQ2 mice with age range of 2 to 11 months. The AE^oDQ2 mice showed moderate to strong diffuse and focal capillary wall staining for IgM and also segmental granular staining for IgG. In addition to IgM and IgG, the AE^oDQ2 mice showed mesangial and focal capillary wall staining for IgA. All AE^oDQ2 mice that were examined developed glomerular deposits as early as 2 months of old which intensified with age. Conversely, AE^oDQ6 mice (2–12 months of age) had no significant glomerular staining for IgA ($N=4$) and only trace fine granular mesangial staining for IgM and IgG (Figure 7).

Direct immunofluorescent staining also revealed diffuse granular mesangial and capillary wall deposition of C1q in 9 of the 10 AE^oDQ2 mice (age range 2 to 11 months) that were examined (Figure 7D); whereas, no C1q deposition was detected in either young or old AE^oDQ6 mice (N=4, age range 2–12 months) (Figure 7H). The C1q deposition increased in intensity as the mice aged (Figure 8). No staining for C3b and C3a was detected in the kidneys of either AE^oDQ2 mice or the controls.

Electron Microscopy

Glomeruli of AE^oDQ2 mice contained extensive immune complex-type deposits within mesangial regions and along the subendothelial aspect of the peripheral capillary loop basement membranes. On high power magnification, the deposits had “fingerprint” substructure characteristic of lupus nephritis. {Tojo, 1993 #174}^{22, 23} Tubuloreticular structures were identified within endothelial cells (Figure 9).

Serology

Serum levels of anti-dsDNA antibodies were measured in AE^oDQ2 and AE^oDQ6 mice at mean age of 11 months. Both anti-dsDNA IgM and anti-dsDNA IgG were significantly higher among AE^oDQ2 mice as compared to the controls (Anti-dsDNA IgM: 1.9 ± 0.5 versus 0.7 ± 0.3 OD, $P < 0.001$; Anti-dsDNA IgG: 1.3 ± 0.8 versus 0.4 ± 0.2 OD, $P < 0.001$) (Figure 10). There was no statistically significant difference in the level of autoantibodies between male and female of each genotype group (analysis not shown). Total IgG and IgM sera levels were also evaluated (Fig 10C–D). AE^oDQ6 had a significantly greater level of total IgG than AE^oDQ2 mice (17.9 ± 2.16 vs. 8.2 ± 2.61) ($P < 0.01$), whereas there was no significant difference in the total IgM sera levels (2.86 ± 0.4 vs 2.84 ± 0.3) ($P = 0.49$). These latter results would indicate that the increased titers of autoantibodies in the AE^oDQ2 transgenic mice did not associate with hyperglobulinemia.

Discussion

In the present study, we report the generation of a novel autoimmune lupus-like disease in humanized transgenic mice that carry the genes encoding HLA-DQ2 on a complete endogenous class II knockout background (AE^o). These AE^oDQ2 mice spontaneously develop a lupus-like syndrome characterized by immune complex mediated skin lesions and nephropathy together with anti-dsDNA antibodies.

The genetic susceptibility to lupus involves the contribution of both MHC and non-MHC genes.^{24–29} Prior studies in different populations have reported several different HLA class II genes in association with SLE;^{30–32} however, the haplotype DR3/DQ2 has the most consistent association with SLE, particularly among Caucasians.^{1, 2, 33, 34} A large study on predominantly Caucasian families showed an association of HLA-DRB1*0301/DQB1*0201 (Relative Risk=2.3) and HLA-DRB1*1501/DQB1*0602 (Relative Risk=1.5) haplotypes with SLE.³⁵ A smaller study on Italian SLE patients showed a strong association with HLA-DR3-DQ2 (DRB1*03-DQA1*0501-DQB1*0201) (odds Ratio=6.5) but not with DR2-DQ6 (DRB1*1501-DQB1*0602) or DR4-DQ8 (DRB1*04-DQA1*0301-DQB1*0302) haplotypes.³⁴ HLA-DRB1*0301 and HLA-DQA*0501 alleles were also significantly more frequent in Spanish SLE patients as compared to the controls; particularly among those with diffuse proliferative glomerulonephritis.¹ In Japanese and African-Americans, the MHC class II association with SLE was found to be with HLA-DR2.^{32, 36} Nonetheless, the strong linkage disequilibrium that exists within and beyond the MHC class II region prevents definitive analysis of how each of these alleles affects the development, progression, and outcome of the disease autonomously.^{1, 37}

Despite this strong linkage disequilibrium between HLA-DR3 and DQ2 alleles, the majority of studies have focused on the association of SLE with HLA-DR3.^{38–40} However, a few studies that evaluated an association with HLA-DQ2 showed interesting findings.^{1, 3} One study showed that the strength of association with HLA-DQA*0501 was higher than any DR allele as measured by aetiological fraction value (δ).¹ In the same study, HLA-DQ2 had the strongest association with both Ro and La antibodies ($\delta=1$). Interestingly, the absence of HLA-DRB1*0301 and presence of HLA-DQB1*0201 (in African-Americans) have been found to be the predictors of disease activity in SLE patients.⁴¹

Our data suggest that the DQ2 transgene is probably the contributing factor in the development of autoimmune disease in AE^oDQ2 mice. This HLA molecule has a strong association not only with SLE but also with other autoimmune disorders.^{1–10} A principal finding that supports the particular role of DQ2 gene in disease development is the absence of disease in the DQ6 transgenic mice that had the same knockout background (AE^o) as the DQ2 transgenic mice. The absence of spontaneous lupus-like disease in AE^oDR3 mice⁴² is also suggestive of an independent role of DQ2 genes in the development of this disease.

The contribution of individual HLA molecules in disease phenotype has also been studied in other HLA transgenic mouse models.⁴³ In our humanized model for rheumatoid arthritis DR4 mediates the sex bias and production of IgG rheumatoid factor. In that model, DQ8 is also able to induce production of rheumatoid factor but does not lead to sex predisposition, similar to the finding of this study.⁴⁴ In addition to the specific role of each HLA molecule in the disease phenotype, the sum of interactions between individual gene products within the MHC region also plays an important role. Previously, AB^oDR3/DQ2 transgenic mice have been generated to investigate the effects of these interactions. Although these animals express HLA genes with functional antigen-presenting characteristics and are able to mediate T cell selection, they do not develop any spontaneous disease or pathology.^{45, 46} There are several differences between this transgenic model and ours which may have contributed to the difference in the disease phenotype. First, our model carries only one HLA molecule. Therefore, we were able to avoid any possible interaction between DR and DQ molecules. Second, the endogenous mouse MHC class II (α chain, β chain and E molecule) has been completely knocked out in our model resulting in the generation of T cells exclusively specific to human MHC class II.¹⁵ Third, there may be a difference in the pattern of expression of class II molecules between the two models. This may be another contributing factor to the development of the disease in the AE^oDQ2 mice. In fact, the decreased level of MHC class II expression is reported in active SLE and other immune diseases.^{47–50} Nonetheless, the age-control AE^o (null) mice with virtually no class II molecule do not develop lupus-like disease (data not shown). Generation of AE^oDQ2 mice with a high level of expression of this gene would be necessary to investigate the role that MHC expression level has upon the activity and pathogenesis of the lupus-like syndrome in these mice.

The autoimmune disease detected in these AE^oDQ2 mice resembles SLE in many aspects. The characteristic skin pathology with IgG, IgM and IgA deposition along the basement membrane resembles cutaneous lesions of lupus. Presence of skin lesions particularly on non-fur-bearing sites may be suggestive of a possible photosensitive characteristic of these lesions similar to cutaneous lesions of human SLE.^{51, 52} The glomerular deposition of immune complexes containing predominantly C1q, “fingerprint” substructures, and tubuloreticular structures, along with increased levels of anti-dsDNA antibodies in AE^oDQ2 mice, are characteristic features of lupus nephropathy.^{22, 23, 53–55} However, there are a few differences between this model and other mouse models of lupus. For example, the gender-specific susceptibility, which is observed in other mouse models of SLE, was not present in AE^oDQ2 mice.^{56–59} Moreover, no C3 deposits were detected in the skin or glomeruli of AE

$^{\circ}$ DQ2 mice. Finally, other spontaneous mouse models of lupus have primarily IgG deposits in the mesangia and glomerular capillary walls,⁶⁰ whereas IgA and IgM were the predominant immunoglobulins of the glomerular deposits found in AE $^{\circ}$ DQ2 mice.

It is also noted that immunoglobulin deposits appear in the kidney at a very young age in the AE $^{\circ}$ DQ2 mice and this precedes the development of proteinuria and hematuria. The increased amounts of C1q over time would indicate that C1q accumulatively binds to the Ig slowly over a prolonged period of time. It would appear then that the development of nephritis in the AE $^{\circ}$ DQ2 mice occurs over a very long period of time with an initial event of Ig deposition. Several studies have reported an accelerated development of symptoms in animal models of lupus using anti-glomerular basement membrane antibodies.⁶¹ Further experiments are essential to enhance the development of disease in AE $^{\circ}$ DQ2 mice in order to make it a more useful model.

In conclusion, the AE $^{\circ}$ DQ2 mouse line will be a useful humanized model for autoimmune lupus-like disease. These animals should offer insight into the role of genetic components and environmental risk factors in the pathogenesis of SLE in the context of the human DQ genotype and will be a valuable tool for investigating the treatment options for this potentially fatal disease.⁶²

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Figure 1.

Chromosomal insertion sites of the DQ2 and DQ6 transgenes. (A) The Fluorescent in situ Hybridization (FISH) of metaphase chromosome slides from the spleens of AE°DQ2 mice (A) showed integration of probe on one chromosome (arrow). Samples from AE°DQ6 mice (B) had integration on three chromosomes (arrows). (C) Spectral karyotyping (SKY) revealed that the human HLA probe labeled in Vysis Spectrum Orange™ was integrated on one copy of mouse chromosome 8 in AE°DQ2 mice (arrow). (D) Evaluation of AE°DQ6 mice showed that the human HLA probe was integrated on both copies of mouse chromosome 2 and one copy of mouse chromosome 8 (arrows).

Figure 2.

Expression of DQ2 and DQ6 molecules in AE°DQ2 and AE°DQ6 mice. (A–H) Reflective expression of cell surface DQ2 as determined by FACS analysis by splenocytes from five evaluated AE°DQ2 (A–D) and five AE°DQ6 (E–H) mice. A pan HLA-DQ, DR, DP antibody (TU39-green line) was used to detect DQ2 and DQ6. The isotype control (Mouse IgG2a) is the purple-filled area. Gates were set for B220+ cells (A, C, E, G) or CD11b+ cells (B, D, F, H), Splenocytes were cultured in vitro with media alone for 24 hours (A–B, E–F) or activated with LPS for 24 hours (C–D, G–H).

Figure 3.

Development of cutaneous lesions in AE^oDQ2 mice. (A) Erosions, ulcerations and crusting are present on the ears and muzzle. (B) H&E staining (10X) shows, thickening of the epidermis, hyperkeratosis as well as subepidermal blistering with hydropic degeneration and lymphoid infiltration in the subepidermal/dermal area (Reflective of 10 cutaneously affected mice). (C) Higher magnification (40x) shows the presence of vacuoles.

Figure 4.

Direct immunofluorescence analysis of the skin. A thick band of IgG (A) and IgM (B) and a thin band of IgA (C) deposition with granular pattern was detected in the basement membrane of lesional ear in AE°DQ2 mice. There was no C3 (E) or C1q (D) deposition in the skin of AE°DQ2 mice (Reflective of five mice evaluated after 6 months of age). The AE°DQ6 mice did not show any IgG, IgM, IgA, C3 or C1q deposition in the skin (F–J).

Figure 6.

Histopathologic alterations in AE°DQ2 and AE°DQ6 mice. Reflective H&E stained kidney sections of AE°DQ2 mice between 2–3 months (A) of age seem completely normal. H&E stained section of the kidney of an AE°DQ2 mouse between 3–15 months of age (B). Glomeruli are hypocellular. Capillary loop lumens are markedly compromised due to subendothelial deposition of immune complex deposits. Tubules and interstitium are unremarkable. H&E stained sections of AE°DQ6 kidneys from 2–3 months of age (C) and 3–15 months of age (D), revealed no significant histopathologic alterations of glomeruli, tubules, interstitial, or vessels. PAS stained kidney section of a 2 month old AE°DQ2 mouse showing minimal mesangial hypercellularity (E). The tubules, interstitium, and vessels are unremarkable. (F) PAS stained section of AE°DQ2 mouse at 14 months of age: Glomeruli show massive subendothelial immune complex deposits. Patchy interstitial lymphocytic infiltrates are identified. Original magnification, $\times 40$. PAS stained sections of AE°DQ6 kidneys from mice aged 2 months (G) and 14 months (H) appear normal.

Figure 7.

Direct immunofluorescent analysis of kidney specimens for presence of IgA, IgM, IgG and C1q in AE°DQ2 (at 11 months of age) and AE°DQ6 (at 12 months of age) mice. (A–D) Glomeruli of AE°DQ2 mice demonstrated strong diffuse granular mesangial and capillary wall staining for IgA (A), IgM (B), weak granular mesangial staining for IgG (C), and strong diffuse granular and capillary wall staining for C1q (D). (E–H) By contrast, AE°DQ6 mice showed no significant staining for IgA (E), IgG (G), C1q (H), and only trace granular mesangial staining for IgM (F). Original magnification, $\times 40$.

Figure 8.

Progression of complement deposition by age in AE^{DQ2} mice. (A) At 2 months of age C1q was present in low intensity in the kidney, however the intensity increased over time as the mice aged (B) 3.5 months of age; (C) 5 months of age; (D) 8 months of age.

Figure 9.

Electron microscopic studies. Electron micrographs were taken of kidneys from an AE^oDQ2 mouse of 11 months of age. (A) Massive mesangial and subendothelial immune complex-type deposits were present. The large subendothelial deposits markedly compromised peripheral capillary loop lumens, and were reminiscent of the “wire loop” lesions observed in lupus glomerulonephritis (Direct magnification, $\times 11,000$). (B) On high power magnification the immune complex deposits had a vaguely paracrystalline appearance with a “fingerprint” (FP) substructure (Direct magnification, $\times 42,000$). (C) Tubuloreticular (TR) structures were identified within endothelial cells (Direct magnification, $\times 21,000$). (D) TR on high power (Direct magnification, $\times 67,000$).

Figure 10.

Comparison of anti-DNA antibody levels in the sera of AE°DQ2 and AE°DQ6 mice. (A) Anti-DNA IgM (OD) and (B) Anti-DNA IgG (OD) were measured by ELISA method in the serum of AE°DQ2 (Mean age 10.3, range 8–14 months, N=12) and AE°DQ6 (Mean age 11.6, range 11–12 months, N=9) mice. Horizontal bars represent the means. ** $P < 0.001$; Wilcoxon rank sum test. (C–D) Total IgM and total IgG levels in sera of AE°DQ2 and AE°DQ6 mice.