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Generation of an HLA-DQ2.5 Knock-In Mouse

Alisa E. Dewan, Frank Koentgen, Marie K. Johannesen, M. Fleur du Pre, and Ludvig M. Sollid

*KG Jebsen Coeliac Disease Research Centre, Institute of Clinical Medicine, University of Oslo, 0372 Oslo, Norway; †Department of Immunology, Oslo University Hospital-Rikshospitalet, 0372 Oslo, Norway; and ‡Ozgene Pty. Ltd., Bentley, Western Australia 6983, Australia

ABSTRACT

The human MHC class II molecule HLA-DQ2.5 is implicated in multiple autoimmune disorders, including celiac disease, type 1 diabetes, and systemic lupus erythematosus. The pathogenic contribution of HLA-DQ2.5 in many of these disorders is not fully understood. There is thus a need for an HLA-DQ2.5 humanized mouse model with physiological expression of this MHC molecule that can be integrated into disease models. In this article, we report the generation of an HLA-DQ2.5 knock-in mouse strain on a C57BL/6 background in which sequences encoding the extracellular moieties of mouse MHC class II H2-IAa and H2-IAb1 have been replaced with those of HLA-DQA1*05:01 and HLA-DQB1*02:01. In heterozygous knock-in mice, the expression of HLA-DQ2.5 is superimposable with the expression of H2-IA. This was not the case in a regular untargeted HLA-DQ2.5 transgenic mouse. HLA-DQ2.5 in the knock-in animals is functional for T cell development and for Ag presentation to HLA-DQ2.5–restricted and gluten-specific T cells. Because C57BL/6 mice do not express H2-IEa, the only functional MHC class II molecule in homozygous HLA-DQ2.5 knock-in mice is the knock-in gene product. This alleviates the need for crossing with MHC class II knockout mice to study the isolated function of the MHC transgene. Our novel mouse strain provides an important tool to study the involvement of HLA-DQ2.5 in models of diseases with association to this HLA allotype.


INTRODUCTION

Autoimmune diseases are typically associated with certain MHC class II (MHC II) variants. The DR3DQ2 haplotype is over-represented in many diseases, including type 1 diabetes, myasthenia gravis, Sjögren syndrome, systemic lupus erythematosus, and celiac disease (CeD) (1, 2). Although the tight linkage disequilibrium of this haplotype makes it difficult to pinpoint the disease-associated HLA genes, in many instances HLA-DQ2 encoded by DQA1*05:01 and DQB1*02:01 (i.e., HLA-DQ2.5) is a candidate for disease involvement in these diseases. In CeD, the strong disease association of HLA-DQ2.5 has been explained by this HLA allotype’s preferential binding of negatively charged, posttranslationally modified gluten residues that are defining Ags of this disease (3).

Autoimmune diseases with MHC II associations are typically hallmarked by the production of autoantibodies and pathogenic involvement of B cells, underscoring the crucial role for T cell–B cell interactions in disease pathogenesis (4). In studies of such diseases, there is a quest for good in vivo models enabling studies to decipher key pathogenic events.

Many mouse strains transgenic (tg) for various human HLA class II allotypes have been made over the years, including for HLA-DQ2.5 (5–9), but most of these mice have been made by...
untargeted insertion of either cDNA or fragments of genomic human DNA. Untargeted insertion is an effective method of introducing transgenes in mice, but it carries the risk of unintended consequences that can affect the disease model (10). The commonly observed insertion of multiple gene copies can negatively impact expression of the transgene (11) or lead to otherwise inauthentic expression levels. Targeted insertion of transgenes into the homologous locus of the model organism avoids many of the issues with untargeted insertion.

In this article, we report on the generation of an HLA-DQ2.5 knock-in (KI) mouse in which the extracellular domains of H2-I\textalpha\textbeta\textgamma have been replaced by the corresponding parts of HLA-DQ2.5. Untargeted HLA class II tg mice are usually crossed onto MHC II knockout mice to study the effect of the tg HLA molecule. Our HLA-DQ2.5 KI is generated in C57BL/6 mice, which lack expression of H2-IE because of a deletion of the promoter and first exon of the H2-Ea gene (12). When bred to homozygosity for the KI gene, the only MHC II molecule expressed by these mice is HLA-DQ2.5 without the need for further crossing. This novel mouse fills a need for an HLA-DQ2.5 humanized model for studies of HLA-associated disease pathogenesis with physiological transgene expression.

MATERIALS AND METHODS

Generation of HLA-DQ2.5 KI mice

The HLA-DQ2.5 \(\alpha\)- and \(\beta\)-chain (DQA1*05:01, DQB1*02:02) KI mice were generated by Ozgene (Perth, WA, Australia). Briefly, CHORI-502 human BAC COX cell line library clone XXbac-254CII

FIGURE 1. Generation of HLA-DQ2.5 KI mice.

(A) Introduction of HLA-DQA1*05:01 sequences into H2-Aa. Top, Targeting construct with human exons 2 and 3 (gray) and FRT-flanked PGK-hygromycin (HygroR) selection cassette (white). Middle, Mouse H2-Aa locus with indication of replaced segments. Dashed lines indicate mouse-human sequence transition sites. Bottom, Mouse H2-Aa locus with integrated transgene with human exons in gray and mouse exons in black after flippase-mediated excision of the FRT-flanked selection cassette. (B) Introduction of HLA-DQB1*0201 sequences into H2-Ab1. Top, Targeting construct with human sequences for part of exon 1 and exons 2 and 3 (gray) and FRT-flanked PGK-neomycin (Neo) selection cassette (white). Middle, Mouse H2-Ab1 locus with indication of replaced segments. Dashed lines indicate mouse-human sequence transition sites. Bottom, Mouse H2-Ab1 locus with integrated transgene with human exons in gray and mouse exons in black after flippase-mediated excision of the FRT-flanked selection cassette. (C) Detection of HLA-DQ2.5 surface expression. Splenocytes from WT, HLA-DQ2.5 KI heterozygous, and HLA-DQ2.5 KI homozygous mice were stained using anti-mouse MHC II (H2-IA/IE) and anti-human HLA-DQ2.5. Data are representative of more than three different experiments (\(n = 1\) mouse per experiment).
(AL731683; BAC PAC Resource Center) (13) was used as PCR template for generation of amplicons for targeting vectors with HLA-DQA1*05:01 and HLA-DQB1*02:01 sequences. Mouse BACS RP2313B17 and RP2344J210 (Genomics Shared Resource, Roswell Park Comprehensive Cancer Center) (14) were used as PCR templates for amplicons of murine sequences for targeting vectors. Mouse sequences used as reference transcrips for murine genes were ENSMUST00000040655 (H2-Aa) and ENSMUST00000040828 (H2-AbI). The targeting constructs were verified by DNA sequencing. The HLA-DQA1*05:01 targeting construct used the human sequence for exons 2 and 3 downstream of a flipase recognition target (FRT)-flanked phosphoglycerate kinase I (PGK) hygromycin resistance cassette (Fig. 1A). The first, unsuccessful, targeting construct for introduction of HLA-DQB1*02:01 used the human sequence for exons 2 and 3 downstream of an FRT-flanked PGK neomycin resistance cassette (Supplemental Fig. 1A). The second targeting construct for introduction of HLA-DQB1*02:01 was similar to the first, with the FRT-flanked resistance cassette moved further upstream of exon 2 (Supplemental Fig. 1B). The mouse human sequence transitions for both these constructs were in the 1–2 intronic sequence and in the 3–4 intronic sequence. The third and successful targeting construct for introduction of HLA-DQB1*02:01 used the human sequence from HLA-DQB1*02:01 for exon 1 from the initial conserved alanine in the leader sequence, through exons 2 and 3, upstream of the FRT-flanked PGK neomycin resistance cassette in intron 3–4 (Fig. 1B). C57BL/6 Bruce4 embryonic stem (ES) cells were used for insertion of linearized target constructs by electroporation. Southern blotting was used to screen for correct integration of target constructs, and ES cells were injected into blastocysts that were transferred into pseudopregnant females to generate chimeric offspring. For the first two attempts, the HLA-DQ2.5 β-chain tg mice were generated first, and ES cells for humanization of H2-Aa were generated from animals with confirmed germline transmission of tg β-chain sequences. For the successful HLA-DQ2.5 KI, ES cells from α-chain offspring with germline transmission were used for insertion of β-chain targeting constructs, so Germline (15) technology was implemented for the selection of mice with germline transmission. Offspring with double KI α- and β-chain cosegregation and germline transmission were bred to B6J-Gt(ROSA)26Sor<sup>tm1(UbiC–FLPe/Ovg</sup>/Ovg flipase-expressing mice to delete the neomycin and hygromycin selection cassette.

**Sequence analysis of nonfunctional HLA-DQ2.5 KI mice**

Single-cell suspensions from spleens of HLA-DQ2.5 KI founder mice were prepared by carefully grinding and filtering tissue through a 70 μm diameter nylon mesh (BD Biosciences). Total RNA was isolated using the RNeasy Mini kit (Qiagen) and reverse transcribed into cDNA in two steps using a mix of 50 ng/μl random hexamers, 20 nM oligoDT, and 1 mM dNTPs and incubation at 65°C for 5 min, then placed on ice for at least 1 min, before adding 5 mM MgCl₂, 10 mM DTT, RNAsin (Promega), and Superscript III (Invitrogen) and incubating at 25°C for 10 min, 50°C for 50 min, and 85°C for 5 min. cDNA was amplified by PCR (α-fwd 5’-GCAGCAGACCTCTGATTCTG-3’, α-rev 5’-TGTTGGATCCGGTCTCTGA-3’, β-fwd 5’-CATGACTTGTGGTTCTCGAAGGG-3’, β-rev 5’-GTCGGGCTGTACAGATG-3’). PCR products were either directly sequenced or cloned into pGEM Easy Vector System I (Promega) cloning vector. Sanger sequencing was performed by Eurofins Scientific/GATC using PCR primers for directly sequenced PCR products or for vector Sp6 (5’-ATTTAGGTTGACAC TATAAA-3’) and T7 (5’-TAATAAGCCTACTATAGG-3’).

**Mice**

Homozygous HLA-DQ2.5 KI mice were obtained by crossing heterozygous founders. Resulting litters were genotyped by PCR (H2-Iaβ f/wd 5’-TCGTGTACGCTGATTGC-3’, H2-Iaβ rev 5’-GTAAGTTGTGGTGTCGACACCG-3’, HLA-DQ2.5 KI β-fwd 5’-ACCTGAGGACCCCCAGAGGG-3’, HLA-DQ2.5 KI β-rev 5’-CTCCGGGGATTCGCAAAGGCC-3’). Non-tg littermates from heterozygous breedings were used as wild-type (WT) controls. Experiments were performed on mice of both sexes indiscriminately. HLA-DQ2.5 tg mice (7) were crossed to homozygosity, and genotype was confirmed by backcrossing, then maintained as homozygous crossings. Heterozygous HLA-DQ2.5 tg mice were obtained from homozygous crossings to WT mice. Homozygous and heterozygous HLA-DQ2.5 KI mice were interbred with TCR-glia-α2 (16) mice to obtain HLA-DQ2.5 KI TCR-glia double tg mice. Mice were maintained on gluten-free chow (RDI OpenStandard Diet; Research Diets) and bred under specific-pathogen-free conditions at the Department of Comparative Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway. All experiments were approved by the Norwegian Food Safety Authority.

**Flow cytometry**

Single-cell suspensions from spleens were prepared as described above. Bone marrow was flushed from the femur. Erythrocytes were removed by treatment with ammonium-chloride-potassium lysis buffer. Cells were resuspended in PBS supplemented with 2% (vol/vol) FCS and incubated with optimal amounts of Abs against B220 (RA3-6B2; BioLegend; Invitrogen), CD3 (145-2C11; BioLegend, ebioscience), CD4 (GK1.5; BioLegend), CD8a (53-6.7; BioLegend), CD11b (M1/70; BioLegend), CD11c (N418; BioLegend), CD19 (6D8; BioLegend, ID3; ebioscience), CD21 (7G6; BD Pharmingen), CD23 (B3B4; ebioscience), HLA-DQ2.5 [2.12.E11 (17)], IA/IE (M5/114.15.2; ebioscience), IgD (I126c; ebioscience), IgM (11/41; ebioscience), SiglecH (440c; ebioscience), and Vß3/R (Ansell). For all flow cytometry assays, Fc block against mouse CD16/32 (93; BioLegend) was used. Cells were analyzed on FACSCalibur (BD Biosciences) or Attune NxT Flow Cytometer (Thermo Fisher Scientific). Data analysis was performed using FlowJo software (BD).

**T cell proliferation assays**

CD4<sup>+</sup> T cells were isolated from pooled spleens and lymph nodes from HLA-DQ2.5 tg TCR-glia-α2 tg mice by negative selection.
FIGURE 2. H2-IA and HLA-DQ2.5 expression in B cell and dendritic cell subsets in WT, heterozygous HLA-DQ2.5 tg, heterozygous HLA-DQ2.5 KI, and homozygous HLA-DQ2.5 KI mice.

(A) Surface expression of HLA-DQ2.5 and H2-IA in CD19+ B220+ B cells and CD11c+ dendritic cells from HLA-DQ2.5 KI and HLA-DQ2.5 tg animals is different. (B) Representative plots showing H2-IA and HLA-DQ2.5 expression on gated splenic follicular/T2 (B220+ CD19+ CD23+ CD21+) B cells. (C) H2-IA and HLA-DQ2.5 expression on splenic dendritic cells. Splenic CD19- B220- CD11c+ dendritic cells were stained for CD8a, CD11b, and SiglecH to identify cDC1 (CD8a+ CD11b+), cDC2 (CD8a+ CD11b+), and pDC (SiglecH+) subsets. (D) H2-IA and HLA-DQ2.5 expression on...
(EasySep Mouse CD4+ T cell isolation kit; StemCell Technologies). Purity of the isolated T cells was assessed by flow cytometry and was typically >80%. T cell purity was taken into consideration when calculating T cell numbers. Isolated CD4+ T cells were labeled with Cell Trace Violet (Molecular Probes). Splenocytes from heterozygous and homozygous HLA-DQ2.5 KI and HLA-DQ2.5 tg mice were irradiated at 30 Gy and used as APC. CD4+ T cells (7 × 10^6 cells/ml, corrected for purity) were cocultured with irradiated splenocytes (3.3 × 10^6 cells/ml) and deamidated α2-gluten 33mer (33merEEE) (LQLQPFPQPQPELPYPQPELPYPQ; Genscript) at indicated concentrations in RPMI 1640 supplemented with 10% (vol/vol) FCS, 1% penicillin/streptomycin, 1% nonessential amino acids, 1% sodium-pyruvate, and 100 μM 2-ME for 72 h at 37°C.

RESULTS

Generation of an HLA-DQ2.5 KI mouse strain

We generated HLA-DQ2.5 KI mice that express a chimeric MHC II surface protein in which the extracellular domains derive from HLA-DQA1*05:01 and HLA-DQB1*02:01. The introduction of HLA-DQA1*05:01 and HLA-DQB1*02:01 sequences into the mouse genome was done by means of homologous recombination with targeting vectors in two separate and consecutive handlings of C57BL/6 Bruce4 ES cells.

For introduction of HLA-DQA1*05:01, exons 2 and 3 of H2-Aa were replaced by the corresponding HLA-DQA1*05:01 exon sequences, and an FRT-flanked hygromycin selection cassette was introduced upstream of exon 2 (Fig. 1A). For introduction of HLA-DQB1*02:01, altogether three attempts were made to obtain a functional KI mouse, the first two being unsuccessful. In the successful attempt, H2-Ab1 was humanized from the first conserved alanine residue in exon 1, and the neomycin selection marker was placed in intron 3–4 of H2-Ab1 (Fig. 1B). Surface expression of HLA-DQ2.5 was detected by flow cytometry of spleen cells from double tg mice (Fig. 1C). The failed attempts used essentially the same strategy as for HLA-DQA1*05:01 with positioning of an FRT-flanked neomycin selection cassette in two different locations upstream of exon 2 (Supplemental Fig. 1A, 1B). With this strategy, no surface expression of HLA-DQ2.5 was observed in double KI mice, and improper exon–intron splicing of H2-Ab1/DQB1*02:01 was identified as the problem (Supplemental Fig. 2).

The generated HLA-DQ2.5 KI mouse expresses a chimeric human-mouse MHC II molecule with mouse sequences in the transmembrane and intracellular domains. The extracellular part of the chimeric β-chain is identical to that of HLA-DQ2.5, and the chimeric α-chain differs from HLA-DQ2.5 at two exon 1-encoded residues in the very N-terminal part (KI chimeric protein sequence: EDDIEAD; HLA-DQ2.5 protein sequence: E-DIVAD).

Targeted integration of the transgene is important for physiological MHC II expression

We next analyzed expression of HLA-DQ2.5 and H2-IA in different cell subsets, comparing HLA-DQ2.5 KI mice with HLA-DQ2.5 tg animals that have had the transgene introduced in an untargeted manner (7). As expected, CD11c^hi dendritic cells had higher surface expression levels of H2-IA than B220^ B cells. Importantly, HLA-DQ2.5 expression in HLA-DQ2.5 KI animals matched this pattern, whereas in HLA-DQ2.5 tg animals, a lower relative expression of HLA-DQ2.5 was seen on dendritic cells compared with B cells (Fig. 2A).

To further assess expression patterns in our tg and KI animals, we examined splenic APC. B cell subsets defined by CD21/35 and CD23 staining were present in normal proportions and coexpressed HLA-DQ2.5 and H2-IA in heterozygous HLA-DQ2.5 KI animals. In the CD21/35^CD23^ follicular B cell subset from HLA-DQ2.5 tg animals, we identified a small population of cells that expressed only H2-IA (Fig. 2B). Dendritic cell subsets defined by coexpression of CD11c and CD8, CD11b, or SiglecH likewise coexpressed HLA-DQ2.5 and H2-IA in heterozygous HLA-DQ2.5 KI animals, but not in HLA-DQ2.5 tg animals, in which a population of cells in each subset expressed endogenous H2-IA but not the transgene (Fig. 2C).

The disparate expression of HLA-DQ2.5 and H2-IA as observed in untargeted tg animals was even more pronounced during cellular development. We examined expression of H2-IA and HLA-DQ2.5 during B cell developmental stages in the bone marrow as identified by IgD and IgM staining (Fig. 2D). In HLA-DQ2.5 tg mice, we found H2-IA surface expression in immature B cells that did not coexpress HLA-DQ2.5, and a significant proportion of translaitional B cells displayed the same surface MHC II expression patterns. In mature recirculating B cells in the bone marrow, this population made up a small percentage of total B cells, confirming our observations with mature follicular B cells in the spleen. HLA-DQ2.5 KI mice coexpressed surface H2-IA and HLA-DQ2.5 at all B cell developmental stages.

We also saw that in HLA-DQ2.5 KI heterozygous animals there was lower surface expression of H2-IA compared with WT and HLA-DQ2.5 tg mice (Fig. 2E). HLA-DQ2.5 surface expression was also lower in KI animals compared with untargeted tg, even when comparing homozygous KI animals with untargeted tg.
with heterozygous untargeted tg. Untargeted tg mice usually carry an unknown number of transgene inserts, which likely accounts for these observations.

Taken together, our data imply nonnatural expression of the transgene in HLA-DQ2.5 tg animals in several cell types and appears to be most marked in early developmental stages. This suggests that the use of the endogenous mouse promotor for our chimeric HLA-DQ2.5 KI protein ensures normal cell surface expression patterns of the gene product.

**HLA-DQ2.5 KI is functional for T cell presentation**

To evaluate whether knock-in HLA-DQ2.5 was functional and able to present Ag to CD4\(^+\) T cells, we took advantage of an HLA-DQ2.5-restricted gluten-specific TCR tg mouse strain that recognizes the DQ2.5-glia-\(\alpha2\) epitope (16), which we crossed to HLA-DQ2.5 KI mice to obtain TCR-glia-\(\alpha2\) tg HLA-DQ2.5 KI heterozygous or homozygous animals. CD4\(^+\) T cells were present in the spleens of these and non-TCR tg HLA-DQ2.5 KI animals, showing that KI HLA-DQ2.5 is able to support T cell differentiation (Fig. 3A). Splenic cellularity remained consistent across genotypes, but we observed a decrease in the frequency of CD4\(^+\) T cells in the spleens of HLA-DQ2.5 KI homozygous animals compared with heterozygous animals, which may suggest the absence of a population of CD4\(^+\) T cells that are selected on H2-IA.

We isolated gluten-specific CD4\(^+\) T cells from HLA-DQ2.5 TCR-glia-\(\alpha2\) double tg mice and cocultured them with irradiated splenocytes from WT, HLA-DQ2.5 KI heterozygous, or HLA-DQ2.5 KI homozygous mice and cognate gluten peptides for 72 h. We found that gluten-specific CD4\(^+\) T cells proliferate when cocultured with HLA-DQ2.5tg APCs but not with WT APCs (Fig. 3B).

We compared gluten-specific CD4\(^+\) T cell proliferation after 72 h of coculture with APCs that were heterozygous or homozygous for the targeted HLA-DQ2.5 KI or for the above-mentioned untargeted HLA-DQ2.5 tg and found no difference in T cell proliferation between CD4\(^+\) T cells cultured with different HLA-DQ2.5 APCs (Fig. 3C). Of note, in vivo proliferation of adoptively transferred gluten-specific TCR tg CD4\(^+\) T cells in response to orally administered deamidated gluten digest has been observed in HLA-DQ2.5 KI mice (18).

**FIGURE 3.** KI HLA-DQ2.5 is functional for T cell development and gluten peptide presentation.

(A) CD4\(^+\) T cells are present in spleens of HLA-DQ2.5 KI heterozygous and HLA-DQ2.5 KI homozygous mice. Flow cytometric analysis of spleen cells from HLA-DQ2.5 KI mice stained with Abs recognizing CD4. Data are mean ± SEM (\(n = 3\)) and representative of at least two independent experiments. \(*p < 0.05, **p < 0.0005\) as determined by Student t test. (B) In vitro functionality of HLA-DQ2.5 KI protein. CD4\(^+\) T cells isolated from spleens and lymph nodes of HLA-DQ2.5 KI TCR-glia-\(\alpha2\) tg mice were labeled with Cell Trace Violet (CTV) proliferation tracking dye and cultured with irradiated splenocytes from WT, heterozygous, or homozygous HLA-DQ2.5 KI mice and indicated concentrations of other antigenic peptides and gluten. (C) Induction of proliferation of gluten-specific CD4\(^+\) T cells did not differ between APCs from targeted HLA-DQ2.5 KI and unguided HLA-DQ2.5 KI mice. CTV-labeled CD4\(^+\) T cells were cocultured with irradiated splenocytes as described in (A) in the presence of 0.1 \(\mu M\) gluten peptides. Data are pooled from three independent experiments. Bars represent mean ± 95% confidence interval.
DISCUSSION

Most autoimmune disorders display associations to certain HLA allotypes. MHC II molecules play important roles in CD4+ T cell selection in the thymus and in Ag presentation to mature cells in the periphery. To study the involvement of human disease-associated HLA allotypes in disease processes in a humanized mouse, tg introduced human MHC II should optimally be present and operate in a physiological way. In this project, our aim was to create a physiological HLA-DQ2.5 tg mouse by employing KI technology.

For the generation of tg mice, targeted gene KI has several benefits over untargeted transgenes. Untargeted gene insertion carries a risk of off-target effects, like silencing of genes in which the transgene might have inserted (19, 20), large-scale chromosomal rearrangements (21, 22), or effects on surrounding genes (23). Untargeted tg organisms also have an unknown gene copy number, which can affect studies in which gene dose is important. Targeted gene insertion avoids these issues and utilizes natural mouse promoter/enhancer gene regulation, resulting in physiological expression of the transgene. When comparing our novel HLA-DQ2.5 KI mouse to the existing HLA-DQ2.5 tg mouse, we observed some of these effects. We observed differences in quantitative expression of the transgene between the two mice, most likely due to the integration of multiple copies of the transgene into the genome of the untargeted tg mice. Interestingly, we also observed qualitative differences in the expression of H2-IA and HLA-DQ2.5 in the untargeted tg mice, which importantly were not observed in the KI mice. The differences in expression of HLA-DQ2.5 and H2-IA were particularly notable for B cells during their maturation in the bone marrow. Although we did not observe any marked differences related to HLA-DQ2.5 function between the untargeted tg and KI animals in the settings we tested, such differences may well occur in more complex autoimmune disease models. Thus, we believe that the HLA-DQ2.5 KI mice offer advantages over the untargeted HLA-DQ2.5 tg mice. In addition to providing mice with physiological expression of the introduced transgene, the KI mice have the advantage of avoiding crossing to MHC II null mice when breeding the HLA tg mice onto genetically modified mouse strains, which may be required for dissection of immune mechanisms.

In C57BL/6 mice, there is no expression of the H2-IE α-chain, leaving the H2-IE β-chain free to potentially pair with the H2-IA α-chain, or in this KI model, the HLA-DQ2.5 α-chain (12). Given the differences between the intracellular and transmembrane regions of both the DQα- and the DQB-chains and a two residue difference in the extracellular region of the DQα-chain in the HLA-DQ2.5 KI and HLA-DQ2.5 tg mice, it is theoretically conceivable that there would be disparate interactions of the two distinct DQα-chains with H2-IEB. However, we find this possibility unlikely because whereas MHC II αβ-chain pairing preference is dictated by sequence variation in the extracellular N-terminal domains (24), the very N-terminal part of the α-chain is not a region known to affect chain pairing (25). We find it even less likely that the structural differences between HLA-DQ2.5 and the chimeric KI protein are responsible for the observed differences in MHC II expression between the HLA-DQ2.5 KI and HLA-DQ2.5 tg animals.

Potential trans-species effects on molecular interactions between the introduced human molecules and endogenous mouse molecules like CD4 and the invariant chain (CD74) are a general challenge faced by MHC II tg. Previous in vivo studies on the interaction between mouse CD4 and HLA-DQ or HLA-DR transgenes have shown that TCR tg T cell selection proceeds without requiring species-matched CD4, but less efficiently (26, 27). In this study, we observed positive selection of HLA-DQ2.5–restricted gluten-specific TCR tg T cells, which also made vigorous responses in the periphery when stimulated with Ag. As we have not made a side-by-side comparison of these T cells with and without human CD4, we are unable to conclude whether the lack of human CD4 in our system leads to inferior T cell selection or peripheral responses.

In the generation of the HLA-DQ2.5 KI mice, the introduction of HLA-DQB1*02:01 was not straightforward. The two first attempts were unsuccessful because of improper exon–intronsplicing of the KI gene. We have no obvious explanation for this faulty behavior, as the two initial designs of the targeting construct for HLA-DQB1*02:01 were very similar to that of HLA-DQA1*05:01, and the DQA1 construct was working properly.

Taken together, we present the making of a novel HLA-DQ2.5 KI mouse, which should be useful for the study of the pathogenesis of CeD and other HLA-DQ2.5–associated disorders.

DISCLOSURES

F.K. is the Chief Executive Officer of Ozgene Pty. Ltd., which provided KO mice under commercial contracts with Oslo University Hospital and the University of Oslo. The other authors have no financial conflicts of interest.

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