Induction of human humoral immune responses in a novel HLA-DR-expressing transgenic NOD/Shi-scid/γcnull mouse

Makiko Suzuki1,2, Takeshi Takahashi1,3,*, Ikumi Katano3, Ryoji Ito3, Mamoru Ito3, Hideo Harigae2, Naoto Ishii1 and Kazuo Sugamura1,4

1Department of Microbiology and Immunology, Tohoku University Graduate School of Medicine, 2-1 Seiryo-cho, Aoba-ku, Sendai 980-8575, Japan
2Division of Hematology and Rheumatology, Tohoku University Graduate School of Medicine, 2-1 Seiryo-cho, Aoba-ku, Sendai 980-8575, Japan
3Immunology, Laboratory Animal Research Department, Central Institute for Experimental Animals, 3-25-12 Tono-machi, Kawasaki-ku, Kawasaki 210-0821, Japan
4Division of Cancer Biology and Therapeutics, Miyagi Cancer Center Research Institute, Natori 981-1293, Japan

*Correspondence to: T. Takahashi; E-mail: takeshi-takahashi@ciea.or.jp

Received 9 November 2011, accepted 13 February 2012

Abstract

Mounting evidence has demonstrated that NOD-Shi/scid/γcnull (NOG) mice are one of the most suitable mouse strains for humanized mouse technologies, in which various human cells or tissues can be engrafted without rejection and autonomously maintained. We have characterized and analyzed various features of the human immune system reconstituted in NOG mice by transplanting human hematopoietic stem cells (hu-HSC). One of the problems of the quasi-immune system in these hu-HSC NOG mice is that the quality of immune responses is not always sufficient, as demonstrated by the lack of IgG production in response to antigen challenge. In this study, we established a novel transgenic NOG sub-strain of mice bearing the HLA-DRA and HLA-DRB1:0405 genes, which specifically expresses HLA-DR4 molecules in MHC II-positive cells. This mouse strain enabled us to match the haplotype of HLA-DR between the recipient mice and human donor HSC. We demonstrated that T-cell homeostasis was differentially regulated in HLA-matched hu-HSC NOG mice compared with HLA-mismatched control mice, and antibody class switching was induced after immunization with exogenous antigens in HLA-matched mice. This novel mouse strain improves the reconstituted human immune systems that develop in humanized mice and will contribute to future studies of human humoral immune responses.

Keywords: adaptive immunity, HLA-DR, humanized mice, NOG mice, transgenic

Introduction

Recent advances in the development of novel mouse models that develop human hematopoietic systems have enabled the direct analysis of human hematopoiesis and immune responses with few constraints (1, 2). Such humanized mouse models are considered relevant for the study of basic human hematolymphoid and immunology as well as for translational research (3,4,5). For example, studies of leukemia stem cells largely depend on humanized mouse technology (6, 7). Some human-specific viral diseases, such as EBV or human T-cell leukemia virus-1 infection, can be also recapitulated in such mice (8,9,10,11). Furthermore, this technology makes it possible to evaluate the effects of therapeutic drugs on various human diseases (12,13,14).

The mouse strains NOD/Shi/scid/γcnull (NOG), NOD/LtSz-scid/IL-2Rγnull (NSG) and BALB/RAG-1 KO/γc KO (BRG) are the most suitable platforms for the in vivo reconstitution of human hematolymphoid systems, as the total deficiency of the endogenous murine immune systems in these mice enables long-term survival of various xenogenic grafts (1, 15). Mounting evidence has demonstrated that multiple lineages of human lymphocytes can develop in situ in these mice by transferring human hematopoietic stem cells (HSC) in the absence of other human-derived tissues (2, 16). Several groups have also examined the functionality of these quasi-human immune systems and have demonstrated that significant immune responses are possible in humanized mice (2, 15, 17). For example, human CD8+ T cells in humanized mice exhibited effective cytotoxic activity and cleared infection with EBV (2). Additionally, human B cells produced antigen-specific IgM upon immunization with various
Humoral immune responses in humanized mice

Gladbach, Germany). We used a biotin-conjugated anti-CD34+ from conventional humanized mice (hu-HSC NOG) were unable to respond to antigenic stimulation in vitro to the same extent as normal human T cells from healthy donors; do; the T cells from the hu-HSC NOG mice neither proliferated in response to anti-CD3 and anti-CD28 antibodies nor produced IL-2 (17). These human T cells were also susceptible to apoptosis (17, 20). These results raised the possibility that weak humoral responses in the hu-HSC NOG mice can be attributed, at least in part, to the suboptimal function of the human T cells. Although the precise cellular and molecular mechanisms involved in the impairment of these T cells are yet unclear, it is possible that T cells that are positively selected by mouse MHC in the mouse thymus are rendered anergic by the human antigen-presenting cells (APC) that express HLA in the periphery.

In this study, we established a novel transgenic NOG strain (NOG/HLA-DR4) that expresses the human HLA-DR4 and HLA-DRB1:0405 genes via a mouse MHC class II (mMHC II) promoter (21). We also generated NOG/HLA-DR4/I-AB KO mice that express the transgenic HLA-DR as the sole functional class II MHC so as to eliminate possible interference from mMHC II. Upon engraftment with HLA-DR:0405-positive HSC, but not with HLA-DR:0405-negative HSC, the humanized NOG/HLA-DR4/I-AB KO mice mediated effective humoral immune responses, as demonstrated by the accumulation of a significant amount of antigen-specific IgG in the sera after immunization. Our results indicate that HLA-restricted human immune responses could be provoked in this new NOG/HLA-DR4/I-AB KO strain. This new humanized mouse strain contributes to the study of the human immune system and the development of new drugs to manipulate human immune responses.

Methods

CD34+ hematopoietic stem cells

The cord blood from full-term deliveries was obtained from the Miyagi Cord Blood Bank, following the institutional guidelines approved by the Tohoku University Committee on Clinical Investigations. Some CD34+ cell samples were obtained from the RIKEN Bioresource Center Cell Bank (Tsukuba, Japan). HSC were isolated, as described elsewhere (17). Briefly, mononuclear cells were isolated using Lymphocyte Separation Medium (MP Biomedicals, Solon, OH, USA) after eliminating phagocytes with silica (Immuno Biological Laboratories, Takasaki, Japan). CD34+ HSC were purified by magnetic cell sorting (MACS; Miltenyi Biotech, Bergisch Gladbach, Germany). We used a biotin-conjugated anti-human CD34 mAb (Serotec, Oxford, UK), a blocking reagent for human Fc receptor (Miltenyi Biotech), and anti-biotin microbeads (Miltenyi Biotech) to label the cells and an AutoMACS pro separator (Miltenyi Biotech) to purify the labeled HSC. The typical purity of the CD34+ fraction was >95%. The purified CD34+ HSC were cryopreserved in Cell Banker (Juji Field, Tokyo, Japan) at –80°C in a deep freezer until use. The haplotype of the HLA-DRB1 locus of the cord blood samples was determined by HLA laboratory (Kyoto, Japan) to identify HLA-DRB1:0405-positive HSC. We collected HSC from 236 different donors in total and 38 individuals among them were positive for HLA-DRB1:0405.

Mice and reconstitution with human stem cells

Six-week-old female NOG mice were obtained from the Central Institute for Experimental Animals (Kawasaki, Japan). To establish a transgenic strain of NOG mice that express human class II (HLA-DR), we used the pDOI-5 vector (kindly provided by Drs Mathis and Benoist of Harvard Medical School, Boston, MA, USA) (21) so that the expression of the HLA-DR and HLA-DRB1:0405 genes were regulated by the mouse class II promoter. The DNA constructs were microinjected into fertilized eggs of NOD mice by conventional methods. The founder mice were screened for the expression of HLA-DR on mouse B cells by flow cytometric analysis. The HLA-DR-expressing transgenic NOG mice (NOG/HLA-DR4) were then crossed with NOG I-AB−/− mice (17, 22) to create NOG/HLA-DR4/I-AB−/− mice. All mice were maintained in the animal facility at Tohoku University School of Medicine under specific pathogen-free conditions, and all animal experiments were properly conducted according to institutional guidelines. NOG/HLA-DR4/I-AB−/− mice were irradiated with 120 cGy X-rays and grafted with 1 × 10^5 CD34+ cells with the appropriate HLA-DR haplotype.

Antibodies and flow cytometric analysis

The following mAbs were used. To identify mouse cells, anti-I-A^b^-FITC (also reactive for I-A^p^ in NOD), anti-CD11c–PE, anti-CD11b–PE, anti-CD19–PE, anti-CD4–PE and anti-CD45–allophycocyanin (APC) were purchased from BD Pharmingen (San Jose, CA, USA). To identify human cells, anti-CD24–FITC and purified anti-HLA-DR were purchased from eBioscience (San Diego, CA, USA) and anti-CD19–FITC, anti-CD34–FITC, anti-CD45RA–FITC, anti-CD5–PE, anti-CD8–PE, anti-CD38–PE, anti-IL-2–PE, anti-IL-4–PE, anti-IL-6–PE, anti-IL-10–PE, anti-mIFN–PE and anti-CD45–APC–Cy7 were purchased from BD Pharmingen. Anti-CD4–Pacific Blue and anti-CD8–Pacific Blue were purchased from Biolegend (San Diego, CA, USA). Anti-CD34–biotin was purchased from AbD Serotec (Kidlington, UK).

To analyze human lymphocytes in hu-HSC NOG/HLA-DR4/I-AB−/− mice, multicolor flow cytometric analysis was performed using a FACS Canto II flow cytometer (BD Biosciences). Peripheral blood was obtained from the retro-orbital venous plexus through heparinized pipettes to periodically monitor reconstitution. At the time of sacrifice, single-cell suspensions were prepared from the spleen or bone marrow by conventional methods. The cells were stained with the relevant mAbs for 15 min on ice, then washed with cold PBS containing 2% FCS and stained with the appropriate secondary antibodies. We used Cytofix/Cytoperm solution.
(BD Biosciences) for intracellular staining according to the manufacturer’s instructions. After the final wash, the cells were subjected to flow cytometric analysis. The proportion of each cell lineage was calculated using FACS Diva software (BD Biosciences).

**The ELISA**

The concentration of human IgM and IgG in the sera of reconstituted NOG mice was measured using a human Ig assay kit (Bethyl, Denver, CO, USA). For the detection of ovalbumin (OVA)-specific human IgM and IgG antibodies, humanized NOG/HLA-DR4/I-Ab<sup>−/−</sup> mice were immunized once a week for 4 weeks with an emulsion of 20 µg OVA whole protein (Bioresearch Technologies, Novato, CA, USA) with alum (Cosmo Bio, Tokyo, Japan) in a total volume of 100 µl by intra-peritoneal injection. Sera from the immunized mice were harvested 3 days after the final immunization. Specific antibodies against OVA were measured by a standard ELISA. Briefly, 96-well plates were coated with 10 µg ml<sup>−1</sup> OVA at 4°C overnight. After washing and blocking with PBS containing 1% BSA, the collected serum samples were loaded. Sera were serially diluted three-fold with blocking solution. HRP-conjugated anti-human Ig antibody was used as a secondary antibody. Both anti-IgG-specific and anti-IgM-specific antibodies were purchased from Bethyl (Montgomery, TX, USA). o-Phenylenediamine was used as a substrate for detection. The absorbance at 450 nm was measured by a microplate reader. Serum titers were defined as the dilution at which the absorbance of the sample was equivalent in intensity to that of non-immunized mice (background signal).

**Immunohistochemistry**

Tissue samples were fixed with 4% PFA, dehydrated with graded alcohol and embedded in paraffin. After sectioning, the specimens were treated with heated citrate buffer and stained with mouse anti-HLA-DR antibody overnight at 4°C. Subsequently, endogenous peroxidase activity was quenched by incubating the specimen for 20 min with 0.3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in methanol at room temperature. The samples were further incubated with labeled polymer (Dako EnVision System; Dako, Glostrup, Denmark) as a secondary reagent for 40 min at room temperature. Staining was completed after a 5- to 10-min incubation with 3,3′-diaminobenzidine plus the chromogen substrate. Hematoxylin was used for counter staining.

**In vitro cultures**

Single-cell suspensions of the spleen from hu-HSC NOG/HLA-DR4/I-Ab<sup>−/−</sup> mice were prepared, as described above, ~16 to 24 weeks after reconstitution. For T-cell stimulation, total spleen cells of the mice were labeled with carboxylfluorescein succinimidyl ester (CFSE; Molecular probes, Eugene, OR, USA) according to a standard protocol. Then, the cells were seeded at 1 × 10<sup>5</sup> T cells per well in a 48-well plate and cultured with 10 µg ml<sup>−1</sup> soluble anti-CD3 (clone OKT3; BD Pharmingen) and 1 µg ml<sup>−1</sup> anti-CD28 (BioLegend) antibodies in 500 µl per well RPMI 1640 medium supplemented with 10% FCS and 50 µM 2-mercaptoethanol (Nacalai Tesque, Kyoto, Japan). The cells were collected on day 6, and T-cell proliferation was assessed by flow cytometry after staining with fluorochrome-conjugated anti-CD4 and anti-CD8 antibodies. To determine cytokine production, cells were stimulated with 50 ng ml<sup>−1</sup> phorbol myristate acetate (PMA) and 1 µg ml<sup>−1</sup> ionomycin for 4 h in the presence of Golgi plug (BD Pharmingen), and the accumulated intracellular cytokines were stained with the relevant mAbs according to the manufacturer's instructions, followed by flow cytometric analysis.

**Results and discussion**

To investigate whether the transgenic expression of human class II (HLA II) molecules in humanized NOG mice influenced the function of the reconstituted human immune system, we developed a novel transgenic strain, NOG/HLA-DR4, which expresses the HLA-DR and HLA-DRB1:0405 genes. This HLA haplotype comprises one of the highest frequencies (13.62%, according to the HLA laboratory; http://www.hla.or.jp/, as of 2010) in the Japanese population and increases the likelihood of identifying donor HSC with this HLA haplotype. The expression of the HLA-DR genes was under the control of the mMHC II promoter (21). We first examined whether mature HLA-DR molecules were properly formed and expressed on the mouse cell surface. Flow cytometric analysis revealed that a substantial number of mMHC II-positive APC [i.e. B cells, dendritic cells (DC) and macrophages (Mφ)] in the immunosufficient NOD/HLA-DR4 transgenic mice expressed the HLA-DR protein at significant levels (Fig. 1A) and that the expressions of I-A and HLA-DR were well correlated (Supplementary Figure 1, available at International Immunology Online). Additionally, thymic epithelial cells (TEC), which are responsible for the positive selection of T cells, also expressed HLA-DR (Fig. 1A). Immunohistochemistry confirmed that HLA-DR-positive cells resided in the stromal area (Fig. 1B). APC (DC and Mφ) from immunodeficient NOG/HLA-DR4/I-Ab<sup>−/−</sup> mice also expressed HLA-DR (Fig. 1C). Collectively, the expression pattern of transgenic HLA-DR was consistent with that of mMHC II.

The NOG/HLA-DR4/I-Ab<sup>−/−</sup> mice were reconstituted with HSC purified from cord blood. Both DRB1:0405-positive and negative HSC were used to compare the effects of matching, or not matching, the HLA haplotype. The chimerism of human hematopoietic cells in the bone marrow progressively increased over time, irrespective of the HLA haplotype of the donor HSC, and there were no significant differences between these groups (data not shown). The chimerism of human CD45<sup>+</sup> cells in the spleen was also comparable between these two groups (Supplementary Figure 2, available at International Immunology Online). Human B and T cells were detected in the spleen ~3 months after HSC transplantation, as seen in conventional NOG mice (Supplementary Figure 2, available at International Immunology Online), and the thymus was also colonized by human T cells, which indicated proper differentiation (Supplementary Figure 3, available at International Immunology Online). The number of thymocytes was not significantly influenced by the presence of HLA-DR transgene (Supplementary Figure 3, available at International Immunology Online). Our previous work demonstrated that the majority of human B cells in hu-HSC NOG...
mice had immature phenotypes, which were similar to the transitional 1 B-cell phenotype of normal human B cells. Our analysis of human B cells in the hu-HSC NOG/HLA-DR4/I-A\textsuperscript{b}/C0 mice revealed that the introduction of HLA-DR did not influence the differentiation of human B cells in the transgenic NOG mice irrespective of the HLA haplotype of the donor HSC (Supplementary Figure 4, available at International Immunology Online).

There was no statistically significant difference in the total cellularity of the spleens from the DRB1:0405\textsuperscript{+} HSC-grafted mice versus the DRB1:0405\textsuperscript{-} HSC-grafted mice at various time points after reconstitution (Fig. 2A). The frequency and

Fig. 1. Expression of HLA-DR in the transgenic mouse strain, NOG/HLA-DR4. (A) Expression of HLA-DR by various APC from immunosufficient NOD/HLA-DR4 (NOD/DR4) transgenic mice. Spleen cells from HLA-DR transgenic or non-transgenic NOD mice were stained with anti-HLA-DR antibody and anti-CD19 antibody for B cells, anti-CD11c antibody for DC, anti-CD11b antibody for M\textsubscript{Φ}, or anti-6C3 antibody for TEC. The expression of HLA-DR in various APC subgroups (red plots) is shown in the left columns, with histograms in the right columns. Histogram values represent the mean fluorescent intensity. Representative flow plots are shown (n = 5). (B) Histological analysis of the thymus of a NOG/DR4 mouse. The thymi from hu-HSC NOG/DR4 I-A\textsuperscript{b}/C0 mice or control hu-HSC NOG mice were isolated 20 weeks after reconstitution, fixed with 10% formalin and processed for immunohistochemical analysis for the expression of HLA-DR. Red arrows represent HLA-DR-positive TEC. Representative results are shown (n = 4). (C) Expression of HLA-DR in the APC of NOG/DR4. Spleen cells from HLA-DR transgenic or non-transgenic NOG mice were stained with anti-HLA-DR together with anti-CD11c or CD11b antibodies. Representative flow plots are shown (n = 4).
the absolute number of CD3\(^+\) splenic T cells were also compared between these two groups (Fig. 2B). At early time points (i.e. ~4 months) after HSC transplantation, the frequency of CD3\(^+\) T cells was significantly higher in DRB1:0405\(^-\) HSC-grafted mice than in DRB1:0405\(^-\) HSC-grafted mice, though this difference became less evident at later time points (Fig. 2B). Accordingly, the absolute number of CD3\(^+\) T cells was higher in the DRB1:0405\(^-\) HSC-grafted mice than in the DRB1:0405\(^-\) HSC-grafted mice 4–5 months after reconstitution, although the latter group reached a number of CD3\(^+\) T cells equivalent to the former group by 6 months (Fig. 2B). The frequency and the absolute number of CD4\(^+\) splenic T cells showed a similar pattern (Fig. 2C), in which DRB1:0405\(^-\) HSC-grafted mice had higher numbers of CD4\(^+\) T cells than DRB1:0405\(^-\) HSC-grafted mice at 4 and 5 months posttransplantation. The analysis of CD8\(^+\) T cells showed the mild increase of the proportion in DRB1:0405\(^-\) HSC-grafted mice compared with that in DRB1:0405\(^-\) HSC-grafted mice (Fig. 2D), which was in contrast with the prominent increase of CD4\(^+\) T cells.

In accordance with the increase of CD3\(^+\) T cells, there was a tendency that the frequency and the number of human B cells became lower in DRB1:0405\(^-\) HSC-grafted mice than in DRB1:0405\(^-\) HSC-grafted mice at 6 months (Supplementary Figure 4, available at International Immunology Online). This did not, however, reach statistical significance.

We next analyzed the composition of human CD4\(^+\) T cells in the hu-HSC NOG/HLA-DR4/I-A\(^+\)/I-k\(^-\) mice in detail. There was a strikingly low frequency of CD4\(^+\)CD45RA\(^-\)/CD62L\(^+\)/CD62L\(^+\)/CD62L\(^-\) naive T cells (T\(_{naive}\)) and a corresponding high frequency of CD4\(^+\)CD45RA\(^-\)/CD62L\(^-\)/CD62L\(^+\)/CD62L\(^-\) effector memory-like T cells (T\(_{EM}\)) and CD4\(^+\)CD45RA\(^-\)/CD62L\(^+\)/CD62L\(^+\)/CD62L\(^-\) central memory-like T cells (T\(_{CM}\)) in the humanized mice compared with normal human T cells from peripheral blood (Fig. 3A). The frequency of T\(_{EM}\) significantly increased in the DRB1:0405\(^-\) HSC-grafted mice, but not in the DRB1:0405\(^-\) HSC-grafted mice, over the course of reconstitution (Fig. 3B), while the absolute number of T\(_{EM}\) significantly increased in both groups. In contrast, T\(_{naive}\) in the DRB1:0405\(^-\) HSC-grafted mice gradually decreased in number and frequency, although this did not reach statistical significance (Fig. 3B) due to large variance, which may be largely attributable to the variation in donor HSC. The analysis of T-cell sub-populations revealed the remarkable expansion of T\(_{EM}\) and the corresponding reduction of T\(_{naive}\) and T\(_{CM}\) in DRB1:0405\(^-\) HSC-grafted mice, whereas the frequencies of these T-cell sub-populations were not significantly altered in the DRB1:0405\(^-\) HSC-grafted mice (Fig. 3C). These results suggested that under HLA-matched conditions, human CD4\(^+\) T\(_{naive}\) cells in NOG/HLA-DR4/I-A\(^+\)/I-k\(^-\) mice vigorously proliferated in an HLA-dependent manner and differentiated into T\(_{EM}\) cells.

We next examined the function of the reconstituted human immune system in hu-HSC NOG/HLA-DR4/I-A\(^+\)/I-k\(^-\) mice. NOG/HLA-DR4/I-A\(^+\)/I-k\(^-\) mice reconstituted with HLA-matched or HLA-mismatched HSC were immunized with OVA protein plus alum adjuvant. After four weekly immunizations, the sera were collected and the presence of OVA-specific antibodies was examined by ELISA (Fig. 4A). As expected, a large quantity of OVA-specific human IgM was detected in all the mice (Fig. 4B left). High levels of OVA-specific human IgG were also detected in the sera of 6/10 immunized mice with HLA-matched HSC (Fig. 4B right). Considerable variance was observed among the mice, and the titer of OVA-specific
Fig. 3. Analysis of the CD4+ T-cell sub-population in hu-HSC NOG/DR4/l-A−/− mice. (A) The expression pattern of CD45RA and CD62L in human CD4+ T cells from humanized mice. Splenocytes from hu-HSC NOG/DR4/l-A−/− mice were isolated 20 weeks after HSC reconstitution and stained with anti-CD4, anti-CD45RA and anti-CD62L antibodies. Representative flow plots from hu-HSC NOG/DR4/l-A−/− mice and normal human peripheral blood are shown (n > 10). (B) The frequencies and the absolute cell numbers of T naïve and TEM in hu-HSC NOG/DR4/l-A−/− mice. The frequencies (left panels) and absolute numbers (right panels) of T naïve and TEM in hu-HSC NOG/DR4/l-A−/− mice. The frequencies (left panels) and absolute numbers (right panels) of CD45RA+/CD62L− T naïve (top) and CD45RA−/CD62L− TEM (bottom) populations, which were obtained by flow cytometry, are shown (left panels). (C) The proportion of each CD4+ T-cell sub-population in hu-HSC NOG/DR4/l-A−/− mice. The mean frequencies of CD45RA+/CD62L− T naïve, CD45RA−/CD62L− TEM, and CD45RA−/CD62L− T CM populations are shown over time.
IgG in one mouse reached to >6250-fold higher than that of non-immunized control mice. OVA-specific IgG was not detected in hu-HSC NOG/HLA-DR4/I-A<sup>b</sup>/C<sup>0</sup>/C<sup>0</sup> mice with HLA-mismatched HSC, although they showed OVA-specific IgM production (Fig. 4B). As for the total amount of IgM and IgG, there was no significant difference between the mice.
with HLA-matched HSC and HLA-mismatched HSC before immunization (Fig. 4C, white symbols). After immunization, total IgM and IgG was increased in several mice in HLA-matched group, but none in HLA-mismatched group (Fig. 4C, gray symbols) and the increase was statistically significant for IgM, but not for IgG. These results suggested that humanized HLA transgenic mice with HLA-matched HSC transplantation mounted immune responses sufficient to induce antibody class switching in the human B cells.

We next analyzed T-cell function in the hu-DRB1:0405* HSC NOG/HLA-DR4/I-Ab\(-/-\) mice. The human T cells showed significant proliferation in response to anti-CD3 and anti-CD28 antibodies in vitro, and the activated T cells produced IL-2 and IFN-\(\gamma\) upon stimulation with PMA and ionomycin (Fig. 4D and E). These results suggested the presence of human T cells with normal function in the hu-HSC NOG/HLA-DR4/I-Ab\(-/-\) mice. However, the magnitude of T-cell proliferation was not as robust as that of human T cells from normal healthy donors (Fig. 4D), and the amount of human IL-2 in the culture supernatants was not as high as that produced by normal human T cells (data not shown).

In the present study, using the novel mouse strain NOG/HLA-DR4/I-Ab\(-/-\), we demonstrated that human lymphocytes that developed in situ in the humanized mice caused human humoral immune responses in an HLA-DR-restricted manner in cases that used HLA-matched HSC for transplantation. This is a significant advance in humanized mouse technology, as there has yet to be a reliable model in which properly functioning adaptive human immune responses occur without the need for xenotransplantation of human tissues (e.g. fetal liver and thymus in the BLT model) (23).

It has been speculated that the mismatch between the mMHC II responsible for the positive selection of human T cells in the mouse thymus and the HLA II expressed by human B cells in the periphery is the major obstacle to inducing functional human adaptive immune responses in conventional humanized mice (17). Our results demonstrated that this problem was overcome, even if partially, by the introduction of HLA II and the elimination of mMHC II. Recently, Danner et al. reported that antigen-specific human IgG was produced in NSG/HLA-DR mice that expressed a human/mouse chimeric molecule, in which the peptide-binding domain of the mouse I-E\(\beta\) chain was substituted with the corresponding domain of HLA-DRB1:0401 to mimic the structure of an HLA-DR4 molecule (24, 25). Because they used I-A\(\alpha\) sufficient NSG mice, the elimination of mMHC II was not necessary for the elicitation of human immune responses. However, it is noteworthy that the I-A\(\alpha\) sufficient NOG/HLA-DR4/I-Ab\(-/-\) mice in our mouse colony did not induce human IgG responses (data not shown). One plausible explanation for these discrepant results is that mMHC II still played a dominant role in the positive selection of human T cells in I-A\(\alpha\)-I\(\beta\) mice because of the abundance of mMHC II\* TEC. This could render the size of the T-cell repertoire restricted by HLA II too small to induce detectable human immune responses. If this is the case, the elimination of mMHC II is critical to maximize the HLA-restricted T-cell repertoire.

Matching the HLA-DR haplotypes of the recipient NOG/DR4 transgenic mouse and donor HSC significantly influenced human T-cell homeostasis. In particular, HLA-matched reconstitution resulted in a large TEM population, suggesting the HLA-dependent rapid proliferation of human T cells. Considering the extremely lymphopenic environment in NOG mice, the expansion of TEM is reminiscent of lymphopenia-induced proliferation of T cells, which is a well-known phenomenon typically seen when a small number of T\(_{naive}\) are seeded into chronically lymphopenic environments (e.g. RAG gene deficiency or scid mutation) (20, 26). Thus, it is possible that a few human thymic immigrants proliferated to restore the T-cell compartment in NOG mice. This mechanism would explain the higher frequency and higher number of CD4\(^+\) T cells in the HLA-matched HSC group, compared with the mismatched group, at early time points. Although the same mechanism would also regulate the relatively slower increase of T cells in the HLA-mismatched group, in this case, mouse DC and M\(\phi\) in NOG/DR4/I-A\(-/-\) mice predominantly stimulate the T cells, whereas both mouse and human APC would stimulate T cells in the HLA-matched HSC group. The difference in the abundance of APC is one reason for the difference in T-cell homeostasis between the HLA-matched and HLA-mismatched groups.

We previously demonstrated that human T cells in conventional hu-HSC NOG mice had extremely low proliferative capacity in response to antigenic stimulation (17). The accumulation of TEM and the rapid decrease of T\(_{naive}\), which have the largest capacity for proliferation, may be one possible explanation. Indeed, when the frequency of T\(_{naive}\) was increased by the transplantation of fetal thymic lobes from NOD mice into the renal capsules of NOG mice, human T cells showed strong proliferation in response to in vitro stimulation that was comparable to that of human T cells from healthy human adults (data not shown). This result suggests that high numbers of T\(_{naive}\) in NOG/DR4/I-A\(-/-\) mice will enable the augmentation of immune responses. There are two major mechanisms that regulate the size of the T\(_{naive}\) pool: the supply from the thymus (27, 28) and homeostatic proliferation in the periphery (29, 30). To enhance the function of the thymus (27), transgenic expression of keratinocyte growth factor or Il-3 ligand in TEC, which can enhance the regeneration of TEC after irradiation, should be tested (31,32,33,34,35). Regarding the homeostasis in the periphery, reconstitution of secondary lymphoid organs in NOG mice, which have a significant deficiency of lymph nodes (LN) (36,37,38), is important, as IL-7, a survival factor for T\(_{naive}\), is provided by LN-resident reticular fibroblastic cells (39).

Humanized mice are an excellent tool with which to study human immunology. The reconstitution of a functional human adaptive immune system in hu-HSC NOG/HLA-DR4/I-Ab\(-/-\) mice offers unique opportunities to test and utilize human immunity. For example, the capability to produce antigen-specific IgG in hu-HSC NOG/HLA-DR4/I-Ab\(-/-\) suggests that this model has great potential for generating mAbs against various exogenous substances, such as viral or bacterial proteins. Such mAbs could work as therapeutic drugs for prevention of infection or allergy (18, 19). Along with further improvements of humanized mouse technologies, e.g. introduction of human cytokine genes (40,41,42), this mouse model will contribute to the development of new therapeutic strategies for human disease.
Supplementary data

Supplementary data are available at International Immunology Online.

Funding

Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan; a Grant-in-Aid for Scientific Research on Priority Areas awarded to K.S. (#19059001); a Grant-in-Aid for Scientific Research (S) from the Japan Society for the Promotion of Science awarded to M.I. (#18100005); a Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science awarded to T. T. (#23590561).

Acknowledgements

We thank M. Konno and E. Saijyo for their technical assistance.

Disclosure

The authors have no financial conflicts of interest.

References


