Kidney360

Kidney Immune Cell Characterization of Humanized Mouse Models

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Key Points

- Experimental studies often fail to translate to clinical practice. Humanized mouse models are an important tool to close this gap.
- We immunophenotyped the kidneys of NOG (EXL) and NSG mouse strains engrafted with human CD34$^+$ hematopoietic stem cells or PBMCs and compared with immune cell composition of normal human kidney.
- Human CD34$^+$ hematopoietic stem cell engraftment results in steady renal immune cell populations in mouse kidney with key similarities in composition compared with human kidney.

Successful translation of experimental mouse data to human diseases is limited because of biological differences and imperfect disease models. Humanized mouse models are being used to bring murine models closer to humans. However, data for application in renal immune cell–mediated diseases are rare. We therefore studied immune cell composition of three different humanized mouse kidneys and compared them with human kidney. NOG and NOGEXL mice engrafted with human CD34$^+$ hematopoietic stem cells were compared with NSG mice engrafted with human PBMCs. Engraftment was confirmed with flow cytometry, and immune cell composition in kidney, blood, spleen, and bone marrow was analyzed in different models. The results from immunophenotyping of kidneys from different humanized mouse strains were compared with normal portions of human kidneys. We found significant engraftment of human immune cells in blood and kidney of all tested models. huNSG mice showed highest frequencies of hTCR$^+$ cells compared with huNOG and huNOGEXL in blood. huNOGEXL was found to have the highest hCD4$^+$ frequency among all tested models. Non-T cells such as hCD20$^+$ and hCD11c$^+$ cells were decreased in huNSG mice compared with huNOG and huNOGEXL. Compared with normal human kidney, huNOG and huNOGEXL mice showed representative immune cell composition, rather than huNSG mice. In summary, humanization results in immune cell infiltration in the kidney with variable immune cell composition of tested humanized mouse models and partially reflects normal human kidneys, suggesting potential use for translational studies.

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Introduction

Experimental studies in mice are important for medical research for discovery, mechanistic work, and to safely test novel treatment options. However, animal models only partially mimic human diseases. Translation from bench to bedside is often challenging.[1] Humanized mouse models are one approach to bring the mouse experiments closer to human relevance, with the goal to replace specific murine cells with human cells. Humanized mouse models have been successfully used in the fields of infectious disease, oncology, transplant medicine, allergology, and immunology.[2] To avoid rejection of engrafted human cells, immunocompromised mouse strains are preferred. Nonobese diabetic (NOD) mouse strains with severe combined immunodeficiency (SCID), such as NSG(NOD-*scid* IL2Rgamma^{null}), NOG(EXL) (NOG: NOD/Shi-*scid*/IL-2Ry^{null}; NOGEXL: hGM-CSF/hIL-3 NOG), NOD-*Rag1*^{null} *IL2rg*^{null}, and BRGC. Cg-*Rag2*^{tm1Fwa} *Il2rg*^{tm1Sug} mice, are most commonly used to study immune-mediated diseases like organ transplant rejection. Human CD34$^+$ hematopoietic stem cells (HSCs) or human Peripheral Blood Mononuclear Cells (PBMCs) are often used to humanize these mouse strains to mimic the human immune system.[3–5]

Several mouse studies demonstrate important pathogenic and reparative roles of different immune cells during AKI.[6–8] These studies used wild type mice and

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mice with specific immune cell or immune regulatory molecule deficiency (or overexpression) to define immune cell functions in AKI.[9–12] Although mouse studies significantly improved our understanding on immune cell functions during AKI and other kidney diseases, recent data show that mouse immune cells poorly represent human immune cell responses.[13] Thus, studies have started to use humanized mice to directly assess human immune cell responses in kidney diseases. For example, in an ischemic AKI model, a protective role of IL-33 through type 2 innate lymphoid cell (ILC2) axis was found in a classical murine model and subsequently investigated in a humanized mouse model using NSG mice engrafted with hematopoietic progenitor cells.[14]

In a study focusing on idiopathic nephrotic syndrome, NOD/SCID mice were engrafted with either $CD34^+$ stem cells or PBMCs from patients with FSGS or minimal-change nephrotic syndrome compared with healthy control donors. Engraftment of $CD34^+$ HSCs led to albuminuria in recipient mice, whereas the engraftment of PBMCs did not, suggesting that immature immune cells mediate disease pathophysiology.[15] Despite these studies, there is limited information on immune cell composition of the kidney between different humanized mouse models and how it compares with normal human kidney. Furthermore, most studies using humanized mice rely on the engraftment data obtained from peripheral blood, which is likely a good indicator of tissue engraftment, but does not inform the level of engraftment within the kidneys. In this study, we compared kidney immune cell reconstitution characteristics of different humanized mouse models and compared with normal human kidney.

Methods

Animals

Female humanized (hu)NOG mice (NOD.Cg-Prkdcscid Il2rgtm1Sug/JicTac) and huNOGEXL mice (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug} Tg(SV40/HTLV-IL3,CSF2)10-7Jic/JicTac) were purchased from Taconic (Rensselaer, NY). NOD SCID γ (NSG; NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) and NOD mice were purchased from Jackson lab (Bar Harbor, ME). Control NSG mice and NOD were purchased from Jackson lab (Bar Harbor, ME). Mice were kept in gram negative and specific pathogen-free facility of Johns Hopkins University. Johns Hopkins University institutional animal care and use committee approved all animal protocols of this study.

Engraftment of NOG, NOGEXL, and NSG Mice

In brief, 3–4-week-old NOG/NOGEXL mice were irradiated with 110 cGy from x-ray irradiator and injected intravenously within 24 hours 1×10^5 human $CD34^+$ cord blood–derived HSC through tail vein. Engraftment was confirmed by flow cytometric analysis of peripheral blood 16 weeks after injection (Taconic; Rensselaer, NY). Animals were killed at 20 weeks postengraftment and organs collected. Control NOG mice were not engrafted with HSCs.

Table 1. Details of humanized mouse strains used in this study

Mouse Strain	Engraftment	Strain Nomenclature	Characteristics
NOG mouse	Human $CD34^+$ hematopoietic stem cells	NOD.Cg-Prkdc^{scid} Il2rg^{tm1Sug}/JicTac (Taconic)	• Extremely immunodeficient mouse lacking mature T, B, and NK cells, defects in cytokine signaling, and innate immunity • NOD genetic background • SCID=Prkdc mutation=DNA repair complex protein → B cell and T cell deficiency • Partial deletion of IL2 receptor common γ chain → prevents cytokine signaling through multiple receptors → deficiency in functional NK cells • Model for engraftment of human cells ($CD34^+$ HSC, PBMCs)
NOGEXL mouse	Human $CD34^+$ hematopoietic stem cells	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug} Tg(SV40/HTLV-IL3,CSF2)10-7Jic/JicTac (Taconic)	• NOG mouse expressing human GM-CSF and IL-3 cytokines transgene • Model for engraftment of human cells ($CD34^+$ HSC, PBMCs) • Supports the differentiation of human myeloid cell lineages and potentiates increased efficiency of HSC and human immune system engraftment compared with the core NOG mouse
NSG (NOD SCID γ) mouse	Human PBMCs	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ Stock No: 005557 (Jackson lab)	• Extremely immunodeficient mouse lacking mature T, B, and NK cells, defects in cytokine signaling, and innate immunity • NOD genetic background • SCID=*Prkdc* mutation=DNA repair complex protein → B and T cell deficiency • Null allele of IL2 receptor common γ chain → prevents cytokine signaling through multiple receptors → deficiency in functional NK cells • Model for engraftment of human cells ($CD34^+$ HSC, PBMCs)

HSC, hematopoietic stem cell; NOD, nonobese diabetic; SCID, severe combined immunodeficiency; NK, Natural Killer; PBMCs, Peripheral Blood Mononuclear Cells; GM-CSF, granulocyte-macrophage colony-stimulating factor.

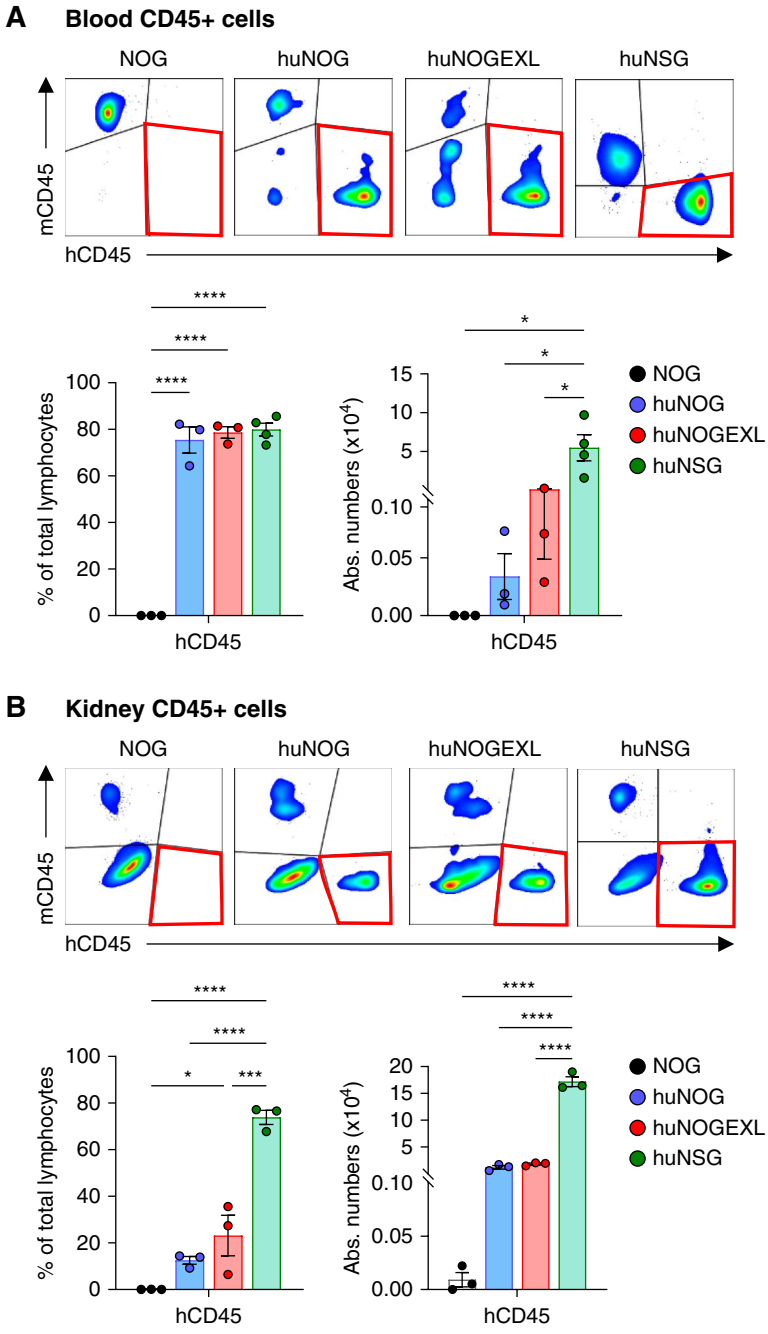


Figure 1. Assessment of CD34$^+$ HSCs engraftment in humanized mouse blood and kidney. (A) Representative flow plots showing hCD45$^+$ cells in peripheral blood of NOG (n=3), huNOG (n=3), huNOGEXL (n=3), and huNSG mice (n=4). Corresponding graphs showing percentage of hCD45$^+$ cells among total lymphocytes (left) and absolute number (right) in the blood of huNOG (n=3), huNOGEXL (n=3), and huNSG mice. (B) Representative flow plots showing hCD45$^+$ cells in kidneys of huNOG (n=3), huNOGEXL (n=3), and huNSG mice (n=3). Corresponding graphs showing percentage of hCD45$^+$ cells among total lymphocytes (left) and absolute number (right) in the kidneys of huNOG (n=3), huNOGEXL (n=3), and huNSG (n=3) mice. Data are expressed as mean±SEM and compared by one way ANOVA followed by Tukey's *post hoc* analysis. *$P \leq 0.05$, ***$P \leq 0.001$, ****$P \leq 0.0001$. CD, cluster of differentiation; h, human; HSC, hematopoietic stem cell; hu, humanized; m, murine.

Three-week-old NSG mice were irradiated with 100 cGy and engrafted with 10×10^6 human PBMCs. PBMCs were isolated from healthy individuals by Ficoll gradient centrifugation. Seventeen days later, immunophenotyping of the blood was performed by flow cytometry. Nineteen days after engraftment, humanized NSG (huNSG) mice were sacrificed and after whole-body perfusion with PBS immunophenotyping of spleen, kidney and bone marrow was performed. Different engraftments and characteristics of the humanized mice are summarized in Table 1.

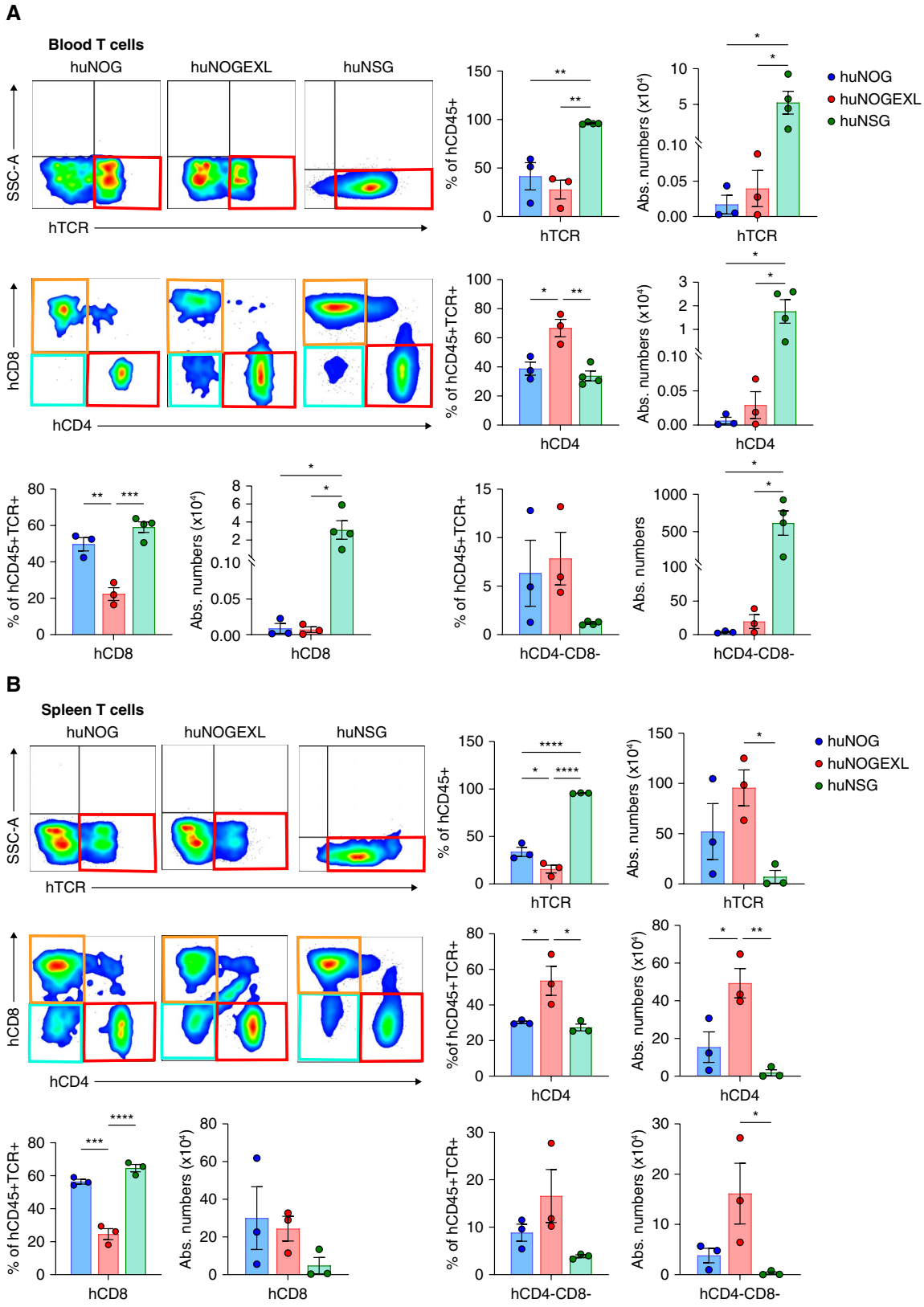
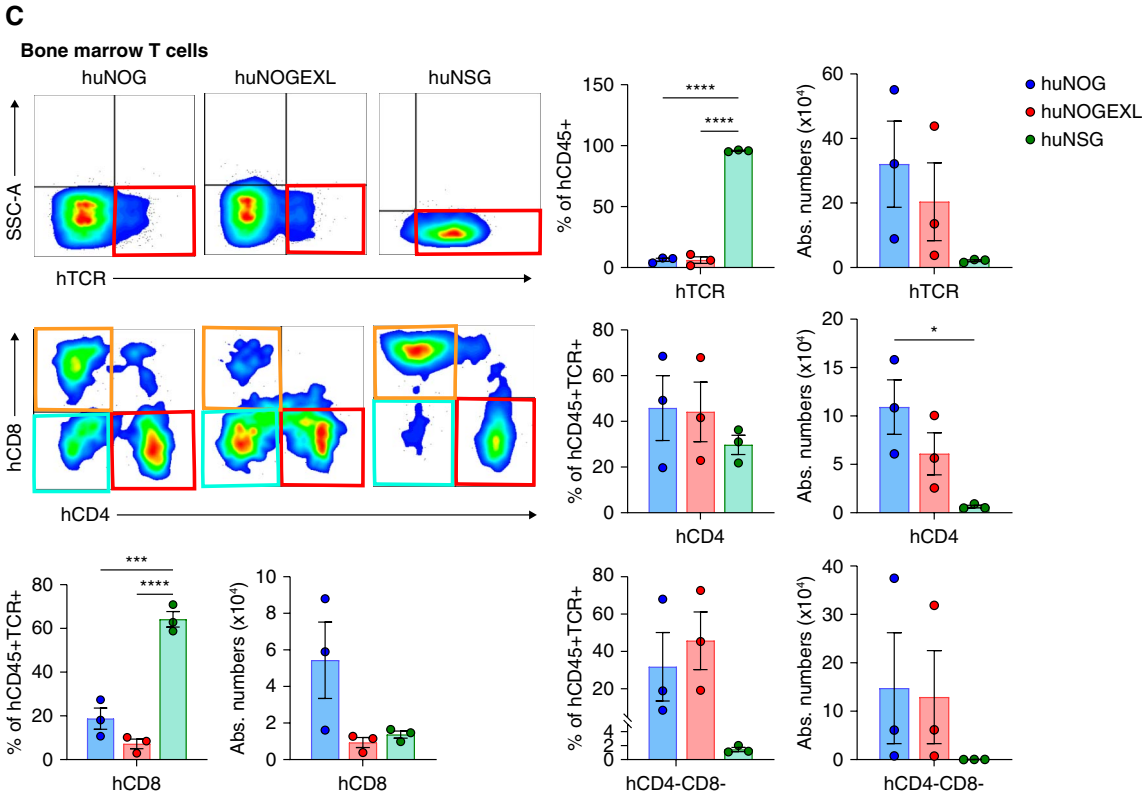


Figure 2. Human T cells in blood, spleen, and bone marrow of HSCs engrafted humanized mice. Representative flow plots and corresponding graphs showing percentage and absolute numbers of hTCR$^+$ T cells among total hCD45$^+$ cells (top panel) and hCD4$^+$, hCD8$^+$, and hCD4$^-$CD8$^-$ DN T cells among hCD45$^+$TCR$^+$ cells (lower panel) in (A) blood, (B) spleen, and (C) bone marrow of huNOG (n=3), huNOGEXL (n=3), and huNSG (n=3) mice. Data are expressed as mean±SEM and compared by one way ANOVA followed by Tukey's *post hoc* analysis. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$. DN, double negative.



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Immune Cell Isolation from Blood, Spleen, and Bone Marrow and Kidney from Mice

Euthanasia was conducted using ketamine hydrochloride (200 mg/kg) and xylazine (15 mg/kg), and after whole-body perfusion with PBS, organs and femur bones were harvested, immune cells were isolated, and immune phenotyping by flow cytometry was performed.

Blood samples were collected before perfusion and diluted in 500 mM EDTA solution. In a next step, samples were mixed with 2% Dextran solution and incubated for 30 minutes at 37°C. Supernatant was harvested and spun at 1450 rpm. After discarding the supernatant, red blood cell (RBC) lysis was performed for 5 minutes (Quality Biological). Suspension was washed and resuspended in FACS buffer.

Spleens were passed through a 70 μm strainer (BD Biosciences). After washing, RBC lysis (Quality Biological, Gaithersburg, MD) was performed for 5 minutes. Cells were washed again and resuspended in FACS buffer.

Bone marrow was collected from femur bones by flushing with PBS. Cells were washed, RBC lysis (Quality Biological) was conducted for 5 minutes, washed again, and resuspended.

Mouse kidneys were harvested and capsules removed. The protocol of isolating kidney mononuclear cells (KMNCs) from kidney has previously been published elsewhere.[16] Kidney samples were sliced, and after adding collagenase D solution (2 mg/ml; Sigma-Aldrich, St. Louis, MO), incubation at 37°C for 30 min was performed. The digested material was ground through a 70 μm strainer (BD Biosciences). Using established Percoll density gradient, KMNCs were separated, washed, and Roswell Park Memorial Institute 1640 media with 5% FBS was added (GE Healthcare, Chicago, IL).

Immune Cell Isolation from Human Kidney

Human kidney samples were obtained from nephrectomies for renal cell carcinoma (RCC) after informed consent and as per Johns Hopkins Review Board guidelines, and normal portion adjacent to the cancer tissue was carefully collected. KMNCs were isolated from human kidney samples as per our published protocol.[17,18]

Immunophenotyping

Cells isolated from different organs of humanized mouse strains and human kidneys were analyzed by flow cytometry. Briefly, cell suspensions were incubated with human Fc Block (clone: Fc1.3216, BD Biosciences) for 15 minutes at 4°C and then stained with antibodies specific for mouse (m)CD45-BUV395 (clone: 30-F11, BD Biosciences), m$\alpha\beta$TCR-FITC (H57-597, BD Biosciences) and human (h) CD45-PacBlue (HI30, Biolegend), h$\alpha\beta$TCR-APC-Cy7 (IP26, Biolegend), hCD4-PerCp-Cy5.5 (RPA-T4, ThermoFisher Scientific), hCD8-BV605 (SK1, Biolegend), hCD20-FITC (2H7, Biolegend), hCD16-BV605 (398, BD Biosciences), hCD14-PE (HCD14, Biolegend), hCD11c-BV395 (B-ly6, BD Biosciences), and hCD206-APC (15-2, Biolegend). Samples were run on LSRII flow cytometer (BD Biosciences) and analyzed with FlowJo software (BD Biosciences). Fluorescence minus one and unstained control samples

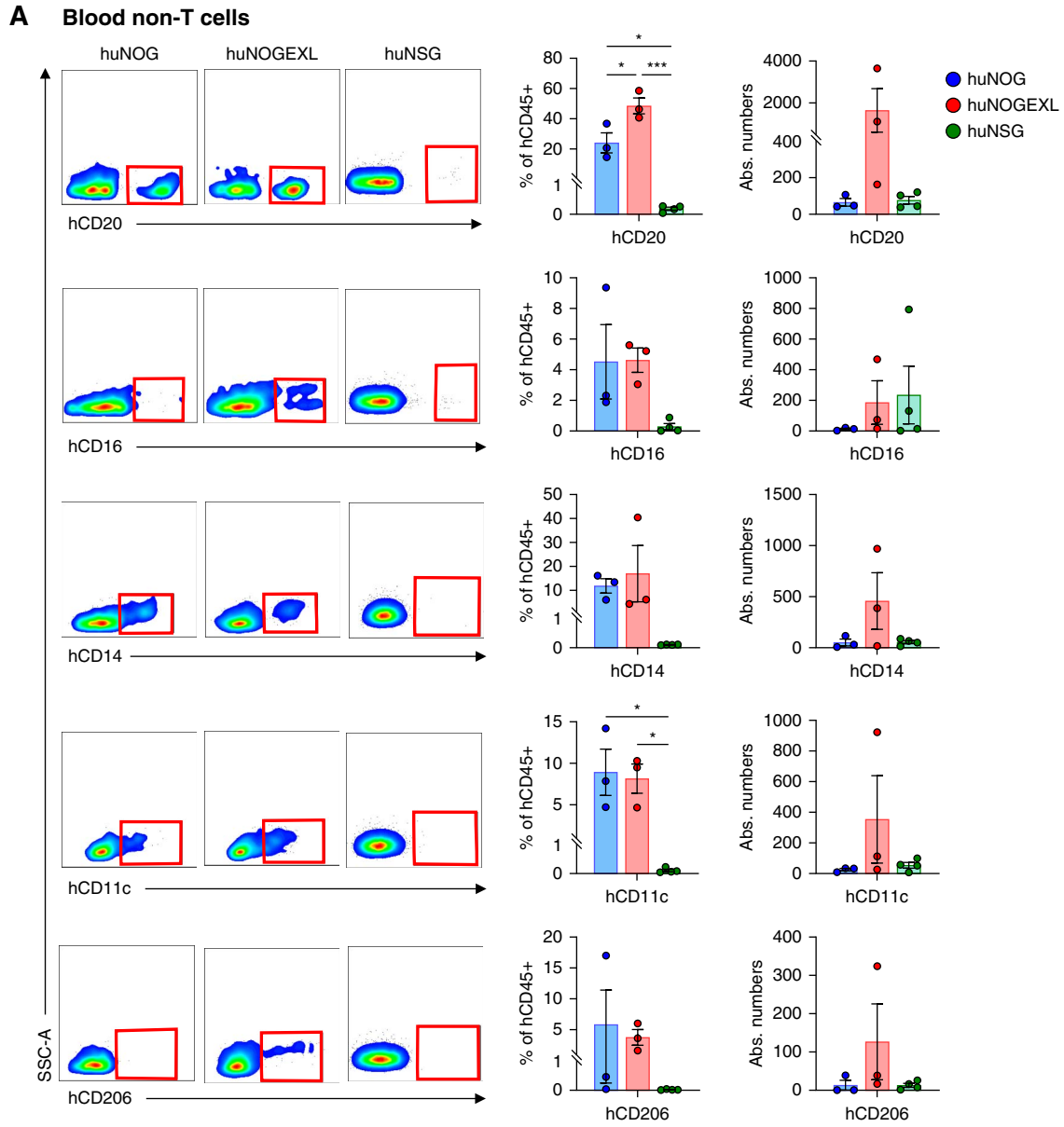


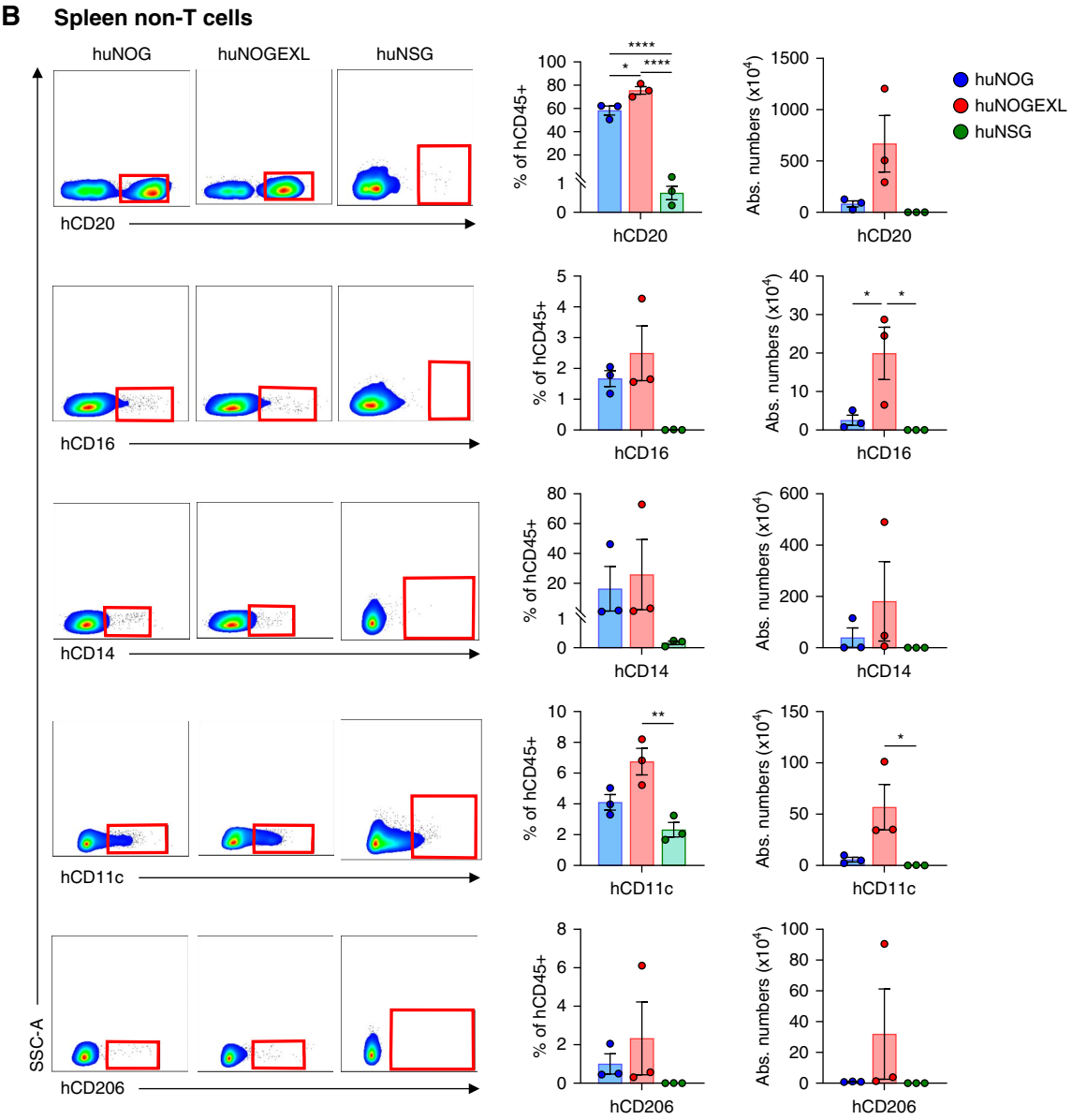
Figure 3. Analysis of non–T-cell populations of human origin in different humanized mouse strains. Representative flow plots and corresponding graphs showing percentage of hCD20$^+$ B cells, hCD16$^+$ neutrophils, hCD14$^+$ monocytes, hCD11c$^+$ dendritic cells, and hCD206$^+$ M2 macrophages among total hCD45$^+$ cells and absolute numbers in (A) blood, (B) spleen, and (C) bone marrow of huNOG (n=3), huNOGEXL (n=3), and huNSG (n=3) mice. Data are expressed as mean±SEM and compared by one way ANOVA followed by Tukey's *post hoc* analysis. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$.

were used for gating purposes during acquisition. Gating strategy for analyzing T cell and non-T-cell populations has been provided in the Supplemental Figure 1.

Briefly, lymphocytes/leukocyte population was identified on the basis of forward scatter and side scatter followed by doublet removal using single-cell gates. Next, human CD45$^+$ cells were identified and analyzed for T-cell (hTCR$^+$, hCD4$^+$, hCD8$^+$, and hCD4$^-$CD8$^-$) and non–T-cell (hCD20$^+$ B cell, hCD16$^+$ neutrophil, hCD14$^+$ monocyte, hCD11c$^+$ dendritic cell, and hCD206$^+$ M2 macrophage) populations. The absolute cell number was calculated by dividing the number of cells in a cell subset during flow analysis using FlowJo by the total number of cells acquired by flow cytometer and multiplying by the number of cells counted with the hemocytometer.

Statistical Analysis

Data are stated as mean±SEM. Data were analyzed using Graph Pad Prism 10 or Microsoft Excel 2016, and comparison between groups was performed with one-way ANOVA with Tukey's *post hoc* analysis. Statistical significance was defined with P value < 0.05.



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Results

Engraftment of CD34$^+$ HSCs Resulted in Significant Human CD45$^+$ Immune Cells in the Kidney of huNOG, huNOGEXL, and huNSG Mice

Immunophenotyping of the huNOG, huNOGEXL, and huNSG mice revealed significant engraftment in all tested models in blood (Figure 1A). A significant increase of human CD45$^+$ immune cells in peripheral blood was detected in huNOG (75.4%±5.6%, $P \leq 0.0001$), huNOGEXL (78.6%±2.5%, $P \leq 0.0001$), and huNSG mice (79.9%±2.7%, $P \leq 0.0001$), confirming successful engraftment. Analysis of absolute numbers showed similar results with huNSG mice showing the highest numbers compared with huNOG and huNOGEXL mice.

Examination of the kidney tissue showed increased proportions of kidney hCD45$^+$ immune cells in all humanized mouse strains (Figure 1B). The percentage of hCD45$^+$ immune cells was similar in huNOG (12.5%±1.7%, $P = 0.31$) and huNOGEXL mice (23.2%±8.7%, $P \leq 0.05$). We observed highest hCD45$^+$ frequency in the huNSG mice both regarding frequency (73.9±3.0%, $P \leq 0.0001$) and absolute numbers (17.19±0.91×10^4) among all humanized strains studied.

huNOGEXL had Increased Proportions of T Cells Compared with huNOG and huNSG Mice in Blood, Spleen, and Bone Marrow

We then compared hTCR$^+$ human T cells in blood, spleen, and bone marrow of different humanized mouse strains. In blood, the frequency of hTCR$^+$ T cells was significantly increased in huNSG mice (96.1%±0.5%) compared with huNOG (41.5%±14.0%, $P \leq 0.0001$) and huNOGEXL (27.8%±9.7%, $P \leq 0.0001$), which was confirmed in absolute numbers (Figure 2A). Among hTCR$^+$ cells, huNOGEXL

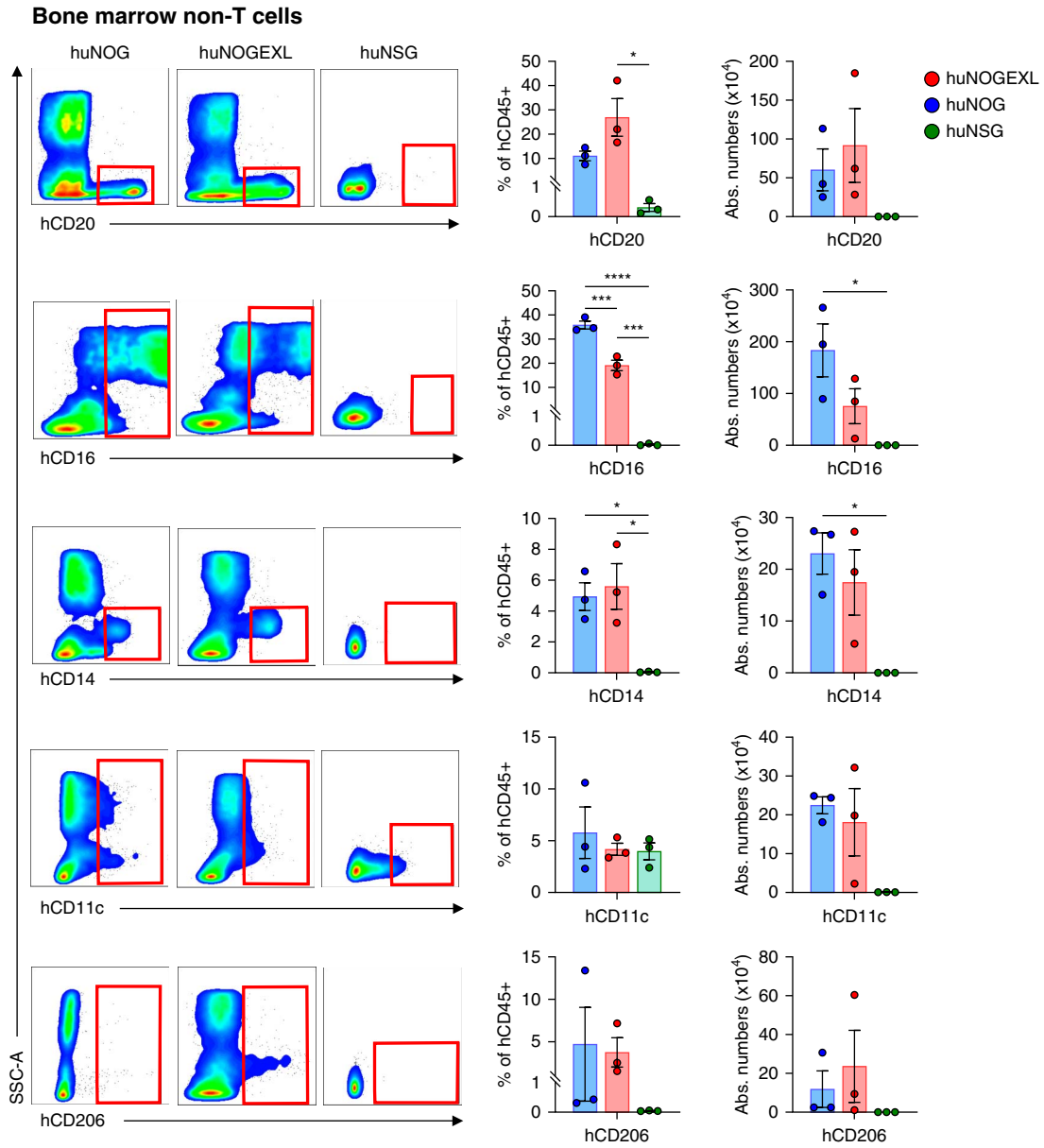


Figure 3. (Continued).

presented with a higher percentage of hCD4$^+$ T cells (66.7% ±6.0%) compared with huNOG (38.8%±4.5%, $P \leq 0.05$) and huNSG (33.8%±3.4%, $P \leq 0.01$) mice. However, when assessing absolute numbers, there was a significant increase of hCD4$^+$ T cells in huNSG compared with huNOG and huNOGEXL mice.

We observed a reduced percentage of hCD8$^+$ T cells in huNOGEXL (22.3%±3.5%) compared with huNOG (49.7% ±3.7%, $P \leq 0.01$) and huNSG (59.1%±2.9%, $P \leq 0.001$) mice. Absolute numbers showed huNSG mice to have significantly higher numbers of hCD8$^+$ cells compared with other mouse strains.

Assessment of splenic immune cell composition revealed higher percentage of hTCR$^+$ T cells (96.6%±0.2%) in huNSG mice compared with huNOG (33.8%±4.8%, $P \leq 0.0001$) and huNOGEXL mice (15.6%±4.1%, $P \leq 0.0001$). However, huNOGEXL mice had highest absolute numbers of hTCR$^+$ cells in the spleen. The percentage of hCD4$^+$ T cells was increased in huNOGEXL (53.6%±8.2%) compared with huNOG (30.2%±0.7%, $P \leq 0.05$) and huNSG (27.3%±1.9%, $P \leq 0.05$), which was confirmed in absolute number assessment. By contrast, percentage of hCD8$^+$ T cells was decreased in huNOGEXL (24.6%±3.4%) in comparison with huNOG (56.4%±1.4%, $P \leq 0.001$) and huNSG mice (64.6%±2.2%, $P \leq 0.0001$). The absolute numbers of hCD8$^+$ T cells showed no significant differences among the groups (Figure 2B).

Investigation of bone marrow showed greater numbers of hTCR$^+$ T cells in huNSG mice (95.8%±0.4%) compared with huNOG (6.3%±1.3%, $P \leq 0.0001$) and huNOGEXL (6.1%±2.7%, $P \leq 0.0001$) mice (Figure 2C). Absolute numbers of hTCR$^+$ T cells were not different among the groups.

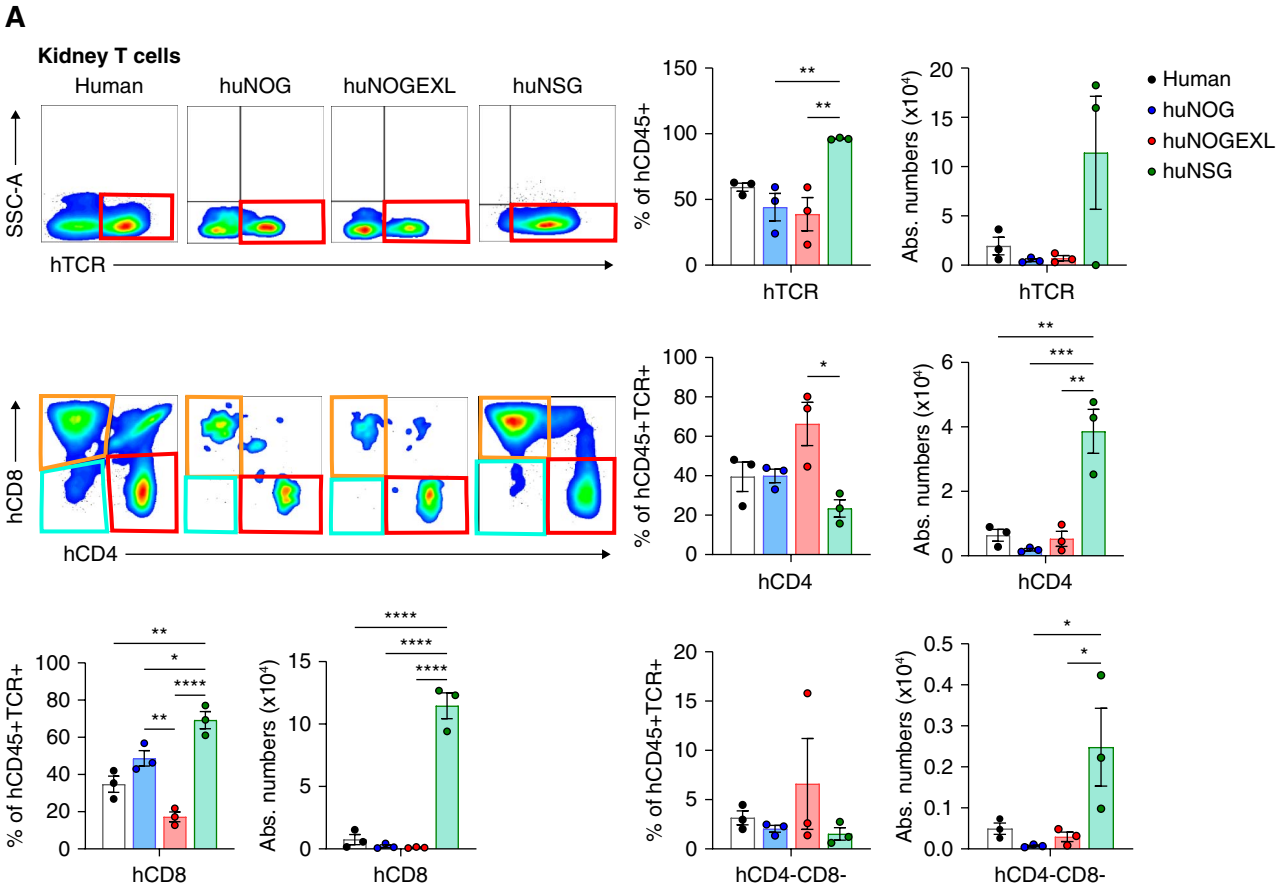


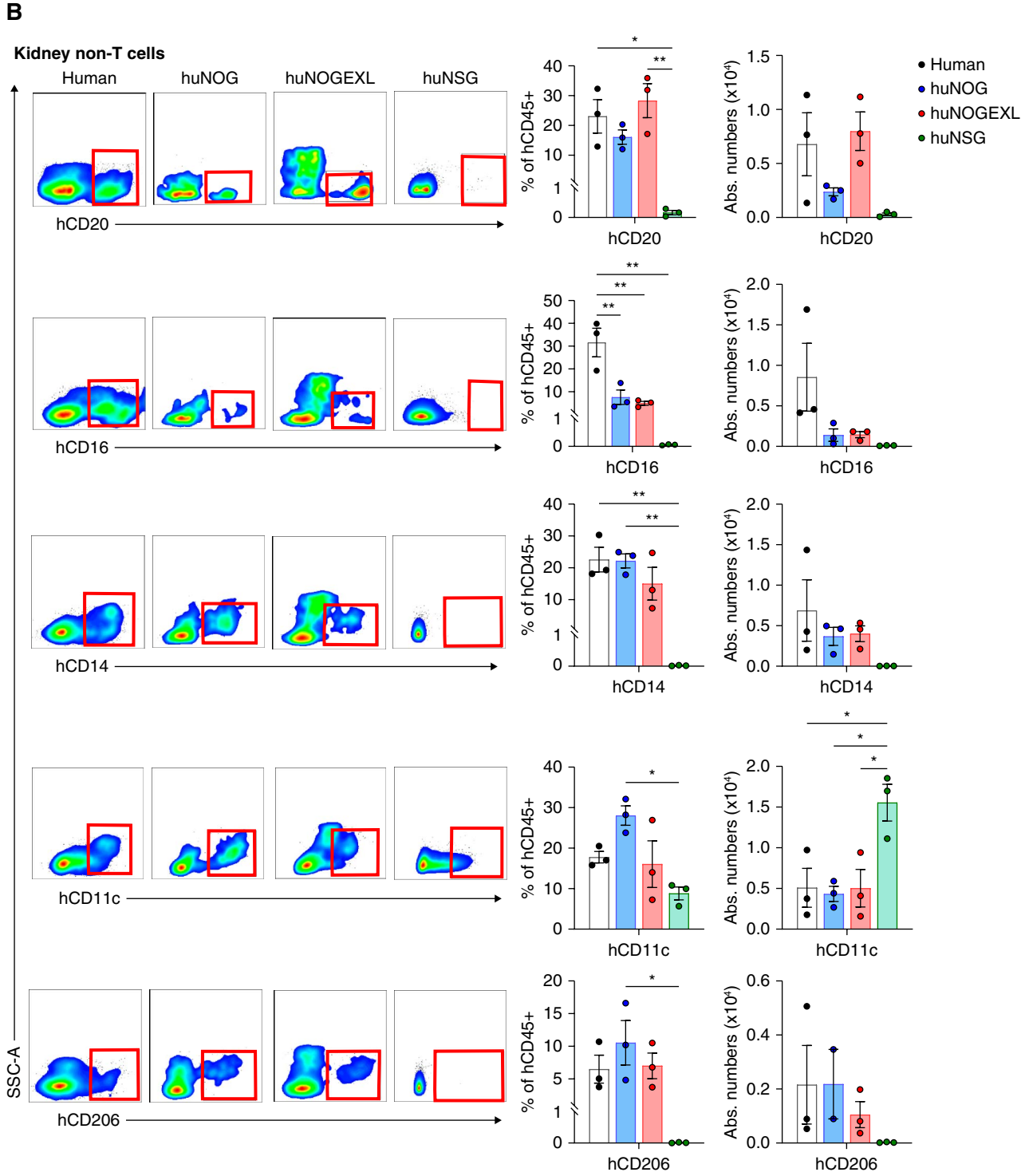
Figure 4. Immunophenotyping of kidney immune cells in different humanized mouse strains compared with normal human kidney. (A) Representative flow plots and corresponding graphs showing percentage and absolute numbers of hTCR$^+$, hCD4$^+$, hCD8$^+$, and hCD4$^-$CD8$^-$ DN T cells in human kidney and huNOG, huNOGEXL, and huNSG mice (n=3/group). (B) Representative flow plots and corresponding graphs showing percentage and absolute numbers of hCD20$^+$ B cells, hCD16$^+$ neutrophils, hCD14$^+$ monocytes, hCD11c$^+$ dendritic cells, and hCD206$^+$ M2 macrophages in in human kidney and huNOG, huNOGEXL, and huNSG mice. Data are expressed as mean±SEM and compared by one way ANOVA followed by Tukey's *post hoc* analysis. *$P \leq 0.05$, **$P \leq 0.01$.

The percentage of hCD8$^+$ T cells were higher in bone marrow of huNSG (64.2%±3.6%) mice compared with huNOG (18.7%±4.8%, $P \leq 0.001$) and huNOGEXL (7.2% ±2.2%, $P \leq 0.0001$) mice. However, absolute number assessment revealed significantly less hCD4$^+$ T cells in huNSG mice compared with huNOG mice, while hCD8$^+$ and hCD4$^-$CD8$^-$ T cells were comparable between groups (Figure 2C).

huNSG had Reduced Proportions of Non-T Cells (Innate Immune Cells) Compared with huNOG and huNOGEXL Mice in Blood, Spleen, and Bone Marrow

Assessment of hCD45$^+$ non-T cells showed significantly reduced proportions in huNSG mice in all tested organs compared with huNOG and huNOGEXL (Figure 3). hCD20$^+$ B cells in huNSG mice were significantly decreased compared with huNOG and huNOGEXL in blood (huNSG: 0.2%±0.1% versus huNOG: 24.0%±6.6%, $P \leq 0.05$; versus huNOGEXL: 48.5%±5.3%, $P \leq 0.001$; Figure 3A) and spleen (huNSG: 0.7% ± 0.2% versus huNOG: 58.2%±3.9%, $P \leq 0.05$; versus huNOGEXL: 75.5%±3.3%, $P \leq 0.0001$; Figure 3B). Assessment of absolute numbers revealed low numbers of hCD20$^+$ in huNSG mice compared with huNOGEXL in blood and spleen (not significant). However, hCD20$^+$ absolute numbers in huNOG mice appeared on similar levels as huNSG. Similarly, reduced hCD11c$^+$ dendritic cells were found in huNSG mice in blood (huNSG: 0.1%±0.0% versus huNOG: 8.9%±2.8%, $P \leq 0.05$; versus huNOGEXL: 8.2% ±1.8%, $P \leq 0.05$) and spleen (huNSG: 2.3%±0.5% versus huNOG: 4.1%±0.5%, $P \leq 0.05$; versus huNOGEXL: 6.7% ±0.9%, $P \leq 0.01$). Absolute number measurements confirmed huNSG to have the lowest levels of hCD11c$^+$ in those organs (not significant).

In bone marrow, hCD16$^+$ neutrophils and hCD14$^+$ monocyte frequencies in huNSG mice were significantly decreased compared with huNOG and huNOGEXL, which was confirmed when assessing absolute numbers (Figure 3C). Because a subset of hCD14$^+$ monocyte/macrophage population also expresses hCD16, we further analyzed the proportions of hCD16$^+$CD14$^-$ neutrophils in these humanized mice. The percentage of hCD16$^+$CD14$^-$ neutrophils in the bone marrow of huNSG mice showed significant decrease with a clear declining trend in the absolute number compared with huNOG and huNOGEXL mice. We also



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observed significantly reduced percentage of hCD16$^+$CD14$^-$ neutrophils in the blood of huNSG mice; however, the absolute numbers were comparable, especially compared with huNOGEXL mice. The percentage of hCD16$^+$CD14$^-$ neutrophils in the spleen of huNSG was significantly higher than huNOG mice, but absolute number was comparable with huNOG but significantly less compared with huNOGEXL mice (Supplemental Figure 2).

Comparison with Normal Human Kidney

We next assessed kidney T cell and non–T cell populations in huNOG, huNOGEXL, and huNSG mice and compared them with normal human kidneys (Figure 4). Percentage and absolute numbers of hTCR$^+$ T cells were comparable between human kidney and different humanized mouse kidneys (Figure 4A). huNSG mice had increased percentage of hTCR$^+$ cells compared with

Table 2. Summary of known findings for humanized mouse strains in kidney diseases

Humanized Mouse Strain	Kidney Disease	Study Observation/Findings	References
NSG mice engrafted with human CD34$^+$ HSCs	SLE	Development of novel SLE model including lupus nephritis	24
NOD/SCID mice engrafted with CD34$^+$ HSC or CD34$^-$ PBMCs	Idiopathic nephrotic syndrome	Development of experimental model for idiopathic nephrotic syndrome after CD34$^+$ HSC engraftment	15
NSG mice engrafted with Lin-CD34$^+$ HPCs	IRI-AKI	IL-33 treatment for AKI prevention in IRI model	14
NSG engrafted with PDX and (CAR) T cells	RCC	CAR T-cell therapy secreting human anti–PD-L1 antibodies	25
NSG engrafted with PDX and huPBMCs	RCC	Testing of carbonic anhydrase IX inhibitors in RCC	26,27
SCID/beige engrafted with PDX and huPBMCs	RCC	Testing of TGF-β–intensive CD8$^+$ T cells into humanized mice bearing huRCC (PDX)	28
NSG mice engrafted with PDX and huCD34$^+$ HSC	Urological malignancies	Engrafted tumors were not rejected. Pembrolizumab in CD8$^+$ T-cell mediated tumor growth inhibition was tested	29,30
NOG/SCID engrafted with PDX and human lymphocytes	Urological malignancies	Testing of PDL-1 blocker durvalumab	31
NSG mice engrafted with huPBMCs	Transplant	Assessment of allogeneic responses in organ transplantation	32
NSG, NOG, NRG, or BRG mice engrafted with human HSC	Transplant	Methodological approaches for transplant research	3
NSG mice engrafted with human T cells or dendritic cells of hypertensive individuals versus controls	Hypertensive nephropathy	Differences in activation status of engrafted T cells	33
NSG mice engrafted with CD34$^+$ HSC	Cytomegalovirus infection	Assessment of replication, latency, and reactivation	34–36
NSG, NOG, NRG, or BRG mice engrafted with human HSC	Immunology	Methodological approaches for immunology research	37

HPC, hematopoietic progenitor cell; HSC, hematopoietic stem cell; NOD, nonobese diabetic; PDX, patient-derived xenograft; SCID, severe combined immunodeficiency; IRI-AKI, ischemia-reperfusion-injury induced acute kidney injury; AKI, acute kidney injury; RCC, renal cell carcinoma; SLE, systemic lupus erythematosus; CAR T cells, Chimeric Antigen Receptor T cells; PBMCs, Peripheral Blood Mononuclear Cells.

huNOG and huNOGEXL kidneys, although the absolute numbers were comparable between groups. Even though percentage of hCD4$^+$ T cell was comparable between human kidney and humanized mice, the absolute numbers were significantly higher in huNSG mice compared with huNOG, huNOGEXL, and human kidneys. The percentage and absolute number of hCD8$^+$ T cells was significantly higher in huNSG kidney compared with normal human kidney and huNOG/huNOGEXL mice. In addition, hCD8$^+$ T cells were significantly decreased in huNOGEXL mouse kidney compared with huNOG and huNSG. There was no difference in the percentage of hCD4$^-$CD8$^-$ double-negative (DN) T cells between human kidney compared with humanized mouse kidneys, although huNSG mice had increased numbers of DN T cells compared with huNOG and huNOGEXL mice.

The percentage of hCD20$^+$ B cells (0.17%±0.08%; $P \leq 0.05$) and hCD14$^+$ monocytes (0.02%±0.0%; $P \leq 0.01$) were significantly reduced in huNSG mice compared with normal human kidneys (hCD20: 23.0%±5.6%; hCD14: 22.6%±3.9%), but huNOG and huNOGEXL mice had comparable percentages with human kidney. The percentage of hCD16$^+$ neutrophils was significantly reduced in both huNOG (7.6%±5.5%, $P = 0.03$), huNOGEXL (4.9%±1.0%, $P = 0.01$), and huNSG kidney compared with normal human kidney (31.5%±6.3%). There was no statistically significant difference in the frequency of hCD206$^+$ M2 macrophages between humanized mice kidney and human kidney (figure 4B).

Discussion

Humanized mouse models have been applied in kidney research.[14] However, a comparison of the immune cell reconstitution of those models and comparison with normal human kidney is lacking. Our data show that humanized mouse kidney of different mouse strains has certain similarities as well as key differences between each other and with normal human kidney. We achieved significant engraftment by delivering HSCs using the intravenous route. This is different from previous studies describing higher levels when using intra osseous injection.[15]

Humanized mouse kidneys have variable rates of humanization depending on the background mouse strain, which needs to be considered when using these models to study kidney diseases. In general, huNSG mice achieved

higher levels of human T cells, whereas non-T cells were lower compared with huNOG and huNOGEXL. More specifically, hCD4$^+$ T cells and B cells were comparable between huNOGEXL and normal human kidneys. Furthermore, human CD34$^+$ HSCs of huNOG and huNOGEXL engraftment resulted in stable kidney resident immune cell populations. However, immune composition in huNSG mice engrafted with PBMCs differed to a higher extent from normal human kidney. We observed significant hCD4, hCD8, and hDN T cells in huNSG mice compared with other humanized mouse strains studied. In addition to kidney, we also observed several differences in the immune cell composition in blood, spleen, and bone marrow from these strains. These variations among humanized mouse strains could be due to the inherent differences between these mouse models that favor development of certain type of immune cells.

For example, both NOG and NSG mice lack mature human T cells, B cells, and functional natural killer cells and are deficient in cytokine signaling, leading to high levels of engraftment of human HSCs.[19] However, NOG mice have IL 2 γ (Il2rg) mutant allele that results in truncated IL2 receptor γ chain, whereas NSG mice are Il2rg null that results in a complete absence of IL2 receptor γ chain and better engraftment properties. In addition, NOGEXL mice additionally express human granulocyte-macrophage colony-stimulating factor and IL3 cytokines transgene that may cause variable engraftment rate compared with NOG.

Furthermore, we found that these mice develop graft versus host disease especially after humanizing NSG mice with human PBMCs. Similar observations have been made in NOG mice that were xenotransplanted with human PBMCs.[20,21] In addition, humanized mouse strains are prone to infections.[5] In this study, we only assessed female humanized mice because engraftment of human HSCs is more efficient in female mice.[22,23] Previous studies have demonstrated sex-associated differences in hCD45$^+$ cell dynamics postengraftment.[22]

The humanized mouse models are being used for various kidney diseases including ischemia-reperfusion-injury induced acute kidnez injury, RCC, systemic lupus erythematosus urologic malignancies, and transplant (Table 2). However, the variability in the kidney immune cell repertoire of different humanized mouse strains identified in this study should be considered carefully before planning an experiment because it can directly affect kidney disease dynamics, response to injury, and infiltration of immune cells to kidneys. On the basis of our results, huNOG mice seem to have a kidney immune cell repertoire that most closely represents the normal human kidney and could be the preferred mode to study important human kidney diseases such as ischemia reperfusion and nephrotoxic AKI, kidney transplant–related complications, and myeloid cell-driven inflammatory kidney damage.

Limitations of this study include lack of comprehensive analysis of immune cell types (*e.g.*, nautral killer, Natural killer T-cells, granulocytes, and innate lymphoid cells) as well as lack of clear distinction between monocytes, macrophages, and dendritic cells.

Moreover, a direct comparison of different immune cells in these humanized mouse models and a normal mouse strain with an intact immune system is lacking and warrants future studies to further assess the immune competence of these models. Another limitation of this study is that it provides only a snap shot of these immune profiles at a single time point. Comparison of immune cells in males versus females would also have been useful. Future studies focusing on the phenotypic and functional properties of intrarenal human immune cells in these models should be performed at different time point postengraftment.

Despite these limitations, the profiling of common humanized mouse kidneys in this study provides a useful tool to researchers for selecting humanized mouse strains for investigating or setting up a specific kidney disease model. Furthermore, these data could help in further refining and selecting humanized mouse models to study kidney immune pathophysiology and lay the groundwork for immune cell therapies for humans.

Disclosures

J.T. Kurzhagen reports the following—Honoraria: Dr. Werner Jackstädt Foundation. P.M. Pierorazio reports the following—Patents or Royalties: UpToDate: Chapter on Small Renal Masses; and Advisory or Leadership Role: Testicular Cancer Awareness Foundation, Board Member (unpaid). H. Rabb reports the following—Ownership Interest: Renibus therapeutics and Advisory or Leadership Role: Scientific advisory board, Renibus and Scientific advisory board, Rapafusyn. All remaining authors have nothing to disclose.

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Data Sharing Statement

All data is included in the manuscript and/or supporting information.

Supplemental Material

This article contains the following supplemental material online at http://links.lww.com/KN9/A408.

Supplemental Figure 1. Gating strategy for identifying and analyzing (A) T cells and (B) non-T cells in different humanized mouse models.

Supplemental Figure 2. Analysis of $CD16^{+}CD14^{-}$ neutrophils in blood, spleen, and bone marrow in different humanized mouse models. $CD16^{+}CD14^{-}$ neutrophil population in huNOG, huNOGEXL, and huNSG kidneys was compared with normal human kidney.

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