Review

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Humanization of Immunodeficient Animals for the Modeling of Transplantation, Graft Versus Host Disease, and Regenerative Medicine

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Abstract. The humanization of animals is a powerful tool for the exploration of human disease pathogenesis in biomedical research, as well as for the development of therapeutic interventions with enhanced translational potential. Humanized models enable us to overcome biologic differences that exist between humans and other species, while giving us a platform to study human processes in vivo. To become humanized, an immune-deficient recipient is engrafted with cells, tissues, or organoids. The mouse is the most well studied of these hosts, with a variety of immunodeficient strains available for various specific uses. More recently, efforts have turned to the humanization of other animal species such as the rat, which offers some technical and immunologic advantages over mice. These advances, together with ongoing developments in the incorporation of human transgenes and additional mutations in humanized mouse models, have expanded our opportunities to replicate aspects of human allotransplantation and to assist in the development of immunotherapies. In this review, the immune and tissue humanization of various species is presented with an emphasis on their potential for use as models for allotransplantation, graft versus host disease, and regenerative medicine.

(*Transplantation* 2020;104: 2290–2306).

INTRODUCTION

For decades, studies in immunology have benefited from the ability to experiment on small animal models. However, it is increasingly clear that the mechanistic gap between human and small animal immune responses is significant, leading to challenges in the translation of findings from animal to human that can, on occasion, have devastating results.[1-3] Humanized laboratory animals have been developed to bridge this gap, and provide a powerful method for the preclinical assessment of human immune responses in the context of transplantation and regenerative medicine. The vast majority of these models are created using immunodeficient mice engrafted with human cells and tissues.

Although humanized mice have been extremely useful in the study of many pathologies, the humanization of other species can also be advantageous. The size of other animals, such as rats, pigs, or nonhuman primates, facilitates more challenging surgical procedures, which would be difficult in mice. Additionally, the anatomy and physiology of larger species more closely resemble that of the human. Before the description of gene-specific nucleases (such as meganucleases, zinc finger nucleases [ZFNs], transcription activator-like effector nucleases [TALENs], and CRISPR/Cas9), targeted genome editing was largely restricted to mice, the only species in which robust embryonic stem (ES) cells were available. However, the recent evolution

Received 13 November 2019. Revision received 22 December 2019.

Accepted 7 January 2020.

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This work was supported by generous funding from Biogenouest by Région Pays de la Loire, IBiSA program, TEFOR (Investissements d'Avenir French Government program, ANRII-INSB-0014), LabCom SOURIRAT project (ANR-14-LAB5-0008), Labex IGO project (Investissements d'Avenir French Government program, ANR-11-LABX-0016-01), IHU-Cesti project (Investissements d'Avenir French Government program, ANR-10-IBHU-005, Nantes Métropole and Région Pays de la Loire), Fondation Progreffe, the ReSHAPE 825392 EU Horizon 2020, Kidney Research UK, the Academy of Medical Sciences, the Wellcome Trust, and the Clarendon Fund and the Restore Research Trust. F.I. is a Wellcome Trust CRCD Fellow, J.H. is a KRUK Senior Fellow, and A.R.C. is an Oxford-Celgene Fellow.

The authors declare no conflicts of interest.

I.A. and F.I. planned and wrote the article. F.I. and J.H. performed the included experiments. G.A., S.M., A.R.C., and J.H. wrote and edited the article as well as performed bibliography searches. G.A., S.M., and A.R.C. contributed equally. F.I. and I.A.are both senior and corresponding authors.

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ISSN: 0041-1337/20/10411-2290

DOI: 10.1097/TP.0000000000003177

of gene-specific nucleases has allowed the generation of immunodeficient animals in all the above mentioned species. In this review, we aim to explore the qualities and benefits of the available immunodeficient animal models, their potential for reconstitution with human tissues, and how this is benefitting preclinical research in the field of transplantation.

MICE

Immunodeficient Mouse Models

Over the past 2 decades, mice have become the dominant rodent model in biomedical research. The prominence of mice owes largely to the development of methods for genetic manipulation, the subsequent establishment and characterization of murine strains—starting with the first knockout mouse in 1987[4]—and the availability of a wide range of transgenic mice as well as antibodies targeting mouse antigens. Further technological advances targeting mouse gene expression have enabled the experimental reproduction of human allogeneic transplantation within in vivo models. For success, such models require: (1) host mice that are rendered genetically immunodeficient, (2) adoptive transfer and engraftment of human immune cells, and (3) transplantation of allogeneic human tissues.

The development of immunodeficient mouse models began following the description of the spontaneously arising severe combined immunodeficiency (*scid*) mutation in C.B-17 mice,[5] which produced mice lacking effective adaptive immunity. The *scid* mutation affects the *Prkdc* gene for the DNA-dependent protein kinase catalytic subunit, which is critical for DNA repair during V(D)J recombination in T and B cell receptor generation. As a result, SCID mice are incapable of producing mature T and B cells. The experimental replication of this immunodeficiency was first achieved by knocking out the *Rag2* gene, which similarly arrests V(D)J recombination and lymphocyte maturation.[6] These immunodeficient models therefore permit hematopoietic reconstitution with adoptively transferred human peripheral mononuclear cells[7] or stem cells,[8] since effective adaptive antihuman responses can no longer be mounted. It later became clear that human cell reconstitution can be significantly enhanced by targeting mouse innate cells to further limit xenoreactivity. Crossing SCID mice with inbred nonobese diabetic (NOD) mice resulted in mice that not only had defective adaptive immunity but also absent complement C5 (rendering these mice deficient in hemolytic complement) and impaired macrophage and dendritic cell function.[9-13] In these NOD-*scid* mice, human cell engraftment was up to 10-fold higher than in C.B-17-*scid* mice.[14]

Further improvement in human cell engraftment and reconstitution came with the null mutation of the interleukin (IL)-2 receptor common γ-chain,[15] blocking the signaling pathways of IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 and, crucially, diminishing mouse natural killer (NK) cell function through impaired IL-15 signaling. Combining mutations of both adaptive (*scid, Rag1/2*) and innate (IL-2Rγ_c) immunity heralded a new wave of severely immunodeficient mice that include NOG (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac), NSG (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ), and BRG (BALB/c *Rag2*$^{-/-}$ *Il2rg*^{tm1Sug}/JicTac) mice,[16] expanding opportunities for successful engraftment of human hematopoietic cells. NOG mice and NSG mice differ in the nature of the IL-2 receptor common γ-chain mutation wherein NOG mice lack the cytoplasmic domain whereas NSG mice have a complete null allele. BRG mice differ from NOG/NSG mice being on the Balb/c rather than the NOD background but like NOG mice, they also lack the cytoplasmic tail of the IL-2 receptor common γ-chain. Current evidence suggests that human cells engraft better in NSG/NOG compared with BRG mice.[17,18] This may be in part due to greater compatibility between human CD47 and NOD SIRPα (discussed below) but perhaps more importantly because the DNA repair defect conferred by the *scid* mutation severely impairs bone marrow stem cell repopulation potential, facilitating exogenous hematopoietic stem cell (HSC) engraftment in a manner not seen in *Rag*-mutant mice.[19] In order to further improve human cell engraftment, later modifications aimed to prevent mouse macrophage-mediated phagocytosis of human hematopoietic cells, which results from impaired "*don't eat me*" signaling via an incompatibility between human CD47 and murine SIRPα. Successful attempts to address this include the transfer of the NOD.*Sirpa* allele, a highly polymorphic variant of SIRPα with high affinity for human CD47,[20,21] or the human *Sirpa*,[22,23] to other genetic backgrounds. A summary of all immunodeficient mouse models used for humanized studies is shown in Table 1.

Immune Humanization of Mice: Peripheral Blood Mononuclear Cell-based Models

Peripheral blood mononuclear cells (PBMCs) or leukocytes are the most commonly used cell product for humanization in transplant studies, providing a method that is both convenient and cost-effective. Mice humanized with human PBMCs robustly reconstitute T cells, which have well-established roles in rejection and tolerance. The early identification of donor-specific memory and the rapid rejection of second-set allografts[45] made adaptive immune cells, especially T cells, the traditional focus of study into pathways of transplant rejection. Indeed, in experimental models, T cells are both necessary and sufficient for the rejection of most allografts.[46,47] Whereas recreation of these features is important, a caveat of PBMC-humanization is that reconstitution is heavily lymphoid-biased: over 90% of human cells are T cells and the majority express an activated or memory phenotype.[48] The disproportionately large fraction of the human leukocyte repertoire (usually ~5%–20%) may misrepresent the global immunologic reaction to transplantation, potentially limiting the relatability of experimental findings to the true human alloresponse.

T cell receptor (TCR) recognition of human major histocompatibility complex (MHC) mismatches drives allograft rejection and human graft versus host disease (GvHD). Curiously, MHC molecules display an inherent cross-species immunogenicity. This is beneficial to xenogeneic models of GvHD where human $CD8^+$ and $CD4^+$ T lymphocytes recognize mouse MHC class I and II, respectively, allowing the transferred lymphocytes to simulate systemic proinflammatory responses.[24] Moreover, the precursor frequency of human lymphocytes responsive to mouse MHC (0.5% in $CD4^+$ cells and 3% in $CD8^+$ cells[24]) is similar to the frequency of alloresponsive lymphocytes (3.9% of $CD4^+$ cells and 2.5% of $CD8^+$ T cells[49]). Yet, xenogeneic

TABLE 1.

Immunodeficient mouse models

Name	Strain	Phenotype	Reference
SCID	B6.CB17-*Prkdc*^{scid}/SzJ	T and B cell deficiency	5
NOD-*scid*	NOD.CB17-*Prkdc*^{scid}/J	T and B cell deficiency Phagocytic tolerance	10
NSG	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ	T, B, and NK cell deficiency Phagocytic tolerance	15
NSG B2M, β2m KO NSG	NOD *scid Il2ry*^{null} *B2m*^{null}	T, B, and NK cell deficiency Xeno-GvHD-resistance	24
NSG HLA-A2/HDD	NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}Tg (HLA-A2/H2-D/B2M)1Dvs/Sz(NSG-HLA-A2/HHD)	T, B, and NK cell deficiency Human HLA-A2 expression	25
NSG HLA-DR	NOG/HLA-DR4/I-Ab$^{-/-}$	T, B, and NK cell deficiency Human HLA-DR expression	26
NSG-SGM3	NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl} Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ	T, B, and NK cell deficiency Human IL-3, GM-CSF and SCF expression	27
hIL-6 Tg NSG	NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl} Tg(BAC1/2-IL6)	T, B, and NK cell deficiency Human IL-6 expression	28
NSGW41	NOD.Cg-*Kit*$^{W-41J}$*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/WaskJ	T, B, and NK cell deficiency, Impaired HSC development	29
NSGWv/+	NOD/SCID*Il2rg*$^{-/-}$ *Kit*$^{Wv/+}$	T, B, and NK cell deficiency, Impaired HSC development	30
NSGWv	NOD/SCID*Il2rg*$^{-/-}$ *Kit*$^{Wv/Wv}$	T, B, and NK cell deficiency, Impaired HSC development	30
NBSGW	NOD,B6.SCID *Il2rγ*$^{-/-}$ *Kit*$^{W41/W41}$	T, B, and NK cell deficiency, Impaired HSC development	31
NOG	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac	T, B, and NK cell deficiency Phagocytic tolerance	15
IL-15-NOG Tg	NOD.Cg-*Prkdc*^{scid} *IL-2Rrgc*^{m1Sug} Tg (CMV-IL2/IL15)1-1Jic/JicTac	T, B, and NK cell deficiency Phagocytic tolerance, human IL-15 expression	32
NOG-EXL	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug} Tg(SV40/HTLV-IL3,CSF2)10-7Jic/JicTac	T, B, and NK cell deficiency Phagocytic tolerance, human GM-CSF & IL-3 expression	33
NOG-IL2 Tg	NOD.Cg-*Prkdc*^{scid} *IL-2rgc*^{m1Sug} Tg(CMV-IL2)4-2Jic/JicTac	T, B, and NK cell deficiency Phagocytic tolerance, human IL-2 expression	34
DRAG	NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl} Tg(HLA-DRA,HLA-DRB1*0401)39-2Kito/ ScasJ	T, B, and NK cell deficiency Phagocytic tolerance, human HLA class II expression	35
NRG	*NOD.Cg-Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl}	T, B, and NK cell deficiency Phagocytic tolerance	36
SRG	Tg(hSIRPA)*Rag2*$^{-/-}$*Il2rg*$^{-/-}$	T, B, and NK cell deficiency Phagocytic tolerance	22
SRG-W41	NOD, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$*Kit*$^{Wv/Wv}$	T, B, and NK cell deficiency	37
BALB *scid*	CBySmn.CB17-*Prkdc*^{scid}	Phagocytic tolerance, Impaired HSC development	38
BRG	BALB/c *Rag2*$^{-/-}$ *Il2rg*^{tm1Sug}/JicTac	T, B, and NK cell deficiency	20,39
BRG hIL-3 hGM-CSF	BALB/c *Rag2*$^{-/-}$ *IL-2Rgc*$^{-/-}$ IL3^{h} CSF2^{h}	T, B, and NK cell deficiency Human IL-3 & GM-CSF expression	40
BRGS	BALB/c *Rag2*$^{-/-}$ *IL-2Rgc*$^{-/-}$ NOD.*sirpa*	T, B, and NK cell deficiency NOD SIRPα expression	20,21
BRGSF	BALB/c *Rag2*$^{-/-}$ *IL-2Rgc*$^{-/-}$Flt3$^{-/-}$	T, B, and NK cell deficiency, NOD SIRPα expression, impaired HSC development	41
BRgWv	BALB/c*Rag2*$^{-/-}$*Il2rg*$^{-/-}$*Kit*$^{Wv/Wv}$	T, B, and NK cell deficiency, impaired HSC development	30
MISTRG	M-CSF,IL-3,Sirpa, TPO, *Rag2*$^{-/-}$ *IL-2Rgc*$^{-/-}$	T, B, and NK cell deficiency, human M-CSF, IL-3, GM-CSF, SIRPα, and TPO expression	42
HUMAMICE	C57BL/6 *HLAA2*$^{+/+}$*/DR1*$^{+/+}$*/ H2b2m*$^{-/-}$*/IAb*$^{-/-}$*/Rag2*$^{-/-}$*/IL2Rg*$^{-/-}$*/perf*$^{-/--}$	T, B, and NK cell deficiency No mouse MHC expression, human HLA expression	43
Nude mouse, athymic nude*Foxn1*^{nu}		T and NK cell deficiency	44

GvHD, graft vs host disease; HSC, hematopoietic stem cell; MHC, major histocompatibility complex; NK, natural killer; NOD, nonobese diabetic; SCID, severe combined immunodeficiency.

models of GvHD can also be confounded by the absence of indirect recognition, abundance of nonhuman antigens, and the differing distribution of mouse MHC. For example, mice do not constitutively express MHC class II in the vasculature, unlike humans; therefore, humanized GvHD models may underestimate CD4^{+} T cell allorecognition in vascular interactions. However, the development of anti-mouse responses is undesirable in many transplantation studies because it invariably leads to lethal xenogeneic GvHD,[15,24] an expanding human leukocyte compartment that is not directed at the tissue in question, and a limited experimental window. One strategy to address this has

been to produce NSG mice deficient in MHC class I and II expression.[50] Intraperitoneal injection of human PBMC in such mice results in the long-term engraftment of functional CD4$^+$ and CD8$^+$ T cells, which retain the capacity to reject mismatched human islets.[50]

The additional incorporation of non-T cell components in experimental models is desirable for replicating a more complete immune system and human alloresponse. The importance of innate-mediated rejection is of increasing interest.[51,52] In clinical studies, T cell depletion (eg, with alemtuzumab) has been shown to be insufficient to prevent renal or intestinal allograft rejection. In these studies, rejection was instead associated with monocytic and eosinophilic inflammation, respectively.[53,54] Experimental cardiac allotransplantation experiments in alymphatic mice results in leukocyte infiltration and proinflammatory cytokine production, highlighting the presence of innate responses that can develop to allografts in the absence of T cells.[55] Moreover, an array of innate immune cells, such as dendritic cells,[56] NK,[57] and mast cells,[58] have been shown to display immunoregulatory properties important for tolerance induction, and their reconstitution in humanized animals is therefore of interest. Another important consideration is that, as humanized mouse models currently used in transplantation research do not support reconstitution of functional human antigen presenting cells in the recipient host, in vivo assessment of immune alloreactivity is limited to responses triggered by presentation of antigen via the direct pathway. Successful engraftment of functional professional human antigen presenting cells may therefore also ensure that all allorecognition pathways are integrated into the experimental model. To be able to incorporate the entire spectrum of innate and adaptive human immune responses into experimental models of transplantation, support for multilineage human hematopoietic cell reconstitution is key.

An important additional requirement of humanized animal models is engraftment or development of mature human B cells capable of effective antigen presentation and immunoglobulin production. We and others have demonstrated the ability of PBMC-humanized immunodeficient mice to engraft mature human B cells and produce human IgG and IgM (Figure 1A)[7,59] however, the frequency of these antibody-producing cells is consistently low and highly variable. Our experience is that immunodeficient mice humanized with UCB HSCs generate significant numbers of human B cells[60] (Figure 1B). Despite this, analyses of human B and T cells generated within different HIS mice models have revealed these B cells to be developmentally blocked,[61] with defective peripheral maturation and humoral responses.[16,62] UCB-humanized mice produce low levels of human IgM and IgG that increase with the development of T lymphocytes, an effect that is enhanced by the introduction of autologous (CD34$^-$ CD45$^+$) leukocytes (Figure 1C–E). Initially, this was thought to result from an inability of mouse B lymphocyte stimulator (BAFF) to signal human B cells, suggested by the observation that administration of recombinant human BAFF to NOD-*Rag1*^{null}*Prf1*^{null} mice humanized with PBMCs increased B cell engraftment and the ability to mount an antipneumococcal polysaccharide response.[63] It has been reported that pre-B and immature B cells differ from mature B cells in not requiring BAFF for their survival.[64-66] This is supported by a recent study in which expression of full-length human BAFF from cDNA in the endogenous mouse locus did not improve maturation of human B cells in HIS mice.[67] An alternative strategy involves the induction of IL-6, which has previously been shown to increase IgG1 expression up to 400-fold.[68] Knock-in of human IL-6 into HIS BRG mice increases levels of total and antigen-specific human IgG, with a concordant rise in memory and IgG$^+$ human B cells, thymocytes, and peripheral T cells,[69] the latter of which are essential for B cell maturation. Additional strategies being investigated include attempts to improve peripheral lymph node development in humanized mice, which among other benefits can increase CXCL-13 signaling.[70] This has the potential to induce CD4$^+$ cells to become follicular T helper cells during antigen stimulation,[71,72] in turn providing stimulatory signals to B cells to mediate positive selection of high affinity B cells and differentiation of plasma cells in germinal centers.[71,73] In transplantation research, as in cancer, infection, and autoimmunity, recapitulation of the human B cell response to disease and treatment in humanized animal models will be key in enhancing the accuracy of empirical research outputs that can safely be translated into clinical studies.

Immune Humanization of Mice: Stem Cell-based Models

PBMCs are characterized by a low frequency of self-renewing pluripotent hematopoietic cells and a high proportion of mature lineage-committed cells. Multilineage reconstitution must, therefore, be achieved with the use of HSC-based products rather than PBMCs. Sources of HSCs include human umbilical cord blood,[30] adult bone marrow, fetal liver,[74] and granulocyte colony-stimulating factor-mobilized adult PBMCs.[75]

NSG and BRG mice can support long-term multilineage hematopoietic reconstitution following human CD34$^+$ HSC transplantation; however, studies have shown that the human T cells that develop are unable to recognize human HLA and mount human-restricted T cell responses because of selection on mouse MHC in the thymus.[39,76] To overcome this obstacle, it is possible to implant human fetal liver and thymus tissue beneath the kidney capsules of SCID mice to produce "thymic organoids" capable of supporting human T cell development.[77] To improve systemic reconstitution of T cells and other immune cell types while preserving this principle, the bone marrow-liver-thymus mouse model was created in the more supportive NSG strain, by transplanting human CD34$^+$ cells intravenously and implanting human fetal thymus and liver tissue beneath the kidney capsule.[78,79] Cells developing within this model show functional human-directed immune responses.[80,81] To reduce the requirement for fetal tissue, a recent model instead utilized neonatal thymus, which has produced human cells capable of rejecting skin xenografts.[82]

Limitations in HSC humanization include (1) the requirement for myeloablative preconditioning of host mice to create a bone marrow niche in which human HSCs can engraft and (2) the challenges that remain in reconstituting entire the spectrum of immune cells.[18,83] To overcome these, microenvironmental alterations to favor human hematopoiesis have been described (Figure 2). Methods include (1) mutation of critical murine growth

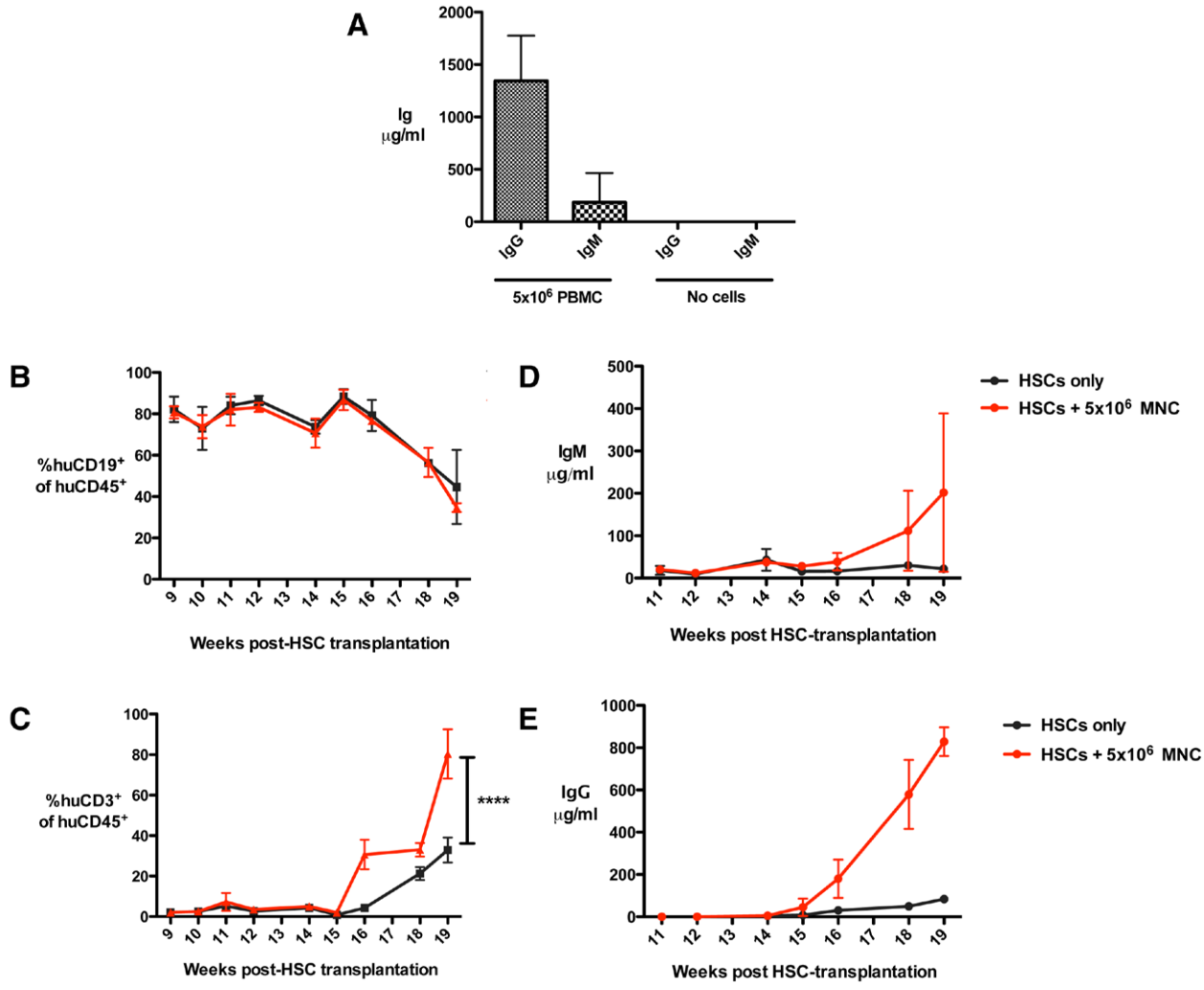

FIGURE 1. Antibody production following BALB/c-Rag2$^{-/-}$cγ$^{-/-}$ mouse transplantation with human PBMC or HSC and a human skin graft. A, BALB/c-Rag2$^{-/-}$cγ$^{-/-}$ mice received a human skin graft followed by 5 × 10^6 allogeneic PBMC (n = 9 mice) or no cells (n = 3 mice) 5 wk later. At the point of skin graft rejection (for those receiving PBMC) or long-term survival (>100 d, for those not receiving cells), mice were sacrificed and serum levels of human IgG and human IgM determined by ELISA. All mice receiving 5 × 10^6 PBMC achieved human leukocyte chimerism levels of >1% in the spleen. B–E, BALB/c-Rag2$^{-/-}$cγ$^{-/-}$ mice received 4 Gy total body irradiation within 24 h of birth, followed shortly by an intrahepatic injection of 5 × 10^4 CD34$^+$CD45$^-$Lin$^-$-enriched cells (derived from human UCB by magnetic bead isolation to a purity of over 92%). Six weeks later, mice received a human skin graft from a donor allogeneic to the HSC donor. One group received no further cells (n = 5 mice) and another group received an intraperitoneal injection of 5 × 10^6 mononuclear CD34$^-$CD45$^+$ cells (MNCs) from the same UCB donor 5 wk postskin transplantation (n = 4 mice). Peripheral blood was monitored from 9 wk onwards for the development of (B) live human 7AAD$^-$CD45$^+$CD19$^+$ and (C) 7AAD$^-$CD45$^+$CD3$^+$ leukocytes. Serum from peripheral blood was monitored from 9 wk onwards for the development of human (D) IgG and (E) IgM, measured by specific ELISA. Immunoglobulin was only detectable from wk 11 onwards. Data are represented as mean ± SD. HSC, hematopoietic stem cell; PBMC, peripheral blood mononuclear cell.

factor receptors, such as the c-kit receptor (NSGW41, NSGWv/+, NSGWv, NBSGW, SRG-W41, and BRgWv mice),[23,30] (2) inhibition of growth factor receptor function (eg, anti-c-kit receptor antibody),[84] (3) exogenous human cytokine administration (eg, B lymphocyte stimulator for mature human B cell reconstitution[63] and IL-7 analogues for T cell reconstitution[75]), and (4) knock-in of human cytokine genes, such as in SGM3,[27] MISTRG,[42] SRG-15,[85] and hIL-6 Tg NSG[28] strains, which include knock-ins of IL-3, IL-15, GM-CSF, M-CSF, or IL-6 to support engraftment of the wider human hematopoietic repertoire, including innate cells and regulatory T cells (Treg).[27]

Tissue Transplantation Into Humanized Mice

Once the challenge of human immune reconstitution is overcome, the interactions of these immune cells with specific tissues can be assessed. While responses are simulated by tumor engraftment, alloresponses are simulated by engraftment of human cells or tissues; the most common models being those that engraft human skin,[86] islets of Langerhans,[87] or blood vessels.[88] The most widely used model is that of human skin allotransplantation.[89-93] Skin grafts benefit from tissue accessibility permitting continuous visible monitoring and from an established progression of rejection in skin architecture and leukocyte infiltration. Moreover, skin is easily obtained as discarded tissue, with a single donor being able to provide sufficient tissue for multiple mice, providing a useful internal control. For example, our experience has shown that we are able to transplant skin to up to 50 mice from a single human donor.[48] Acute and chronic rejection of human solid-organ transplants, such as kidney, heart, or

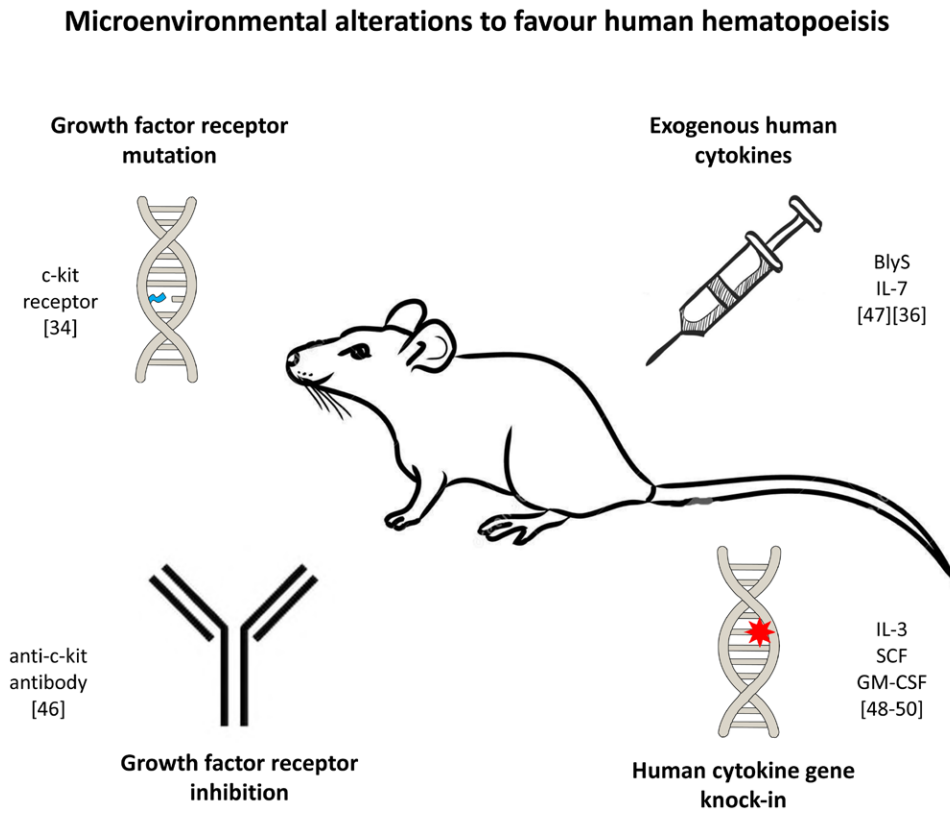

FIGURE 2. Microenvironment alterations to enhance human hematopoiesis. BlyS, B lymphocyte stimulator; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; SCF, stem cell factor.

lungs, is characterized by vascular injury.[94] Rejection of the interposed artery segment in immune humanized mice is a highly relevant model, yet fails to represent the entire vascular tree. Capillaries of human origin are maintained in adult skin grafts on immunodeficient mice, while the graft is simultaneously permeated by mouse capillaries within the first 21 d posttransplantation (Figure 3A–C and Pober et al[92]). Immune humanization with PBMC selectively destroys human microvasculature, in a process that can be halted by the cotransfer of $CD4^+$ Treg (Figure 3A–E). The humanization of mice with both PBMC and Treg can enable the long-term maintenance of human microvasculature in these skin grafts up to at least 100 d (Figure 3D and E). Some studies use alternate sources of capillaries, such as synthetic microvessels derived from endothelial colony-forming cells in cord blood.[95-97]

Human organs and tissues demonstrate unique immune functions and immune compartments that would ideally be modeled in homogenous tissues in vivo. This is clearly impractical in rodents, whose small size has traditionally limited the pool of suitable human tissues. Yet studies have ingenuously overcome this obstacle by generating or transferring human muscle, cartilage, bone, liver, kidneys, and intestines. Tissues such as cartilage,[98] muscle,[99] and ossicles[100,101] may be of value in understanding the immunogenicity of vascularized composite allografts. Human satellite cells obtained from skeletal muscles can integrate with NSG mouse muscle to successfully produce muscle fibers and self-renew in situ.[99] Allogeneic human articular chondrocytes in an agarose scaffold could successfully produce a cartilage matrix in NSG mice reconstituted with $CD34^+$ HSC, without signs of antidonor responses.[98] Recent advances in tissue engineering have generated protocols for creating 3-dimensional complex organoids from HSC or primary cells. The human bone niche can be reproduced by the subcutaneous differentiation of mesenchymal stromal cells that produce vascularized ossicles, to which human HSCs successfully home and reside.[100,101] These ossicles have normal bone marrow architecture, a diverse cell repertoire including osteoclasts and osteoblasts, and organized hematopoietic clusters around sinusoids.

Human intestinal organoids have been produced from pluripotent stem cells; true to form, these organoids develop crypt-villus structures and are populated by Paneth and goblet cells.[102] After implantation into the mesentery, organoids grow a vascular pedicle and can be joined to the murine intestine by anastomosis.[102] Engraftment of fetal intestine into the mesentery of SCID mice is also a successful approach generating human intestinal architecture and supporting an enteric nervous system.[103] Reconstruction of the mouse biliary tree with human extrahepatic cholangiocyte organoids has been demonstrated. Human cholangiocyte organoids grown on scaffolds could be surgically applied to repair and replace the gall bladder wall and common bile ducts.[104] Engraftment of mice with human livers has been achieved by implantation of fetal liver[105] or by the repopulation of immunodeficient mouse livers with human hepatocytes or pluripotent stem cells.[106] Immunodeficient mice are genetically modified to impair hepatic homeostasis, creating space in the hepatic niche and a regenerative stimulus. Urokinase-type plasminogen activator transgenic mice or fumarylacetoacetate

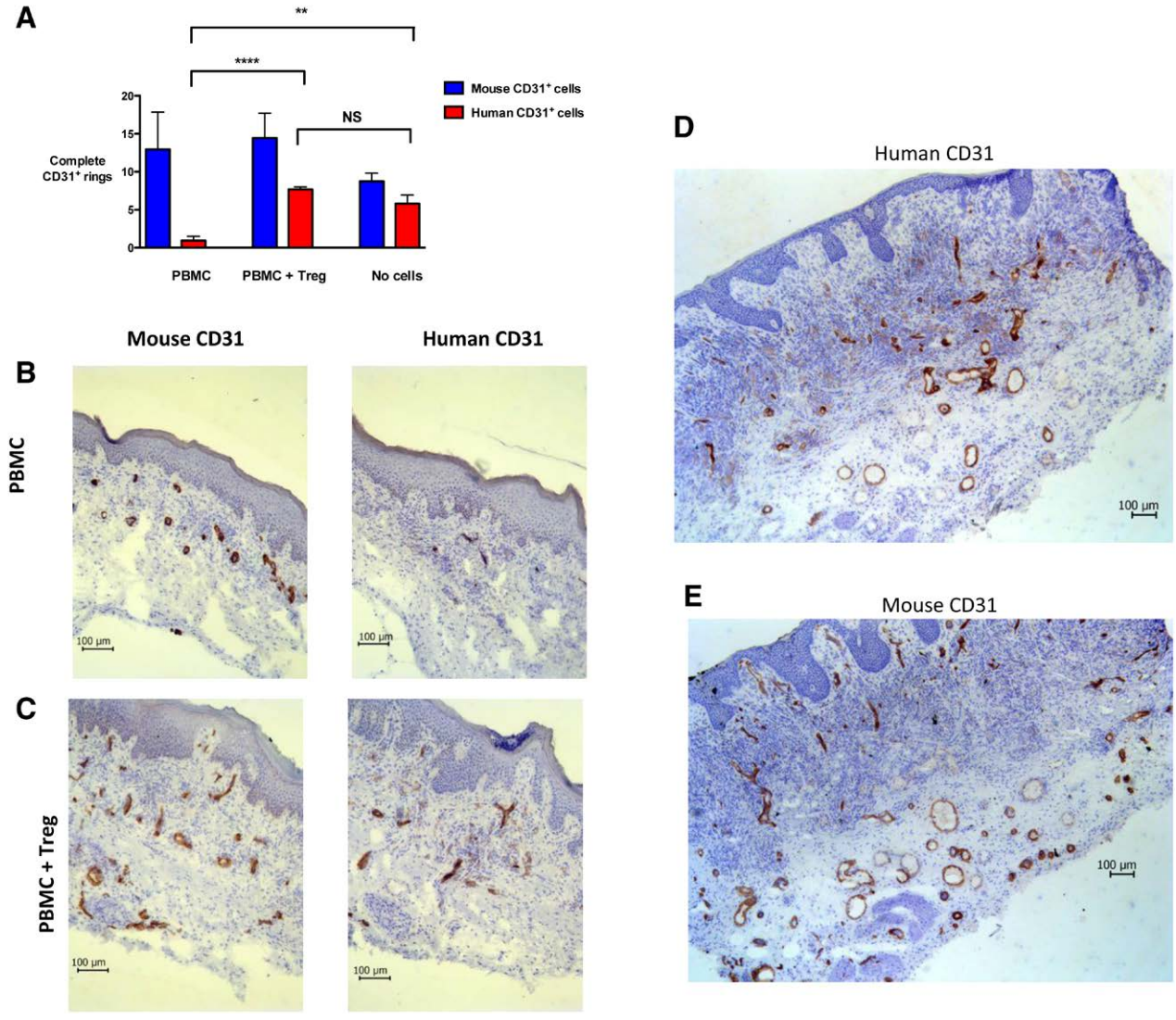

FIGURE 3. Human microvessels in human skin allografts are preserved in Treg-treated humanized BALB/c-Rag2$^{-/-}$cγ$^{-/-}$ mice. BALB/c-Rag2$^{-/-}$cγ$^{-/-}$ mice received a human skin graft and 5 wk later were injected with 5 × 10^6 allogeneic PBMCs (n = 3 mice) or 5 × 10^6 allogeneic PBMCs with 5 × 10^6 ex vivo-expanded CD127^{lo}CD25$^+$CD4$^+$ human Treg derived from the PBMC donor (ie, autologous Treg, n = 3 mice). A further group did not receive any cells (RPMI-1640 medium only, n = 2 mice). A, Twenty-one days postadoptive cellular transfer, skin grafts were procured and analyzed by immunohistochemistry for the number of complete cellular rings staining positive for either human CD31 or mouse CD31 within the dermis and epidermis. Complete rings were counted in the entirety of 3 separate sections (ie, in triplicate) per skin sample. Data are represented as mean values of the average of these triplicate values ± SD from a single in vivo assay using 1 PBMC donor and 1 skin donor, $^{**}P < 0.005$, $^{****}P < 0.0001$, NS, not significant. Representative photomicrographs are shown of human skin sections procured 21 d postadoptive cellular transfer and stained by immunohistochemistry to visualize human CD31$^+$ or mouse CD31$^+$ cells in (B) mice receiving 5 × 10^6 PBMCs only and (C) mice receiving 5 × 10^6 PBMCs with 5 × 10^6 ex vivo-expanded Treg. Sections are counterstained with hematoxylin. The histology shown is representative of 6 stained sections for each type of stain for each of n = 3 mice for each group. Representative photomicrographs are shown of human skin sections from mice receiving PBMCs with Treg, procured >100 d postadoptive cellular transfer and stained by immunohistochemistry for (D) human CD31$^+$ cells and (E) mouse CD31$^+$ cells. Sections are counterstained with hematoxylin. Multiple complete human and mouse CD31$^+$ rings are visible. Photomicrographs are representative of 6 sections from each of n = 4 mice. PBMC, peripheral blood mononuclear cell; Treg, regulatory T cell.

hydrolase (Fah) knockouts are common modifications for inducing toxicity in murine liver cells.[107] The transfer of human hepatocytes has variable engraftment rates, but repopulation of up to 95% has been reported alongside a conserved liver microstructure.[108] The Fah$^{-/-}$Rag2$^{-/-}$Il 2rg$^{-/-}$ (FRG) mice combined with the NOD strain has been used to engraft both an immune system and repopulate the liver with human hepatocytes, creating dual chimerism and permitting the study of human hepatocyte-immune interactions.[109] As an alternative to models requiring endogenous liver damage, induced pluripotent stem cells (iPS) can produce liver organoids comparable with adult human tissue in gene expression, protein secretion, and drug metabolism. After implantation into the livers of NSG mice, these organoids integrate with the native tissue and become vascularized within 4 wk.[110]

Of interest in modeling renal conditions, complex kidney organoids can be generated from pluripotent stem cells. Kidney organoids successfully contain organized compartments dedicated to nephrons, collecting ducts, stroma, and an endothelial network.[111] Human kidney organoids can be transplanted into NSG mice, where they join onto the

murine vasculature and undergo glomerular and tubular epithelium maturation.[112]

Preclinical Models for Immunotherapies

Control of immune responses is a requirement following allotransplantation to ensure graft function and survival. Faithful models of human biology lend themselves to the study of pathogenesis as well as the development of novel therapeutics. In the last 10 y, humanized mouse models have been broadly applied in preclinical studies for the development of the next generation of immunotherapies including costimulation and cytokine modulation, adoptive regulatory cell transfers, chimeric antigen receptor T cells, and nanotechnology for targeted drug delivery.

Immune humanization produces robust models of GvHD. This platform has provided the basis for the efficacy of adoptive $CD4^+$ and $CD8^+$ Treg therapy,[90,113,114] mesenchymal stem cell transfers,[115] costimulatory blockade such as anti-CD28 or anti-CD3 monoclonal antibodies,[116,117] blockade of chemokine receptors,[118] and small molecule drugs for the inhibition of JAK1/2 signaling.[119]

The advances in bioengineering human tissues and organs have the potential to enable studies of tissue-specific immune interactions with concurrent assessment of drug metabolism and off-target effects. However, the simultaneous engraftment of immune cells with adult allogeneic skin or vessels is currently the most prevalent model of tissue transplantation. Costimulatory and cytokine modulatory therapies have shown promise in these human tissue transplant models.[89,120] Nanotechnology was recently applied to humanized models where arteries perfused with nanoparticles carrying silencing RNA could reduce endothelial MHC class II expression and could prevent alloimmune-mediated arteriopathy.[121]

Cell therapies rely on humanized models where differences in murine and human biology could have myriad effects on the fate of the transferred cell, different immune subsets, and the allogenic tissue. The adoptive transfer of polyclonal $CD4^+$ and $CD8^+$ Treg has been shown to prevent the rejection of human skin and vessel grafts[48,86,88,90,122]; these studies have inspired clinical trials into adoptive Treg transfer. Treg transfer therapies have been shown in humanized models to be modulated by their migratory potential,[86] antigen-specificity,[123] and number.[86] To this end, the development of Treg bestowed with chimeric antigen receptors specific for alloantigens have been implemented in skin graft models to reduce alloimmune injury.[93,124,125] Importantly, a number of national medical regulatory agencies have now started to acknowledge data from humanized models in submissions for clinical trial approval.

RATS

Immunodeficient Rat Models and Immune Humanization

Rats provide several advantages to mice in experimental studies in transplantation. First, rats are up to 10 times larger than mice, providing an advantage for technically demanding surgical studies. Their size enables the precise implantation of human tumors into relatively small anatomical locations, such as the prostate or specific areas of the brain, as well as enabling the implantation of organoids in orthotopic locations. Size of rats could benefit grafts that require immediate vascularization, such as human fetal vascularized organs, skin, and vessels. Second, there are no reports of shortened lifespan in rat strains used for generating immunodeficient models. This is unlike most NOD-derived immunodeficient mice, since they show reduced survival due to thymoma beginning at month 8,[13] although survival may not be reduced for NSG mice.[75] Third, rats share some immune characteristics with humans that mice do not.[126] Although some of these are not relevant to immunodeficient models where rat T cells are absent, other characteristics remain pertinent such as macrophage expression of CD4 and/or CD8 or the expression of MHC class II on endothelial cells.[127] A further advantage of immunodeficient rats is their potential to sustain a larger number of human cells, therefore allowing larger numbers of human immune cells to be procured from the spleen for functional or molecular studies ($50–150 \times 10^6$ cells compared with $2–10 \times 10^6$ cells in mice[128]).

For many years, the only immunodeficient rats were nude rats established from a spontaneous mutation in the *Foxn1* gene[129] (Table 2). Similar to nude mice, nude rats are only deficient in T cells, while B and NK cell compartments are normal. Furthermore, they have a leaky phenotype whereby older rats produce T cells that develop from a spontaneous rearrangement of their TCRs.[204,205] Novel immunodeficient rats have been generated with mutations in key genes, including *Rag1*,[166-168] *Rag2*,[169-171] and *Il2rg*.[172] *Rag1* and *Rag2* KO rats have normal NK cell generation, and there are residual B and T cells in single *Rag1*, *Rag2*, or *Il2rg* mutants. The severity of immunodeficiency has been increased by combining mutations in the aforementioned genes and/or *Prkdc*.[167,174,176,177]

Complement levels in most inbred mouse strains,[9,11] including NOD-derived immunodeficient mice,[12,13] are undetectable or very low. This led to the recent development of a complement-sufficient NSG strain.[206] In contrast, rats have complement levels comparable with humans, as previously shown for several strains,[11] including Sprague-Dawley (SD) rats deficient for Rag-1 and Il2rg (SD-RG) and expressing hSIRPα (RRGS).[128]

Rats selectively deficient for B cells and consequently immunoglobulin of all isotypes have been generated by deleting the *Igh6* gene (orthologue of the IgM human gene) using ZFNs[178] or CRISPR/Cas9[179] technologies. Using ZFNs, rats have been generated that lack not only heavy chain immunoglobulins but also both kappa and lambda light chains. These animals were then subsequently humanized by transgenic expression of human immunoglobulin coding sequences allowing the production of fully human monoclonal antibodies of high affinity.[180,181]

A limitation of humanized allo-GvHD models in immunodeficient mice is the need for total body irradiation to observe clear clinical GvHD, which is increasingly disparate to clinical practice. This irradiation is not required in an immunodeficient rat model in which allo-GvHD was studied.[128] Additionally, *Prkdc* mutations to obtain a SCID phenotype in many mouse strains (like all NOD-derived immunodeficient strains such as NSG and NOG) increase toxicity due to irradiation in certain models, such as in cancer treatments, since PRKDC is an enzyme essential in DNA repair and its absence generates uncontrolled toxicity in the host tissues. Some[174,176,182] but not all[177] immunodeficient rats also carry a mutation in the *Prkdc* gene.

TABLE 2.

Transplantation and regenerative medicine models using immunodeficient species other than mice

Species	Mutated genes	Immune phenotype	Transplantation model	References
Rat				
	Foxn1	T– (leaky) B+ NK+	Human kidney stem cells, MSCs, neural stem cells, smooth muscle progenitors, retinal cells, pancreatic progenitors, hepatocytes, osteoblasts, astrocytes, oligodendrocytes, stromal stem cells in rotator cuff, bone regeneration, dental follicular cells, cardiomyocytes, intestinal cells	130-165
	Rag1	T– (partially) B– (partially) NK+	Human hepatocytes	166-168
	Rag2	T– (partially) B– (partially) NK+	Human fetal kidneys	169,170,208
	Il2rg	T– (partially) B– (partially) NK+	Not described	171,172,173
	Prkdc	T– B– NK+	Human iPS-derived neural precursors	174,175,209
	Il2rg and *Prkdc*	T– B– NK–	Human iPS, tumor cells and hepatocytes	174
	Rag1, *Rag2* and *Il2rg*	T– B– NK–	Human cancer cells	176
	Rag1 and *Il2rg*	T– B– NK–	Human skin, tumor and hepatocytes	177
	IgM	T+ B– NK+	Rat transplantation models	178,179
	IgM, *Igκ*, *Igλ*hulgs	Human antibodies	Not described	180,181
	Prkdc, *Il2rg*, *hSIRPa*	T– B– NK– Mo inhibition	Immune humanization, human cancer cells, iPS	182
	Rag1, *Il2rg*, *hSIRPa*	T– B– NK– Mo inhibition	Immune humanization, human GvHD	128
Nonhuman primate	*IL2RG*	T– B– NK–	Not described	183
Pig				
	IL2RG	T– B– NK–	Not described	184-186
	RAG1 and 2	T– B– NK+	Not described	187
	RAG1	T– B– NK+	Not described	188
	RAG2	T– B– NK+	Human iPS	189-191
	RAG2 and IL2RG	T– B– NK–	Not described	192
	ARTEMIS	T– B– NK+	Human skin	193,194
Zebra fish				
	Rag1	T– B– NK+	Human tumors	195
	Rag2	T– B– NK+	Not described	196
	Prkdc and Il2rg	T– B– NK-	Not described	197
	Prkdc and Il2rg	T– B– NK–	Human tumors	198
Rabbit				
	RAG1 and RAG2	T– B– NK+	Human tumors	199
	RAG1, RAG2 and IL2RG	T– B– NK–	Not described	200
	FOXN1, RAG1, RAG2, IL2RG	T– B– NK–	Not described	201
	IgH	T+ B– NK+	Not described	202
Syrian hamster				
	RAG1	T– B– NK+	Not described	203

GvHD, graft vs host disease; iPS, induced pluripotent stem cell; NK, natural killer.

As in mice, the molecular incompatibility between rat SIRPα and human CD47 could lead to the elimination of certain types of human cells by macrophages. A recent publication described a rat strain combining mutations in *Prkdc*, *Il2rg*, and the expression of human SIRPα, which allowed better immune humanization compared with animals without human SIRPα.[182] Immune humanization in these animals was performed using $CD34^+$ cells from fetal liver alone or in combination with autologous fetal thymus but not with human PBMCs. Despite immune humanization, this report did not describe their use to explore any immune response (such as skin rejection or antihuman tumor responses). Animals from the RRG line, with *Rag1* and *Il2rg* mutations, indefinitely accepted human skin, tumors, and even hepatocytes, but did not accept human PBMCs.[177] However, RRG animals crossed with rats expressing human SIRPα[207] (RRGS animals) or with macrophage depletion allowed immune humanization using human PBMC.[128] These rats humanized with PBMCs could develop GvHD and reject tumor cells. Furthermore, the presence of normal complement levels in RRGS animals allowed the successful prevention of acute GvHD by using a new depleting antihuman T cell polyclonal antibody. Immune humanization of RRGS animals with cord blood $hCD34^+$ cells allowed immune humanization (unpublished). Altogether, these studies indicate that as for mice, immune humanization is more difficult than tissue or tumor humanization and that inhibition of macrophage-mediated phagocytosis of human immune cells by $CD47^-$SIRPα interactions is necessary.

Tissue Humanization of Rats

Nude *Foxn1*^{RNU} rats have been transplanted with a variety of human stem cells or organs in different models of transplantation and regenerative medicine, including models of intestinal transplantation,[130] bronchus grafts,[131] nephropathy,[132] liver,[133,134] cardiac infarct,[135-137] bone and chondrocyte healing and regeneration,[138-142] rotator cuff lesions,[143,144] tendon lesions,[145,146] brain or spinal trauma,[147-155] urethral dysfunction,[156,157] retinal degeneration,[158-162] in vivo differentiation of ES cell-derived pancreatic beta cells,[163] multiciliated airway cells for the generation of an artificial trachea,[164] and periodontal tissues[165] (Table 2).

The most recently developed models of immunodeficient rats engrafted with solid human tissues are summarized in Table 2. A rat strain, deficient in Fah and *Il2rg*, is suitable for the engraftment of human hepatocytes that demonstrate a competitive proliferative advantage in comparison to the resident rat hepatocytes.[171] Rag1-deficient rats transplanted with human hepatocytes have been able to partially reconstitute the liver.[167] *Il2rg* and *Prkdc* mutated rats have been shown to successfully engraft human iPS, tumors, and hepatocytes.[174] Rag2-deficient rats have also been transplanted with human fetal kidneys (17–18 wk gestation) as a method to explore kidney organogenesis in vivo.[208] Fetal kidneys increased their size through nephrogenesis and were functionally effective in prolonging the survival of nephrectomized recipients. This is a new animal model for the study of kidney ontogeny and preclinical toxicity of therapeutics agents. In a model of neonatal hypoxic brain injury, *Prkdc*-deficient rats transplanted with human neural precursor cells showed amelioration of lesions.[209] It has also been demonstrated that rats from the RRG line bearing *Rag1* and *Il2rg* mutations can indefinitely accept transplanted human skin and tumors.[128]

It is interesting to note that, in some models, immunodeficient rats seem to tolerate xenografts better than immunodeficient mice. This is the case for human pancreatic progenitors that matured faster in rats than mice.[163] It is possible that accelerated maturation of beta cells was due to similar glucose levels in rats and humans during fasting or after glucose challenge, whereas mice showed high fasting glucose levels and dramatic glucose fluctuations peaking at higher levels after glucose challenge.[163] Additionally, human tumors grew significantly faster and larger in SD-RG rats than in NSG mice and several fresh lung squamous tumors from patients (PDX model) were successfully implanted in this immunodeficient rat line.[176] The use of immunodeficient rats for the generation of PDX tumors coupled with the humanization of immune responses will likely represent useful models for cancer research.

Rats do, however, have disadvantages when compared with mice. Their larger size implies higher breeding costs. There are also fewer established genetic modifications applicable in rats when compared with immunodeficient mice, such as expression of human cytokines and human MHC or elimination of dendritic cells. In the near future, the application of new genome editing nucleases, such as meganucleases,[166] ZFNs,[210] TALENs,[211] and CRISPR/Cas9,[212,213] will facilitate the development of rats with disease-specific mutations including with Cre-conditional mutations.[214,215]

IMMUNODEFICIENCY AND HUMANIZATION IN OTHER SPECIES

Pigs are an attractive species for the experimental implantation of human cells or tissues, because of their large size and physiological proximity to humans. Several lines of immunodeficient pigs carrying spontaneous or induced inactivation of *RAG1*, *RAG2*, *ARTEMIS*, or *IL2RG* alone or combined (*RAG2* and *IL2RG*) have been described.[216] However, in pigs that carry mutations of the *IL2RG*,[184-186] *RAG1*,[188] *RAG2*,[191] *RAG2*, and *IL2RG*[192] genes, there are no descriptions of transplantation of human cells or tissues. In T$^{(-)}$B$^{(-)}$NK$^{(+)}$ SCID pigs carrying spontaneous point mutations in the *ARTEMIS* gene,[193] human cryopreserved deceased skin was accepted for at least 28 d.[194] Since the successful in utero engraftment and differentiation of human CD34^{+} progenitors in immunocompetent pigs has been reported,[217] it has been proposed that immunodeficient pigs could be used in this setting to generate large numbers of human cells.[216] Human lymphocytes can recognize porcine MHC molecules and respond at similar levels to human allogenic MHC proteins.[218,219] The homology between human and porcine MHC molecules means that antibodies against HLA class II antigens have a propensity to also bind swine leukocyte antigen class II antigens. In the context of future immune humanization of immunodeficient pigs, it is important to point out that porcine SIRPα binds to human CD47 and thus provides inhibition of pig macrophage phagocytosis of human leukocytes.[220]

Zebrafish offer the attractive characteristic of optical transparency and ease of breeding. Although immunodeficient zebrafish have been described, such as those deficient for Rag2,[196] Rag1,[195] or for both Prkdc and Il2rg,[198] they have only been used for implanting human tumors and not for normal human tissue or cell transplantation. Human CD34^{+} HSCs have been transplanted into immunocompetent zebrafish where they are home to the caudal niche and engage endothelial cells and undergo cell division.[221]

Rabbits have excellent fecundity and a convenient size for many experimental procedures, while remaining small enough for maintenance as a laboratory animal. Immunocompromised rabbits have been produced with deficiencies in RAG1 and RAG2[199]; RAG1, RAG2, and IL2RG[200] FOXN1, RAG1, RAG2, IL2RG,[201] and IgH,[202] but to date they have only been used for implantation of human tumor cells. Similarly, Syrian hamsters are small animal models used in several areas of research.[222]Syrian hamsters deficient for RAG1 have been described but have not yet been humanized.[203]Finally in nonhuman primates, immunocompromised marmosets mutated for *IL2RG* using ZFNs and TALENs have been described,[183] but there have been no reports on immune humanization or transplantation of human tissues in these animals.

CONCLUSIONS

It is clear that the use of humanized animals is important in providing opportunities to understand the mechanisms of human immune responses to tissue transplants in vivo. These models provide an excellent path for the development and assessment of human-based immunotherapies in a human context. Regulatory agencies are starting to accept data from humanized models as indicative of

therapeutic efficacy of a human-specific agent. A number of newer models have been developed to provide a more complete picture of human immune responses or to allow surgical procedures that would otherwise be impossible in mice. For most studies, this is not necessarily the case that the most advanced model should be used, rather that a specific model should be selected that answers the experimental question at hand. It is also important to note the significant limitations that exist. First, none of the models described fully reproduce all elements of a functional human immune system in the peripheral blood, and also in tissues as for macrophages and innate lymphoid cells.[223] Second, human immune responses result from a complex interaction of cells between the peripheral, tissue, and lymphoid systems. Many of these elements do not exist even in the most advanced models. Third, these models do not yet provide a complete substitute for pharmacokinetic studies in larger animal models. However, it is arguable that some safety elements related to the effects on human leukocytes (eg, a cytokine storm) would be observed in a fully humanized animal. Nevertheless, agents that are found to be unsuccessful in a humanized animal are unlikely to be successful after translation, providing a method for filtering out therapies that would fail in expensive and risky early clinical trials.

PERSPECTIVES

In cancer studies, the outcomes of therapeutic adoptive T cell transfers could be modeled in immune-humanized mice engrafted with patient tumor (PDX models) and the therapeutic cell transfer.[224] Patient material could also be used in generating relevant and insightful humanized models for transplantation. An example is a recent study that engrafted mice with pericardiophrenic artery segments obtained from the donor and PBMC from the recipient. Interestingly, histological changes in the artery were associated with development of chronic lung allograft dysfunction in the patient, indicating the presence of alloreactive T cells at time of transplant.[225] Such models could provide a method for developing customized patient-specific therapies.

The importance of the microbiota in immune education is recognized, but the role of the microbiome in tolerance and rejection is an ongoing subject of research.[226] Allotransplantation and immunosuppression are associated with changes in gut microbiota population frequency and diversity.[227] Allogenic animal models have shown the ability of commensal bacteria in the gut or on the skin to influence allograft survival.[228-230] In NSG mice reconstituted by neonatal administration of human CD34$^+$ cells, antibiotics modified skin allograft tolerance and the success of the immunotherapy teplizumab.[117] The impact of microbiota on allograft survival and sensitivity to immunointervention is a problem for the reproducibility of animal work, wherein animals housed in different facilities vary significantly in their microbiome composition.[231,232] Indeed, mice from different animal houses have different capacities to tolerate or reject orthotopic lung allografts and the same principle applies to the tolerance of xenografts in immune humanized animals.[231] Methods do exist to partially engraft human microbiota into mature murine intestinal environments, but the mammalian microbiome is species-specific and symbiotic.[233-235] Not all human intestinal flora is maintained in a mouse intestine after transfer, resulting in bacterial diversity that is not representative of the original donor.

Rats have some characteristics that make them attractive for humanization. Their larger size makes them well suited for orthotopic implantation of human organoids of different tissues differentiated from human iPS or ES cells. New and exciting models are emerging using interspecies chimeras to generate organs from a different species in an animal knockout for a tissue master gene. For these models, the rat is often used.[236,237] Nevertheless, species chimerism decreases with time during the embryo development and this could be due to in embryo immune responses, including SIRPα-CD47 incompatibilities that could benefit from the use of SIRPα humanized rats. Additionally, a number of rat knockout models that reproduce human genetic diseases better than mice, such as in dystrophin[238] or *Aire*-deficient[239] rats, could be used in an immunodeficient-humanized setting.

In nonmouse immunodeficient models, the rat is the only species that has been immune humanized. Yet very little has yet been performed in terms of tissue transplantation outside of current mouse models. It is likely that immune humanized pigs will be developed in the future. Given the recent advances in genetic modification, the tools now exist to produce immunodeficient animals in a range of species. The demand for immunodeficient recipients will likely increase in the future for the implantation of cells derived from human iPS and ES cells, such as hepatocytes, pancreatic beta cells, and retinal cells. Recent years have seen an explosion in the production of genetically humanized transgenic mice to sustain specific and functional components of human cells and tissues, which may move across species. Regenerative medicine is directly contributing to the pool of human tissues and organs that can be incorporated into other species, having already produced kidney organoids, muscle, cartilage, bone, intestines, bladder walls, and liver. The next step will be to apply these models to the study of cell, tissue, and solid organ transplantation and rejection. For example, antibody-mediated rejection could be modeled in transgenic mice with functional B cell antibody responses combined with a vascular transplant, or tissue-specific immunogenicity could be assessed using kidney organoid implantation together with T cell engraftment. The next generation of immunotherapies, such as cellular therapies and immunosuppressant-loaded nanoparticles, will require human immunity and tissues to thoroughly assess their functionality, immunogenicity, and target specificity. The development of advanced humanized animals with greater likeness to functional multilineage human immune systems will therefore permit the study and replication of more complex responses in allotransplantation, GvHD, and regenerative medicine.

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