Humanized Mice in Dermatology Research

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INTRODUCTION

The term "humanized mice" refers to immunodeficient mice containing human cells or tissues or to mice (immunodeficient or not) that have been genetically modified to express human genes. Humanized mouse models are increasingly utilized in many areas of research, such as infectious disease, autoimmune disease, cancer biology, and drug development. Because humanized mice recapitulate human physiology and pathology better than traditional mouse models do, they are employed both in disease modeling and in preclinical investigations of novel therapies. As these models are increasingly utilized in dermatology research, it is important for dermatology researchers and clinicians to have a rudimentary understanding of humanized mice. In this article, we review the basic biology of humanized mice and provide examples of their use in dermatology research.

GENERAL PRINCIPLES OF HUMANIZED MICE

The term "humanized mice" traditionally referred to mice engrafted with human cells or tissues. However, advancements in genetic engineering have resulted in mice genetically programmed to express human genes, also considered humanized mice. Therefore, humanized mice can now be more broadly defined as any mouse containing functional human proteins, cells, tissues, or organs. Overlapping vocabulary has been utilized in the literature to describe humanized mouse models, including the terms "chimera," "xenograft," or "xenotransplant." A chimera is an organism containing two or more genetically distinct cells, and the prefix "xeno" specifically refers to the combination of two species (human and mouse, in this case).

Further complicating matters, a broad range of humanized mouse models are utilized in research, and multiple approaches exist to generate such models. In our view, it is easiest to categorically conceptualize humanized mice in three regards: (i) the type of "host" mouse used, (ii) the type of human cells/tissues engrafted into that host mouse, and (iii) genetic modifications used to improve points (i) and (ii).

CURRENTLY EMPLOYED "HOST" MICE

Immunodeficient mice must be utilized as hosts for human cells/tissues because the immune system of a normal (wild-type) mouse would reject the human cells/tissues. Multiple immunodeficient mouse strains have been established over time, and there is currently no universal immunodeficient mouse strain used for all humanized mouse studies, so researchers must choose the most appropriate immunodeficient strain for the purpose.

A few basic principles are common to currently employed immunodeficient mouse strains. First, mice must lack functional T and B lymphocytes to prevent rejection of human cells/tissues. Some mouse strains have a genetic defect in *recombination-activating gene*s (*Rag*) *1* or *2*, which encode the enzymes responsible for T- and B-cell receptor rearrangement. Alternatively, a genetic defect in *protein kinase, DNA-activated, catalytic polypeptide (Prkdc)* results in severe combined immunodeficiency (SCID) mice. Rag and SCID mice lack mature T and B cells, but still contain functional natural killer cells that can destroy human cells (reviewed in Shultz *et al.*, 2007). Therefore, Rag and SCID mice have been further modified by a targeted mutation leading to either nonfunctional or a complete absence of the IL-2 receptor-γ chain (Il2rg). This completely inhibits natural killer–cell development and impedes development of lymph nodes and T and B cells, while also potentially impairing signaling via other cytokines that share the Il2rg, including IL-4, -7, -9, -15, and -21 (reviewed in Shultz *et al.*, 2007). Finally, immunodefi-

ADVANTAGES

- Humanized mice better recapitulate human disease than traditional mouse models.
- Genetic modifications can be employed to further "humanize" mice.
- Humanized mice can serve as preclinical models to test novel therapeutics; results may better reflect human drug metabolism, side-effect profiles, and efficacy.

LIMITATIONS

- Complete multilineage engraftment of the human hematopoietic system and development of memory T- and B-cell responses are difficult to obtain.
- Cross-reaction between coexpressed mouse and human factors can confound experimental results.
- Absence of additional human factors, such as cytokines, homing ligands, and receptors, may limit accurate modeling of human physiology/pathology.

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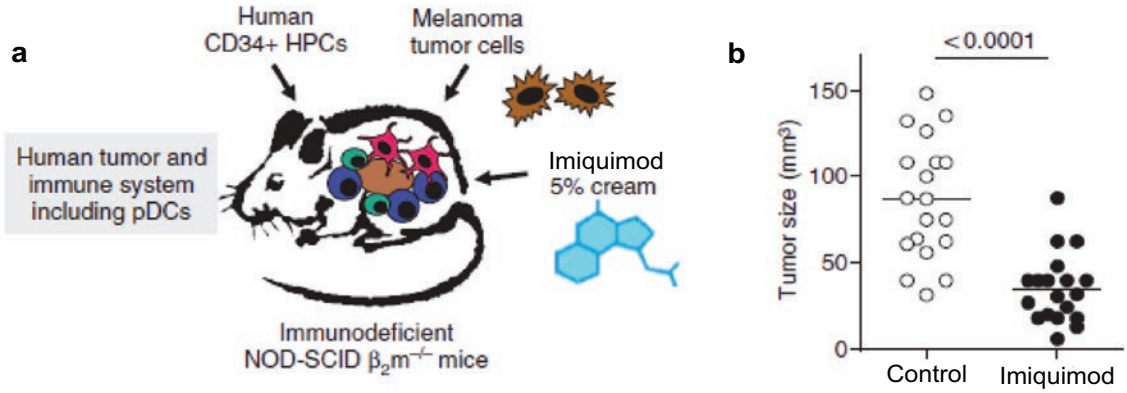

Figure 1. Humanized mouse model demonstrates that imiquimod mobilizes and activates plasmacytoid dendritic cells to inhibit melanoma tumor growth. (**a**) Immunodeficient mice were sublethally irradiated and then injected intravenously with human CD34+ hematopoietic stem cells (HPCs), which include plasmacytoid dendritic cells (pDCs). Melanoma cell lines were injected subcutaneously. Mice were then treated topically (or not, control) with imiquimod, a TLR7/8 agonist. (**b**) Tumor growth was measured and compared between the mice that received imiquimod and the controls. NOD-SCID, nonobese diabetic–severe combined immunodeficient. Reprinted from Aspord *et al.* (2014).

cient mouse strains may also contain defects in dendritic-cell or macrophage function and/or in the complement system. The various immune defects may render a specific mouse strain more or less suited for a particular research study.

Currently, the NSG (NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/*SzJ*) and NOG (NODShi.Cg-*Prkdc*^{scid}*IL2rg*^{tm1Sug}/*Jic*) immunodeficient mouse strains are commonly employed in humanized mouse models owing to enhanced human cell engraftment (reviewed in Shultz *et al.*, 2012). Moreover, the NSG strain is relatively easy to acquire and breed. A more comprehensive list of immunodeficient strains used in humanized mouse models can be found in Shultz *et al.* (2007).

TYPES OF HUMAN CELLS/TISSUES ENGRAFTED

Humanized immune system

There are three basic models of humanized immune systems in immunodeficient mice. First, human peripheral blood mononuclear cells (PBMCs) or human lymphocytes from lymph node or spleen can be injected intravenously (most commonly), intraperitoneally, or intrahepatically into an immunodeficient mouse (reviewed in Brehm *et al.*, 2014; Shultz *et al.*, 2007, 2012). This is commonly referred to as the hu-PBL model. Hu-PBL is the simplest, most rapid engraftment procedure of the three models. However, it results in engraftment of effector and memory T lymphocytes, but not other immune cells. Also, a xenogeneic (cross-species) graft-versus-host disease (GVHD) ensues approximately 4 weeks after human cell injection, which may limit the utility of this model to short-term studies. Notably, GVHD researchers have leveraged this phenomenon into a preclinical model of disease (van Rijn *et al.*, 2003).

The second model is the transfer of human hematopoietic stem cells (HSCs) into an immunodeficient mouse. This is commonly referred to as the Hu-HSC or Hu-CD34 method, since HSCs express and may be selected for via CD34. HSCs are typically isolated from fetal bone marrow, umbilical cord blood or liver, or granulocyte colony-stimulating factor–mobilized peripheral blood (reviewed in Brehm *et al.*, 2014; Shultz *et al.*, 2012). Hu-HSC results in multilineage hematopoiesis, although naive T cells are restricted to mouse rather than human major histocompatibility (MHC) groups (reviewed in Shultz *et al.*, 2012).

The third model, BLT, involves surgical implantation of autologous human fetal liver and thymus fragments under the immunodeficient mouse's renal capsule along with transfer of autologous human HSCs (reviewed in Brehm *et al.*, 2014). This model is the most complex, requiring technical/surgical expertise and a source of fetal tissue (which can be limiting). Because of the presence of human thymic tissue, appropriate T-cell education and HLA restriction occurs (reviewed in Brehm *et al.*, 2014).

Notably, different terms are used for these models in the literature, which may be confusing to the reader. Originally, model names included "SCID" because SCID mice were used prior to the introduction of immunodeficient mice lacking Il2rg. Also, some review articles refer to four models rather than three because coimplantation of human fetal liver and thymus was separated from the transplantation of fetal liver, thymus, and bone marrow (Shultz *et al.*, 2012).

Caution is needed when reading the literature or designing experiments: lineage engraftment, the ability to generate primary and secondary cellular or humoral responses, and T-cell restriction of each of the above models can vary depending on the host mouse utilized. Most publications reference experimentation performed in SCID or Rag-deficient mice, but outcomes may differ if NSG or NOG mice are used.

Transplantation of nonimmune cells/tissues

In addition to immune cells, other types of human cells have been grafted into immunodeficient mice. For example, mice engrafted with human hepatocytes are increasingly employed in drug metabolism studies (reviewed in Strom *et*

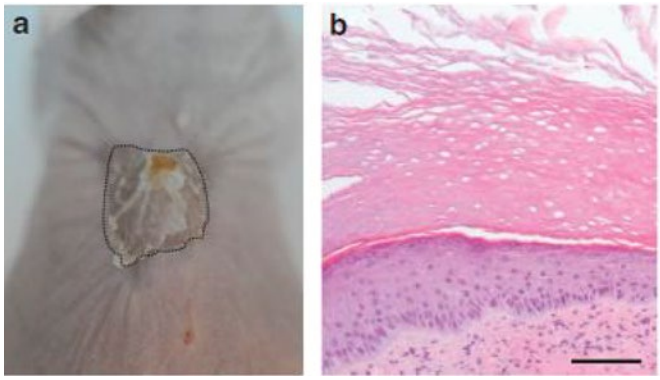

Figure 2. Novel humanized mouse model of pachyonychia congenita using bioengineered skin equivalents generated from patients. Skin keratinocytes and fibroblasts were taken from patients with pachyonychia congenita, due to a keratin 6A mutation, to generate bioengineered skin equivalents that were then grafted onto the backs of immunodeficient mice. (**a**) Gross image and (**b**) histology image (stained with hematoxylin and eosin) demonstrating clinical and pathologic characteristics of pachyonychia congenita after minor trauma. Reprinted from Garcia *et al.* (2011).

al., 2010). Human cancer cells, either patient-derived or cell line–derived, are transplanted into host mice to study cancer biology, metastasis, and novel treatments (reviewed in Zhou *et al.*, 2014). The types of human cancers studied via this mechanism are numerous and include both solid tumors and hematologic malignancies (reviewed in Zhou *et al.*, 2014). Human lung, intestine, pancreatic islet, and tonsil cells have also been engrafted into immunodeficient mice (reviewed in Brehm *et al.*, 2014).

Human tissues and organs can be transplanted into immunodeficient mice as well. In dermatology, researchers have capitalized on the accessibility of healthy and diseased human skin and straightforward surgical techniques to transplant human skin onto immunodeficient mice. This overcomes many physiologic discrepancies between mouse and human skin that challenges traditional mouse models. However, an inevitable issue with this approach is the mismatch between homing molecule expression on human cells and binding partners on murine endothelial cells that supply the transplanted human skin (reviewed in Petersen and Sorensen, 2008). This is problematic, for example, in humanized mouse models of GVHD where human skin is transplanted onto immunodeficient mice and then human PBMCs are transferred into that mouse. Migration of human inflammatory cells into the skin graft may not reflect the types and numbers of cells that actually migrate into patient skin in GVHD.

Genetically modified humanized mice

Genetically modified mice are revolutionizing humanized mouse models because they overcome limitations inherent to a particular model. For example, immunodeficient mice transgenically expressing human MHC class I and/or II have been generated to allow proper MHC:T-cell receptor interaction with engrafted human T cells. Mice transgenically expressing human p53, a tumor-suppressor gene, in place of mouse p53 were generated for cancer studies (reviewed

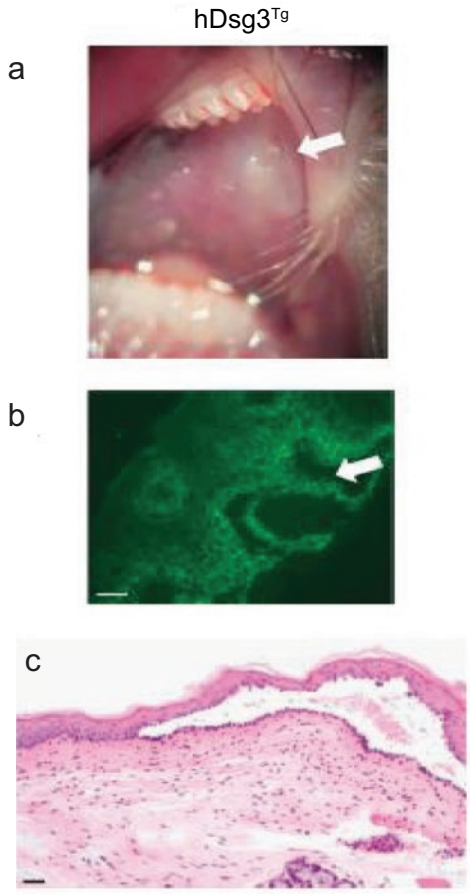

Figure 3. Genetically modified humanized mouse model to study the role of human anti–desmoglein 3 immunoglobulin in pemphigus vulgaris (PV). Human anti-desmoglein 3 (Dsg3) antibodies do not recognize mouse Dsg3. Therefore, mice were genetically modified to transgenically express human Dsg3 ($hDsg3^{Tg}$) in place of mouse Dsg3 and then exposed to anti-Dsg3 immunoglobulin from human patients with PV. (**a**) Humanized mouse mucosa demonstrates clinical erosions characteristic of PV. (**b**) Direct immunofluorescence staining shows positive staining and (**c**) histology depicts classic intraepidermal blistering characteristic of PV. Reprinted from Culton *et al.* (2015).

in Scheer *et al.*, 2013). Mice have been created to transgenically express urokinase plasminogen activator, which inhibits mouse hepatocyte function so that drug metabolism occurs only through engrafted human hepatocytes (reviewed in Strom *et al.*, 2010). Ever-advancing genetic technologies have given researchers new freedom to push the limits of humanized mice (reviewed in Scheer *et al.*, 2013).

HUMANIZED MICE IN DERMATOLOGY RESEARCH

Humanized mice are increasingly employed in dermatology research. This is due in part to the ease of acquiring normal and diseased human skin for experimentation, of surgically transplanting human skin onto a mouse, and of visualizing/assessing cutaneous disease. So far, humanized mice have been employed to study autoimmune/inflammatory dermatoses such as psoriasis, alopecia areata, pemphigus vulgaris, GVHD, delayed-type hypersensitivity, and atopic dermatitis, genodermatoses such as lamellar ichthyosis, pachyonychia

congenita, and epidermolysis bullosa, cutaneous malignancies, including melanoma and squamous cell carcinoma, and wound healing (Aspord *et al.*, 2014; Aufenvenne *et al.*, 2012; Culton *et al.*, 2015; Garcia *et al.*, 2011; Gilhar *et al.*, 2013; Krasagakis *et al.*, 2001; Patel *et al.*, 2012; reviewed in Petersen and Sorensen, 2008; van den Broek *et al.*, 2014; van Rijn *et al.*, 2003). The experimental approaches in these humanized mouse studies varied, demonstrating that, even within dermatologic research, humanized mouse models are diverse.

The figures show three examples of humanized mouse models utilized in dermatology research. Aspord *et al.* (2014) investigated the effect of the drug imiquimod (Aldara) on melanoma, utilizing a humanized mouse model in which sublethally irradiated immunodeficient mice were engrafted with $CD34^+$ HSCs from umbilical cord blood and later injected subcutaneously with melanoma cell lines (Figure 1). Garcia *et al.* (2011) surgically grafted bioengineered skin equivalents from keratinocytes and skin fibroblasts from patients with the genodermatosis pachyonychia congenita onto the backs of immunodeficient mice to develop a preclinical model of disease (Figure 2). Culton *et al.* (2015) generated transgenic mice expressing human desmoglein 3 (Dsg3) in mice lacking Dsg3 to study the role of anti-Dsg3 immunoglobulin in serum from patients with pemphigus vulgaris (Figure 3).

LIMITATIONS

Despite significant advancements made with humanized mice, these models are not without limitations. From a practical standpoint, the technical expertise required for some models can be quite advanced, and human cell/tissues, for example, fetal tissue, may be difficult to obtain. Purchasing, housing, and/or breeding host mice can be expensive. From a scientific perspective, there continue to be impediments to the development of memory T-cell responses and humoral immunity. Similarly, although human neutrophil, red blood cell, and platelet precursors engraft in mouse bone marrow, they do not circulate in mouse blood in any substantial number. These are in part secondary to the inability of mice to produce human cytokines and growth factors and, as mentioned above, the mismatch between homing ligands and receptors on human immune cells and mouse vessels (for further discussion, see Shultz *et al.*, 2012). Although scientists are working to address these limitations, it is important to remember that even though these mice are humanized, they are still mice.

CONFLICT OF INTEREST

The authors state no conflict of interest.

CME ACCREDITATION

This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of the Duke University School of Medicine and Society for Investigative Dermatology. The Duke University School of Medicine is accredited by the ACCME to provide continuing medical education for physicians. To participate in the CME activity, follow the link provided. Physicians should only claim credit commensurate with the extent of their participation in the activity.

To take the online quiz, follow the link below:
http://continuingeducation.dcri.duke.edu/sid-journal-based-translational-education-dermatology-research-techniques-made-simple-2015

QUESTIONS

This article has been approved for 1 hour of Category 1 CME credit. To take the quiz, with or without CME credit, follow the link under the "CME ACCREDITATION" heading.

For each question, more than one answer may be correct.

1. **Hu-PBL refers to which of the following type of humanized mouse models:**
   - A. Mice transplanted with fetal liver and thymus fragments.
   - B. Immunodeficient mice in which PBMCs or lymphocytes are transplanted with engraftment of mature lymphocytes.
   - C. Immunocompetent mice transplanted with human cells lacking IL-2 expression.
   - D. Immunodeficient mice transplanted with PBMCs or lymphocytes with resultant multilineage hematopoiesis.

2. **Why are immunodeficient mice used as "hosts" for humanized mouse models?**
   - A. Immunocompetent mice have dendritic cells that would reject human cells.
   - B. There is space in the host-mouse bone marrow for engraftment of human cells.
   - C. They lack T, B, and natural killer cells that would otherwise reject human cells.
   - D. Their inability to signal through IL-4, -7, -9, -15, and -22 prevents rejection of human cells.

3. **What immunodeficient strains are most commonly employed for humanized mouse models?**
   - A. SCID and nude mice.
   - B. Rag and nude mice.
   - C. NSG and NOG mice.
   - D. NSG and Rag mice.

4. **Humanized mouse models have been used in which of the following areas of dermatologic research?**
   - A. Pemphigus vulgaris and other autoimmune diseases.
   - B. Wound healing.
   - C. Squamous cell carcinoma and other cutaneous malignancies.
   - D. All of the above.

5. **Which is true of humanized mouse models?**
   - A. They are diverse and in constant development.
   - B. The term refers to mice containing functional human proteins, cells, tissues, and organs.
   - C. They may also be called "human–mouse chimeras" or "xenotransplants" in the case of mice containing human cells or tissues.
   - D. All of the above.

SUPPLEMENTARY MATERIAL

A PowerPoint slide presentation appropriate for journal club or other teaching exercises is available at http://dx.doi.org/10.1038/jid.2015.393.

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