Animal Models for Alopecia Areata: What and Where?

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Disease is not limited to humans. Rather, humans are but another mammal in a continuum, and as such, often share similar if not identical diseases with other mammalian species. Alopecia areata (AA) is such a disease. Natural disease occurs in humans, nonhuman primates, many domestic animals, and laboratory rodents. However, to be useful as models of human disease, affected animals need to be readily available to the research community, closely resemble the human disease, be easy to work with, and provide reproducible data. To date, the laboratory mouse (most if not all of the C3H substrains) and the Dundee experimental bald rat fit these criteria. Manipulations using full-thickness skin grafts or specific immune cell transfers have improved the models. New mouse models that carry a variety of genetic-based immunodeficiencies can now be used to recapitulate the human immune system and allow for human full-thickness skin grafts onto mice to investigate human-specific mechanistic and therapeutic questions. These models are summarized here including where they can currently be obtained from public access repositories.

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INTRODUCTION

Alopecia areata (AA) is a cell-mediated autoimmune disease that targets anagen-stage hair follicles in many mammalian species (Tables 1 and 2) (Sundberg *et al.*, 1995; McElwee and Hoffmann, 2002) and possibly even feathers in chickens (Smyth and McNeil, 1999). In humans, this disease has been subdivided clinically into alopecia areata (patchy hair loss), alopecia totalis (hair loss on the head), and alopecia universalis (total body hair loss). Similar forms exist in other mammals affected with this disease. While reported in a variety of domesticated species, these are usually single-case reports that do not make them useful as animal models for drug efficacy trials (Table 2). However, small groups can be found through dermatologists at many veterinary colleges and, as such, may provide additional species for limited drug efficacy testing before going to human clinical trials.

RODENT MODELS FOR ALOPECIA AREATA

A useful animal model should accurately recapitulate the human disease, be readily available, and provide reproducible results. A number of such models have been developed, primarily in laboratory rodent species, several of which are commonly used and validated by many different laboratories, most notably the C3H/HeJ mouse (Sundberg *et al.*, 1994; McElwee *et al.*, 1998) and Dundee experimental bald rat (DEBR) (Michie *et al.*, 1991; Oliver *et al.*, 1991). All animal models provide benefits as well as limitations. Therefore, new models are constantly being sought to address shortcomings, but also to determine whether results can be reproduced before proceeding to human clinical trials.

What are the models of choice and how does one find them? Table 3 summarizes those that are available and repositories where the animals can be obtained. Some contract services are available that will perform preclinical screening for those without such resources.

HUMAN XENOGRAFT MODELS

Human xenografts offer the potential for grafting skin onto immunodeficient mice and then providing human immune system cells (Gilhar *et al.*, 2012, 2013). The initial studies have yet to be reproduced in other laboratories and rigorously confirmed, partly because few investigators have access to human tissue and facilities for housing immunodeficient mice. New combinations of mutations causing immunodeficiency are being created on genetically selected (congenic or inbred mouse strains) and pathogen-free stocks, which are already opening up new directions for xenografts (patient-derived xenografts for human tumors onto immunodeficient mice). Those currently heavily used are the NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (commonly referred to as the NSG mouse) or NOD.Cg-Rag1^{tm1Mom} *Il2rg*^{tm1Wjl}/SzJ (NRG mouse). A significant advancement in the creation of humanized mice occurred with the introduction of targeted mutations within the interleukin (IL) 2 receptor common gamma chain (*Il2rg*) gene into inbred strains carrying mutations for specific

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Abbreviations: AA, alopecia areata; DEBR, Dundee Experimental Bald Rat; Il2rg^{tm1Wjl}*, interleukin 2 receptor, gamma chain null; Lyst*^{bg}*, lysosomal trafficking factor also known as the beige mouse allele; NRG, NOD.Cg-Rag1*^{tm1Mom}*, Il2rg*^{tm1Wjl}*/SzJ; NSG, NOD.Cg-Prkdc*^{scid} *Il2rg*^{tm1Wjl}*/SzJ; Prkdc*^{scid}*, protein kinase, DNA activated, catalytic polypeptide, severe combined immunodeficiency allele*

Table 1. Animal models with AA or for use with xenografts

Species	Spontaneous	Graft induced	Cell induced	Xenograft	References
C3H/HeJ[1] mouse	Adult onset Wax and wanes	Full-thickness skin graft, 8–10-week progressive	See below	NA	Sundberg *et al.*, 1994; McElwee *et al.*, 1998; Silva and Sundberg, 2013
C3H/HeN[1] mouse	Adult onset	NT	NT	NA	McElwee *et al.*, 1999
C3H/HeJ mouse	NA	NA	8–12 week progressive	NA	Wang *et al.*, 2015
A/J mouse	12–18 months	25+ weeks	NA	NA	McElwee *et al.*, 1999
Prkdc^{scid}, *Lyst*^{bg}	NT	NT	NA	Human skin graft plus human immune cells	Gilhar *et al.*, 2013
NOD/ShiLtSz/J-*Prkdc*^{scid}, *Il2rg*^{tm1Wtl} (NSG)	NT	NT	NA	Human skin graft plus human immune cells	Predicted, not tested
NOD/ShiLtSz/J-*Rag1*^{tm1Mom}, *Il2rg*^{tm1Wtl} (NRG)	NT	NT	NA	Human skin graft plus human immune cells	Predicted, not tested
NSG/NRG plus other mutations	NA	Future	Future	Future	Future
DEBR	Adult onset Wax and wanes	NT	NT	NA	Michie *et al.*, 1990; McElwee, 2004

Abbreviations: AA, alopecia areata; DEBR, Dundee experimental bald rat; NA, not applicable or recipients are not histocompatible with xenografts; NT, not tested.

[1]Most if not all C3H substrains react in a similar manner.

Table 2. Case studies suggesting alternative species for preclinical trials with small numbers

Species	Spontaneous	References
Dogs	Adult onset	Tobin *et al.*, 2003; Tobin and Olivry, 2004
Horses	Adult onset	Colombo *et al.*, 2004; Bruet *et al.*, 2008; Hoolahan *et al.*, 2013; Rosychuk, 2013
Cattle	Adult onset	Timm *et al.*, 2010; Valentine *et al.*, 2012
Nonhuman primates	Adult onset	Beardi *et al.*, 2007

Table 3. Sources of animal models for AA research

Animal	Source	Special order/Contract labs
C3H/HeJ normal (JR# 659)	The Jackson Laboratory, Bar Harbor, ME (http://jaxmice.jax.org/)	Production strain readily available.
C3H/HeNCrl normal (stain code 025)	Charles River Laboratories, Wilmington, MA (http://www.criver.com/products-services/)	Production strain readily available. No information available on AA in colony.
C3H/HeNHsd normal (strain code 040)	Harland Laboratories (http://www.harlan.com/)	Production strain readily available. No information available on AA in colony.
A/J normal (JR# 646)	The Jackson Laboratory, Bar Harbor, ME (http://jaxmice.jax.org/)	Production strain readily available.
C3H/HeJ with AA	Production strain readily available.	Special order for either spontaneous or graft induced AA. The Jackson Laboratory, *In Vivo* Services, Sacramento, CA
A/J with AA	Production strain readily available.	Special order for spontaneous AA.
NOD/ShiLtSz/J-*Prkdc*^{scid}, *Il2rg*^{tm1Wtl} (NSG, JR#5557)	The Jackson Laboratory, Bar Harbor, ME (http://jaxmice.jax.org/)	Production strain readily available.
NOD/ShiLtSz/J-*Rag1*^{tm1Mom}, *Il2rg*^{tm1Wtl} (NRG; JR#7799)	The Jackson Laboratory, Bar Harbor, ME (http://jaxmice.jax.org/)	Production strain readily available.
DEBR rat	Dr HC Hennies, Center for Dermatogenetics, Medizinische Universität Innsbruck, Innsbruck, Austria (h.hennies@i-med.ac.at)	Embryos frozen at Charles River Laboratories (quantities very limited)
Dogs	NRA	Contact dermatologists at veterinary colleges
Horses	NRA	Contact dermatologists at veterinary colleges
Cattle	NRA	Contact dermatologists at veterinary colleges
Nonhuman primates	NRA	Contact zoos or nonhuman primate centers

Abbreviations: AA, alopecia areata; DEBR, Dundee experimental bald rat; NRA, not readily available.

immunodeficiencies including *Prkdc*^{scid}, *Rag1*^{tm1Mom}, or *Rag2*^{tm1Fwa} (Shultz *et al.*, 2007). The Il2RG chain is a critical component of the IL2, IL4, IL7, IL15, and IL21 high-affinity ligand-binding receptors (Sugamura *et al.*, 1996; Rochman *et al.*, 2009). The absence of this gene leads to severe impairments in T- and B-cell development and function, completely prevents natural killer (NK) cell development, and results in multiple other defects in innate immune function (Cao *et al.*, 1995; Shultz *et al.*, 2005). Immunodeficient mice bearing a mutated *Il2rg* gene support greatly enhanced engraftment of human hematopoietic stem cells (Shultz *et al.*, 2005), peripheral blood mononuclear cells (King *et al.*, 2008), and tissues (Stoddart *et al.*, 2011) as compared with previous models. The base NSG and NRG mouse strains are continuing to be improved by genetic manipulation that results in the introduction of targeted mutations or genes encoding human-specific growth factors and cytokines. For example, the beige mutation (*Lyst*^{bg}) was recently added to the NSG mouse model (L. Shultz, personal communication). Variations of these models in which specific human HLA genes are added, and the corresponding mouse H2 alleles removed, provide even more promise to address very specific immunological questions. These and other immunodeficient mice will provide novel tools for unraveling the complexity of the pathogenesis of AA in the near future.

CONFLICT OF INTEREST

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