A New Humanized Mouse Model for Alopecia Areata

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Although alopecia areata (AA) is not life threatening, it may lead to severe psychological disturbances, reducing the quality of life in all ages. Thus, a new animal model is needed for shedding more light onto the pathogenesis of this cell-mediated, organ-specific autoimmune disease to identify more effective therapeutic strategies. Recently, we succeeded in developing a new humanized mouse model of AA, which includes transplantation of healthy human scalp skin obtained from normal volunteers on to severe-combined immunodeficient mice. This is followed by intradermal injection of either autologous or allogeneic peripheral blood mononuclear cells, which had been cultured with high dose of IL-2 and enriched for natural killer group 2D–positive (NKG2D+) and CD56+ cells. This protocol leads to rapid and predictable development of focal hair loss, with all the characteristic clinical, histological, and immunohistochemical features of AA. This humanized mouse AA model underscores the functional importance of NKG2D+ and CD56+ cells in AA pathogenesis and promises to be instrumental for identifying novel AA treatment strategies.

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Although animal models are an important tool in the ongoing challenge to understand the pathogenesis of various autoimmune diseases, for many of these, optimal animal models with sufficient relevance to the human condition remain to be developed. Alopecia areata (AA) exemplifies this problem, as the best-established mouse model (Sundberg *et al.*, 1994) of this CD8+ T cell–dependent, organ-specific autoimmune disease (Bertolini *et al.*, 2012; Gilhar *et al.*, 2012a), whose pathogenesis has not been fully resolved yet, and is not entirely satisfactory.

For example, spontaneously occurring AA–like hair loss develops only very late and rather unpredictably, and in a very low percentage of mice; also, in the aging C3H/HeJ mouse model of AA, the inflammatory infiltrate occurs in an untypical perifollicular location; even though the occurrence of AA–like lesions can be accelerated by transplantation of lesional skin to as yet unaffected young mice (McElwee *et al.*, 1998; Freyschmidt-Paul *et al.*, 2006), the problem remains that C3H/HeJ mice display major constitutive immunological abnormalities, such as a toll-like receptor-4 signaling defect (e.g., Gosemann *et al.*, 2010; Harlow *et al.*, 2012) that is not a feature of human AA. Disease onset, which is characterized by—typically focal—loss of hair in any hair-bearing area (Gilhar *et al.*, 2012a)—is followed by an unpredictable clinical course, and current treatment options are limited, purely symptomatic, and overall unsatisfactory (Harries *et al.*, 2010; Gilhar *et al.*, 2012a). Although the disease is not life threatening, it may lead to severe psychological disturbances, reducing the quality of life in patients of all age groups and both genders (Inui *et al.*, 2013), which is aggravated by the lack of satisfactory therapy. While hair follicle immune privilege collapse, CD8+ T cells, CD56+ natural killer (NK) cells, and other natural killer group 2D-positive (NKG2D+) cell populations as well as IFN-γ secretion by these cells are all appreciated to have a key role in AA pathogenesis (Paus *et al.*, 2005; Freyschmidt-Paul *et al.*, 2006; Ito *et al.*, 2008; Kang *et al.*, 2010; Petukhova *et al.*, 2010), the exact pathogenesis of AA remains unknown (Bertolini *et al.*, 2012; Gilhar *et al.*, 2012a).

Thus, a new, clinically relevant animal model is needed for shedding more light on the pathogenesis of AA and as a preclinical screening tool for probing novel, more effective therapeutic strategies. Recently, we succeeded in developing humanized mouse model that meets these aims (Gilhar *et al.*, 2012b). This model is based on three previous observations: (1) AA can be transferred to human scalp skin transplants on severe-combined immunodeficient (SCID) mice by injection of lesional T cells, namely CD8+ lymphocytes, which had been cultured with human hair follicle homogenate enriched for melanocyte-related autoantigens (Gilhar *et al.*, 2001). (2) AA patients exhibit an overexpression of NK–activating receptors (NKG2D) and NKG2D and their activating ligands (MICA, ULBP3) (Ito *et al.*, 2008; Petukhova *et al.*, 2010). (3) The NKG2D receptor, which is expressed by NK, NKT cells, and subsets of other, mostly CD8+, T cells is known to be involved in the pathogenesis of some other autoimmune diseases including diabetes mellitus and rheumatoid arthritis (Gilhar *et al.*, 2012a).

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Abbreviations: AA, alopecia areata; NK, natural killer; NKG2D, natural killer group 2D; PMBC, peripheral blood mononuclear cell; SCID, severe-combined immunodeficient

In the new humanized AA mouse model, healthy human scalp skin obtained from normal volunteers is transplanted onto SCID mice, followed by intradermal injection of either autologous or allogeneic peripheral blood mononuclear cells (PBMCs). The PBMCs had been cultured with high dose of IL-2 before the injections into the grafts. Flow cytometric analysis and *in vitro* study revealed that incubation of PBMCs with high dose of IL-2 induced lymphocytes to express NK phenotype, including high levels of NKG2D and CD56 (Gilhar *et al.*, 2012b).

Injection of these NK–like cells into allogeneic or autologous hair-bearing human skin grafts transplanted onto SCID mice led to the rapid and predictable development of hair loss lesions with all the characteristic clinical, histological, and immunohistological features of human AA. Hair loss was observed only in grafts injected with the NK–like cells 3–5 weeks following injections but not in the control groups. (These controls included PBMCs cultured with phytohemagglutinin, which induces polyclonal PBMCs activation, instead of high-dose IL-2 (PBMCs depleted of CD56+ cells; for details, see Gilhar *et al.*, 2012b)).

The humanized mouse model of AA permits the predictable and rapid induction of a clinical, histological, and immunohistological phenocopy of human AA, notably in previously healthy human hair follicles within their natural tissue habitat *in vivo* (albeit on immunocompromised SCID mice). Moreover, this animal model is relatively easy to setup as it requires only blood and scalp skin from healthy volunteers, rather than from AA patients. Furthermore, this novel animal model of human AA provides the first functional evidence that NKG2D+ cells as well as NKG2D–stimulating ligands such as MICA and ULPB3 have a crucial role in primary AA pathogenesis (Ito *et al.*, 2008; Petukhova *et al.*, 2010). In addition, as normal, IL-2-stimulated PBMCs from healthy volunteers suffice to induce an AA phenocopy, this questions how important constitutive genetic abnormalities really are in primary AA pathogenesis and refocuses attention on the secretory activities of the (IFN-γ-secreting) perifollicular infiltrate in AA (Gilhar *et al.*, 2012b).

Finally, and perhaps most importantly, this novel mouse model provides an instructive new preclinical screening device of unparalleled clinical relevance for exploring the therapeutic effects of various candidates as future anti-AA agents, and has already allowed us to identify Kv 1.3 blockers as a promising novel class of candidate AA therapeutics (Gilhar *et al.*, 2013).

This new model also provides an intriguing opportunity to instructively probe the effects of different types of stressors leading to neurogenic skin inflammation (e.g., Arck *et al.*, 2006; Paus and Arck, 2009) on the development of AA.

Systematic use of this model is expected to quickly identify additional new candidates, ideally including drugs that are already in clinical use and could be easily repositioned for AA management.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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