The Evolving Pathogenesis of Alopecia Areata: Major Open Questions

Ralf Paus[1,2,3]

Journal of Investigative Dermatology Symposium Proceedings (2020) **20,** S6–S10; doi:10.1016/j.jisp.2020.04.002

INTRODUCTION

As each time before, the 2018 National Alopecia Areata Foundation Summit Meeting once again provided an excellent opportunity to reconsider important frontiers in current alopecia areata (AA) research. In the following, I comment on selected major open questions in the rapidly evolving field of AA pathobiology (Dainichi and Kabashima, 2017; Gilhar et al., 2016; McElwee et al., 2013; Paus et al., 2018; Pratt et al., 2017), with emphasis on a much-needed, pathobiology-based reclassification of AA, biomarkers, and the potential role of atopy in AA.

ALOPECIA AREATA: HOW MANY DISEASES?

Although typically viewed as a single disease entity, the variable presentation phenotypes and rather different prognostic features of various clinical presentations of AA have long questioned that AA represents a single disease entity. The perhaps somewhat eclectic classification of different AA types that Ikeda had suggested more than 50 years ago (Ikeda, 1965) has not caught on in the field, which continues to distinguish only phenotypic variants based on the extent and distribution of hair loss lesions (focal AA, multifocal AA, AA totalis, AA universalis, diffuse AA) (Gilhar et al., 2012; Strazzulla et al., 2018; www.naaf.org). However, the rather striking differences in AA phenotype and prognosis (see below) that we encounter in AA patients render it unlikely that the traditional concept of AA as a single disease entity, which still transcends most reviews on the topic, is a viable one.

The characteristic AA hair loss phenotype consists of initially focal alopecia in clinically normal-appearing skin, cadaver and/or exclamation mark hairs, and regrowth of white hairs, often associated with nail pitting or nail dystrophy (Gilhar et al., 2012). What we do know so far is that the acute AA hair loss phenotype requires the following: (i) the presence of an IFNγ-secreting perifollicular inflammatory cell infiltrate around the bulb of anagen hair follicles (HFs), (ii) collapse of the anagen HFs' physiological immune privilege, (iii) major HF dystrophy leading to hair shaft shedding, and typically associated with (iv) premature HF regression (catagen induction).

All currently available evidence suggests that the characteristic AA hair loss phenotype simply does not occur without this peculiar constellation (Gilhar et al., 2012; Paus et al., 2018). Notably, however, with increasing chronicity, the character of the AA phenotype dynamically changes from an anagen effluvium in acute, rapidly progressing AA into a telogen effluvium with premature exogen in the chronic stage (Zhang et al., 2020). We also know that CD8+ T cells alone suffice to elicit AA-like hair loss lesions, whereas autoantibodies directed against HF antigens fail to do so (de Jong et al., 2018; Gilhar et al., 2012,1998).

However, certain subpopulations of autologous activated NK cells can also induce AA lesions in previously healthy human skin in vivo, without the requirement of a defined disease-predisposing genetic constellation and probably even in an autoantigen-independent manner (Gilhar et al., 2013, 2016; Paus et al., 2018). That a Koebner-like triggering of AA lesions can sometimes be seen (d'Ovidio, 2013) further supports that (auto-)antigen-specific autoimmunity is not a *conditio sine qua non* for developing the AA hair loss phenotype and that rather non-specific HF damage suffices to elicit AA—as long as this leads to a collapse of HF immune privilege and above-threshold dystrophy of the affected anagen HF. This finding has invited the hypothesis that AA is not necessarily a distinct disease entity, but primarily represents a *stereotypic response pattern* that even healthy anagen HFs will demonstrate if conditions 1–4 (see above) coincide, irrespective of genetic predisposition and autoantigen expression status (McElwee et al., 2013; Paus et al., 2018).

AAA VERSUS NON-AAA

This hypothesis further stipulates that only in those AA patients, where there is evidence for a specific CD8+ T cell-dependent autoimmune response (e.g., where specific intralesional autoreactive CD8+ T cells against HF-associated autoantigens can be demonstrated [Bertolini et al., 2015; de Jong et al., 2018]), this AA response pattern results from a genuine autoimmune disease. For this variant, we have suggested the term "autoimmune alopecia areata" (AAA). Patients with a positive personal and/or family history of other autoimmune disorders, frequently relapsing and/or rapidly progressive AA may be top candidates

[1]*Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, Florida, USA;* [2]*Centre for Dermatology Research, University of Manchester, and NIHR Biomedical Research Centre, Manchester, United Kingdom; and* [3]*Monasterium Laboratory, Muenster, Germany*

Correspondence: Ralf Paus, Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, 1600 NW 10th Avenue, RMSB, Room 2023A, Miami, FL 33136, USA. E-mail: rxp803@med.miami.edu

Abbreviations: AA, alopecia areata; AAA, autoimmune alopecia areata; AD, atopic dermatitis; HF, Hair Follicle

for showing the AAA variant (Paus et al., 2018).

Only in this subpopulation of AA patients, it makes sense to search for pathogenic autoantigens and autoreactive T cells, whether or not these autoantigens are melanocyte and/or melanogenesis-related (Gilhar et al., 2001; Paus et al., 1993) or are derived from other antigens produced by anagen HFs (Lindestam Arlehamn et al., 2018; Wang et al., 2016). That preexisting endogenous T cell immunity against melanocyte-associated antigens has recently been detected in the PBMCs of healthy human donors (Przybyla et al., 2019) and that CD8-T cell-driven autoimmune responses against human epidermal melanocytes in vitiligo and halo nevi as well as against human melanoma cells are well-documented phenomena (Strassner and Harris, 2016), only underscores that peptides derived from (stressed?) HF melanocytes in melanin-producing anagen HFs remain very plausible contenders as pathogenic autoantigens in AAA patients (Paus et al., 2018).

BIOMARKERS

To identify reliable biomarkers for distinguishing the AAA subgroup from other pathobiologically distinct AA patient subpopulations is clinically important. Only in the former the development of curative therapy (i.e., therapeutic strategies aimed at re-establishing peripheral tolerance against HF-associated autoantigens [Oelert et al., 2017] and/or at eliminating autoreactive CD8+ T cells) makes sense and is indeed required to achieve lasting treatment success (Bertolini et al., 2015); instead, in all other AA subpopulations, symptomatic therapy should be sufficient, namely one that manages to re-establish and maintain HF immune privilege, a prerequisite for protecting the HF from further immune attacks and thus for lasting hair regrowth (Paus et al., 20018, 2005). Therefore, the field is challenged to develop molecular biomarkers that permit one to reliably distinguish AAA patients from other non-autoimmune forms of AA with a good prognosis (see below), in which intermittent, less aggressive, and topical forms of AA treatment may well suffice.

A relatively short treatment course with Jak inhibitors (Wang et al., 2018) may be indicated and justified even in patients that belong to the non-AAA group, if they show extensive, therapy-resistant hair loss and rapid disease progression, so as to facilitate the re-establishment of HF immune privilege. Instead, the potential adverse effects of these rather non-specific immunoinhibitory agents (Gilhar et al., 2019) question whether systemic long-term therapy can be justified in the non-AAA subgroup, not the least since relapse after therapy discontinuation is the rule. Therefore, even though Jak inhibitors may work in all variants of AA, at least in a sizable majority of patients (Phan and Sebaratnam, 2019; Wang et al., 2018), it is clinically important to be able to robustly distinguish pathobiologically distinct AA subgroups through the analysis of sensitive, affordable, and predictive biomarkers. Hopefully, the availability of such biomarkers will overcome the limitations of the currently practiced, unsatisfactory AA classification scheme and permits one to replace the proposed Ikeda classification (Ikeda, 1965) with a new, pathobiologically more convincing and better-defined one.

However, for the time being, indicators derived from clinical examination, such as the extent, distribution and progression of hair loss lesions, associated ophiasis or nail dystrophy, and the presence of atopy, along with thorough history taking (e.g., age of first AA onset, family history of AA, associated autoimmune disease) (Gilhar et al., 2012; Strazzulla et al., 2018) contain to provide the most reliable prognostic indicators. Therefore, any proposed biomarkers of disease severity, therapy response, and/or prognosis (e.g., Czarnowicki et al., 2019; Gong et al., 2020; Jabbari et al., 2016; Jang et al., 2017; Song et al., 2018) are challenged to demonstrate that they are indeed more informative, more reliable, and also more cost-efficient than unglamorous and non-molecular, but highly instructive clinical examination and history taking.

WHAT IS THE MOLECULAR AND CELLULAR BASIS OF THE DIVERGENT PROGNOSIS IN AA?

AA research is challenged with having to come up with plausible explanations why not only the extent and distribution of AA lesions but also the disease prognosis differs so substantially between affected individuals. For example, this ranges from a relatively high chance of spontaneous hair regrowth in non-atopic patients whose first hair loss episode occurred after puberty and without associated nail dystrophy or family history for AA and other autoimmune diseases, to a poor prognosis for spontaneous disease remission in pre-pubertal atopic patients with a family history for AA or other autoimmune diseases (Gilhar et al., 2012; Strazzulla et al., 2018). In addition, we still lack a satisfactory mechanistic explanation why exactly AA lesion topography (e.g., the presence of ophiasis or nail involvement) are reliable prognostic markers (Lee et al., 2019; Roest et al., 2018), and why various comorbidities (Lee et al., 2019a, 2019b) such as Down Syndrome and lupus erythematosus are associated with a negative prognosis.

Elucidating the molecular and cellular basis for these prognostic differences predictably will also shed further light on AA pathobiology in general and is expected to support further that the AA phenotype cannot be viewed as one homogeneous disease entity (Ikeda, 1965; McElwee et al., 2013; Paus et al., 2018). Personal history for atopic diseases, namely atopic dermatitis (AD), in a given patient with AA, is widely accepted as a robust negative prognostic marker (Gilhar et al., 2012; Ikeda, 1965; Strazulla et al., 2018). However, again, the underlying pathobiologic link remains quite obscure.

One central problem here is that the molecular nature of "atopy" and the "atopic diathesis" remains very poorly understood (Röcken et al., 1998) and that even the most authoritative and comprehensive reviews on AD (e.g., Nakajima et al., 2019; Weidinger et al., 2018) tend to ignore some important neurophysiological facets, such as the defective beta$_2$-adrenergic signaling in many AD patients (Archer et al., 1985; Röcken et al., 1998; Schallreuter et al., 2007; Sivamani et al., 2007). Until it has been clarified whether or not each of the candidate key players in AD pathobiology plays into the worse prognosis of those AA patients who also have AD, it appears prudent to not rule

out any potential contributing factor, including neurophysiological abnormalities like a beta$_2$-adrenergic defect.

Mast cells and eosinophils may be a good starting point for dissecting mechanistically why atopic individuals with AA have a worse prognosis. Although the presence of eosinophils may not be of much help for distinguishing AA from other differential diagnoses upon biopsy of long-standing AA cases (Yoon et al., 2014), the number of perifollicular eosinophils and mast cells is significantly increased in a large percentage of lesional AA skin samples (Bertolini et al., 2014; Zhang et al., 2015, 2013). This finding raises the possibility that these classical atopy- and AD-associated immunocytes (Weiniger et al., 2018) play a more important role in AA pathobiology than has previously been appreciated, and may contribute to the poorer prognosis seen in atopic AA patients—perhaps mediated in part also by beta$_2$-adrenergic signaling defects in mast cells and/or eosinophils (Schallreuter et al., 2007).

BEYOND THE CD8+ T CELL HORIZON

Not much attention in the field has been given yet to the fact that the number and percentage of degranulated perifollicular mast cells are significantly higher in lesional compared with non-lesional AA skin, that these cells proliferate abnormally, show greatly increased physical contacts with CD8+ T cells, and switch from their physiological, immunoinhibitory to a profoundly proinflammatory, autoimmunity-promoting phenotype in human lesional AA skin (Bertolini et al., 2014). This intriguing observation further encourages one to systematically explore the relative functional contribution of mast cells to AA prognosis, namely in atopic patients, and their impact on the response to therapy. This finding is further supported by most recent insights from the murine system on the intimate relationship between mast cells, the skin microbiome, and the presence and/or absence of a functional HF epithelium (Wu et al., 2019).

I expect that one of the most intriguing but essentially uncharted frontiers in AA immunopathobiology will arise from a deeper exploration of the role that the—as yet insufficiently characterized—human HF microbiome and its dysbiosis may play in the maintenance, damage, collapse and restoration of HF immune privilege. A more decidedly microbiological focus in AA research is further supported by the potential therapeutic effect of fecal microbiota transplants (Rebello et al., 2017) and the currently discussed role of intestinal dysbiosis in AA (Borde and Åstrand, 2018). Recent murine studies on the key role commensal microbes and the HF-derived chemokine production controlled by them in the recruitment of regulatory T cells into neonatal skin (Scharschmidt et al., 2017) and the demonstration that HF keratinocyte-derived cytokines maintain skin-resident innate lymphocyte subpopulations which subsequently modulate the skin microbiome by regulating sebocyte functions and sebum production (Kobayashi et al., 2019) encourage one only further to explore the as yet unknown role of the HF microbiome and its dysbiosis in human AA pathobiology (Polak-Witka et al., 2019; Lousada et al., 2020).

Mast cells and eosinophils are not the only cells urging us to look beyond the CD8+ T cell horizon in AA research. It has been demonstrated in the C3H/HeJ mouse and in the humanized AA mouse model that CD8+ T cells suffice to elicit the disease, but that AA severity and extent are driven by additional inputs from CD4+ T cells (Gilhar et al., 2002, 1998; McElwee et al., 2005). In this, regulatory T cells may play an important modulatory role (Bae et al., 2017; McElwee et al., 2005; Zöller et al., 2018). Given the focal nature of AA, and the not uncommon recurrence of hair loss lesions in some of the same locations as during the preceding hair loss event, one can't help suspecting that resident memory T cells (Boniface et al., 2018; Clark, 2015; Wu et al., 2018) also are involved in AA pathobiology and deserve to be targeted. However, the functional contribution of regulatory T cells and resident memory T cells to human AA and its prognosis is currently unknown and awaits systematic analysis.

Evidence from human skin biopsies (Ito et al., 2008), GWAS analysis (Petukhova et al., 2010), and the humanized mouse model of AA (Gilhar et al., 2016) has already pointed to a major functional role of NK cells in AA pathobiology, given that distinct subpopulations can either promote or inhibit AA in vivo (Ghraieb et al., 2018; Gilhar et al., 2013; Kaufman et al., 2010). As yet unpublished work from my group suggests that gamma and/or delta T cells also play an important, non-antigen-specific role in triggering HF immune privilege collapse and AA hair loss lesions (Uchida et al., unpublished study/under review). Given that skin-resident innate lymphocyte subpopulations suffice to induce psoriasis lesions in healthy human skin transplants on SCID mice (Keren et al., 2018), the Gilhar Laboratory currently examines whether innate lymphocyte subpopulations are also be involved in AA pathobiology and prognosis.

Thus, the more one shifts the attention in AA research away from antigen-specific autoreactive α/ß T cells that recognize intrafollicular, major histocompatibility complex Class I-presented autoantigens (be they melanocyte-associated or not), to "innate" immunocytes, especially when investigating non-AAA variants, the more one is likely to uncover that there are many different routes along which the four-pronged HF damage constellation can be generated that ultimately promotes the AA hair loss phenotype (see above). As a consequence, an optimal personalized medicine approach to future AA management would seem to require that the chosen therapy is tailored to the specific pathogenesis pathway, which has induced the AA hair loss phenotype in a given patient (Bertolini et al., 2020).

That, in turn, would require a set of—as yet unavailable—diagnostic biomarkers that permit the identification of the distinct pathogenesis pathways underlying the AA response pattern at hand in any given patient. Developing such a set of reliable, AA subtype-specific diagnostic biomarkers, some of which may double as prognostic biomarkers, is a tall order, but should be methodologically achievable in principle. Given that AA is a profoundly territorial hair loss phenotype, where "location" and topo(patho)biology is all that matters in terms HF immunopathology (Paus et al., 2018, 2005; Bertolini et al., 2020), this will require a skin biopsy, while blood and/or serum-derived biomarkers may serve mainly as auxiliary parameters.

THE CENTRAL IMPORTANCE OF HF IMMUNE PRIVILEGE PROTECTION AND MAINTENANCE

Irrespective of when truly convincing AA biomarkers will eventually emerge, all currently available evidence suggests that the HF damage response pattern that we over-simplistically call "AA" does not develop without the relative physiological immune privilege of the HF having collapsed, first. Therefore, therapeutically, it remains the primary challenge in successful AA management to prevent immune privilege collapse (to stop phenotype progression) and to restore it lastingly (to enable the anagen HF to recreate an immune milieu in which it can safely generate a pigmented hair shaft without being immediately attacked again by a peribulbar inflammatory cell infiltrate) (Gilhar et al., 2012; McElwee et al., 2013; Paus et al., 2018). Although this remains to be formally documented, it is likely that Jak inhibitors achieve this quite effectively—albeit not in all AA patients, typically only transitorily, and not without the risk of adverse effects (Gilhar et al., 2019).

Therefore, in my personal view, the top priority of therapy-oriented translational AA research still is to develop universally effective, well-tolerated, and cost-efficient HF immune privilege guardians and to understand much better than we currently do how the intrafollicular production of such agents is regulated at the molecular level. I remain convinced that, among exogenous agents, topical FK506 (tacrolimus), if administered in a vehicle that manages to transport an effective drug concentration to the anagen hair bulb, and systemic "superpotent" α-melanocyte-stimulating hormone analogs are particularly attractive candidate therapeutics in this regard (Ito et al., 2004; Paus et al., 2018).

Perhaps, the most appealing therapeutic strategy is however to develop topically applicable agents that upregulate the intrafollicular production of potent endogenous immune privilege guardians such as α-melanocyte-stimulating hormone, TGFb1/2, IL-10, and IGF-1 by HF keratinocytes, or that promote the release of immunoinhibitory neuropeptides such as vasoactive intestinal peptide and calcitonin gene-related peptide from perifollicular nerve endings in and around lesional AA skin (Bertolini et al., 2016, 2020; Kinori et al., 2012; Paus et al., 2018). The systematic search for such agents promises to be quite productive for improving future AA management.

No matter how many key questions in AA pathobiology will remain open, by when we shall finally have elaborated a satisfactory, pathobiology-based reclassification of AA, and will have identified corresponding biomarkers, if all we achieve in the foreseeable future is to gain mastery over the protection and restoration of HF immune privilege, this will predictably usher in a new level of efficacy in the therapeutic management of the AA hair loss phenotype.

ORCID

Ralf Paus: https://orcid.org/0000-0002-3492-9358

CONFLICT OF INTEREST

The author states no conflict of interest.

ACKNOWLEDGMENTS

This article is published as part of a supplement sponsored by the National Alopecia Areata Foundation.

Funding for the Summit and publication of this supplement was provided by the National Alopecia Areata Foundation. This Summit was supported (in part) by the National Institute of Arthritis and Musculoskeletal and Skin Diseases under Award Number R13AR074890. The opinions or views expressed in this professional supplement are those of the authors and do not necessarily reflect the official views, opinions or recommendations of the National Institutes of Health or the National Alopecia Areata Foundation.

REFERENCES

Archer CB, Hanson JM, Morley J, MacDonald DM. Adrenoceptor function in atopic dermatitis: in vitro and in vivo observations. Acta Derm Venereol Suppl (Stockh) 1985;114:93–7.

Bae JH, Hwang WS, Jang YJ, Lee YH, Jang DE, Kim JS, et al. CD80CD86 deficiency disrupts regulatory CD4+FoxP3+T cell homoeostasis and induces autoimmune-like alopecia. Exp Dermatol 2017;26:1053–9.

Bertolini M, Pretzlaff M, Sulk M, Bähr M, Gherardini J, Uchida Y, et al. Vasoactive intestinal peptide, whose receptor-mediated signalling may be defective in alopecia areata, provides protection from hair follicle immune privilege collapse. Br J Dermatol 2016;175:531–41.

Bertolini M, Uchida Y, Paus R. Toward the clonotype analysis of alopecia areata-specific, intralesional human CD8+ T lymphocytes. J Investig Dermatol Symp Proc 2015;17:9–12.

Bertolini M, Zilio F, Rossi A, Kleditzsch P, Emelianov VE, Gilhar A, et al. Abnormal interactions between perifollicular mast cells and CD8+ T-cells may contribute to the pathogenesis of alopecia areata. PLOS ONE 2014;9:e94260.

Bertolini M, McElwee K, Gilhar A, Bulfone-Paus S, Paus R. Hair follicle immune privilege and its collapse in alopecia areata. Exp Dermatol 2020; https://doi.org/10.1111/exd.14155 (accessed 9 September 2020).

Boniface K, Jacquemin C, Darrigade AS, Dessarthe B, Martins C, Boukhedouni N, et al. Vitiligo skin is imprinted with resident memory CD8 T cells expressing CXCR3. J Invest Dermatol 2018;138:355–64.

Borde A, Åstrand A. Alopecia areata and the gut-the link opens up for novel therapeutic interventions. Expert Opin Ther Targets 2018;22:503–11.

Clark RA. Resident memory T cells in human health and disease. Sci Transl Med 2015;7:269rv1.

Czarnowicki T, He H, Leonard A, Kim HJ, Kameyama N, Pavel AB, et al. Blood endotyping distinguishes the profile of vitiligo from that of other inflammatory and autoimmune skin diseases. J Allergy Clin Immunol 2019;143:2095–107.

Dainichi T, Kabashima K. Alopecia areata: what's new in epidemiology, pathogenesis, diagnosis, and therapeutic options? J Dermatol Sci 2017;86:3–12.

de Jong A, Jabbari A, Dai Z, Xing L, Lee D, Li MM, et al. High-throughput T cell receptor sequencing identifies clonally expanded CD8+ T cell populations in alopecia areata. JCI Insight 2018;3:pii:121949.

D'Ovidio R. Koebner phenomenon in alopecia areata: rapid appearance after trichogram. Implications for the pathogenesis and therapy. G Ital Dermatol Venereol 2013;148:225–9.

Ghraieb A, Keren A, Ginzburg A, Ullmann Y, Schrum AG, Paus R, et al. iNKT cells ameliorate human autoimmunity: lessons from alopecia areata. J Autoimmun 2018;91:61–72.

Gilhar A, Etzioni A, Paus R. Alopecia areata. N Engl J Med 2012;366:1515–25.

Gilhar A, Keren A, Paus R. JAK inhibitors and alopecia areata. Lancet 2019;393:318–9.

Gilhar A, Keren A, Shemer A, d'Ovidio R, Ullmann Y, Paus R. Autoimmune disease induction in a healthy human organ: a humanized mouse model of alopecia areata. J Invest Dermatol 2013;133:844–7.

Gilhar A, Landau M, Assy B, Shalaginov R, Serafimovich S, Kalish RS. Melanocyte-associated T cell epitopes can function as autoantigens for transfer of alopecia areata to human scalp explants on Prkdc(scid) mice. J Invest Dermatol 2001;117:1357–62.

Gilhar A, Landau M, Assy B, Shalaginov R, Serafimovich S, Kalish RS. Mediation of alopecia areata by cooperation between CD4+ and CD8+ T lymphocytes: transfer to human scalp explants on Prkdc(scid) mice. Arch Dermatol 2002;138:916–22.

Gilhar A, Schrum AG, Etzioni A, Waldmann H, Paus R. Alopecia areata: animal models illuminate autoimmune pathogenesis and novel immunotherapeutic strategies. Autoimmun Rev 2016;15:726–35.

Gilhar A, Ullmann Y, Berkutzki T, Assy B, Kalish RS. Autoimmune hair loss (alopecia areata) transferred by T lymphocytes to human

scalp explants on SCID mice. J Clin Invest 1998;101:62–7.

Gong Y, Zhao Y, Zhang X, Qi S, Li S, Ye Y, et al. Serum level of IL-4 predicts response to topical immunotherapy with diphenylcyclopropenone in alopecia areata. Exp Dermatol 2020;29:231–8.

Ikeda T. A new classification of alopecia areata. Dermatologica 1965;131:421–45.

Ito T, Ito N, Bettermann A, Tokura Y, Takigawa M, Paus R. Collapse and restoration of MHC class-I-dependent immune privilege: exploiting the human hair follicle as a model. Am J Pathol 2004;164:623–34.

Ito T, Ito N, Saatoff M, Hashizume H, Fukamizu H, Nickoloff BJ, et al. Maintenance of hair follicle immune privilege is linked to prevention of NK cell attack. J Invest Dermatol 2008;128:1196–206.

Jabbari A, Cerise JE, Chen JC, Mackay-Wiggan J, Duvic M, Price V, et al. Molecular signatures define alopecia areata subtypes and transcriptional biomarkers. EBiomedicine 2016;7:240–7.

Jang YH, Choi JK, Jang YH, Moon SY, Lee WJ, Lee SJ, et al. Increased blood levels of NKG2D+CD4+ T cells in patients with alopecia areata. J Am Acad Dermatol 2017;76:151–3.

Kaufman G, d'Ovidio R, Kaldawy A, Assy B, Ullmann Y, Etzioni A, et al. An unexpected twist in alopecia areata pathogenesis: are NK cells protective and CD49b+ T cells pathogenic? Exp Dermatol 2010;19:e347–9.

Kinori M, Bertolini M, Funk W, Samuelov L, Meyer KC, Emelianov VU, et al. Calcitonin gene-related peptide (CGRP) may award relative protection from interferon-γ-induced collapse of human hair follicle immune privilege. Exp Dermatol 2012;21:223–6.

Lee S, Kim BJ, Lee CH, Lee WS. Topographic phenotypes of alopecia areata and development of a prognostic prediction model and grading system: a cluster analysis. JAMA Dermatol 2019a;155:564–71.

Lee S, Lee H, Lee CH, Lee WS. Comorbidities in alopecia areata: a systematic review and meta-analysis. J Am Acad Dermatol 2019b;80:466–77.e16.

Lindestam Arlehamn CS, Paul S, Wang EHC, de Jong A, Christiano AM, Sette A. Large scale epitope identification screen and its potential application to the study of alopecia areata. J Invest Dermatol 2018;19:S54–6.

Lousada MB, Lachnit T, Edelkamp J, Rouillé T, Ajdic D, Uchida Y, et al. Exploring the human hair follicle microbiome. Br J Dermatol 2020; https://doi.org/10.1111/bjd.19461 (accessed 9 September 2020).

McElwee KJ, Freyschmidt-Paul P, Hoffmann R, Kissling S, Hummel S, Vitacolonna M, et al. Transfer of CD8(+) cells induces localized hair loss whereas CD4(+)/CD25(-) cells promote systemic alopecia areata and CD4(+)/CD25(+) cells blockade disease onset in the C3H/HeJ mouse model. J Invest Dermatol 2005;124:947–57.

McElwee KJ, Gilhar A, Tobin DJ, Ramot Y, Sundberg JP, Nakamura M, et al. What causes alopecia areata? Exp Dermatol 2013;22:609–26.

Nakajima S, Nomura T, Common J, Kabashima K. Insights into atopic dermatitis gained from genetically defined mouse models. J Allergy Clin Immunol 2019;143:13–25.

Oelert T, Gilhar A, Paus R. T-cell "induced-self" MHC class I/peptide complexes may enable "de novo" tolerance induction to neo-antigens occurring outside of the thymus: lessons from the hair follicle. Exp Dermatol 2017;26:529–31.

Paus R, Bulfone-Paus S, Bertolini M. Hair follicle immune privilege revisited: the key to alopecia areata management. J Investig Dermatol Symp Proc 2018;19:S12–7.

Paus R, Nickoloff BJ, Ito T. A 'hairy' privilege. Trends Immunol 2005;26:32–40.

Paus R, Slominski A, Czarnetzki BM. Is alopecia areata an autoimmune-response against melanogenesis-related proteins, exposed by abnormal MHC class I expression in the anagen hair bulb? Yale J Biol Med 1993;66:541–54.

Petukhova L, Duvic M, Hordinsky M, Norris D, Price V, Shimomura Y, et al. Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. Nature 2010;466:113–7.

Phan K, Sebaratnam DF. JAK inhibitors for alopecia areata: a systematic review and meta-analysis. J Eur Acad Dermatol Venereol 2019;33:850–6.

Polak-Witka K, Rudnicka L, Blume-Peytavi U, Vogt A. The role of the microbiome in scalp hair follicle biology and disease. Exp Dermatol 2020;29:286–94.

Pratt CH, King LE Jr, Messenger AG, Christiano AM, Sundberg JP. Alopecia areata. Nat Rev Dis Primers 2017;3:17011.

Przybyla A, Zhang T, Li R, Roen DR, Mackiewicz A, Lehmann PV. Natural T cell autoreactivity to melanoma antigens: clonally expanded melanoma-antigen specific CD8+memory T cells can be detected in healthy humans. Cancer Immunol Immunother 2019;68:709–20.

Rebello D, Wang E, Yen E, Lio PA, Kelly CR. Hair growth in two alopecia patients after fecal microbiota transplant. ACG Case Rep J 2017;4:e107.

Röcken M, Schallreuter K, Renz H, Szentivanyi A. What exactly is "atopy"? Exp Dermatol 1998;7:97–104.

Roest YBM, van Middendorp HT, Evers AWM, van de Kerkhof PCM, Pasch MC. Nail involvement in alopecia areata: a questionnaire-based survey on clinical signs, impact on quality of life and review of the literature. Acta Derm Venereol 2018;98:212–7.

Schallreuter KU, Wei Y, Pittelkow MR, Swanson NN, Gibbons NC, Wood JM. Structural and functional alterations in the beta 2-adrenoceptor are caused by a point mutation in patients with atopic eczema. Exp Dermatol 2007;16:807–13.

Scharschmidt TC, Vasquez KS, Pauli ML, Leitner EG, Chu K, Truong HA, et al. Commensal microbes and hair follicle morphogenesis coordinately drive Treg migration into neonatal skin. Cell Host Microbe 2017;21:467–77.e5.

Sivamani RK, Lam ST, Isseroff RR. Beta adrenergic receptors in keratinocytes. Dermatol Clin 2007;25:643–53, x.

Song T, Pavel AB, Wen HC, Malik K, Estrada Y, Gonzalez J, et al. An integrated model of alopecia areata biomarkers highlights both TH1 and TH2 upregulation. J Allergy Clin Immunol 2018;142:1631–4.e13.

Strassner JP, Harris JE. Understanding mechanisms of autoimmunity through translational research in vitiligo. Curr Opin Immunol 2016;43:81–8.

Strazzulla LC, Wang EHC, Avila L, Lo Sicco K, Brinster N, Christiano AM, et al. Alopecia areata: disease characteristics, clinical evaluation, and new perspectives on pathogenesis. J Am Acad Dermatol 2018;78:1–12.

Wang EHC, Sallee BN, Tejeda CI, Christiano AM. JAK inhibitors for treatment of alopecia areata. J Invest Dermatol 2018;138:1911–6.

Wang EHC, Yu M, Breitkopf T, Akhoundsadegh N, Wang X, Shi FT, et al. Identification of autoantigen epitopes in alopecia areata. J Invest Dermatol 2016;136:1617–26.

Weidinger S, Beck LA, Bieber T, Kabashima K, Irvine AD. Atopic dermatitis. Nat Rev Dis Primers 2018;4:1.

Wu CC, Kim JN, Wang Z, Chang YL, Zengler K, Di Nardo A. Mast cell recruitment is modulated by the hairless skin microbiome. J Allergy Clin Immunol 2019;144:330–3.e6; pii:S0091-6749(19)30413-0.

Wu H, Liao W, Li Q, Long H, Yin H, Zhao M, et al. Pathogenic role of tissue-resident memory T cells in autoimmune diseases. Autoimmun Rev 2018;17:906–11.

Yoon TY, Lee DY, Kim YJ, Lee JY, Kim MK. Diagnostic usefulness of a peribulbar eosinophilic infiltrate in alopecia areata. JAMA Dermatol 2014;150:952–6.

Zhang B, Zhao Y, Cai Z, Caulloo S, McElwee KJ, Li Y, et al. Early stage alopecia areata is associated with inflammation in the upper dermis and damage to the hair follicle infundibulum. Australas J Dermatol 2013;54:184–91.

Zhang X, Ye Y, Zhu Z, Yang Y, Cao H, McElwee KJ, et al. Sequential cyclic changes of hair roots revealed by dermoscopy demonstrate a progressive mechanism of diffuse alopecia areata over time. Exp Dermatol 2020;29:223–30.

Zhang X, Zhao Y, Ye Y, Li S, Qi S, Yang Y, et al. Lesional infiltration of mast cells, Langerhans cells, T cells and local cytokine profiles in alopecia areata. Arch Dermatol Res 2015;307:319–31.

Zöller M, Zhao K, Kutlu N, Bauer N, Provaznik J, Hackert T, et al. Immunoregulatory effects of myeloid-derived suppressor cell exosomes in mouse model of autoimmune alopecia areata. Front Immunol 2018;9:1279.