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 autologus 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...
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., 2001, 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; Strazzulla 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 beta2-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 beta2-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 beta2-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 mast cells (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 beta2-adrenergic signaling defects in mast cells and/or eosinophils (Schallreuter et al., 2007).

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 AA 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 involved in AA pathobiology and prognosis.

Thus, the more one shifts the attention in AA research away from antigen-specific autoreactive CD8 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(path)obiology 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 immediately attacks a pigmented hair shaft, 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).

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, TGFβ1/2, IL-10, and IGF-1 by HF keratinocytes, or that promote the release of immunoinhibitory neuuropeptides 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 we all 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.

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CONFLICT OF INTEREST
The author states no conflict of interest.

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