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What causes alopecia areata?

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Abstract

The pathobiology of alopecia areata (AA), one of the most frequent autoimmune diseases and a major unsolved clinical problem, has intrigued dermatologists, hair biologists and immunologists for decades. Simultaneously, both affected patients and the physicians who take care of them are increasingly frustrated that there is still no fully satisfactory treatment. Much of this frustration results from the fact that the pathobiology of AA remains unclear, and no single AA pathogenesis concept can claim to be universally accepted. In fact, some investigators still harbour doubts whether this even is an autoimmune disease, and the relative importance of $CD8^+$ T cells, $CD4^+$ T cells and $NKGD2^+$ NK or NKT cells and the exact role of genetic factors in AA pathogenesis remain bones of contention. Also, is AA one disease, a spectrum of distinct disease entities or only a response pattern of normal hair follicles to immunologically mediated damage? During the past decade, substantial progress has been made in basic AA-related research, in the development of new models for translationally relevant AA research and in the identification of new therapeutic agents and targets for future AA management. This calls for a re-evaluation and public debate of currently prevalent AA pathobiology concepts. The present Controversies feature takes on this challenge, hoping to attract more skin biologists, immunologists and professional autoimmunity experts to this biologically fascinating and clinically important model disease.

Prologue

The aetiology of AA is unknown, even though most evidence is consistent with an autoimmune disease to which both genetic predisposition and environmental factors contribute: in genetically predisposed individuals, triggering factors cause a predominantly CD8-driven, Th1-type T-cell autoimmune reaction against the hair follicles that results in acute hair loss (1). The hair follicle is an immune-privileged site with low levels of major histocompatibility complex expression. Recent evidence indicates that development of alopecia areata requires a breakdown in immune privilege with infiltration of the hair follicle by T lymphocytes (2).

Alopecia areata occurs in genetically predisposed individuals, in which proinflammatory signals, including substance P and inter-feron-c, upregulate expression of major histocompatibility complex class Ia inside the hair follicle (2,3). It was demonstrated that interferon-γ (IFN-γ) is the most potent inducer of ectopic MHC class I expression in murine anagen hair bulbs *in vivo* (4). Systemic treatment with IFN-c also accelerated the development of AA in genetically susceptible C3H/HeJ mice associated with expression of MHC class I and class II in the follicular epithelium (5). As the primary source of IFN-c is activated T cells, an activation of the immune system may occur prior to loss of immune privilege.

Besides genetic factors leading to autoimmunity, environmental triggers such as viruses, bacteria and other infectious pathogens are thought to play a major role in the development of autoimmune diseases such as diabetes and systemic lupus erythematosus. Recent studies have shown molecular mimicry between early events in lupus autoimmunity and specific Epstein–Barr virus (EBV) responses, as well as increased EBV load and association of the presence of Epstein–Barr virus DNA in patients with systemic lupus erythematosus compared with controls (6). It has also been postulated that patients with AA harbor a $CD8^+$ T-cell deficiency and that EBV infection leads to infection of a target organ, a clonal expansion of EBV and antibodies against EBV, leading to infiltration of autoreactive T cells and development of ectopic lymphoid follicles (7).

The possibility that AA was caused by cytomegalovirus (CMV) was first suggested by Skinner *et al*. (8) after detection of CMV DNA sequences using polymerase chain reaction (PCR) in scalp biopsies of AA. However, this hypothesis was not confirmed by other authors (9–12). Recently, Rodriguez and Duvic (13) described 12 patients who self-reported infectious mononucleosis <6 months before sudden onset of AA.

The seasonal aspect of AA is another intriguing factor that can be explained by viral infections. D'Ovidio demonstrated the seasonal cycle of the disease, with relapses mostly occurring in February–March, independent of atopic disease. The relative increase of relapses in the early spring could be explained as an effect of the seasonal increase in potential provocation factors such as viral infections (14).

In the recent years, multiple mechanisms have been postulated through which host infection can lead to autoimmunity (15,16) as (i) *molecular mimicry*; (ii) epitope spreading; (iii) bystander activation (various parts of the immune system respond to invading pathogens, after which the inflammatory environment damages self-tissue in a non-specific manner); and (iv) *subdominant cryptic self-antigens*, normally invisible to the immune system which are processed by the APCs secondary to the inflammatory environment.

All mechanisms mentioned above suggest a perpetuation of the inflammatory response that can interact with the hair follicle and produce a collapse of its immune privilege. In this way, the lack of detectable viral DNA in scalp biopsies of patients with AA does not exclude a viral aetiology, as the virus may just act as a trigger factor and the process then perpetuated by the new environment of self-antigens and cytokines.

Viewpoint 1

The question would be better stated as ‘What causes alopecia areatas?’ It is unlikely that what we describe today as a single disease will be shown to have a common disease pathogenesis in all patients and other mammalian species that display the alopecia areata (AA) phenotype. The distinct phenotypic presentations, from patchy AA, through alopecia totalis and alopecia universalis, to diffuse AA and ophiasis AA, may be indicators of different underlying disease development mechanisms or variations thereof (1). For example, lichen planopilaris and pseudopelade of Brocq are widely regarded as clinical variants of the same disease mechanism (2). Yet microarray analysis of scalp biopsies reveals active signalling pathways unique to each respective diagnosis (3). It is also possible that individuals presenting with similar AA pheno- types will be shown to have different causal pathogeneses. Thus, while we all have our favoured hypotheses of AA development and argue as to which is correct, it is unlikely that exactly the same mechanism is involved in every case identified within the AA disease collective.

The onset of AA likely requires input from multiple factors [genetics (4), stress (5,6), hormones (7), diet (8), infectious agents (9), vaccinations (10), etc.] that determine the physical and biochemical status of the immune system and hair follicles. Different factors will be prevalent in different respective subsets of patients. If so, there are several sequelae.

(i) Attempting to define a common genetic basis for AA using a heterogeneous population of patients will be difficult without detailed consideration of the variations in disease presentation. (ii) Identifying a common, primary environmental trigger for AA is unlikely. (iii) Attempting to find a single inflammatory target antigen common to all cases will prove elusive. Rather than wade into the quagmire of possible triggers for AA onset, a focus on identifying general principles of AA development applicable to most cases is more appropriate at this stage in our, still limited, understanding of AA.

While many, myself included, have claimed that the evidence to support an autoimmune scenario for AA is ‘compelling’ (11), the current evidence does not meet the requirements to prove AA is truly an autoimmune disease (12,13). Objectively, the research data produced thus far are best described as ‘consistent with’ autoimmune mechanism(s) in AA. The key issue, to define the primary antigen targets of attack as self-antigens derived from the hair follicle unit, remains to be proven, although studies have provided tantalizing clues (14–16). With the aid of several AA animal models, indirect, functional evidence has been produced to show that the immune system dominates in determining the expression of AA-like hair loss (17–22). If there is no functional immune system/inflammation, there is no AA-like hair loss in these models.

One can argue as to how comparable these models are with the human disease. However, functional studies with humans are not possible, and AA models are the best practical alternative (23). Only coincident case reports hint at the significance of inflamma-tory cells in humans with reports of AA developing after bone marrow transplantation (24,25), development of AA in selectively immunosuppressed individuals (26–28) and AA onset in HIV-positive people (29–31). Data should be considered from any and every source and

synthesized into realistic hypotheses. In the absence of any hard data that would force a ‘paradigm shift’ (32) in thinking, an autoimmune scenario is currently the best fit for the facts at hand.

Many hypotheses for autoimmunity onset have been put forward involving cross-reactive antigens; central tolerance failure and forbidden clone release; impaired apoptotic cell clearance; environment-induced antigen deformation; genetic mutation to antigens or immune system components; cryptic epitopes and epitope spreading; haptenization; failure of peripheral tolerance through loss of lymphocyte ignorance or anergy; hormone- or cytokine-induced phenotypic skewing; failure of active suppression via T-regulatory cells or tolerogenic dendritic cells; and so on (23,33–36). Most of these scenarios have not been considered in the context of AA. Instead, a rather unique dermatologist-friendly, skin-centric hypothesis has been developed based on hair follicle immune privilege (37). If hair follicles have immune privilege, then loss of that immune privilege will result in targeting by the immune system (38). This breach in immune privilege, and exposure of unique hair follicle antigens, may come about through follicle injury and/or deficiency in immune-privilege-conferring factors. This model's simplicity is alluring, but AA is not a simple disease.

Although there is evidence that hair follicles have a degree of immune privilege (39), we have no direct evidence of immune privilege failure causing AA development. Recently, hair follicles distant from AA lesions in people with patchy AA were shown to have select differences in gene and product expression related to immune privilege (40). However, it is notable that there was no actual AA lesion, suggesting perturbations in hair follicle immune privilege may not be enough on their own to elicit an immune response. In the genetically susceptible AA mouse model, where AA is transferred from affected to unaffected mice by skin grafting (17), injury to follicles through the sham grafting of normal skin in comparative control mice, and the associated induction of follicle damage and MHC expression, does not induce AA (41,42). Only a transient injury response is observed before returning to normal (41). Although IFN-γ injection has previously been suggested to promote MHC expression in hair follicles apparently inducing AA (43), these injections were systemic, and the primary mode of action was likely to be on leucocytes distant from the skin consistent with research on other autoimmune diseases (44–46). Further, IFN-γ-induced AA onset could not be replicated in other laboratories (47). While hair follicle immune-privilege collapse may be required to permit AA onset, additional AA-inducing events are also needed.

Hair follicle immunoprotection is transient, limited to the anagen growth stage, because of the nature of the hair follicle cycle. Regression of the hair follicle in catagen involves significant apoptosis and remodelling of the transient portion of the hair follicle (48). Immune cells infiltrate around catagen-stage hair follicles (49–52). This normal process of hair follicle cycling may constantly expose the immune system to low levels of hair follicle-derived antigens. Autoimmunity is not an all-or-nothing event. Autoreactivity is a progressive scale of response with a threshold level above which overt autoimmune disease is observed (4). An example of this may be the low level of hair follicle-specific antibodies found in some humans and animal models in the absence of overt AA (53–56).

Langerhans cells and dendritic cells are capable of presenting cell apoptosis-derived antigens to lymphocytes and stimulating autoimmunity (57–59). If catagen regression became disordered and the associated immune cell infiltrate inappropriately expressed antigenic peptides plus co-stimulatory molecules, antigen presentation to the immune system might breach the threshold for the onset of overt AA (60,61). Studies that have examined the lymph nodes and skin of mice reveal pro-inflammatory events occur in the lymph nodes in advance of significant lymphocyte infiltration into the skin and subsequent hair loss onset (41,42,62). As such, the fate decision for AA onset may occur in the draining lymph nodes rather than in the skin (60,63). Once hair follicle autoreactive lymphocytes are activated and migrate to the skin, it seems they have little trouble overcoming putative immune privilege mechanisms. Injections of $CD8^+$ cells from AA mice to normal-haired littermates quickly result in localized hair loss (21).

To fully explain the causes of AA, one must take a holistic approach. The AA phenotype may present in the skin, but that does not mean the entire disease mechanism is *limited* to the skin. In short, we are dealing with the immune system in its entirety, influenced by multiple genetic and environmental factors, with variations pertinent to different subsets of patients with AA.

Viewpoint 2

The mosaic of autoimmunity has been described (1–4). This data combined with the considerable evidence regarding alopecia areata (AA) may lead us to the assumption that AA is a tissue-specific autoimmune disease (5,6).

Immunosuppressive agents, such as systemic corticosteroids and cyclosporine, as well as immunotherapy with contact sensitizers demonstrated a beneficial effect in AA (7–13). The disease is associated with various autoimmune and inflammatory disorders (14) (Table 1). The most characteristic histological feature of AA is a perifollicular lymphocytic infiltrate around the hair follicle (HF) hair bulb comprised primarily of $CD4^+$ T cells, whereas the few intrafollicular lymphocytes tend to be $CD8^+$ T cells. Mast cell numbers were also shown to be increased in lesional AA skin (15,16). Evidence of immune activation includes the expression of human leucocyte antigen (HLA)-DR, HLA-A, HLA-B, HLA-C normally not expressed and the intercellular adhesion molecule (ICAM)-1 on the proximal follicular epithelium (17,18). The follicular upregulation of HLA-A, HLA-B, HLA-C, HLA-DR and ICAM-1 is most likely induced by interferon (IFN)-γ produced by T cells (18–20). Further support for an autoimmune pathogenesis has been derived from human scalp graft/severe combined immunodeficiency disease (SCID) mouse studies and other animal models (21,22).

Grafting of bald scalp from patients with AA to nude mice results in hair regrowth, suggesting that hair growth is prevented by immunological factors (23). The significance of autoantibodies to the HF epithelium in AA is not clear (24). Injections of patient's serum into human skin transplanted onto nude mice failed to induce hair loss (25).

On the other hand, there is a strong evidence for the role of T cells in the pathogenesis of AA by the ability to transfer hair loss to human scalp grafts on SCID mice by injecting

activated T cells derived from AA lesions (26). Prior to the transfer of cells, the scalp T cells were cultured with homogenized HFs as a source of autoantigen as well as autologous antigen-presenting cells and IL-2. Thus, HF homogenate is essential to activate the T cells to induce hair loss, presumably functions as autoantigen. McElwee demonstrated hair regrowth in the DEBR model for AA following in vivo depletion of $CD4^+$ or $CD8^+$ T cells (27,28). Still, maximal hair loss in the humanized/SCID mouse model of AA requires cooperation between $CD4^+$ and $CD8^+$ T cells and has a T-helper cytokine profile (29), although a localized hair loss could be detected in C3H/HeJ mice injected with $CD8^+$ T cells alone, isolated from lymph nodes and spleen of diseased mice (30).

A central issue in AA pathogenesis is the immune privilege (IP) of the hair follicle. Indeed, several internal organs were found to have varying degrees of IP (13,31–35). These include the brain, testes, the pregnant uterus, the anterior chamber of the eye and the cornea and also the proximal HF hair bulb. All these organs share similar mechanisms for providing protection from immune-mediated inflammation. Such inflammation might impair the function of vital tissues and threaten the survival of the host (31). Mediators of HF IP include immunosuppressive cytokines, such as alpha-MSH, TGF-beta1 and IGF-1, which are prominently expressed by the follicular epithelium (13,33,36,37). Furthermore, the lower part of the follicular epithelium is deficient in expression of the major histocompatibility complex (MHC) class I and class II molecules, and only a few Langer-hans cells are observed around and within the HFs. These cells are functionally impaired because they do not express DR molecules (38).

The absence of MHC class I molecules may create an immuno-logical dilemma, as it may activate natural killer (NK) cells, which are programmed to produce lyses of MHC class I–negative cells (39). Macrophage migration inhibitory factor (MIF) was found to produce a profound and immediate inhibition of NK cell–mediated cytolysis (40–42).

Ito *et al*. (43) showed the presence of this strong expression of the potent NK cell inhibitor MIF in normal HFs, versus a dramatically decreased MIF expression in AA, facilitating the accumulation of NK cells around and within the HFs. Recent literature reveals that NK cells posses a regulatory effect on autoreactive effector T cells (44,45). The role of these cells in autoimmune disorders remains controversial, as in different mouse models such as colitis or EAE, NK cell depletion increased disease incidence, whereas other studies showed the opposite (46–49). Recent studies distinguish between NK and NKT cells' activity, and it seems that both populations might play a role in the pathogenesis of AA (Gilhar A & Keren A, unpublished data). MHC class I polypeptide–related sequence A (MICA) protein and UL 16-binding protein (ULBP3) were found in recent GWAS to be associated with AA (50,51). MICA and ULBP3 are ligands of NKG2D, an activating receptor of lymphocytes bearing NK receptors. Immunohistology and flow cytometric analysis (FACS) revealed that MICA and NKG2D as well as $CD56^+$ cells are greatly upregulated in AA (50,52).

Many autoimmune diseases are investigated through the use of animal models (53). Such models should express elements of the disease as they are observed in humans and thus demonstrating characteristic features of the human disease. The model may illuminate more intensely the pathogenesis of the disease along with detection of the therapeutic efficacy of

various molecules. During the last two decades, the C3H/HeJ mouse model was widely used as a model for AA and is considered as the best one for the disease (30,54–57). On the other hand, deeper understanding of immune-related diseases was achieved by using humanized mouse model. The scientific literature, for example, reports that the best model for psoriasis is based on mice containing human components (58). Such a model should be characterized by a number of basic features as follows: reproducible, easily distributed to other institutes, responsive to external stimuli in a similar manner as human patients and above all, inclusive of the characteristic changes of the disease (58,59).

Recently, we have succeeded in establishing such a model for AA (59). Peripheral blood mononuclear cells (PBMCs) from healthy individuals were cultured with high dose of interleukin-2 (IL-2). Flow cytometric analysis revealed a high expression of $NKG2D^+$/$CD56^+$ cells following this PBMCs/IL-2 culture. Thereafter, the cultured cells were transferred to the SCID mice, which had been transplanted with normal human skin obtained from people who underwent plastic surgery but had no AA. The interaction between the human $NKG2D^+/CD56^+$-enriched PBMCs and the normal human HFs led to the development of characteristic features of AA (59), strongly supporting the concept that NKG2D stimulation plays an important role in the pathogenesis of AA (43).

This new humanized AA mouse model enables us not only to further investigate AA pathogenesis, but also to explore new therapeutic strategies and to test the therapeutic efficacy of a range of candidates at the level of preclinical research. The new model is much easier to perform than its predecessor described above. There is no need to obtain skin or blood samples from patients with AA, whereas the precursor model required both (26). This new AA model also illustrates that an autoimmune disease can be induced in healthy human skin under certain conditions even in the absence of a defined genetic predisposition or peripheral blood abnormalities (59).

The new humanized mouse model possesses advantages even over the C3H/HeJ mice. In contrast to the C3H/HeJ model, the histological staining and immunohistochemical staining of the humanized AA mouse model are almost identical to that observed in patients with AA (59). C3H/HeJ mice have a spontaneous mutation at the lipopolysaccharide response locus (mutation in the Toll-like receptor 4 gene, Tlr4Lps-d) and thus may display some major immunological abnormalities (60). In addition, while SCID mice can be cross-bred with other mutant subtypes with SCID background, C3H/HeJ mice cross-breeding is more restricted. Some therapeutics are efficacious in animals, but not humans, such as FK 506 (61,62). Most recently, we have observed a beneficial effect of KV1.3-blocking agents in the new AA humanized mouse model, which raises new hope for a successful therapy of human AA (63).

The puzzle regarding the pathogenesis of AA has not been completed yet, and there are some unresolved questions regarding the aetiology. For example, ‘Which are the follicular autoantigens that are exposed to the immune system, following the collapse of the IP?’ In AA, autoantigens could be associated with melanocytes, keratinocytes and dermal papilla-related peptides. Pigmented HFs are preferentially lost in active disease, whereas regrowth

frequently demonstrates white hair. For this reason, it has been suggested that melanogenesis-related peptides may be the targets of an immunological attack (64–67).

It has been observed that melanocyte-derived peptides are capable of serving as autoantigens for inducing AA in the humanized SCID mouse (68). Furthermore, the histological features seen in the alopecia associated with Vogt–Koyanagi–Harada disease (VKH) are consistent with AA (69). VKH disease is a rare autoimmune disease primarily involving pigmented tissues. Animal models support the notion that melanocytes play a role in the induction of AA. For example, Becker *et al*. (70) showed induction of AA-like in C57BL/6 mice, following immunotherapy for melanoma. The histological features demonstrated the presence of dense $CD8^+$ T-cell infiltrates around the HFs. Passive transfer of the $CD8^+$ T cells to naïve mice led to the development of AA. Nagai *et al*. (64) showed hair loss and vitiligo-like appearance in mice bearing interleukin-12-producing B16 melanoma cells by the depletion of $CD4^+$ T cells. The Smyth chicken model (71) demonstrated loss of pigmented feathers combined with the development of non-scarring, inflammatory, AA-like hair loss.

Melanoma-associated antigens such as GP100 and MART-1 can activate T cells for transfer of AA to autologous scalp skin grafts on SCID mice, indicating that melanocyte-associated autoantigens can be pathogenic (68). Recently, McElwee and Wang (72) showed new data that pathogenic lymphocytes of C3H/HeJ mice respond *in vitro* not only to these melanogenesis-associated proteins (Tyr, TRP2, MART, GP100), but also to trichohyalin- and keratin 16-related peptides. Interestingly, this enriches the list of candidate autoantigens in AA.

There are also anecdotal reports supporting the role of melanocytes in AA. Yousefi *et al*. (73) described a patient with AA associated with regression of cutaneous melanoma. Others (74) demonstrated the development of AA following adjuvant therapy with alpha-IFN in malignant melanoma. All these observations do not rule out the possibility of other autoantigens, which might be located outside the melanocytes. Furthermore, the studies do not negate possibility that non-functional melanocytes are capable of serving as autoantigens. Some investigators have even questioned the primary role of melanocytes in the pathogenesis of AA (75).

Additional unresolved questions regarding the pathogenesis of AA are as follows: (i) The role of hair follicle autoantibodies in patients with AA; (ii) the potential role of the innate immune system to be investigated more systematically, for example, in view of the identification of a gene regulating innate immunity (NALP1) in vitiligo (76); (iii) the role of Tregs (77); (iv) the role of NK and NKT cells; and above all, (v) the poor response to the various treatments for severe cases with AA including the novel biological immunomodulators (78).

Irrespective of these open questions, re-establishment of IP has been proposed as a promising therapeutic manipulation to induce hair regrowth and to prevent disease progression (5,6,9,13). In summary, while we do believe that AA is an autoimmune

phenomenon, the questions raised above should be answered before definitive conclusions can be drawn.

Viewpoint 3

Several lines of evidence have revealed that alopecia areata is an organ-restricted, T-cell-mediated autoimmune disease of hair follicles (1,2). Disease induction is associated with collapse of hair follicle immune privilege in both humans and animal models. T lymphocytes, cytokines, neuropeptides and genetic background all play important roles in the induction of alopecia areata. To address which of these factors most contribute to the pathogenesis, we will focus on the configuration and pattern formation (3) of alopecia areata, which directly reflect its pathogenesis.

The configuration of alopecia areata

Why does alopecia areata (AA) usually exhibit a round alopecia lesion rather than a sphenoid or a rectangular shape? The configu-ration and distribution of skin eruptions faithfully reflect the pathogenesis of the diseases. For example, patients with a creeping disease show a linear eruption, perhaps somewhat comparable with the movement of skin lesions in larva migrans. Therefore, for understanding the pathogenesis of AA, it is worthwhile to address the mechanism underlying its formation in a round or an ovoid pattern.

Randomly spreading T-lymphocyte hypothesis

A cuboidal nodule is a representative morphological phenotype of pigmented nevi. This configuration is supposed to reflect the proliferation of nevus cells in a randomly directed manner. In patients with AA, $CD4^+$ and $CD8^+$ T lymphocytes infiltrate around the hair follicles which have lost their immune privilege and show ectopic MHC class I and class II expression in the follicular epithelium (4). A study of purified T-cell injection into the mice suggested that both $CD4^+$ and $CD8^+$ T cells have a crucial role in the pathogenesis of AA. $CD8^+$ cells act as the main effector cells and $CD4^+$ cells serve as the regulatory players (5). We have confirmed that infiltrating $CD4^+$ T cells consist mostly of $CCR5^+$ cells with few $CCR4^+$ cells, suggesting a dominant role of Th1 cells in the alopecic lesion (6). Provided that the infiltration of T lymphocytes begins at one hair follicle and T lymphocytes migrate randomly to the adjacent hair follicles, the attack of hair follicles by T cells might result in a round alopecia (Fig. 1a).

Cytokine diffusion hypothesis

In 2006, Sick *et al*. identified WNT and its inhibitor DKK as the main molecular players in determining hair follicle spacing through a reaction–diffusion mechanism first proposed by Alan Turing (7,8). Overexpression of the activator (WNT) increases the follicular density, whereas moderate expression of the inhibitor (DKK) during the initial inductive wave increases the inter-follicular spacing. Although this model for hair follicle morphogenesis cannot be directly transferred to the pathogenesis of multifocal AA, a similar ‘activator–inhibitor reaction–diffusion’ mechanism might determine the shape and distribution pattern of AA.

The most likely candidate of activator in AA is the Th1 cytokine, interferon γ (IFNG). Ifng mRNA expression was significantly increased in the region with alopecia compared with the hair-bearing skin in the AA mouse model C3H/HeJ mice (6). Injection of rat anti-mouse IFNG improved the hair growth index more efficiently than control rat IgG in C3H/HeJ mice (6). Moreover, human AA can be successfully treated with neutralizing anti-IFNG antibody injections (9). Given that the Th1 cells surrounding an affected hair follicle secrete IFNG and that IFNG diffuses in a randomly directed fashion to induce a chain autoimmune reaction in adjacent hair follicles, the alopecia might become round (Fig. 1b).

Perspective—It is an important, but unelucidated question in dermatology how the configuration and distribution of skin eruptions are determined (3). Why do the discoid patch in lupus erythematosus and the erythematous plaque in psoriasis vulgaris display a round phenotype? In contrast, what determines the shape of ovoid erythema following skin tension lines in patients with pityriasis rubra? These are at least two types of erythema configuration with unknown pathomechanisms.

If a T-cell diffusion hypothesis or a cytokine diffusion theory can be applied to the formation of the erythema, velocity of the T-cell or cytokine movement may differ depending on the direc tions. The orientation of dermal fibroblasts and/or skin tension may affect the diffusion efficiency of T cells and/or cytokines. Moreover, movement in the horizontal direction in dermis may be dependent on the vertical depth. Therefore, further three-dimensional studies will be required to explain the round-pattern formation in skin eruptions, including those of AA, where both the randomly spreading T-lymphocyte hypothesis and the reaction–diffusion hypothesis may be at play.

Viewpoint 4

The actual epitope(s) involved in initiating alopecia areata (AA), a cell-mediated autoimmune disease, in humans and other susceptible species such as mice, rats, dogs, horses, cattle and chicken, remain(s) elusive, although serendipitously one was recently identified for the mouse (1). This new data from studies on a strain of mice resistant to spontaneous AA suggest none of the current hypotheses to explain the aetiology of AA may be correct.

Whether or not these new observations hold up under intense scientific scrutiny to ensure that the data are applicable to humans and other species will be the focus of future work. Based solely on existing published hypotheses on the source of the initiating epitopes, aberrant responses to infectious agents and melanogenesis-related proteins remain the most widely accepted candidates. Unfortunately, especially in humans, the observations supporting these hypotheses are based on data derived from patients who already have AA rather than data obtained at the time of initiation of AA.

Historically, a variety of infectious agents were considered to be the cause of AA (2). Cytomegalovirus (CMV) as an inciting agent of AA has been a source of controversy since it was initially proposed in 1995 (3,4). CMV infects most humans at or near birth and resides in their tissues throughout life. Therefore, it was not surprising that this herpesvirus

was found using very sensitive molecular tools in skin from patients with AA. Numerous subsequent studies, both in humans and in laboratory mice that spontaneously develop AA, found no conclusive evidence to link CMV with AA (5–9). Rather, detecting CMV appeared to be a concurrent and unrelated event in humans with AA and had absolutely no presence, much less any influence, in the mouse model of AA.

Similarly, there are numerous clinical reports suggesting a link between hepatitis B (HBV) and C virus (HCV) infections or even vaccinations to prevent HBV or HCV with onset of AA (10–15). Acute onset of AA after HBV immunization, with recurrence after re-immunization (16), prompted a large-scale study in the AA mouse model that showed no induction of AA by HBV immunization (17). The actual frequency of AA in the immunized human population was similar to that reported in many studies for the epidemiology of AA in a large human population unrelated to this or any other treatments. Although an infectious agent, possibly expressing a superantigen, such as seen with streptococcal infections and rheumatic heart disease (18) would fit such a hypothesis nicely, this scenario remains unsupported.

The hypothesis that melanogenesis-associated proteins are the epitope(s) that initiate AA is based largely on the observations that in humans, white hair is spared from developing AA and that if hair regrows after clinical AA, it may regrow white (unpigmented). Very limited studies using human skin grafted to immune-deficient mice (xenografts) suggested that melanogenesis-associated proteins might be involved in the initiation of AA (19), although this has not been reproduced by others. By contrast, the spontaneous C3H/HeJ inbred mouse strain develops AA predictably when young immune-competent mice receive full-thickness skin grafts from affected mice (20,21), as documented by a number of different laboratories (22–26). Using this method, when affected skin is grafted onto C3SnSmn.CB17-*Prkdc*^{scid}/*Prkdc*^{scid}/Sz immunodeficient mice (congenic mice in which the *Prkdc*^{scid} allele was moved from CB17 onto C3H/HeJ by 10 back-crosses), white hair grows back in the graft site (20). Our interpretation of these data was not that the melanogenesis-associated protein epitopes were destroyed in the affected skin by the auto-immune disease, but the melanocyte ‘stem cells’ were damaged by the surgery and during the subsequent healing process. This regrowth of white hair in areas of injured skin is seen in many different pigmented inbred strains. For example, freeze branding, applying liquid nitrogen to the skin, is a common method to identify cattle with pigmented hair. If freeze branding is used in C3H/HeJ agouti mice to produce defined areas of white hair, and these mice then receive skin grafts from AA mice, both the pigmented and non-pigmented areas of skin and hair are affected by AA, which does not support the argument that melanin-related proteins are the inciting epitope (27). A number of albino strains of mice, especially A/J, also develop AA (28). Albino mice lack tyrosinase and therefore do not produce typical melanin pigments, even though the biochemical pathway to make melanin, including melanosomes, is present.

Another possible mechanism is a humoral immune response to structural proteins, hard keratins and/or keratin-associated proteins within the hair follicles. Initial studies in humans with AA and control groups were expanded to mice, rats, dogs and horses, species that also develop AA (29–32). Investigators found a loose correlation between the presence of autoantibodies against a variety of hair keratins and keratin-associated proteins in the

patients with AA compared with controls. While these antibodies were more frequently found in patients with AA, these autoanti-bodies were also found to various degrees, albeit at a lower frequency, in clinically normal patients in all species that develop AA (33–37). A detailed study was conducted using 105 C3H/HeJ females in which sera were collected on a monthly basis from weaning until past 1 year of age to blindly assess onset of autoantibodies against hair follicle proteins (Tobin DJ & Sundberg JP, unpublished data). There was no consistent correlation between the development of these autoantibodies and the onset of AA.

Transcriptome analyses provide a method to observe regulation of humoral autoimmune responses. The first published transcriptome analysis of human and mouse AA skin using Affymetrix microarrays showed that epitope spreading (developing autoantibodies to countless epitopes as revealed by upregulation of transcripts) was a late-stage feature of AA (38). As in AA-affected humans, C3H/HeJ mice with spontaneous or graft-induced AA make transcripts for antibodies directed against many self-antigens (29,33,36,37). The Affymetrix probes recognize transcripts previously characterized to produce antibodies against antigens such as DNA (39,40). Furthermore, the antibody transcripts are directed towards virtually every immunoglobulin isotype. This suggests that the C3H/HeJ mouse has a global propensity towards autoimmunity that is initiated within the hair follicle, but then quickly spreads to many self-antigens and is therefore unlikely to be the inciting cause (38). The relevance of these autoantibodies occurring late in the course of AA remains uncertain, but they may actually help define variations in severity of AA, the anatomical sites affected and response to therapy.

The evolving evidence indicates that AA is a very complex polygenic disease with different clinical subtypes. Human gene association studies continue to show a group of major histocompatibility genes (MHC; human lymphocyte antigens, HLA) are linked to susceptibility to AA and specific clinical types of AA with differing severity (41–47). However, additional genes identified in human AA studies appear only related by association with other specific genetic diseases weakly linked to AA (48–51). Subsequent genome-wide screening for quantitative trait loci (QTLs) defined multiple loci in humans, mice and rats that develop AA (52–54). Using haplotype mapping techniques or, more recently, genome-wide association mapping techniques, many more genes, both within the QTL that included the MHC in mice and many others, were identified (Sundberg *et al*. in press).

One group claimed that interferon-gamma (IFNG) was a key molecule in the pathogenesis of AA (55) and used recombinant IFNG to induce AA in C3H/HeJ and other strains of mice. A second group, using larger numbers of mice and more controls, showed that the occurrence of AA after IFNG injections was no different than that seen in spontaneously occurring AA (56). The first group also reported that IFNG induced AA when injected in C3H/HeJ, but not in C3H/HeN (55), the major difference between these substrains being a mutation in the Toll-like receptor 4 (*Tlr4*) gene for C3H/HeJ mice (57,58). Their observation was contrary to the published data that both substrains develop spontaneous AA at comparable rates (28). Perhaps, the different dose and source of INFG between the studies may account for the discrepancy in results. Much more work in this area is needed to sort out the role(s) of IFNG and other INF proteins in the pathogenesis of AA.

In summary, over the last two centuries, many hypotheses have been proposed to explain how or why AA develops, none of which have been proven so far. Interesting results were reported, but have not been reproduced by follow-up data.

The actual cause of AA will likely not be found in humans or other animals that develop AA as long as studies focus on clinically established AA. The rodent models of AA, even with their perceived flaws, are reproducible and able to be studied from time of initiation to established AA. As the genetic studies in the mouse and rat models of AA progress and molecular markers are found to predict which individual animals will develop AA, we will observe the very earliest events that define the cause of AA. These same molecular markers may predict which humans will most likely develop familial AA, and used to document the onset, severity and response to therapy in those individuals.

Viewpoint 5

‘There is no such uncertainty as a sure thing’

Robert Burns Both direct and indirect evidence support an autoimmune aetiology for alopecia areata (AA) (1,2). Indirect evidence includes the increased prevalence of other autoimmune diseases with concomitant organ-specific antibodies, the association with specific human leucocyte antigens (HLA) and the response to immunosuppressive agents, especially systemic steroids. Direct evidence includes the presence of antibodies targeting the anagen hair follicle (HF), changes in the amount and/or ratio of T cells, dense lymphocytic peribulbar infiltrate affecting the anagen follicle and, recently, the immune privilege collapse theory.

Does alopecia areata really have an autoimmune aetiology?

The characteristics of patients with AA vary from those seen in patients with other classical autoimmune diseases. For example, AA can present in very young patients, as young as 1 year of age (3), it is most prevalent in young patients under the age of 20 (4,5), and both sexes are affected equally (4,6). In contrast to other autoimmune diseases, there is a positive family history in up to 42% of patients with AA (7). Even though there are reports on increased prevalence of other autoimmune diseases in patients with AA, this is unusual. In fact, one study showed no increased prevalence of autoimmune diseases in 300 patients with AA in Israel (5). Atopic dermatitis, the ‘autoimmune disease’ most commonly associated with AA (8), has recently been described as an epidermal structural filaggrin-mediated genetic disease (9). Data are accumulating that psoriasis, which is also suggested to be an autoimmune-mediated disease, has a strong genetic basis, and again, some of the susceptibility genes are located outside of the MHC loci (10–12).

The autoantibodies reported to target the HF are heterogeneous, with different patterns of antibodies targeting different structures of the HF (13). Indeed, passive transfer of serum from patients with AA to nude mice did not elicit hair loss in grafted human scalp skin (14). In addition, initial reports of increased levels of organ-specific autoantibodies have not been confirmed in subsequent studies (e.g. thyroid microsomal antibodies) (15). Moreover, the presence of autoantibodies does not necessarily confirm the presence of an autoimmune

disease. For example, antinuclear and antismooth muscle antibodies can be found in duodenal ulcer patients (16), a disease caused by Helicobacter pylori infection. Certain class I and class II HLA antigens have been associated with the presence of AA in addition to disease severity and/or length (17). However, certainly, they are not diagnostic of AA.

The presence of a peribulbar Th1-predominant lymphocytic inflammation is believed to be the characteristic finding in the diagnosis of AA (18). However, this infiltrate is found in only one-third of patients with AA and quickly resolves after the acute phase of the disease (18). The situation is even more complicated in recurrent episodes, when a mixture of findings is to be expected (18). Furthermore, immune cells are commonly absent in areas of severe follicular damage (19,20). Assuming the disease targets a HF-associated antigen, one would expect an abundance of lesions, that is alopecia totalis or universalis, and not circumscribed AA. Additionally, there are long periods of complete clinical remission and resolution of histological findings. This is not usually seen in other autoimmune diseases such as vitiligo, systemic lupus erythematosus, myasthenia gravis, insulin-dependent diabetes mellitus and others.

Although the new biological treatments are effective treatments for several systemic autoimmune diseases (21), they have consistently failed to show any efficacy in AA (etanercept, efalizumab and, most recently, alefacept and adalimumab) (22–27). On the contrary, there is growing evidence of biological agents triggering AA (28,29).

Although highly auspicious, the immune privilege collapse theory still has many key open questions (30). For example, what is the exact trigger heralding this collapse and is it genetically modulated? In the healthy non-disease state, what are the precise factors maintaining immune privilege and which factors inhibit natural killer cell–mediated HF attack? What governs which HFs lose their immune privilege? What factors lead to the restoration of immune privilege and to spontaneous recovery from AA? In addition, although nail involvement may be seen in up to 66% of patients with AA (1), the immune privilege collapse theory by itself cannot explain this connection.

Alopecia areata is a genetic disease

Is it possible to completely explain AA without adopting the autoimmune theory? The answer is: ‘Yes, we can!’ There remains no doubt that AA is predominantly a genetic disease. Table 2 reviews the large volume of documented evidence supporting this hypothesis.

Of special interest is the recent work by Petukhova *et al*. (31) who performed an extensive genome-wide association study (GWAS), involving more than 1000 patients with AA and 3278 controls. This was the first GWAS to be performed in patients with AA, and it provided some interesting insights into the mechanisms of disease. In total, eight regions were found to be associated with AA, pointing to genes related to the immune system as well as for genes unique to the HF itself. Summary of the findings in this GWAS is presented in Fig. 2.

Is AA only a genetic disease and what type of genetic disease is it?

Although the disease prevalence is increased in first-degree family members of patients with AA, direct transmission of the disease is not always evident. Among first-degree relatives of 348 severely affected patients, van der Steen *et al*. (32) found that one of the parents was affected in 7%. Among the siblings, 3% had developed AA, while AA was present in 2% of the children. In addition, a variety of clinical presentations exist. This points to a complex genetic inheritance pattern involving several susceptibility genes. However, because in many families we noticed gradual worsening of the disease in consecutive generations (5), a nucleotide expansion may also explain the incomplete and variable penetrance and severity issues. The fact that AA has such a wide clinical heterogeneity lends further support to the idea that genetic complexity is to be expected in this disease (33).

The idea that a complex of susceptibility genes is involved in AA may also help to explain some of its clinical manifestations. It is possible that during the course of disease, one of the susceptibility genes will be expressed in the nails, leading to the nail involvement (1). In case a susceptibility gene is expressed in the thyroid, thyroid disease, reported to be present in higher frequencies in patients with AA, might occur (1). Similarly, it is conceivable that many of the disease associations reported so far in patients with AA are actually organ-specific manifestations of differentially expressed susceptibility genes (e.g. rheumatoid arthritis, vitiligo).

Although different modifying gene expression patterns may explain the varied clinical manifestations of AA, such as the initial site, age of presentation and disease severity, contributing mechanisms may add to the disease (Fig. 3). AA may be a structural genetic disease caused by mutations in genes such as *filaggrin*. Epidermal integrity is best maintained in non-exposed skin regions, while as seen most often in AA, the exposed regions of the scalp and face are more frequently affected (34). Is this due to a repeated minor trauma such as combing or shaving? Is it possible that these minor traumatic events lead to penetration of an infectious agent?

The short fractured hairs in the periphery of active AA lesions (exclamation mark hairs) are thought to result from disintegration of the hair shaft due to precortical cell degeneration, leading to abnormal moulding, and mechanical collapse (19). Recurrence may be explained by these mechanisms, as in streptococcus causing cellulitis. Infectious mechanisms are also proposed based on the slow and steady expansion of AA lesions. Recurrent episodes seen in AA are not pathognomonic for autoimmune diseases, but seen also with inflammatory diseases, for example, gout, which is manifested by sudden episodes of inflammation and regression of symptoms either spontaneously or with treatment, where a toxic metabolite is the provoking trigger. It is of interest that recently three different gene loci were found to be connected to the risk for gout (35).

Summary and outlook

Alopecia areata is a recurrent, unpredictable form of non-scarring hair loss that is generally felt to be mediated by T lymphocytes targeting HFs (2) and hence an autoimmune disease. Although it is not easy to raise doubts on the autoimmune theory, the data does not

necessarily support this ‘general feeling’. This is especially true when one considers that a target antigen remains to be identified, and for that reason, until the antigen is found, the autoimmune concept still remains a hypothesis (36).

Although not as common as the prevailing theory, several other hypotheses are sometimes revisited in the medical literature. These include, for example, the genetic, infectious, trophoneurotic, toxic and endocrinological hypotheses. In this short viewpoint, we have emphasized the importance of genetic factors in the pathogenesis of the disease. AA is clearly a genetic disease, although other factors may play a role in disease aetiology. In this regard, it is important to keep in mind that there may be several genetic subsets of AA, and one or more of them may be autoimmune, evident, for example, the association of AA with the autoimmune polyglandular syndrome (37). Another subset may be associated with a gene located on chromosome 21, as manifested by the increased prevalence of AA in patients with Down's syndrome (38). Nevertheless, it is not necessary that all AA subsets are auto-immune in origin. For example, most patients with AA do not have any additional autoimmune-related conditions.

In view of the fact that the autoimmune hypothesis of the AA aetiology has been lately regarded as a ‘sure thing’, the uncertainties still existing in this theory mandate a different look at this disease.

Taken together, we believe that AA should be viewed as a complex disease, influenced by a large number of genes. When a specific disease liability threshold is exceeded, based on the individual set of genes, the disease breaks out. The environment is engaged in the disease process, either by shifting the disease threshold or by changing the genetic contribution of a subset of polymorphisms (39). Considering the fact that complex diseases should be conceptualized as a non-linear amalgamation of multiple interacting factors, it seems that the journey to finding the whole range of factors that contribute to AA pathogenesis is only beginning.

Viewpoint 6

‘It is common error to infer that things which are consecutive in order of time have necessarily the relation of cause and effect.’

Jacob Bigelow, US botanist (1787–1879)

The Editor's invite to join esteemed colleagues in an unfettered discussion on the cause(s) of alopecia areata (AA) was too tempting to resist. Tempting for me especially, as this confounding condition was my passport into the world of scientific research and hair follicle biology in particular. Fresh from an undergraduate degree in immunology, my postgraduate studies were on this form of alopecia – long feted for its hallmark ‘swarm of bees’ (1). Under the guidance of an open/independent-minded clinical supervisor (David Fenton), I become acquainted with the ‘exclamation mark hairs’ of patients with AA – How could one ignore these microshouts for help? – and AA’s associated pigmentary anomalies.

With an awe-inspiring transmission electron microscope at my disposal, so began my attempt to understand the ‘cause’ of the clinical features of AA via an analysis of the structure of the tissues involved. As Szent-Györgyi aptly put it, ‘If structure does not tell us anything about function, it means we have not looked at it correctly’ (2). With this penetrating entreatment in mind, I sought to peer directly into the heart of the AA-affected anagen hair follicle bulb. However, my attention therein was soon diverted to the hair bulb melanocyte. These cells were frequently observed to undergo an apoptosis-like degeneration, but often with little other evident tissue disruption (e.g. a scarce or undetectable immunocyte infiltration or keratinocyte damage), and only rarely in direct contact to immunocytes. Remarkably, healthy-appearing melanocytes may be found adjacent to these degenerating melanocytes (3). Upon closer examination, many of these ‘AA-vulnerable’ melanocytes appeared to exhibit aberrant melanogenesis and melano-some transfer. Their affected cell cytoplasm was commonly filled with numerous vacuolated melanosomes, indicative of an ‘aborted’ melanogenesis and other evidence of oxidative stress (Fig. 4) (3).

In some affected anagen hair bulbs, the melanocytes appeared to have been completely deleted, while adjacent keratinocytes still continued to proliferate. Meanwhile, ‘housekeeping’ or potentially antigen-presenting macrophages were present to remove remnants of damaged melanocytes from the hair follicle, commonly via the follicular dermal papilla or dermal sheath – an appropriate and ‘normal response’, I presumed. Could this suggest the melanocyte effect predates the characteristic premature precipitation of the hair follicle into catagen?

These ultrastructural studies presented to me a choice of take-home message: either (i) aberrantly functioning melanocytes in AA-affected hair follicles are an important early target for an appropriate immune response or (ii) normally functioning melanocytes are an important early target for an inappropriate immune response in AA (4), perhaps due to an inappropriately activated immunosurveillance machinery (5–7).

I used to favour predominantly the former possibility, as on one level the melanocyte defect appears *intrinsic*. By that I mean patients with AA may indeed have some primary defect/alteration of their melanogenesis biochemistry, against which an immune response can subsequently be raised in susceptible individuals (e.g. in those with permissive MHC haplotypes). In this way, they may resemble individuals with leukoderma due to vitiligo, although in the latter, the focus is largely the epidermal melanocytes, and leukotrichia is much less common. Thus, an (auto) immune response may only be secondary to a primary precipitating factor (e.g. redox imbalance 8), especially in those with vulnerable immune system-associated genetics. Both AA and vitiligo may be associated with poorly regulated or aberrant melanogenesis, with an associated potential cytotoxicity that is likely to be significantly immunogenic. This is particularly true for differentiated melanocytes located in the melanogenic region of the anagen hair bulb, which may explain why poorly differentiated melanocytes/melanoblasts in the outer root sheath and stem reservoir appear to be spared, permitting recovery of pigmentation after initial hair regrowth in AA.

However, I am now also more open to the view that the initial stimulus for an AA ‘attack’ may be against one or a relatively small number of early anagen hair follicles that become ‘recognizable’ to the immunosurveillance systems of the body during a very defined phase of early anagen. In most cases, this phase is (or is contemporaneous with) the reconstruction of the follicular melanin unit. This short time window may be associated with some unmasking of potentially dominant antigens, which for most of us is ignored by our immune system.

The initial lesion in a single early anagen hair follicle may only involve a very narrow and restricted anti-self-recognition repertoire, which is facilitated to spread or expand due to the lowering of the antigenic threshold to anagen hair follicles spatially proximal to the first-hit ‘bad apple follicle’. This then allows a broader antigen recognition of close-by anagen hair follicles and may indeed underlie the apparent random movement of multiple small lesions across the scalp and body – each with their own first-hit ‘bad apple hair follicle’.

Whether this ‘antigenic threshold’ is set abnormally low in AA individuals appears to be supported by their well-characterized susceptibility/severity genetic associations. Antigenic thresholding may also determine whether patchy AA ever becomes more severe and/or A. totalis or universalis and whether remission is more or less likely. At least for the most common patchy variant of AA, it would appear that systemic immune factors in blood, either lymphocytes or anti-hair follicle antibodies, do not recognize (or seek out) all anagen hair follicles equally despite apparent similar access.

While much has been written about melanocytes in AA, keratinocytes may not be innocent bystanders in this pigment-fuelled battle (9). While the preferential targeting of pigmented hair follicles together with sparing of white hair follicles in AA is indeed impressive, it may not be absolute. However, involvement of the precortical keratinocyte population may still be secondary, an overspill from adjacent melanocytes. In this context, I would be very interested to hear from clinician colleagues whether AA is as common in individuals with tyrosinase-negative oculocutaneous albinism compared with those with normal pigmentation. If not (as I would daringly predict), messy melanin could be a culprit/accomplice in the pathogenesis of AA.

Alopecia areata studies tend to throw up as many questions as answers. For me, I am curious why ‘CD8/CD4 T cell bees’ are not always prominent, if these are the principal ‘effectors’? Why if present are they not always clearly evident at the site of follicular damage? Are infiltrating T lymphocytes the *sine qua non* for all of the tissue changes we see in acute AA or do they drive the short circuiting of the follicular growth cycle in long-standing but also weakly- or even non-inflammatory alopecia totalis? I am especially curious to learn how lymphocytic infiltrates avoid regrowing hair follicles located so close to areas of active hair loss? This tells me that the hair follicle retains at least some control of its destiny. The successful transfer of hair loss in AA scalp biopsies injected with $CD8^+$ T cells upon transplantation to mice is clearly impressive (10), although this may still somehow represent a secondary ‘effect’. Under normal (non-experimental) conditions, $CD8^+$ T cells may be drawn to the scene of some earlier crime, that is, an elusive primary event, and literally inflames this situation.

Maybe the true primary effector in AA is something altogether more subtle – a humoral factor perhaps? Pro-inflammatory cytokines (IL-1, IL-6, TNF-a, TGF-ß), MHC-II-activating interferons, etc. are all attractive possibilities, but surely something still needs to activate immunocytes (or indeed follicular keratinocytes) to produce these factors.

And what about antibodies? While these are B-cell products, T cells are essential in their activation to plasma cells (Fig. 5). Antibodies can be found in abundance in AA (sometimes in extremely high titres) and more importantly can be specifically directed to antigens expressed in anagen hair follicles, despite the latter's close histological similarity with cell types of the epidermis and dermis (11,12). Anti-hair follicle antibody titres can increase even before onset of overt clinical hair loss (13). Even if these ‘AA antibodies’ have no or only limited role in the pathogenesis of AA, they may still serve as useful guide dogs in our search for target antigens whose expression modulates (creating variable antigenic thresholds) during early to late anagen phase of the hair growth cycle.

Different antigenic dominos to fall during this period of the cycle may either trigger or perpetuate the immune-mediated helter-skelter of AA. We have previously reported that patients with AA have antibodies that can immunoprecipitate hair folliclespecific keratins (recognized by the AE13 monoclonal antibody) (14). Moreover, sera taken from patients with AA or vitiligo contain circulating antibodies that can preferentially bind antigens extracted from primary cultures of hair follicle versus epidermal melanocytes, respectively (15). More recently, we have also shown using a proteomic approach that the structural protein trichohyalin (a crucial assembler of keratin intermediate filaments and richly expressed in the inner root sheath; IRS) is a very common and possibly immunodominant antigen in this anagen-specific component of the hair follicle (16). It would be very interesting to see whether this protein (or associated or cross-reactive peptide or amino acid mimic) can also stimulate/activate T cells in transfer studies of AA. Interestingly, evidence for the involvement of trichohyalin as a potential immunodominant antigen in AA has also been reported by McElwee (17). I was somewhat surprised therefore that trichohyalin did not appear in the very large GWAS of AA individuals reported by Christiano's laboratory (18) though some gene encoding a trichohyalin ‘mimic’ fragment may have.

My prediction would be that the inner root sheath (IRS) is a prominent target/lesion in AA and in other conditions with hair follicle cycle defects, as this sheath appears to be a veritable gate-keeper of the hair follicle's main product – the fibre. But what of a connection between hair bulb melanocytes and IRS? Nothing obvious yet springs to mind...except that trichohyalin expression, like the reconstruction of the follicular pigmentary unit, is one of the earliest differentiation events of the early anagen hair bulb (19). Could a melanocyte lesion in anagen III/IV AA follicles disrupt IRS differentiation (either directly or via a consequent inflammatory response) leaving entry into a premature catagen the follicle's only secure option?

In summary, I am hugely aware that in these few lines, I have bypassed a mountain of corroborating data that place the T cell as lead actor on the AA stage (regardless of who has fed him his lines), as well as sidestepping the relevance of applying today's apparently looser definition of ‘autoimmunity’. I remain perplexed, however, by attempts to conclude

that this heterogeneous condition represents indeed a single disorder (with a single ‘cause’) or whether is it even ‘(HF)-organ specific’ as many are seduced into describing it (note that several other melanocyte-containing tissues like eyes, nails, etc. can also be affected).

Just when I think that this is quite enough to deal with, I reflect on the Editor’s intended remit of ‘cause’ – thus the focus is on the nature of the fuse that was lit just before the AA ‘big bang’, that is, what are the aetiological factors? Is the hair follicle's ‘antigenic threshold’ variable such that it can be influenced by antigenic mimicry after viral infection, etc.? Or should we rather focus on quite diffuse polygenetic associations and how these may enter their subsequent epigenetic grooves (20)? However, it is much less interesting for the patient and clinician to worry about how the train leaves the station than the damage it can cause en route, and more so if it ultimately goes off the rails. More serenely put, perhaps:

‘The thinker makes a great mistake when he asks after cause and effect. They both together make up the indivisible phenomenon’

Johann Wolfgang von Goethe (1749–1832)

Viewpoint 7

After such a scholarly, spirited and thought-provoking debate, perhaps, I can best avoid being merely repetitive by revisiting my own tortuous journey through the AA pathobiology labyrinth.

As a postdoc with Kurt Stenn at Yale in the late 1980s, fresh out of German medical school, I was blessed with a supreme hair biology mentor. However, he abhorred being bothered with murky hair follicle (HF) immunology issues. This turned out to be a blessing in disguise as it encouraged me to find a hair research niche not yet fully explored by others when I returned to Berlin for my dermatology residency and opened the first hair clinic of our hospital. When that clinic got quickly overrun by patients with AA, I tried to make sense of the numerous and partially conflicting HF immunopathology observations on AA that had been reported by the early 1990s in patients and animals (e.g. 1–12), but failed to recognize a persuasive AA pathogenesis concept.

It struck me as logical to, first, characterize the immunology of healthy HFs before trying to understand what goes wrong in AA. Thus, we began to explore the immunobiology of normal HFs in mice and humans (reviewed in 13). We did so following the footsteps of Billingham (14) and Westgate (15) who had revived the concept of HF immune privilege (IP), and Harrist et al. (16) who had shown that human anagen HF epithelium downregulates MHC class Ia expression, but had missed the relevance of this observation for HF IP. Our first simple studies on murine HF immunobiology (17–19) then provided a fertile ground, in which the hypothesis arose that AA results from a collapse of the HF's MHC class I-based IP. This, we postulated, exposes previously hidden HF-associated autoantigens to autoreactive $CD8^+$ T cells (20).

This novel concept stated clearly that IP collapse alone does not suffice to induce AA lesions and that several other events have to conspire to produce the disease (e.g. the HF has

to be in anagen to be attacked; there have to be pre-existing autoreactive T cells, to which – normally sequestered – peptides cleaved from melanogenesis-related and/or other anagen-associated autoantigens produced by the HF epithelium are ectopically presented by MHC class Ia molecules; and there must be sufficient co-stimula-tory signals and possibly help from other immunocytes) (20). Also, our hypothesis proposed that a large variety of secondary immunological parameters (from predisposing genetic factors determining immune response patterns via HF autoantibodies to $CD4^+$ T cells) may all impact greatly on the clinical AA pheno-type seen in a given patient, on the course and prognosis of the disease and on a patient's response to therapy (reviewed and further developed in 13).

Thus, right from its inception, this AA theory (20) accounted for the well-known heterogeneity and complexity of AA pheno-types (21) as well as for multiple other AA factoids: for example, that $CD8^+$ T cells are routinely the first *intrafollicular* lymphocytes seen in AA, that autoantibodies are incapable of transferring the disease, that aberrant MHC class II expression of AA HFs is a secondary phenomenon, that different pathways (incl. viral infection and IFNg) can induce HF IP collapse and that, rarely, AA can also affect non-pigmented HFs (see previous essays and 22). Moreover, our theory integrated well with the central role of autoreactive $CD8^+$ T cells and MHC class I-presented autoantigens recognized in other ‘organ-specific’, T-cell-mediated autoimmune diseases (37). Thus, this theory was appealingly simple, but not simplistic.

However, back in the 1990s, it ran contrary to the (then domi-nant) belief that autoantibodies or MHC class II-presented autoantigens recognized by $CD4^+$ T cells lie at the heart of AA pathogenesis –thus was duly ignored by most AA experts. It was to no avail when I pointed out that nobody had ever been able to induce AA by antibodies; that it made little sense to focus on MHC class II-presented (auto)antigens recognized by $CD4^+$ T cells, because the ectopic MHC class II expression of HFs observed in AA is a secondary event that only follows after $CD4^+$ T cells have appeared on the AA ‘crime scene’ (12); and that the excellent DEBR rat model of AA unequivocally documented a key role of $CD8^+$ T cells in AA pathogenesis (23) soon after publication of our hypothesis. These arguments were nonchalantly brushed aside, and many esteemed colleagues, who readily grant today that healthy HFs do enjoy some form of IP and that this is compromised in AA (e.g. 24,25), long questioned whether HF IP was more than just a fanciful and wildly speculative notion.

Only several years after Gilhar *et al*. (26) showed in their first humanized mouse model of AA that characteristic hair loss lesions can be induced within transplanted human skin by the transfer of $CD8^+$ T cells alone, but not by $CD4^+$ T cells or serum alone, the IP collapse theory of AA gained more credibility, yet ever so slowly [characteristically, it was not even cited in (26)]. Our hypothesis was further backed up by additional studies from the Gilhar laboratory, including evidence in support of melanogenesis-associated proteins as likely candidate autoantigens in AA and IFNg as a key AA-triggering cytokine (e.g. 27,28). To my delight, the bulk of subsequently published AA investigations also directly or indirectly buttressed key tenants of the HF IP collapse concept and the intimately connected key role of $CD8^+$ cells in AA patho-biology (see, e.g. 25,29–31). In fact, it was recently confirmed that MHC class I-restricted $CD8^+$ T cells are sufficient to induce AA-like lesions in mice (32), which could hardly have happened without a prior breakdown of HF IP.

That AA also affects the nail (frequently) and the eye (rarely) does not argue against AA being an organ-specific autoimmune disease: both eye and nail matrices are very special in that, like the HF, they express melanocyte- and melanogenesis-associated antigens and harbour defined immunoprivileged compartments (33,34). Thus, the definition holds if one employs the term ‘organ specific’ in the sense of ‘specific for tissues that are immunoprivileged and express melanocyte-related antigens’. Further, if one accepts that HF autoantibodies found in patients with AA (and healthy individuals!) have no role in the primary pathogenesis of AA, it is unreasonable to expect that they can be useful guides to the pathogenically important *primary* autoantigens in AA. To identify those, it is much more logical and promising to isolate and characterize intralesional autoreactive $CD8^+$ cells and the MHC class I-presented (auto)antigenic peptides recognized by these lymphocytes (13,20,22).

The apparently never-ending debate on whether or not AA really is an autoimmune disease, in my eyes, had ceased to be productive ever since the landmark studies of McElwee et al. (23) and Gilhar et al. (26). In addition to the obvious association of AA with other autoimmune diseases [recently confirmed again (35)] and HLA haplotypes also found in other autoimmune diseases (see previous viewpoints), in my perception, the autoimmunity question has long been settled by these facts: (i) AA can be adoptively transferred with MHC class I-restricted $CD8^+$ T cells alone (23,26), which attack an autoantigen-expressing tissue that ectopically expresses normally suppressed MHC class I molecules [as the latter are almost never expressed ‘empty’, they are expected to present [auto]antigens (37)], (ii) AA can be suppressed by eliminating these $CD8^+$ T cells, (iii) AA can be induced by bone marrow transplantation (36) and (iv) reasonable candidate intrafollicular autoantigens do exist (e.g. melanogenesis-related proteins). In the face of this evidence, I felt it to be a scholastic exercise to continue debating the autoimmune nature of AA, akin to philosophizing over ‘how many angels can dance on the head of a pin’.

Instead, a much more pertinent problem vexed me: If healthy human anagen HFs suppress MHC class I as one key mechanism of maintaining their IP [the other central mechanism is the creation of an immunoinhibitory signalling milieu in and around the HF epithelium (13,22)], why are they not under constant attack by NK cells? After all, these are primed to recognize and eliminate MHC class I-negative cells (37).

Taisuke Ito in my laboratory, then in Hamburg, first provided a plausible answer (38): Normal human anagen HFs suppress the expression of agonists of NK cell-activating surface receptors (NKG2D), such as MICA [NKG2D are also expressed on NK T cells and $CD8^+$ T-cell subpopulations (37)]; in addition, healthy HFs appear to secrete agents (e.g. MIF) that suppress the functions of $NKG2D^+$ cells. Instead, AA HFs massively overexpress MICA and downregulate MIF immunoreactivity; moreover, the number of $NKG2D^+$ cells is significantly increased, both in the blood and in the perifollicular skin of patients with AA (38).

The novel concept that $NKG2D^+$ cells (incl. NK cells) are insufficiently controlled in AA (38) sat well with previously published genetic evidence of an association of MICA with AA (39) and was subsequently supported by a gold standard GWAS from Angela

Christiano's laboratory. This study confirmed an association of AA with NKG2D-activating MICA family members, most notably ULBP3, and overexpression of NKG2D ligands in patients with AA (40). Shortly thereafter, the first functional proof for the importance of IFNg-secreting NKG2D$^+$ cells in AA pathogenesis was provided by a new humanized mouse model of AA (41).

However, this new model from the Gilhar laboratory (41) raised a fresh set of fundamental questions. While it would be foolish to question the major role that genetic factors surely play at some stage in the course of AA (see viewpoints above, and 39,40,42–50), this mouse model demonstrates that a classical AA phenotype can be robustly induced in normal human skin and with cells that were both obtained from apparently healthy individuals without a personal or family history of AA. This hardly supports the notion that the genetic basis of AA is all important. The fact that characteristic AA lesions have by now been induced in previously normal-appearing human skin and with 'normal' PBMCs from several genetically very distinct individuals in this model (41,51) makes it quite unlikely that a certain genetic makeup is *essential* for the AA phenotype to develop.

Therefore, I can not really see why it would be justified to define AA as a 'genetic disease' in the strict sense – beyond the truism that that genes are somehow involved in (almost) everything. Evidently, an individual's genetic make-up strongly plays into disease susceptibility, phenotype, course and/or response to therapy, in AA as much as in almost any other disease one can think of. But that does not make AA a 'genetic' disease (whatever that means exactly). And let us not forget that, besides genes, environmental parameters (e.g. stress, diet, infectious agents) can greatly impact the AA phenotype and course. Thus, can we, please, get a bit more restrictive with branding a complex disease (like AA) as 'genetic' ...?

Put bluntly, I interpret the new Gilhar AA model (41) to suggest that most, if not all, anagen HFs, *irrespective* of their specific genetic make-up, will respond with a collapse of their IP and with HF dystrophy, premature catagen entry and hair shaft shedding (22), if they are attacked by an overwhelming inflammatory infiltrate that secretes IP collapse-inducing signals, such as IFNg. Furthermore, as AA lesions can be experimentally induced in normal human scalp skin, that is, in HFs with a presumably intact IP, by IL-2-activated, but otherwise normal PBMCs enriched for NKG2D$^+$ and CD56$^+$ cells from healthy donors (41,51), this questions whether pre-existing *auto-reactive* CD8$^+$ T cells are really *indispensable* for AA lesions to develop.

Of course, the injected cell cocktail also contained plenty of CD8$^+$ T cells (41), some of which may have recognized MHC class I-presented HF autoantigens that could have been exposed by HFs whose IP already had become labile during skin surgery, transplantation and healing, only to collapse completely after the intra-cutaneous injection of a mass of IFNg-secreting cells. However, even the transfer of CD4$^+$ T cells alone suffices to cause autoimmune hair loss and hair depigmentation in RAG$^{-/-}$ mice in a manner that is reminiscent of AA (52). Along with the fact that an AA phenotype can be induced in human skin by NKGD2G$^+$/CD56$^+$ cells, only a minority of which is CD8$^+$ (41,51), this suggests

that, at least under experimental conditions and in mice, autoreactive $CD8^+$ T cells may not be indispensable for AA lesions to develop.

Therefore, I wonder: 'Does the AA phenotype only reflect an end-stage *stereotypic response pattern of healthy HFs* to the onslaught of a massive, IFNg-secreting inflammatory cell infiltrate, irrespective of the precise composition and origin of this infiltrate, rather than a single disease entity?' In other words, does the AA phenotype initially represent a normal response pattern of the HF to a defined spectrum of inflammatory tissue damage, aimed at restoring HF homoeostasis? In those many individuals who show spontaneous remission of AA (associated with restoration of HF IP!), the damage repair may have been successful, at least temporarily. In this hypothetical scenario, a genuine autoimmune disease develops only once the AA response pattern has permanently failed to restore HF IP.

Just as the diverse manifestations of AA all share defined clinical characteristics that we exploit to make the diagnosis (22,53), both autoantigen-specific, $CD8^+$ T-cell-dependent *and* non-antigen-specific events appear to be capable of inducing an AA pheno-type – provided that these events culminate in HF IP collapse and HF dystrophy. (I will leave it to the scholastics among us to debate until the cows go home whether non-antigen-specific variants of the HF's AA response pattern qualify as a genuine cell-mediated autoimmune disease, even if they are induced by T or NK cells and reflect autoaggressive immunity.)

This brings me to my closing credo, which has helped to keep me oriented within the AA pathobiology labyrinth. HF IP collapse is clearly not the only event required to induce visible AA lesions (13,20,22), and there are many ways to induce it. However, all currently available evidence suggests that the stereotypic HF *response pattern* that we call 'AA' never occurs *without* HF IP collapse. Thus, if there is one *conditio sine qua non* in AA pathobiology, it is this: HF IP collapse.

The essays for this Controversies feature on 'What causes AA?' were invited with the aim to refocus our attention on key mecha- nisms in AA pathobiology that are particularly promising targets for more effective future therapy. From this perspective, my own travels through the AA research landscape got me convinced that it is very important to systematically search for the elusive autoantigens of AA and to characterize both the autoreactive *intracutaneous* $CD8^+$ T cells that recognize them and the $CD4^+$ T cells (incl. Tregs) and possibly other immunocytes such as mast cells that interact with and regulate $CD8^+$ T cell (54–56). And I fully agree that it is important to define and therapeutically target principal co-stimulatory signals and to restore defective T cell-inhibitory signals in AA, such as CTLA4 (40,44,57–59); to explore the role of $NKG2D^+$ cells in AA and to learn how to suppress or antagonize NKG2D-stimulating endogenous ligands such as MICA and ULPB3 (22,38–40); and to systematically dissect the role of potential cellular co-players in both normal hair growth regulation and AA pathobiology, such as mast cells and cd T cells (18,19,54,60,61).

These approaches not only will help to define at which stage this putatively 'healthy' HF response pattern to inflammatory damage becomes a genuine autoimmune disease, but will also greatly enrich our unsatisfactory therapeutic arsenal in AA management, and may even

identify a curative therapy one day. Yet, in my view, the relative weight of all this is dwarfed by the paramount importance of restoring a collapsed HF IP in AA (13,20,22,62).

Digesting the published literature leaves me with little doubt that IFNg, both in mice and in most patients with AA, is the key instigator of HF IP collapse (9,62–67). Also, if viral infection plays any role as a trigger factor in some patients with AA, IFNg is a key candidate cytokine through which such a trigger effect is likely mediated (37). The prototypic stress-induced neuropeptide, substance P, also induces HF IP collapse and activates HF-attacking $CD8^+$ T cells (68,69). Thus, substance P-dependent neurogenic HF inflammation (70) may well expose the HF to additional IFNg. Antagonizing and downregulating intracutaneous IFNg- and substance P receptor (NK1)-mediated signalling, in my book, therefore remains one of the two most promising therapeutic strategies for more effective AA management – irrespective of the various pathogenic roads that coalesce in the HF's AA response pattern.

The second strategy is preventing this HF IP collapse from happening so as to halt disease progression (13,22,62). This may best be done by ‘learning from the HF’, that is, by using endogenous neuropeptides and growth factors that healthy anagen HFs employ to maintain their IP: aMSH, CGRP, TGFß1, IGF-1 (62,71) and likely many other ‘HF IP guardians’ waiting to be uncovered. There even is a routinely prescribed immunosuppressive drug that effectively restores a collapsed human HF IP *in vitro*: FK 506 (tacrolimus) (62). (That this has not worked after topical application in patients with AA so far most likely reflects insufficient drug penetration to the anagen hair bulb, which we should be able to overcome.)

Our patients distressed by AA and their partners/relatives could not care less how sophisticated and holistic our approach is to the pathobiology of AA. What they want is satisfactory therapeutic results. With this in mind: Is there really a more promising, more effective and less risky AA management strategy than the one I have now advocated for two decades, that is, trying our utmost to restore HF IP in these patients, pursuing all the available leads on how this may be accomplished (13,20,34,62,71–75)...?

Epilogue

To conclude this *Controversies* feature, let us return to practical issues in the actual management of AA – namely to as yet insufficiently appreciated indications that antihistamines may be effective in AA therapy. The latter supports the concept that mast cells play an additional role in AA pathogenesis that deserves to be dissected in more detail and puts a welcome practical spin on the AA pathobiology discussion: here, we may simultaneously be looking at an underinvestigated and potentially important ‘new’ player in AA pathogenesis, while enriching our therapeutic tools in AA management.

Efficacy of antihistamines for AA has been reported from Japan (1–3). Recently, we have reported two cases of AA responsive to an antihistamine fexofenadine chloride (4) and moreover carried out a retrospective chart review to study its effect for AA. Our retrospective study showed that fexofenadine chloride enhances hair regrowth, compared with the controls, by 0.862 of SALT score (S0-5), corresponding to 21.6% area of the scalp, during the one year immunotherapy. This supportive effect was seen in patients with a

background of atopy, but not in those without it (5). Moreover, Ohyama *et al*. showed that another antihistamine, ebastine, significantly recovered AA lesions in C3H/HeJ mice (6).

Autoreactive T lymphocytes secrete IFN-γ (6), which induces the expression of MHC class I and II, ICAM-1 and HLA-DR on the hair epithelium and dermal papilla (7), causing the collapse and restoration of MHC class I-dependent immune privilege of the hair follicles in AA (8). Additionally, degranulation of mast cells in AA (9), triggered by substance P (10), can induce the catagen phase of the hair cycle (11) (Fig. 6). As fexofenadine reportedly suppresses substance P-induced mast cell degranulation in the skin (12), mast cells are the possible therapeutic targets of fexofenadine in AA.

Therefore, one possible reason why the fexofenadine effect could be detected only in atopic patients with AA (5) may be the greater potential and likelihood of mast cell activation in atopic cases. One feasible reason why the fexofenadine effect was limited to an additive or supportive level could well be that this antihista-mine suppresses only the pathogenic AA pathway that involves mast cells (Fig. 6), but not the autoimmune pathobiology itself (13).

Taken together, we propose that the beneficial effects of antihistamines seen in AA management not only provide additional therapeutic options in atopic patients with AA, but also point to an additive influence of mast cells in AA pathogenesis.

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References

1. McDonagh AJ, Tazi-Ahnini R. Clin Exp Dermatol. 2002; 27:405–409. [PubMed: 12190641]
2. Gilhar A, Paus R, Kalish RS. J Clin Invest. 2007117:2019–2027. [PubMed: 17671634]
3. Yazdan P. Semin Cutan Med Surg. 2012; 31:258–266. [PubMed: 23174496]
4. Rückert R, Hofmann U, van der Veen C, et al. J Invest Dermatol. 1998; 111:25–30. [PubMed: 9665382]
5. Paus R, et al. Yale J Biol Med. 1993; 66:541–554. [PubMed: 7716973]
6. James J A et al. Curr Opin Rheumatol. 2006; 18:462–467.
7. Pender MP. Autoimmune Dis. 2012; 2012:189096. Epub 2012 Jan 24. [PubMed: 22312480]
8. Skinner RB Jr. J Invest Dermatol. 1995; 104:3S–4S. [PubMed: 7738384]
9. Tosti A. 107. J Invest Dermatol. 1996:443. [PubMed: 8751985]
10. Garcia-Hernández MJ, et al. J Invest Dermatol. 1998; 110:185. [PubMed: 9457919]
11. McElwee KJ. J Invest Dermatol. 1998; 110:986–987. [PubMed: 9620311]
12. Offidani A. J Cutan Med Surg. 2000; 4:63–65. [PubMed: 11179926]
13. Rodriguez TA, Duvic M. J Am Acad Dermatol. 2008; 59:137–139. [PubMed: 18329131]
14. d'Ovidio R, d'Ovidio F. 130. Giornale Italiano di Dermatologia. 1995:295.
15. Koyama S. Cytokine. 2008; 43:336–341. [PubMed: 18694646]
16. Ercolini AM, Miller SD. The Clin Exp Immunol. 2008; 155:1–15.

References

1. Ikeda T. Dermatologica. 1965; 131:421–445. [PubMed: 5864736]
2. Ross EK. J Am Acad Dermatol. 2005; 53:1–37. quiz 38–40. [PubMed: 15965418]

3. Yu M. J Dermatol Sci. 2010; 57:27–36. [PubMed: 19932600]
4. McElwee K. Eur J Dermatol. 2001; 11:11–16. [PubMed: 11174130]
5. Zhang X. J Invest Dermatol. 2009; 129:1527–1538. [PubMed: 19020552]
6. Elenkov IJ, Chrousos GP. Ann N Y Acad Sci. 2002; 966:290–303. [PubMed: 12114286]
7. McElwee KJ. Exp Dermatol. 2001; 10:420–429. [PubMed: 11737261]
8. McElwee KJ. Exp Dermatol. 2003; 12:30–36. [PubMed: 12631244]
9. Rodriguez TA, Duvic M. J Am Acad Dermatol. 2008; 59:137–139. [PubMed: 18329131]
10. Ikeda T. Dermatologica. 1967; 134:1–11. [PubMed: 6030798]
11. Kalish RS, Gilhar A. J Investig Dermatol Symp Proc. 2003; 8:164–167.
12. McElwee KJ. Exp Dermatol. 1999; 8:371–379. [PubMed: 10536963]
13. Rose NR, Bona C. Immunol Today. 1993; 14:426–430. [PubMed: 8216719]
14. Gilhar A. J Invest Dermatol. 2001; 117:1357–1362. [PubMed: 11886495]
15. Lueking A. Mol Cell Proteomics. 2005; 4:1382–1390. [PubMed: 15939964]
16. Tobin DJ. J Investig Dermatol Symp Proc. 2003; 8:176–181.
17. McElwee KJ. J Invest Dermatol. 1998; 111:797–803. [PubMed: 9804341]
18. McElwee KJ. Br J Dermatol. 1999; 140:432–437. [PubMed: 10233262]
19. McElwee KJ. Br J Dermatol. 1996; 135:211–217. [PubMed: 8881662]
20. Gilhar A. Arch Dermatol. 2002; 138:916–922. [PubMed: 12071819]
21. McElwee KJ. J Invest Dermatol. 2005; 124:947–957. [PubMed: 15854035]
22. Carroll JM. J Invest Dermatol. 2002; 119:392–402. [PubMed: 12190862]
23. McElwee KJ. Dermatology. 2005; 211:47–53. [PubMed: 15983437]
24. Barahmani N. J Am Acad Dermatol. 2003; 49:1192. [PubMed: 14639420]
25. Sanli H. Acta Derm Venereol. 2004; 84:86–87. [PubMed: 15040493]
26. Phillips MA. J Am Acad Dermatol. 2005; 53:S252–S255. [PubMed: 16227102]
27. Kanitakis J. Transpl Int. 2012; 25:e117–e119. [PubMed: 23013211]
28. Zuk DM. Am J Transplant. 2011; 11:163–168. [PubMed: 21199356]
29. Stewart MI, Smoller BR. J Cutan Pathol. 1993; 20:180–183. [PubMed: 8100574]
30. Cho M. South Med J. 1995; 88:489–491. [PubMed: 7716609]
31. Baranda L. Acta Derm Venereol. 2005; 85:277–278. [PubMed: 16040426]
32. Kuhn TS. Science. 1962; 136:760–764. [PubMed: 14460344]
33. Theofilopoulos AN. Immunol Today. 1995; 16:150–159. [PubMed: 7718089]
34. Theofilopoulos AN. Immunol Today. 1995; 16:90–98. [PubMed: 7888073]
35. Goodnow CC. Nature. 2005; 435:590–597. [PubMed: 15931211]
36. Goodnow CC. Cell. 2007; 130:25–35. [PubMed: 17632054]
37. Paus R. J Investig Dermatol Symp Proc. 1999; 4:226–234.
38. Paus R. Trends Immunol. 2005; 26:32–40. [PubMed: 15629407]
39. Breitkopf T. J Invest Dermatol. 2013; 133:1722–1730. [PubMed: 23370538]
40. Kang H. J Invest Dermatol. 2010; 130:2677–2680. [PubMed: 20613773]
41. Zoller M. J Invest Dermatol. 2002; 118:983–992. [PubMed: 12060392]
42. McElwee KJ. J Invest Dermatol. 2002; 119:1426–1433. [PubMed: 12485450]
43. Gilhar A. J Invest Dermatol. 2005; 124:288–289. [PubMed: 15654992]
44. Schroder K. J Leukoc Biol. 2004; 75:163–189. [PubMed: 14525967]
45. Hu X, Ivashkiv LB. Immunity. 2009; 31:539–550. [PubMed: 19833085]
46. Boehm U. Annu Rev Immunol. 1997; 15:749–795. [PubMed: 9143706]
47. Sundberg JP. J Exp Anim Sci. 2007; 43:265–270.
48. Botchkareva NV. J Invest Dermatol. 2006; 126:258–264. [PubMed: 16418734]
49. Weedon D, Strutton G. Acta Derm Venereol. 1981; 61:335–339. [PubMed: 6173989]
50. Lindner G. Am J Pathol. 1997; 151:1601–1617. [PubMed: 9403711]
51. Eichmuller S. J Histochem Cytochem. 1998; 46:361–370. [PubMed: 9487118]

52. Parakkal PF. J Ultrastruct Res. 1969; 29:210–217. [PubMed: 5362393]
53. McElwee KJ. Br J Dermatol. 1996; 134:55–63. [PubMed: 8745887]
54. Tobin DJ. Arch Dermatol. 1997; 133:57–61. [PubMed: 9006373]
55. Tobin DJ. J Invest Dermatol. 1997; 109:329–333. [PubMed: 9284100]
56. Tobin DJ. J Invest Dermatol. 1994; 102:721–724. [PubMed: 8176253]
57. Ueno H. Immunol Rev. 2007; 219:118–142. [PubMed: 17850486]
58. Albert ML. Nature. 1998; 392:86–89. [PubMed: 9510252]
59. Mehling A, Beissert S. Crit Rev Biochem Mol Biol. 2003; 38:1–21. [PubMed: 12641341]
60. McElwee KJ. J Investig Dermatol Symp Proc. 2003; 8:182–187.
61. Lu W. Expert Rev Mol Med. 2006; 8:1–19. [PubMed: 16787552]
62. McElwee KJ. Vet Pathol. 2003; 40:643–650. [PubMed: 14608017]
63. McElwee KJ, Hoffmann R. Clin Exp Dermatol. 2002; 27:410–417. [PubMed: 12190642]

References

1. Shoenfeld Y. Isr Med Assoc J. 2008; 10:13–19. [PubMed: 18300564]
2. Shoenfeld Y. Isr Med Assoc J. 2008; 10:8–12. [PubMed: 18300563]
3. Shoenfeld Y. Isr Med Assoc J. 2008; 10:3–7. [PubMed: 18300562]
4. Rahamim-Cohen D, Shoenfeld Y. Isr Med Assoc J. 2001; 3:381–382. [PubMed: 11411207]
5. Paus R. Yale J Biol Med. 1993; 66:541–554. [PubMed: 7716973]
6. Paus R. Trends Immunol. 2005; 26:32–40. [PubMed: 15629407]
7. Alkhalifah A. J Am Acad Dermatol. 2010; 62:191–202. [PubMed: 20115946]
8. Ito T. Eur J Dermatol. 2010; 20:126–127. [PubMed: 19822483]
9. Harries MJ. BMJ. 2010; 23:c3671. [PubMed: 20656774]
10. Gordon KA, Tosti A. Clin Cosmet Investig Dermatol. 2011; 4:101–106.
11. Hordinsky MK. Dermatol Ther. 2011; 24:364–368. [PubMed: 21689246]
12. Miteva M, Tosti A. Expert Opin Pharmacother. 2012; 13:1271–1281. [PubMed: 22594679]
13. Gilhar A. N Engl J Med. 2012; 366:1515–1525. [PubMed: 22512484]
14. Rashtak S, Pittelkow MR. Curr Dir Autoimmun. 2008; 10:344–358. [PubMed: 18460895]
15. Cetin ED. Am J Dermatopathol. 2009; 31:53–60. [PubMed: 19155726]
16. Bertolini M. Exp Dermatol. 2012; 21:477–479. [PubMed: 22621196]
17. Gilhar A. Clin Immunol Immunopathol. 1993; 66:120–126. [PubMed: 8095867]
18. Mcdonagh AJ. Br J Dermatol. 1993; 3:250–256. [PubMed: 7506926]
19. Rückert R, et al. J Invest Dermatol. 1998; 111:25–30. [PubMed: 9665382]
20. Ito T. Am J Pathol. 2004; 164:623–634. [PubMed: 14742267]
21. Kalish RS, Gilhar A. J Invest Dermatol Symp Proc. 2004; 8:164–167.
22. Gilhar A, Kalish RS. Autoimmun Rev. 2006; 5:64–69. [PubMed: 16338213]
23. Gilhar A, Krueger GG. Arch Dermatol. 1987; 123:44–50. [PubMed: 3800422]
24. Tobin DJ. Investig Dermatol Symp Proc. 2003; 8:176–181.
25. Gilhar A. Br J Dermatol. 1992; 126:166–171. [PubMed: 1536782]
26. Gilhar A. Clin Invest. 1998; 101:62–67.
27. McElwee KJ. Br J Dermatol. 1999; 140:432–437. [PubMed: 10233262]
28. McElwee KJ. Br J Dermatol. 1996; 135:211–217. [PubMed: 8881662]
29. Gilhar A. Arch Dermatol. 2002; 138:916–922. [PubMed: 12071819]
30. McElwee KJ. J Invest Dermatol. 2005; 124:947–957. [PubMed: 15854035]
31. Niederkorn JY. Nature Immunology. 2006; 7:354–359. [PubMed: 16550198]
32. Paus R. J Invest Symp Proc. 2003; 8:188–194.
33. Wahl SM. Immunol Rev. 2006; 213:213–227. [PubMed: 16972906]
34. Mellor AL, Munn DH. Nat Rev Immunol. 2008; 8:74–80. [PubMed: 18064049]

35. Forrester JV. Mucosal Immunol. 2008; 1:372–381. [PubMed: 19079201]
36. Gilhar A. J Clin Invest. 2009; 117:2019–2027. [PubMed: 17671634]
37. Kinori M. Exp Dermatol. 2012; 21:223–226. [PubMed: 22379970]
38. Christoph T. Br J Dermatol. 2000; 142:862–873. [PubMed: 10809841]
39. Ljunggren HG. Int J Cancer Suppl. 1991; 6:38–44. [PubMed: 2066183]
40. Apte RS. Invest Ophthalmol Vis Sci. 1997; 38:1277–1282. [PubMed: 9152248]
41. Apte RS. J Immunol. 1996; 156:2667–2673. [PubMed: 8609381]
42. Apte RS. J Immunol. 1998; 160:5693–5696. [PubMed: 9637476]
43. Ito T. J Invest Dermatol. 2008; 128:1196–1206. [PubMed: 18160967]
44. Terme M. J Immunol. 2008; 180:4679–4686. [PubMed: 18354191]
45. Lu L. Immunity. 2007; 26:593–604. [PubMed: 17509909]
46. Winkler-Pickett R. J Immunol. 2008; 180:4495–4506. [PubMed: 18354171]
47. Kitaichi N. J Leukoc Biol. 2002; 72:1117–1121. [PubMed: 12488492]
48. Fort MM. J Immunol. 1998; 161:3256–3326. [PubMed: 9759840]
49. Zhang B. J Exp Med. 1997; 186:1677–1687. [PubMed: 9362528]
50. Petukhova L. Nature. 2010; 466:113–117. [PubMed: 20596022]
51. Petukhova L. Dermatol Ther. 2011; 24:326–336. [PubMed: 21689242]
52. Ito T. J Dermatol Sci. 2010; 60:67–73. [PubMed: 20943348]
53. Taneja V, David CS. Nat Immunol. 2001; 2:781–784. [PubMed: 11526385]
54. McElwee KJ. J Invest Dermatol. 1998; 111:797–803. [PubMed: 9804341]
55. Sundberg JP. J Invest Dermatol. 2004; 123:294–297. [PubMed: 15245428]
56. Sundberg JP. J Invest Dermatol. 2011; 131:2323–2324. [PubMed: 21753782]
57. Duncan FJ. J Invest Dermatol. 2013; 133:334–343. [PubMed: 23014334]
58. Gudjonsson JE. J Invest Dermatol. 2007; 127:1292–1308. [PubMed: 17429444]
59. Gilhar A. J Invest Dermatol. 2013; 133:844–847. [PubMed: 23096715]
60. Gosemann JH. Injury. 2001; 41:1060–1067. [PubMed: 20591432]
61. Price VH. J Am Acad Dermatol. 2005; 52:138–139. [PubMed: 15627095]
62. Freyschmidt-Paul P. Eur J Dermatol. 2001; 11:405–409. [PubMed: 11525945]
63. Gilhar A. J Invest Dermatol. 2013; 133:2088–2091. [PubMed: 23636064]
64. Nagai H. Arch Dermatol Res. 2006; 298:131–134. [PubMed: 16786344]
65. Tobin DJ. Microsc Res Tech. 1997; 38:443–451. [PubMed: 9297694]
66. Khoury EL, Price VH. J Invest Dermatol. 1995; 104(Suppl):24S–25S. [PubMed: 7738380]
67. Hann SK. J Dermatol. 1996; 23:100–103. [PubMed: 8839236]
68. Gilhar A. J Invest Dermatol. 2001; 117:1357–1362. [PubMed: 11886495]
69. DiPreta EA. Cutan Med Surg. 2000; 4:156–160.
70. Becker JC. J Invest Dermatol. 1996; 107:627–632. [PubMed: 8823372]
71. Smyth JR, McNeil M. J Invest Dermatol Symp Proc. 1999; 4:211–215.
72. Wang E, McElwee KJ. Dermatol Ther. 2011; 24:337–347. [PubMed: 21689243]
73. Yousefi M. Int J Dermatol. 2006; 45:314–315. [PubMed: 16533238]
74. Radny P. Dermatology. 2004; 209:249–250. [PubMed: 15459547]
75. McElwee KJ. Exp Dermatol. 2001; 10:420–429. [PubMed: 11737261]
76. Jin Y. N Engl J Med. 2007; 356:1216–1225. [PubMed: 17377159]
77. McElwee K. Investig Dermatol Symp Proc. 2005; 10:280–281.
78. Price VH. Am Acad Dermatol. 2008; 58:395–402.
79. Brown WH. Br J Dermatol Syph. 1929; 41:299–323.
80. Tan RS. Proc R Soc Med. 1974; 67:195–196. [PubMed: 4820815]
81. Adams BB, Lucky AW. Pediatr Dermatol. 1999; 16:364–366. [PubMed: 10571834]
82. Hordinsky M, Ericson M. J Investig Dermatol Symp Proc. 2004; 9:73–78.
83. Sanli H. Dermatology. 2008; 216:349–354. [PubMed: 18285686]

84. Wang SJ. Am J Med Genet. 1994; 51:234–239. [PubMed: 8074151]
85. Werth VP. Arch Dermatol. 1992; 128:368–371. [PubMed: 1550369]
86. Singh AH, Werth VP. J Clin Rheumatol. 1997; 3:343–345. [PubMed: 19078223]
87. Cunliffe WJ. Br J Dermatol. 1969; 81:877–881. [PubMed: 5359449]
88. Kurtev A, Iliev E. Int J Dermatol. 2005; 44:457–461. [PubMed: 15941431]
89. Kasumagić-Halilović E. Acta Dermatovenerol Croat. 2008; 16:123–125. [PubMed: 18812059]
90. Suzuki S. Eur J Neurol. 2005; 12:566–570. [PubMed: 15958099]
91. Corazza GR. Gastroenterology. 1995; 109:1333–1337. [PubMed: 7557104]
92. Thompson DM. Proc R Soc Med. 1974; 67:1010–1012. [PubMed: 4154458]
93. Brenner W. Dermatologica. 1979; 159:356–360. [PubMed: 478074]
94. Muller SA, Winkelmann RK. Arch Dermatol. 1963; 88:290–297. [PubMed: 14043621]
95. Treem WR. Gastroenterology. 1993; 104:1187–1191. [PubMed: 8462807]

References

1. Gilhar A. J Clin Invest. 2007; 117:2019–2027. [PubMed: 17671634]
2. Nakamura M. J Dermatol Sci. 2013; 69:6–29. [PubMed: 23165165]
3. Chuong CM. Exp Dermatol. 2006; 15:547–564. [PubMed: 16761964]
4. Paus R. Trends Immunol. 2005; 26:32–40. [PubMed: 15629407]
5. Gilhar A. Arch Dermatol. 2002; 138:916–922. [PubMed: 12071819]
6. Nakamura M. Am J Pathol. 2008; 172:650–658. [PubMed: 18245811]
7. Maini PK. Science. 2006; 314:1397–1398. [PubMed: 17138885]
8. Sick S. Science. 2006; 314:1447–1450. [PubMed: 17082421]
9. Skurkovich S. J Invest Dermatol Symp Proc. 2005; 10:283–284.

References

1. Alli R. J Immunol. 2012; 188:477–486. [PubMed: 22116824]
2. McElwee KJ. Exp Dermatol. 1999; 8:371–379. [PubMed: 10536963]
3. Skinner RB. J Am Med Assoc. 1995; 273:1419–1420.
4. Skinner RB. J Invest Dermatol. 1995; 104(Suppl):3s–4s. [PubMed: 7738384]
5. Hernandez MJG. 110. J Invest Dermatol. 1998:185. [PubMed: 9457919]
6. Tosti A. J Invest Dermatol. 1996; 107:443. [PubMed: 8751985]
7. Jackow C. J Am Acad Dermatol. 1998; 38:418–425. [PubMed: 9520023]
8. Offidani A. J Cutan Med Surg. 2000; 4:63–65. [PubMed: 11179926]
9. McElwee KJ. J Invest Dermatol. 1998; 110:986–987. [PubMed: 9620311]
10. Gruppo Italiano Studi Epidemiologici in Dermatologia. Arch Dermatol. 1991; 127:688–691. [PubMed: 2024987]
11. Podanyi B. Orv Hetil. 1998; 139:2633–2637. [PubMed: 9842236]
12. Paoletti V. Panminerva Med. 2002; 44:349–352. [PubMed: 12434117]
13. Conte A. G Ital Dermatol Venereol. 1990; 125:85–89. [PubMed: 2376421]
14. Italian Group of Epidemiological Studies in Dermatology. G Ital Dermatol Venereol. 1990; 125:563–567. [PubMed: 2091979]
15. Jadali Z. Eur J Dermatol. 2006; 16:94–95. [PubMed: 16491522]
16. Wise R. JAMA. 1997; 278:1176–1178. [PubMed: 9326478]
17. Sundberg JP. Vet Dermatol. 2009; 20:99–104. [PubMed: 19175564]
18. Proft T. Clin Exp Immunol. 2003; 133:299–306. [PubMed: 12930353]
19. Gilhar A. J Invest Dermatol. 2001; 117:1357–1362. [PubMed: 11886495]
20. McElwee KJ. J Invest Dermatol. 1998; 111:797–803. [PubMed: 9804341]
21. Silva KA, Sundberg JP. Comp Med. in press.

22. McElwee KJ. Exp Dermatol. 2003; 12:30–36. [PubMed: 12631244]
23. Freyschmidt-Paul P. J Invest Dermatol Sym Proc. 2003; 8:104–108.
24. Freyschmidt-Paul P. J Invest Dermatol. 2002; 119:980–982. [PubMed: 12406350]
25. Freyschmidt-Paul P. Br J Dermatol. 2006; 155:515–521. [PubMed: 16911275]
26. Nakamura M. Am J Pathol. 2008; 172:650–658. [PubMed: 18245811]
27. McElwee KJ. Exp Dermatol. 2001; 10:420–429. [PubMed: 11737261]
28. McElwee KJ. J Invest Dermatol Symp Proc. 1999; 4:202–206.
29. Tobin DJ. J Invest Dermatol. 1997; 109:329–333. [PubMed: 9284100]
30. Tobin DJ. Exp Dermatol. 1998; 7:289–297. [PubMed: 9832317]
31. Tobin DJ. Br J Dermatol. 2003; 149:938–950. [PubMed: 14632797]
32. Tobin, DJ.; Olivry, T. Spontaneous canine model of alopecia areata.. In: Chan, LS., editor. Animal Models of Human Inflammatory Skin Diseases. CRC Press; Boca Raton: 2004. p. 469-481.
33. Tobin DJ. J Invest Dermatol. 1994; 102:721–724. [PubMed: 8176253]
34. Tobin DJ. J Invest Dermatol. 1995; 104:13–14.
35. Tobin, DJ. Alopecia areata is associated with antibodies to hair follicle-specific antigens located predominantly in the proliferative region of hair follicles.. In: Vanneste, D.; Randall, V., editors. Hair Research for the Next Millennium. Excerpta Medica Int Congress; Amsterdam: 1996. p. 237-241.
36. Tobin DJ. Arch Dermatol. 1997; 133:57–61. [PubMed: 9006373]
37. Tobin DJ. J Invest Dermatol. 1997; 108:654.
38. Carroll J. J Invest Dermatol. 2002; 119:392–402. [PubMed: 12190862]
39. Mohan C. J Clin Invest. 1998; 101:1362–1372. [PubMed: 9502778]
40. Sobel ES. J Immunol. 1999; 162:2415–2421. [PubMed: 9973523]
41. Duvic M. Arch Dermatol. 1991; 127:64–68. [PubMed: 1670917]
42. Welsh EA. Invest Dermatol. 1994; 103:758–763.
43. Duvic M. J Invest Dermatol. 1995; 104(Suppl):5s–6s. [PubMed: 7738397]
44. deAndrade M. J Invest Dermatol Symp Proc. 1999; 4:220–223.
45. deAndrade M. J Invest Dermatol. 2001; 117:519.
46. Barahmani N. J Invest Dermatol. 2006; 126:74–78. [PubMed: 16417220]
47. Barahmani N. J Invest Dermatol. 2008; 128:240–243. [PubMed: 17637820]
48. Tazi-Ahnini R. Tissue Antigens. 2002; 60:489–495. [PubMed: 12542742]
49. Tazi-Ahnini R. Hum Genet. 2003; 112:400–403. [PubMed: 12589427]
50. Kemp EH. Human Immunol. 2006; 67:535–539. [PubMed: 16829308]
51. Wengraf DA. Tissue Antigens. 2008; 71:206–212. [PubMed: 18194361]
52. Martinez-Mir A. Am J Hum Genet. 2007; 80:316–328. [PubMed: 17236136]
53. Sundberg JP. J Invest Dermatol. 2004; 123:294–297. [PubMed: 15245428]
54. Sundberg JP. J Invest Dermatol. 2003; 120:771–775. [PubMed: 12713579]
55. Gilhar A. J Invest Dermatol. 2005; 124:288–289. [PubMed: 15654992]
56. Sundberg JP. J Exp Anim Sci. 2007; 43:265–270.
57. Poltorak A. Science. 1998; 282:2085–2088. [PubMed: 9851930]
58. Qureshi ST. J Exp Med. 1999; 189:615–625. [PubMed: 9989976]

References

1. Madani S, Shapiro J. J Am Acad Dermatol. 2000; 42:549–566. [PubMed: 10727299]
2. Shapiro, J. Alopecia areata: pathogenesis, clinical features, diagnosis and practical management. Hair Loss, Principles of Diagnosis and Management of Alopecia. Martin Dunitz; London, UK: 2002.
3. Kakourou T. J Eur Acad Dermatol Venereol. 2007; 21:356–359. [PubMed: 17309458]
4. Wasserman D. Int J Dermatol. 2007; 46:121–131. [PubMed: 17269961]

5. Nanova K, et al. Alopecia areata in Israel. Basic science thesis in dermatology. 2007
6. Safavi KH. Mayo Clin Proc. 1995; 70:628–633. [PubMed: 7791384]
7. Shellow WV. Int J Dermatol. 1992; 31:186–189. [PubMed: 1568816]
8. Thomas E, Kadyan R. Indian J Dermatol. 2008; 53:70–74. [PubMed: 19881991]
9. Baurecht H. J Allergy Clin Immunol. 2007; 120:1406–1412. [PubMed: 17980411]
10. de Cid R. Nat Genet. 2009; 41:211–215. [PubMed: 19169253]
11. Nair RP. Nat Genet. 2009; 41:199–204. [PubMed: 19169254]
12. Zhang XJ. Nat Genet. 2009; 41:205–210. [PubMed: 19169255]
13. Tobin DJ. Arch Dermatol. 1997; 133:57–61. [PubMed: 9006373]
14. Gilhar A. Br J Dermatol. 1992; 126:166–171. [PubMed: 1536782]
15. Puavilai S. Int J Dermatol. 1994; 33:632–633. [PubMed: 8002158]
16. Pellicano R. Dig Dis Sci. 2004; 49:395–398. [PubMed: 15139486]
17. Colombe BW. J Investig Dermatol Symp Proc. 1999; 4:216–219.
18. Whiting DA. Arch Dermatol. 2003; 139:1555–1559. [PubMed: 14676070]
19. Tobin DJ. Microsc Res Tech. 1997; 38:443–451. [PubMed: 9297694]
20. Cetin ED. Am J Dermatopathol. 2009; 31:53–60. [PubMed: 19155726]
21. Ramos-Casals M. Medicine (Baltimore). 2008; 87:345–364. [PubMed: 19011506]
22. Price VH. J Am Acad Dermatol. 2008; 58:395–402. [PubMed: 18280336]
23. Strober BE. J Am Acad Dermatol. 2005; 52:1082–1084. [PubMed: 15928633]
24. Wendling P. Skin Allergy News. 2008; 39:2.
25. Chaves Y. Dermatology. 2008; 217:380. [PubMed: 18849606]
26. Ferran M. J Eur Acad Dermatol Venereol. 2011; 25:479–484. [PubMed: 20586836]
27. Zschoche C. J Dtsch Dermatol Ges. 2013; 11:450–453. [PubMed: 23279686]
28. Posten W, Swan J. Arch Dermatol. 2005; 141:759–760. [PubMed: 15967923]
29. Tosti A. Arch Dermatol. 2006; 142:1653–1654. [PubMed: 17179002]
30. Paus R. J Investig Dermatol Symp Proc. 2003; 8:188–194.
31. Petukhova L. Nature. 2010; 466:113–117. [PubMed: 20596022]
32. van der Steen P. Acta Derm Venereol. 1992; 72:373–375. [PubMed: 1361288]
33. Dudda-Subramanya R. Eur J Dermatol. 2007; 17:367–374. [PubMed: 17673378]
34. Duvic M. J Investig Dermatol Symp Proc. 2003; 8:219–221.
35. Dehghan A. Lancet. 2008; 372:1953–1961. [PubMed: 18834626]
36. Randall VA. Lancet. 2001; 358:1922–1924. [PubMed: 11747911]
37. Collins SM. Br J Dermatol. 2006; 154:1088–1093. [PubMed: 16704638]
38. Barankin B, Guenther L. J Cutan Med Surg. 2001; 5:289–293. [PubMed: 11907837]
39. Gibson G. DNat Rev Genet. 2009; 10:134–140.
40. Jackow C. J Am Acad Dermatol. 1998; 38:418–425. [PubMed: 9520023]
41. Betz RC. Br J Dermatol. 2008; 158:389–391. [PubMed: 18028494]
42. Entz P. Eur J Dermatol. 2006; 16:363–367. [PubMed: 16935791]
43. Martinez-Mir A. Am J Hum Genet. 2007; 80:316–328. [PubMed: 17236136]
44. Betz RC. J Invest Dermatol. 2007; 127:2539–2543. [PubMed: 17581619]
45. Megiorni F. Br J Dermatol. 2011; 165:823–827. [PubMed: 21692766]
46. Forstbauer LM. Eur J Hum Genet. 2012; 20:326–332. [PubMed: 22027810]
47. Jagielska D. J Invest Dermatol. 2012; 132:2192–2197. [PubMed: 22534877]
48. Conteduca G, et al. Clin Exp Med. 2012 [Epub ahead of print].
49. Alfadhli S, Nanda A. Immunol Lett. 2013; 150:130–133. [PubMed: 23318300]
50. Lee S. PLoS One. 2013; 8:e53613. [PubMed: 23326468]
51. Sundberg JP. J Invest Dermatol. 2004; 123:294–297. [PubMed: 15245428]
52. Wengraf DA. Tissue Antigens. 2008; 71:206–212. [PubMed: 18194361]
53. Kemp EH. Hum Immunol. 2006; 67:535–539. [PubMed: 16829308]

54. Pforr J. Tissue Antigens. 2006; 68:58–61. [PubMed: 16774540]
55. Barahmani N. J Invest Dermatol. 2006; 126:74–78. [PubMed: 16417220]
56. Shimizu T. Genes Immun. 2005; 6:285–289. [PubMed: 15815686]
57. Tazi-Ahnini R. Hum Genet. 2003; 112:400–403. [PubMed: 12589427]
58. Tazi-Ahnini R. Tissue Antigens. 2002; 60:489–495. [PubMed: 12542742]
59. Tazi-Ahnini R. Eur J Immunogenet. 2002; 29:25–30. [PubMed: 11841485]
60. Tazi-Ahnini R. Hum Genet. 2000; 106:639–645. [PubMed: 10942113]
61. Galbraith GM. Hum Hered. 1999; 49:85–89. [PubMed: 10077728]
62. Galbraith GM, Pandey JP. Hum Genet. 1995; 96:433–436. [PubMed: 7557966]
63. Cork MJ. J Invest Dermatol. 1995; 104:15S–16S. [PubMed: 7738374]
64. Welsh EA. J Invest Dermatol. 1994; 103:758–763. [PubMed: 7798612]

References

1. Thies W. Arch Klin Exp Dermatol. 1966; 227:541–549. [PubMed: 5984796]
2. Szent-Györgyi A. Trans 1st Josiah Macy Conference on Connective tissues. 1951
3. Tobin DJ. J Invest Dermatol. 1990; 94:803–807. [PubMed: 2355182]
4. Gilhar A. J Clin Invest. 2007; 117:2019–2027. [PubMed: 17671634]
5. Paus R. Yale J Biol Med. 1993; 66:541–554. [PubMed: 7716973]
6. Christoph T. Br J Dermatol. 2000; 142:862–873. [PubMed: 10809841]
7. Ito T. Am J Pathol. 2004; 164:623–634. [PubMed: 14742267]
8. Schallreuter KU. FASEB J. 2012; 26:2471–2485. [PubMed: 22415306]
9. Messenger AG. Br J Dermatol. 1986; 114:337–347. [PubMed: 3954954]
10. Gilhar A. J Clin Invest. 1998; 101:62–67. [PubMed: 9421466]
11. Tobin DJ. J Invest Dermatol. 1994; 102:721–724. [PubMed: 8176253]
12. Tobin DJ. J Investig Dermatol Symp Proc. 2003; 8:176–181.
13. McElwee KJ. Exp Dermatol. 1999; 8:371–379. [PubMed: 10536963]
14. Tobin, DJ.; Bystryn, J-C. Immunology of alopecia areata.. In: Camacho, FM.; Randall, VA.; Price, V., editors. Hair and Hair Disorders: Research, Pathology and Management. Martin Dunitz; London: 2000. p. 187-201.
15. Tobin DJ, Bystryn JC. Pigment Cell Res. 1996; 9:304–310. [PubMed: 9125754]
16. Leung MC. J Proteome Res. 2010; 9:5153–5163. [PubMed: 20722389]
17. Wang E, McElwee KJ. Dermatol Ther. 2011; 24:337–347. [PubMed: 21689243]
18. Petukhova L. Nature. 2010; 466:113–117. [PubMed: 20596022]
19. Tobin DJ. Pigment Cell Melanoma Res. 2011; 24:75–88. [PubMed: 21070612]
20. Botchkarev VA. J Invest Dermatol. 2013; 133:1918–1921. [PubMed: 23856928]

References

1. Thies W, Klaschka F. Arch Klin Exp Dermatol. 1970; 237:51–58. [PubMed: 5417144]
2. Ledesma GN, York KK. Arch Dermatol Res. 1982; 274:1–8. [PubMed: 6219633]
3. Kalish RS, Johnson KL, Hordinsky MK. Arch Dermatol. 1992; 128:1072–1077. [PubMed: 1497361]
4. Happle R. Arch Dermatol Res. 1980; 267:109–114. [PubMed: 6446265]
5. Galbraith GM. Br J Dermatol. 1984; 110:163–170. [PubMed: 6607739]
6. Perret C. Acta Derm Venereol. 1984; 64:26–30. [PubMed: 6203277]
7. D'Ovidio R. Arch Dermatol Res. 1981; 271:265–273. [PubMed: 6975602]
8. Messenger AG, Bleehen SS. J Invest Dermatol. 1985; 85:569–572. [PubMed: 2415641]
9. Gilhar A. Clin Immunol Immunopathol. 1993; 66:120–126. [PubMed: 8095867]
10. Gilhar A. Br J Dermatol. 1992; 126:166–171. [PubMed: 1536782]

11. Michie HJ. Br J Dermatol. 1990; 123:557–567. [PubMed: 2147388]
12. Khoury EL. J Invest Dermatol. 1988; 90:193–200. [PubMed: 2448391]
13. Paus R. Trends Immunol. 2005; 26:32–40. [PubMed: 15629407]
14. Billingham RE, Silvers WK. J Invest Dermatol. 1971; 57:227–240. [PubMed: 4256544]
15. Westgate GE. J Invest Dermatol. 1991; 97:417–420. [PubMed: 1714928]
16. Harrist TJ. Br J Dermatol. 1983; 109:623–633. [PubMed: 6360195]
17. Paus R. Br J Dermatol. 1994; 131:177–183. [PubMed: 7917980]
18. Paus R. Br J Dermatol. 1994; 130:281–289. [PubMed: 8148267]
19. Paus R. Dev Biol. 1994; 163:230–240. [PubMed: 8174779]
20. Paus R. Yale J Biol Med. 1993; 66:541–554. [PubMed: 7716973]
21. Ikeda T. Dermatologica. 1965; 131:421–445. [PubMed: 5864736]
22. Gilhar A. N Engl J Med. 2012; 366:1515–1525. [PubMed: 22512484]
23. McElwee KJ. Br J Dermatol. 1996; 135:211–217. [PubMed: 8881662]
24. Breitkopf T. J Invest Dermatol. 2013; 133:1722–1730. [PubMed: 23370538]
25. Kang H. J Invest Dermatol. 2010; 130:2677–2680. [PubMed: 20613773]
26. Gilhar A. J Clin Invest. 1998; 101:62–67. [PubMed: 9421466]
27. Gilhar A. Arch Dermatol. 2002; 138:916–922. [PubMed: 12071819]
28. Gilhar A. J Invest Dermatol. 2001; 117:1357–1362. [PubMed: 11886495]
29. McElwee KJ. J Invest Dermatol. 2005; 124:947–957. [PubMed: 15854035]
30. Tsuboi H. J Dermatol. 1999; 26:797–802. [PubMed: 10659500]
31. Nagai H. Arch Dermatol Res. 2006; 298:131–134. [PubMed: 16786344]
32. Alli J. J Immunol. 2012; 188:477–486. [PubMed: 22116824]
33. Ito T. J Invest Dermatol. 2005; 125:1139–1148. [PubMed: 16354183]
34. Kinori M. Invest Ophthalmol Vis Sci. 2011; 52:4447–4458. [PubMed: 21700717]
35. Huang KP. JAMA Dermatol. 2013; 22:1–5.
36. Barahmani N. J Am Acad Dermatol. 2003; 49:1192. [PubMed: 14639420]
37. Murphy, K. Janeway's Immunobiology. 8th edn.. Garland Science; London and New York: 2011.
38. Ito T. J Invest Dermatol. 2008; 128:1196–1206. [PubMed: 18160967]
39. Barahmani N. J Invest Dermatol. 2006; 126:74–78. [PubMed: 16417220]
40. Petukhova L. Nature. 2010; 466:113–117. [PubMed: 20596022]
41. Gilhar A. J Invest Dermatol. 2013; 133:844–847. [PubMed: 23096715]
42. Martinez-Mir A. Am J Hum Genet. 2007; 80:316–328. [PubMed: 17236136]
43. John KK. J Invest Dermatol. 2011; 131:1169–1172. [PubMed: 21346773]
44. Megiorni F, et al. Arch Dermatol Res. 2013 [Epub ahead of print].
45. Sundberg JP. J Invest Dermatol. 2011; 131:2323–2324. [PubMed: 21753782]
46. Jabbari A. Dermatol Clin. 2013; 31:109–117. [PubMed: 23159180]
47. Redler S. Br J Dermatol. 2012; 167:1360–1365. [PubMed: 22897480]
48. Jagielska D. J Invest Dermatol. 2012; 132:2192–2197. [PubMed: 22534877]
49. Betz RC. J Invest Dermatol. 2007; 127:2539–2543. [PubMed: 17581619]
50. Lee S. PLoS One. 2013; 8:e53613. [PubMed: 23326468]
51. Gilhar A. J Invest Dermatol. 2013; 133:2088–2091. [PubMed: 23636064]
52. Furmanski AL. J Invest Dermatol. 2013; 133:1221–1230. [PubMed: 23303453]
53. Harries MJ. BMJ. 2010; 341:c3671. [PubMed: 20656774]
54. Bertolini M. Exp Dermatol. 2012; 21:477–479. [PubMed: 22621196]
55. Stelekati E. Immunity. 2009; 31:665–676. [PubMed: 19818652]
56. McElwee KJ. J Invest Dermatol. 2002; 119:1426–1433. [PubMed: 12485450]
57. Sundberg JP. J Invest Dermatol. 2011; 131:2323–2324. [PubMed: 21753782]
58. John KK. J Invest Dermatol. 2011; 131:1169–1172. [PubMed: 21346773]
59. Zöller M, et al. J Autoimmun. 2004; 23:241–256. [PubMed: 15501395]

60. D'Ovidio R. G Ital Dermatol Venereol. 1988; 123:569–570. [PubMed: 3254330]
61. Kloepper JE. J Invest Dermatol. 2013; 133:1666–1669. [PubMed: 23334345]
62. Ito T. Am J Pathol. 2004; 164:623–634. [PubMed: 14742267]
63. Freyschmidt-Paul P. Br J Dermatol. 2006; 155:515–521. [PubMed: 16911275]
64. Gilhar A. J Invest Dermatol. 2005; 124:288–289. [PubMed: 15654992]
65. Ghoreishi M. Br J Dermatol. 2010; 163:57–62. [PubMed: 20346028]
66. Skurkovich S. J Investig Dermatol Symp Proc. 2005; 10:283–284.
67. Gilhar A. Clin Immunol. 2003; 106:181–187. [PubMed: 12706404]
68. Peters EM. Am J Pathol. 2007; 171:1872–1886. [PubMed: 18055548]
69. Siebenhaar F. J Invest Dermatol. 2007; 127:1489–1497. [PubMed: 17273166]
70. Paus R, Arck P. J Invest Dermatol. 2009; 129:1324–1326. [PubMed: 19434088]
71. Kinori M. Exp Dermatol. 2012; 21:223–226. [PubMed: 22379970]
72. Harries MJ, et al. J Pathol. 2013 doi: 10.1002/path.4233 [Epub ahead of print].
73. Muldoon LL. J Cereb Blood Flow Metab. 2013; 33:13–21. [PubMed: 23072749]
74. Mellor AL, Munn DH. Nat Rev Immunol. 2008; 8:74–80. [PubMed: 18064049]
75. Waldmann H. Nat Rev Nephrol. 2010; 6:569–576. [PubMed: 20717099]

References

1. Ogawa H. Skin Res. 1994; 36:60–68.
2. Ogawa H. Nishinihon J Dermatol. 1995; 57:1206–1211.
3. Yoshizawa Y, Kawana S. Jpn J Dermatol. 2005; 115:1473–1480.
4. Inui S. J Dermatol. 2007; 34:852–854. [PubMed: 18078416]
5. Inui S. J Dermatol. 2009; 36:323–327. [PubMed: 19500180]
6. Ohyama M. J Dermatol Sci. 2010; 58:154–157. [PubMed: 20388588]
7. Gilhar A. J Clin Invest. 1998; 101:62–67. [PubMed: 9421466]
8. Ito T. Am J Pathol. 2004; 164:623–634. [PubMed: 14742267]
9. Toyoda M. Br J Dermatol. 2001; 144:46–54. [PubMed: 11167682]
10. Siebenhaar F. J Invest Dermatol. 2007; 127:1489–1497. [PubMed: 17273166]
11. Maurer M. Lab Invest. 1997; 77:319–332. [PubMed: 9354767]
12. Bae S. Prog Med. 2007; 27:2123–2126.
13. Bertolini M. Exp Dermatol. 2012; 21:477–479. [PubMed: 22621196]

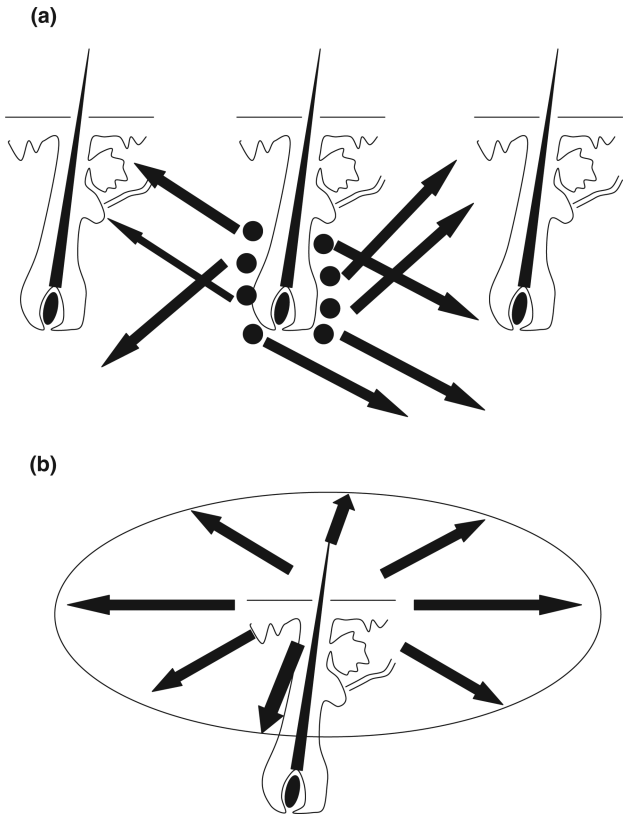

Figure 1.

How does round alopecia get formed in skin regions affected by AA? (a) Random autoreactive T cell diffusion hypothesis. (b) Cytokine (interferon c diffusion hypothesis).

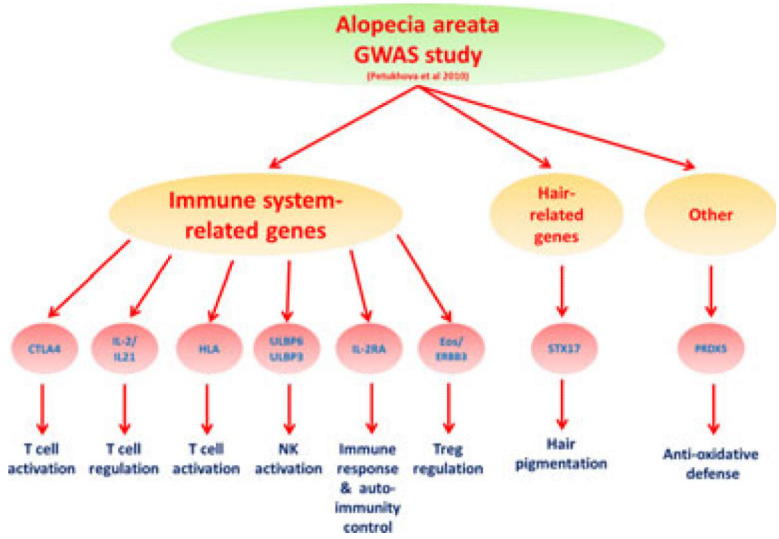

Figure 2.

Schematic representation of the findings in the GWAS performed by Petukhova et al., 2010 (31).

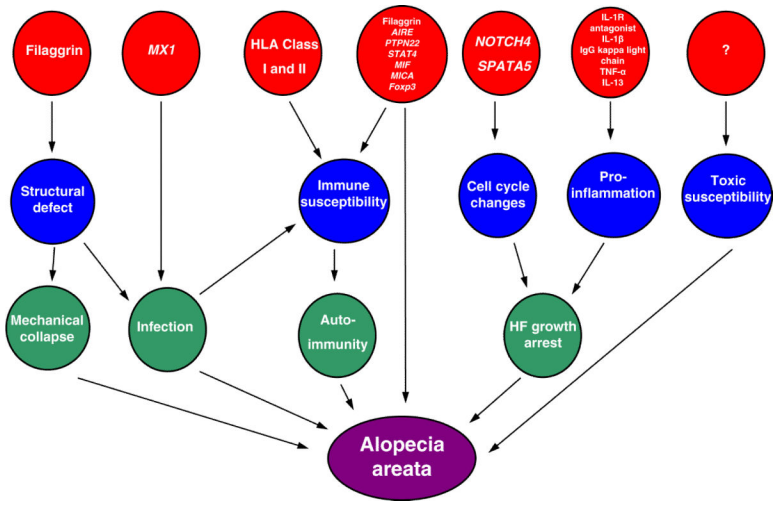

Figure 3.

Suggested susceptibility genes for the development of alopecia areata and their presumed causal mechanism (41,52–64).

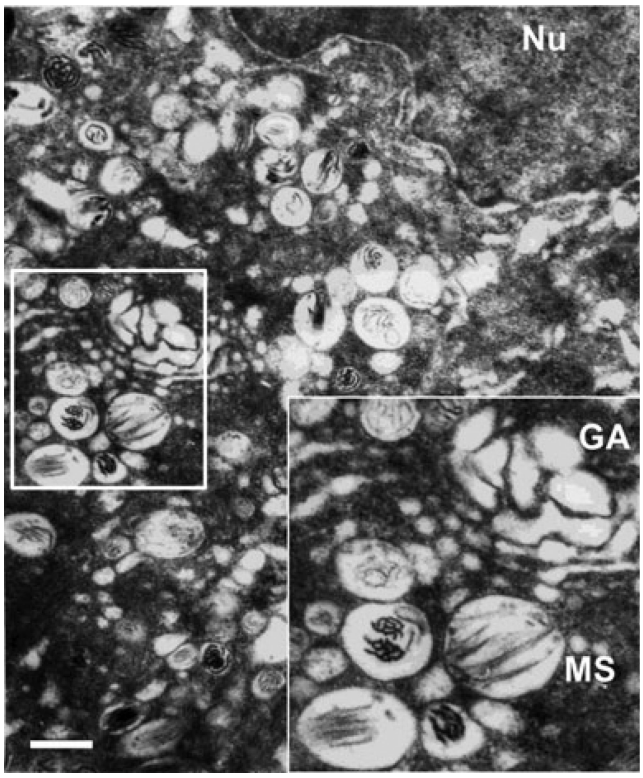

Figure 4.

Transmission electron microscopic view of part of an affected hair bulb melanocyte in acute alopecia areata. Note (a) aberrant melanogenesis, as evidenced by the abnormal deposition of melanin in enlarged abnormal melanosomes (MS), and (b) swollen Golgi apparatus. Nu, melanocyte nucleus. Scale bar 0.5 μm.

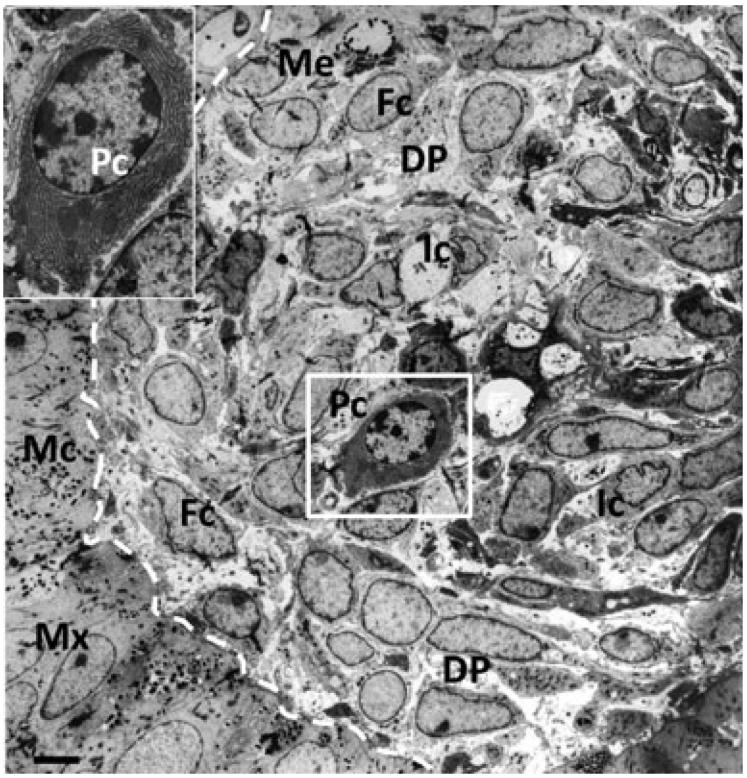

Figure 5.

Transmission electron microscopic view of part of an affected hair bulb in acute alopecia areata with a view through the follicular dermal papilla (DP). Note (a) relatively normal-appearing matrix (Mx) containing melanocytes (Mc), and (b) a more disrupted DP with an active plasma cell (Pc), fibroblasts (Fc) and other cells with immunocyte morphology (Ic). Scale bar 5 μm.

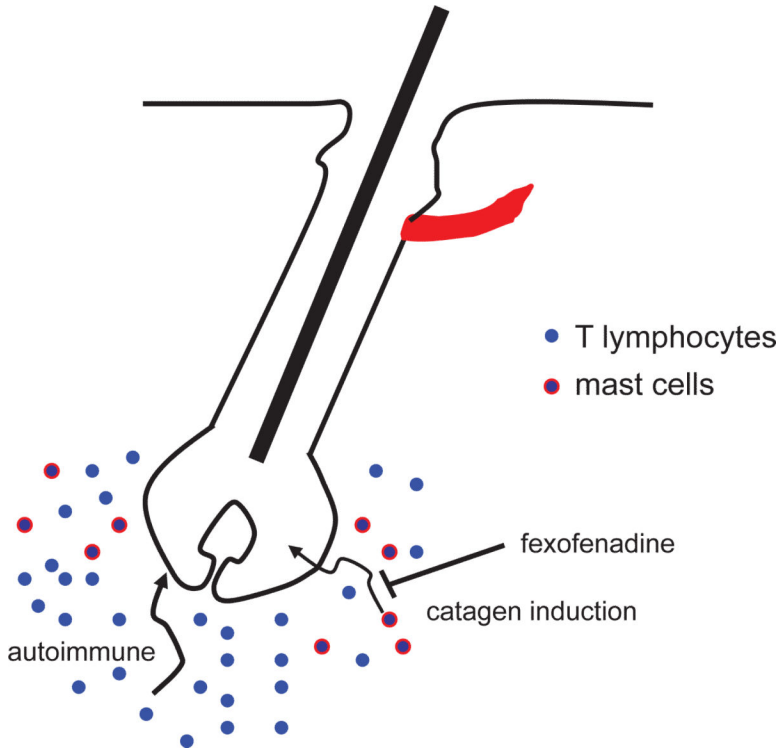

Figure 6.
Possible pathomechanism of AA and effect of fexofenadine.

Table 1

Autoimmune diseases associated with alopecia areata

Autoimmune diseases	References
Vitiligo	(71,79–83)
Type I (insulin-dependent) diabetes	(84)
Lupus erythematosus	(85,86)
Thyroiditis	(87–89)
Myasthenia gravis	(80,90)
Celiac disease	(91)
Scleroderma	(79,92,93)
Rheumatoid arthritis	(94)
Ulcerative colitis	(80,92,95)

Table 2

Clues to the genetic aetiology of alopecia areata

Genetic clue	Details	References
Family history	In all reports, there is a high frequency of a positive family history in first-degree relatives of affected patients, ranging from 10–42% (30% in our group), more obvious if the disease starts at a younger age. This and the equal frequency in men and women, hint to a dominant genetic disease. One small study found a very high concordance of 55% in monozygotic twins. Such a high concordance rate is not seen in any other autoimmune diseases.	(40)
Susceptibility genes	The various HLA associations, for example, an increase in the appearance of DRB1*04 alleles and a significant decrease in the appearance of DRB1 *03 alleles in patients with AA, may be one genetic component predisposing to AA. Recently, following the advances in mapping of complex disorders, genome-wide scans of patients with AA provided evidence for several susceptibility loci, some of which lie outside the HLA loci. These include genomic regions containing genes that control regulatory T cells, cytotoxic T-lymphocyte-associated antigen 4, IL-13 and the *ULBP* gene cluster, which encodes activating ligands of the natural killer cell receptor NKG2D. In addition, the R620W variant of PTPN22 was found to be more common in patients with AA, and the presence of the *filaggrin* gene mutations was found to be associated with a more severe form of AA when it occurred in conjunction with atopic dermatitis. Case-control studies have shown that single nucleotide polymorphisms (SNPs) in Foxp3, NOTCH4 and ICOSLG genes were associated with AA. Additionally, SNPs in some immune-related genes (including HLA-DMB, PMS2 and TLR1) have been found to be associated with alopecia universalis using exomic sequencing. Over the years, polymorphisms in other genes have been reported to be associated with AA. They are summarized in Fig. 3.	(31,41–50)
Animal model based on genetics	The C3H/HeJ mouse develops adult onset disease that resembles AA in adult humans. The disease in mice develops spontaneously, based on four different genetic susceptibility loci. One could postulate that AA in humans could also develop spontaneously, without the need for any external trigger, taking into account the complex gene susceptibility.	(51)
Differential expression of genes	The age of onset and the different expression patterns may be attributable to different expression of genes, some of which might be defected, during different time periods. Indeed, HFs are known to express different genes depending on hair cycle phase.	