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Is Alopecia Areata an Autoimmune-Response Against Melanogenesis-Related Proteins, Exposed by Abnormal MHC Class I Expression in the Anagen Hair Bulb?

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The etiology of alopecia areata (AA), a putative autoimmune disease characterized by sudden hair loss, has remained obscure. It is not understood, how the characteristic inflammatory infiltrate that selectively attacks anagen hair follicles in AA is generated. We hypothesize that this reflects an unexplored form of autoimmunity, a cytotoxic T cell attack on rhythmically synthesized autoantigens normally sequestered by a lack or very low level of MHC class I (MHC I)-expression, and suggest the following mechanism of AA pathogenesis:

Microtrauma, neurogenic inflammation, or microbial antigens cause a localized breakdown of MHC I-"negativity" in the proximal anagen hair bulb *via* proinflammatory cytokines. This exposes autoantigens derived from melanogenesis-related proteins (MRP-DP), which are only generated during anagen, and triggers two successive waves of autoimmune responses: $CD8^+$ cytotoxic T cells initiate AA after recognizing MRP-DP abnormally presented by MHC I molecules on hair matrix melanocytes and/or keratinocytes; a secondary attack, carried by $CD4^+$ T cells and antigen presenting cells, is then mounted against MHC class II - presented additional autoantigens exposed by damaged melanocytes and keratinocytes. The latter causes most of the follicular damage, and extrafollicular disease, and depends greatly on the immunogenetic background of affected individuals. This unifying hypothesis explains the clinical heterogeneity and all salient features of AA, and argues that only the unlikely coincidence of multiple predisposing events triggers AA. The suppression of MHC I- expression and synthesis of MRP in the hair bulb, and the "tolerization" of MRP-DP autoreactive $CD8^+$ T cells may be promising strategies for treating AA.

The etiology of AA[d], an enigmatic form of rapidly developing hair loss with often devastating psychological effects, has long been the subject of animated debate. While early in AA research roles for the nervous system and for infectious agents in the pathogenesis of AA were postulated ("trophoneurotic theory", theory of bacterial "foci"), today

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[d]*Abbreviations used:* AA, alopecia areata; MHC I, major histocompatibility complex class I; MRP, melanogenesis-related proteins; MRP-DP, MRP-derived peptide autoantigens; anagen, growth phase of the hair cycle; MC, melanocytes; KC, keratinocytes; TNF-α, tumor necrosis factor α; IFN-γ, interferon γ; IL-1, interleukin 1; α-MSH, alpha-melanocyte stimulating hormone.

most authors tend to classify AA as an autoimmune disease [1–3]. Yet, this is only supported by circumstantial evidence, and no etiologic concept is generally accepted. A deeper understanding of the etiology of AA is not only important for evident clinical reasons, but is of wider biological interest, since this disease may reflect a still uncharted, general principle of autoaggression relevant to other ill-understood putative "autoimmune" diseases.

The characteristic T cell-dominated inflammatory infiltrate attacking the hair follicle, the association of AA with other autoaggressive diseases, various autoantibodies and other abnormalities of humoral and cellular immune parameters, as well as evidence for immunogenetic influences, suggest an autoimmune response as the underlying pathogenetic principle in AA. The significance of these findings, however, remains uncertain and controversial [1–3]. Neither the presence of autoantibodies nor linkage with certain HLA alleles alone can be accepted as firm evidence for an autoimmune disease [4, 5]. Autoantibodies to hair follicle-associated antigens occur in normal individuals [6], and many autoantibodies may serve physiological functions [5, 7]. No specific follicular autoantigen has been identified as responsible for triggering AA, and neither passive transfer of an AA-like disease state by T cells or serum, nor cell-mediated induction of an experimental analogue of AA have been demonstrated [8, cf. 4]. Initial hopes that the abnormal expression of MHC class II molecules by lesional hair bulb KC may explain the initiation of an autoimmune attack [9] waned when it was shown that the inflammatory infiltrate appears on the scene before HLA-DR expression of hair bulb KC is detected [10] (secondary, aberrant MHC class II expression is seen in many autoimmune diseases [5]).

The search for a convincing etiologic theory is complicated by uncertainty on whether all cases of AA represent manifestations of the same disease [11]. Even in cases with similar presenting signs one sees considerable variation in the associated diseases, immunological and endocrinological laboratory findings, family history, history of atopy, HLA-association, prognosis, course, and response to therapy [1, 2, 11]. As long as one adheres to the view of AA as a single disease entity, in our view, any credible theory should integrate the following salient features of AA.

CLINICAL CHARACTERISTICS OF AA [1, 2]

AA is characterized by the sudden onset of predominantly non-scarring, reversible hair loss with striking absence of visible signs of skin inflammation. Though dramatic forms of rapid disease progression leading to the loss of all body hair do occur, the majority of patients suffers hair loss in peculiarly circumscribed skin areas. Heavily pigmented scalp and beard hair follicles, which display the highest percentage of follicles in anagen (i.e., the growth phase of the hair cycle), are affected most often. Hair loss is often accompanied by nail dystrophy, and AA patients have an increased incidence of abnormal thyroid function and other autoimmune diseases, including vitiligo, and a higher prevalence of other pigmentary defects (e.g., Vogt-Koyanagi-Harada syndrome, ocular depigmentation in the retinal epithelium). Remarkably, white or greying hair follicles are commonly spared in AA, while regrowing hair shafts usually are white before they become repigmented. Attacks of fulminant AA, which affect exclusively pigmented hair

follicles so that only pre-existing grey or white hair is left behind, may underlie the mysterious phenomenon of "overnight greying."

In a minority of cases, AA disease onset coincides with severe emotional stress, surgery, dental manipulations, head trauma, bacteremia and/or the presence of so-called bacterial "foci." The course of AA is entirely unpredictable, with a high rate of spontaneous remission, and a worse prognosis in patients with rapid progression, nuchal hair loss, and onset before puberty. Hints at genetic factors arise from the observation that a worse prognosis is also seen in patients with a positive family history for AA and/or a personal history of atopy, and that the incidence of AA appears to be highest among dark black-haired Japanese, and rather low in fair-haired Scandinavians (reliable epidemiological data are wanting, however). Though a wide spectrum of immunomodulatory treatment strategies all may induce hair regrowth, the response to therapy is often unsatisfactory, and relapses after discontinuation of therapy are very common.

HISTOPATHOLOGY OF AA [1, 2, 13]

The earliest recognized histological sign of AA are peri- and intrafollicular as well as perivascular inflammatory infiltrates composed primarily of activated T lymphocytes and macrophages. Typically, these cells assemble in a "bee-swarm"-like fashion around the proximal hair bulb of affected follicles, and some inflammatory cells invade the hair matrix. Without this infiltrate, there is no AA, and only melanogenically active anagen follicles (anagen III–VI) come under attack. It has not been clarified definitively which anagen substage is assaulted: an arrest of follicles in anagen III-IV is well-documented [12, 13], but also anagen VI may be attacked [14]. In acute AA lesions, intrabulbar inflammatory cells can be detected in close proximity to hair matrix MC [3], and KC of the precortical hair bulb matrix show ultrastructural signs of cell damage [15]. This cellular attack on the follicle terminates anagen prematurely and precipitates the follicle into catagen, often with signs of follicle dystrophy; these include abnormal pigmentation and matrix degeneration [16], but, with rare exception, there is no follicle destruction. Consequently, lesional skin displays an abnormally high percentage of catagen and telogen follicles [13]. Signs of follicular KC and MC degeneration, including more apoptosis of proximal hair bulb KC than one normally finds during follicle regression (catagen) are seen in acute AA [16, 17]. Other structural abnormalities of AA follicles have been described in the dermal papilla and in the epithelial-mesenchymal junction zone between hair bulb and papilla [13, 15, 18, 19].

IMMUNOLOGY OF AA

The inflammatory infiltrate in the acute phase of AA consists of both activated CD4+ and of $CD8^+$ T cells as well as of macrophages and some Langerhans cells, possibly with a relative predominance of helper ($CD4^+$) over cytotoxic ($CD8^+$) T cells [1–3]. The exact order of appearance of these various mononucleas cell populations has not been established, mainly because the pathogenic process is already far advanced at the time of visible hair loss, i.e., when most biopsies are taken, and biopsies from the perimeter of an acute lesion or from clinically uninvolved skin may not accurately reflect the very earliest stage of AA pathogenesis. However, $CD8^+$ T cells can be seen to invade the proximal hair bulb early in acute AA [1].

Strong MHC class I (MHC I) immunoreactivity is found in the proximal hair bulb of AA follicles, which is in striking contrast to the normal MHC I-"negativity" in this region [1–3, 10, 20–22]. In addition, an "ectopic" expression of MHC class II-molecules (e.g., HLA-DR) by hair bulb KC and increased numbers of MHC-class II^+ Langerhans cells are found in many lesional hair bulbs [1–3, 15, 20, 22]. Normal proximal hair bulbs are MHC class II-negative, and Langerhans cells may be absent from the normal proximal anagen hair follicle [1, 21, 22]. Early lesional AA follicles also display aberrant expression of adhesion molecules known to direct hematopoietic cell extravasation and migration, while the epithelium of normal skin and hair follicles seems devoid of ICAM-1 immunoreactivity, the epithelial bulb of AA follicles is strongly ICAM-1^+ [cf. 23].

A large number of, at times, contradictory abnormalities of humoral and/or cell-mediated immunity has been described in AA patients, but the pathogenic significance of these findings remains controversial [1–3]. One fairly consistant finding is a higher incidence of autoantibodies to thyroid microsomal and gastric parietal cell antigens in AA than in controls [1, 2]. Recently, an enrichment of "autoreactive" T lymphocytes with undefined antigen-specificity in lesional AA scalp skin has been reported [24]. The association of AA with HLA-haplotypes is still uncertain. For example, increased frequencies of the MHC I alleles HLA-B8, 9, 12, 18 have been reported in some, but not all AA populations [cf. 25, 26]. Among the MHC class II-associations suggested for AA [1, 2], a linkage with HLA-DR4 appears well-documented [25], while HLA-DRw52a may confer relative protection from AA [26].

Among these features of AA, the exclusive attack on melanogenically active anagen follicles, the distinct pigmentation-phenomena listed above, and the association with vitiligo all point to the follicular MC as a possibly important etiologic factor [cf. 3, 27–29]. In this context, some aspects of normal hair follicle biology must be kept in mind:

a) MC enjoy some form of protection from immune recognition in the proximal anagen hair follicle; in homografts of guinea pig epidermis from black skin transplanted to white skin beds of an incompatible recipient strain, donor MC migrate into the recipient hair bulbs and, in contrast to epidermal MCs, survive there for an extended period of time [cf. 30].

b) The MHC expression pattern in anagen hair follicles is highly unusual and may explain a); in hair follicles of humans, rats and mice, MHC I expression in the proximal hair bulb of anagen, but not telogen (= resting) follicles normally is either absent or below detectablility by immunohistology [21, 31, 32]; this "lack" of classical MHC I expression may be comparable to the situation in the fetotrophoblast [31, 32], where the lack of HLA-A and HLA-B expression is utilized to prevent a maternal cytotoxic T cell attack on paternal antigens generated by the fetal "graft" [33].

c) Melanogenesis and melanosome transferral to cortical human hair bulb KC are strictly coupled to the anagen stage of the hair cycle, are initiated during anagen III-IV, and cease at the end of anagen VI [28, 34, 35].

On this background, we argue that it will prove most incisive to clarify why only melanogenically active anagen follicles are attacked, and why the normal anagen hair follicle displays its peculiar pattern of MHC I-expression, as well as to intensify the search for MHC I-dependent autoantigens in AA. As a working hypothesis to stimulate research

research into this direction, we propose this hypothetical chain of events to result in the development of AA:

HYPOTHESIS

An anagen follicle becomes susceptible to an autoimmune attack only if the unique repression of MHC I-expression in its proximal bulb matrix fails as a result of proinflammatory cytokines like INF-γ, TNF-α and IL-1. These are released in response to rather non-specific stimuli, like localized microtrauma, neurogenic inflammation, infectious agents, or microbial superantigens. In some individuals, this abnormal MHC expression exposes immunogenic peptides derived from melanogenesis-related proteins (MRP-DP) *via* MHC I-molecules to the skin immune system. These peptides are only generated during anagen-associated melanogenesis. If an individual has $CD8^+$ T cells that recognize MHC I-presented MRP-DP and receive a co-stimulatory signal, two successive waves of immune responses are mounted.

The first one actually triggers AA and is driven by autoreactive $CD8^+$ T cells launching a cytotoxic attack on hair matrix MC and/or KC presenting MRP-DP. The cytokines and additional autoantigens released during this initial attack, and the abnormal expression of adhesion molecules induced thereby, subsequently attract a secondary immune response, which involves $CD4^+$ T cells, Langerhans cells, and macrophages. This secondary assault causes most of the follicular damage but varies greatly between affected individuals, depending on their immunogenetic background, on the spectrum of additionally exposed autoantigens, and on the nature of the perturbation of the local cytokine network. Damage to the dermal papilla, and induction of abnormal KC apoptosis are the key factors in determining follicular damage and recovery.

Dependent on the level of damage, premature catagen induction and a reversible follicle dystrophy ensue with the clinical result of hair shaft shedding. Autoantibodies and autoreactive T cell clones generated during the secondary immune response perpetuate the attack on the follicle, which is now also directed against MHC class II-dependent autoantigens presented by ectopic MHC class II expression on hair matrix KC and/or by the locally accumulated antigen presenting cells. These secondary autoantibodies and autoreactive T cells account for the involvement of extrafollicular cells with the same or cross-reacting antigens (e.g., epidermal MC, nail matrix, thyroid gland). Regrowth of depigmented hair shafts reflects both the damage inflicted on follicular MC and a protective blockade of MRP generation, which decreases the likelihood of disease perpetuation.

DISCUSSION

We propose that AA reflects a previously unexplored form of autoimmunity: a cytotoxic T cell attack on rhythmically synthesized, normally sequestered autoantigens. According to our hypothesis, the initiation of AA is not based on an inherent abnormality of hair follicle KC or MC functions, but results from the rare coincidence of several events predisposing to disease development: a cytokine-induced breakdown of MHC I-"negativity" of the anagen hair bulb, the exposure of MRP-DP by MHC I, and the presence of autoreactive $CD8^+$ T cells that recognize MRP-DP and display cytotoxic activity. In other words, different proinflammatory stimuli and pre-existing factors (e.g., presence of autoreactive $CD8^+$ T cells, level of constitutive expression of MHC I) are thought to

increase the likelihood of developing AA, and then to coalesce into a cytotoxic T cell attack that triggers AA.

Once launched, this attack on the follicle is portrayed to be fueled and perpetuated by a cascade of secondary immune responses and proinflammatory cytokine-network alterations. Most follicle damage and most of the immunological abnormalities seen in AA patients are attributed to this set of secondary immune responses (e.g., autoantibodies, $CD4^+$ T cell-dominated infiltrate, ectopic MHC class II-expression, intrabulbar Langerhans cells, abnormal adhesion molecule expression). These are thought to display substantial inter-individual variations, depending on the immunogenetic background (e.g., HLA-DR4), the response to additionally released or presented autoantigens, and on the local damage inflicted by the abnormal peri- and intrafollicular cytokine milieu. This explains the heterogeneity of AA cases, the strict association of AA with melanogenically active anagen follicles and all other salient features of AA outlined above.

This hypothesis integrates previous suggestions that MC play a role in AA pathogenesis [3, 16, 28, 29], and is in line with the concept that hair cycling and the severity of the inflammatory damage to the hair follicle, particularly to the dermal papilla, play critical roles in the pathogenesis of AA [13, 18]. Our scenario works without having to postulate an obscure inherent functional defect of KC or MC [36], or abnormal adhesion molecule expression [23], while it assigns adhesion molecules and chemotactic cytokines an important role in the secondary autoimmune response [cf. 23, 36]. It extends upon the propositions that the intrabulbar changes in MHC expression seen in acute AA lesions facilitate or even are responsible for induction of an immune response against unidentified autoantigens [3] and that the follicular MHC I-"negativity" under physiological circumstances serves to prevent an immune response against potentially harmful antigens generated by melanogenically active MC [32].

The predominance of $CD4^+$ T cells and MHC class II-restricted antigen presenting cells in the inflammatory infiltrate has generally been interpreted as pointing to an MHC class II-dependent antigen as autoimmune target in AA. Yet, it remains unproven that $CD4^+$ T cells appear on the scene before any $CD8^+$ T cells arrive, so the predominance of $CD4^+$ and APC cells could be a secondary event. The occurrence of AA in AIDS patients [37], who suffer from relative $CD4^+$ T cell depletion, questions the relevance of an MHC class II-dependent initiating event. Recently, it was reported that experimental systemic lupus erythematosus cannot be induced in an established murine model if test mice lack MHC I molecules [38]. Since the incidence of lupus is increased in patients with AA [2, 39] this further encourages one to systematically explore the role of MHC I-presented autoantigen(s) in AA.

Several aspects of the primary autoimmune response outlined here need to be clarified:

How is MHC I-expression induced? What are the characteristics and determinants of autoreactive T cell -, MHC I- and MRP-DP interactions? Why are there any autoreactive $CD8^+$ T cells at all, and what is the co-stimulatory signal required for cytotoxicity? Which MRP are candidates for providing antigenic MRP-DP? And why are epidermal MC only exceptionally affected in AA, leading to vitiligo, if joint MHC I expression and melanogenesis predispose to an autoimmune attack on MC?

MHC I-EXPRESSION

Two mechanisms operating side by side could be responsible for abnormal MHC I expression in the anagen hair bulb: stimulation by locally released cytokines known to induce or enhance MHC I-expression, or failure to maintain suppression of MHC I-expression. IFN-γ and TNF-α, for example, stimulate MHC I expression [40], notably also in organ-cultured human hair follicles [20]. Local microtrauma, or the stress-induced release of substance P from the elaborate perifollicular nerve plexus with the consequence of localized neurogenic inflammation [cf. 41–43], could create such a cytokine milieu. MHC I expression as a consequence of neurogenic inflammation in a limited skin area corresponding to the distribution of selected cutaneous sensory nerves would nicely explain why some patients appear to suffer their first attack of AA subsequent to emotional or physical trauma [2, 44], and why the lesions are usually so well-circumscribed.

Individuals, and distinct territories of hairy skin in a given individual, may differ in the constitutive MHC I-repression of anagen hair bulbs. This would account for varying, genetically determined risk levels for developing AA in a specific location and for the preference of AA for some cutaneous territories (e.g., nuchal skin). Likewise, microbial antigens (e.g., streptococcal superantigens) released from infectious foci or during bacteremia or viremia could indirectly account for a higher-than-normal level of MHC I expression *via* the associated immune responses with enhanced, but localized IFN-γ and TNF-α secretion [cf. 7, 45, 46]. In a small minority of AA patients, where the clinical history suggests involvement of an infectious agent/antigen in triggering the disease, microbial superantigens may also act as MHC-nonrestricted polyclonal activators of $CD8^+$ T cells [cf. 7, 45, 46].

Since almost all nucleated somatic cells express MHC I constitutively, its "lack" of expression likely is the result of active MHC I suppression. One candidate class I-repressor is α-MSH, which suppresses epithelial cell MHC I expression *in vitro* [47]. This neuropeptide is found in skin where it may even be generated locally [48]. Considering that the proinflammatory cytokine IL-1 is a potent functional antagonist of α-MSH [48], minor local inflammatory perturbations could easily disinhibit MHC I expression *via* the IL-1-mediated antagonism of α-MSH. Accepting that enhanced IL-1 secretion is an almost universal response to any kind of traumatic or inflammatory stimulus in the skin [49, 50], the delicate milieu of regulatory factors maintaining relative MHC I-"negativity" in the proximal hair bulb may easily be disturbed: MHC I-expression could become upregulated almost explosively when both IL-1 and IFN-γ are present.

Cytokines released during the initial cytotoxic attack may facilitate rapid disease progression by stimulating MHC I-expression in previously uninvolved neighboring follicles ready to switch from early to mid anagen. This centrifugal propagation of cytokine-mediated, abnormal MHC I-expression would be limited by cytokine diffusion and inactivation, and would further explain the development of well-circumscribed AA lesions. Yet, the rather non-specific pro-inflammatory events depicted above must be fairly frequent events, and the vast majority of our scalp hair follicles are in anagen, thus generating MRP. Why then does AA occur in less than 0.1 % of individuals [1], and not each time a follicle switches on melanogenesis during anagen while its level of MHC I expression is dangerously high?

CD8+ / MHC / MRP-DP INTERACTIONS

We propose that AA is relatively rare due to a very low statistical chance of a cytotoxic T cell attack on MRP-DP-presenting MC or KC. MRP-DP-reactive CD8+ T cell clones must have escaped clonal elimination and induction of anergy [5, 7, 45]; they must get into direct contact with an MRP-DP presenting cell in the hair matrix, and - according to current dogma - must receive a co-stimulatory signal necessary to induce cytotoxic activity [7, 45, 51, 53]. Also, intracellular MHC I-molecules must fold properly around MRP-DP to present them on the cell surface [7, 45, 51, 52]. The normal lack or low level of MHC I-expression may serve to dramatically reduce the chance that such a fit is ever made and that the few MHC molecules possibly expressed ever meet an autoreactive T cell. Finally, only individuals expressing certain MHC I haplotypes may be at risk of making an intracellular MHC/MRP-DP fit required for surface expression of the MHC-peptide complex [7, 45, 51, 52].

How do CD8+ T cells get into the hair bulb? Since the follicle is endowed with a rich perifollicular and intrapapillary vasculature, T cell trafficking between the hair matrix and the dermal papilla is a reasonable possibility (reminder: the presence of intraepidermal T cells and of dermo-epidermal T cell trafficking [50] had long escaped notice). T cells probably enter the hair bulb matrix rather infrequently, since immunohistology of normal human anagen hair follicles does not usually reveal T cells in the matrix region. However, the cytokines proposed to enhance MHC I-expression also are potent inducers of adhesion molecules and could thus guide increased T cell numbers into the matrix (e.g., both IFN-γ and TNF-α stimulate the expression of ICAM-1 on KC and MC; [23, 49, 50]. Thus, stronger proinflammatory and MHC I-stimulating local perturbations would greatly increase the chances for intrabulbar contact between MRP-DP and autoreactive T cells.

AUTOREACTIVE, CYTOTOXIC CD8+ T CELLS

Since follicular melanogenesis commences already *in utero*, one would expect that MRP-DP autoreactive thymocytes are eliminated during thymic maturation or are rendered "anergic" after having come into contact with MRP-DP as mature T cells, assuming these peptides are encountered without the required co-stimulatory signal [cf. 5, 7, 51, 53]. However, melanogenically active follicular MC may be shielded from contact with the surrounding mesenchyme by a dense, glycosaminoglycan-rich extracellular matrix that may obstruct [31], though not prevent, immune interactions. Thus, it is conceivable that normally *follicular* MRP-DP are never presented to thymocytes or T cells and that, therefore, circulating MRP-DP autoreactive CD8+ T cells do exist. Whether or not this is the case may again be influenced by immunogenetic factors, namely by the expression and activity of so-called immune response and immune suppression genes [cf. 5, 7, 45] (cf. apparent AA-protective role of HLA-Dw52a [26]). Whenever autoreactive T cells do exist, under normal circumstances, the absence or very low level of MHC I-expression in the hair matrix would prevent MRP-DP recognition by the occasional T cell trafficking through the matrix.

Once MHC I-expression has been stimulated, however, and additional T cells have been attracted to enter the matrix, the situation would change dramatically: the same

cytokines responsible for this could also contribute signals promoting a cytotoxic attack on MRP-DP expressing cells, now recognized for the first time by autoreactive T cells (e.g., upregulation of target cell adhesion molecules; synergism of IL-1 and TNF-α with IL-2 driven $CD8^+$ T cell proliferation; enhancement of CTL formation by IFN-γ). Simultaneously present $CD4^+$ T cells may provide co-stimulatory signals (e.g., IL-2) for autoreactive $CD8^+$ T cells, if the MRP-DP expressing cell does not produce such signals like B7 expression [45, 51, 53]. Note that even human KC can be stimulated to express a B7-like molecule *in vitro* [74]. Again, there is a chance to escape AA, if MHC-presented MRP-DP are not recognized as "foreign", or because no sufficient co-stimulation is offered, thus inducing T cell anergy [cf. 7, 51, 53].

MRP-DP AS AUTOANTIGENS

As outlined, the elusive follicular autoantigens in AA are probably generated only during anagen, and may arise from melanogenically active MC [3, 28, 29]. Though multiple plasma membrane and extramelanosomal cytoplasmic melanocyte proteins, theoretically, could serve as donors for autoantigenic peptides, it is more reasonable to assume that the donor proteins are melanocyte-specific. This designates melanosomal antigens and other MRP transported into melanosomes likely candidates (e.g., tyrosinase, DOPAchrome tautomerase [TRP-2], b-locus protein [TRP-1] and Pmel 17, addressed together as MRP [54, 55] since they are considered to be the only melanocyte-specific proteins [72]. In addition, melanosomes and MRP are synthesized in hair follicle MC, and are transferred to hair matrix KC, only during anagen [28, 35]. This would explain why only anagen follicles come under attack, and why the incriminated autoantigenic MRP-DP can be presented by both KC and MC, provided MHC I-molecules carry them to the cell surface.

That MC-associated antigens can indeed be immunogenic autoantigens is demonstrated by the cytotoxic autoantibodies against normal MC found in vitiligo [56–58], and by the antibodies to unidentified melanoma antigens described in some AA patients [59]. Yet, the very large number of gene products involved in melanogenesis and melanosome construction [54, 55, 72] makes it difficult to narrow down the list of candidate MRP serving as donors for MRP-DP.

The higher incidence of vitiligo in AA patients has been interpreted to suggest that the same MC-derived antigens are autoimmune targets in both vitiligo and AA, and the MC-related autoantibodies found in some AA patients [59], namely in those with both AA and vitiligo, might help to identify the MRP-DP incriminated in AA.

However, there is currently no convincing immunnological evidence availabe that AA and vitiligo share the same pathogenesis: on the contrary, both diseases are too rarely associated (only 1–4% of AA patients have vitiligo [1] and autoantibodies in vitiligo attest to a B cell-mediated autoimmune response; according to the immunological vitiligo theories, a complement-mediated, antibody-dependent cytotoxicity reaction may underlie the development of vitiligo [58, 73], but not the cell-mediated autoimmune-attack in AA [1, 3, 36]. Also, the MRP-DP incriminated in AA may only be generated in follicular, but not epidermal MC. Together, this would explain why epidermal MC are not attacked in the majority of AA patients. If, however, epidermal MC become a target of the proposed

secondary immune response, which may give rise to the mentioned autoantibodies, AA patients would also develop vitiligo. In short, the autoantibodies found in AA or vitiligo probably are misleading guides for identifying the MRP-DP in question.

More pertinent clues arise from the finding that cytolytic T cells can be isolated from patients with certain MHC I expressing melanomas [60, 75]. For example, these cytolytic T cells recognize and respond to an HLA-A2-presented antigen coded for by the tyrosinase gene [60]. Enzymatically active tyrosinase, or protein products of alternatively spliced tyrosinase mRNAs serving as "receptors/transporters" for L-tyrosine or L-DOPA [61], would be present only during active melanogenesis (i.e., anagen III–VI), and, as in melanoma, could be presented by MHC I-molecules. Therefore, we currently favor tyrosinase gene products as sources for the incriminated MRP-DP, since their presentation by MHC I and recognition by autoreactive T cells has been demonstrated conclusively [60]. We recognize that the new research focus suggested here (MHC I-presented melanocyte-associated antigens recognized by CD8+ T cells) will quickly reveal additional candidate proteins [cf. 75].

In summary, though many circumstances and events predispose to developing AA, none of them suffices to induce it, and the chance for all of them to coincide is indeed small. While this explains the low incidence of AA, it also provides a rationale for the frequent recurrence of AA attacks, since now the depicted chain of predisposing events may be linked together more easily, for example due to the local presence of primed autoreactive T cells and the persistance of a high level of MHC I expression in a given skin area.

PERSPECTIVES

Since our hypothesis is quite specific, it can be put to the test. Crucial support would arise from the demonstration that AA patients have $CD8^+$ T cells responding to MHC I-presented MRP-DP *in vitro*. If challenge with MRP or MRP-DP (in an MHC I-presentation setting) triggers AA-like symptoms in the anagen follicles of normal test animals, or of animals with a MHC-haplotype predisposing them to the development of autoimmune disease, this hypothesis would also be supported strongly. The same applies if the administration of IFN-γ, TNF-α or IL-1 to non-lesional skin in AA patients or - ethically more acceptable - in an available rat model of AA [63], induces a more rapid onset of AA lesions than seen spontaneously. If, however, AA-like hair loss can be induced in experimental animals that selectively lack follicular MC, MRP, $CD8^+$ T cells or MHC I molecules, our hypothesis becomes untenable.

Targeting the secondary immune response in AA certainly remains a good approach for limiting secondary follicle damage but, according to this hypothesis, it is insufficient for preventing disease progression and recurrence. The synchronization of all anagen follicles in the perimeter of an acute AA lesion into telogen should prevent progression of the lesion, because this would switch-off of MRP synthesis. Another approach would be to restore the local signal milieu maintaining relative MHC I-"negativity." Local administration of potent analogues of α-MSH [64], a putative MHC I-suppressor [47] is one option, assuming that the suppression of MHC I expression would prevent $CD8^+$ T cell recognition of MRP-DP, even if this should stimulate follicular melanogenesis. The topi-

cal application of drugs, hormones, or cytokines that inhibit melanogenesis or antagonize IL-1, TNF-α and/or IFN-γ should now be considered in AA management, provided these agents do not simultaneously up-regulate MHC I expression. Recently reported liposome preparations that target the hair follicle [65] are particularly promising for the topical delivery of α-MSH agonists, MHC I-stimulators, melanogenesis-inhibitors, and/or IL-1-, IFN-γ- or TNF-α-antagonists.

Corticosteroids have established suppressive effects on inflammatory infiltrates and on cytokine secretion [45] which makes them useful tools for suppressing the proposed secondary attack in AA. In addition, they may switch-off follicular melanogenesis: potent topical corticosteroids suppress anagen development in mice, which entails the suppresion of follicular melanogenesis [66]. However, that even potent corticosteroids are only partially effective in AA and do not prevent its recurrence might reflect an inhibitory effect of these drugs on the local generation of α-MSH [48] so that MHC I expression is not effectively downregulated. Also, corticosteroids are not appreciated as down-regulators of MHC I expression. The unsatisfactory response of AA to immunomodulatory treatment (incl. contact sensitizers, anthralin, cyclosporine A, corticosteroids, UV-irradiation) that is characterized by a high relapse rate after discontinuation of therapy invites to study how these agents modify the expression of MHC I and MRP-DP in the proximal anagen hair bulb. This may provide clues as to why and when these agents work, so they can be used more discriminatingly.

Several T cell-directed management strategies [67] for AA follow logically from our hypothesis. Besides the clinically rather unattractive, non-specific depletion of $CD8^+$ T cells by appropriate antibodies, the putative MRP-DP autoreactive $CD8^+$ T cells may be specifically "tolerized" by administering peptides that make a fit with the incriminated MHC I molecules, but fail to activate the T cells. Even the induction of oral tolerance by feeding of MRP-DP is a rational option, given the encouraging initial results obtained with the oral administration of myelin basic protein in the prevention and treatment of multiple sclerosis and of type II collagen in the management of rheumatoid arthritis [67–69]. Prior to clinical studies, all these approaches can be tested in the DEBR rat model of AA [63].

The AA literature documents the keen interest many dermatologists have long kindled for this fascinating disease [1, 2, 36, 44], but it also shows that AA has not enjoyed much attention from immunologists specializing in autoimmune diseases - most textbooks on clinical immunology or autoimmunity do not even mention AA. This field would greatly profit if professional immunologists finally were to discover the intricacies and surprises of hair follicle immunology in health and disease [21, 22, 30–32, 70, 71]. From a theoretical, experimental and clinical perspective, the study of AA is an ideal point of entry for exploring the immunology of the hair follicle and the concept of autoimmunity to normally sequestered, rhythmically generated, MHC I-dependent antigens.

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