Alopecia Areata Susceptibility in Rodent Models

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With our current view of alopecia areata as an autoimmune disease, it is probable that disease development in an individual is dependent on multiple genetic and environmental factors interacting in a complex system. Rodent models afford the opportunity to investigate alopecia areata development and to define the significance of the different factors involved. Recently, rodent model characterization has been conducted using flow cytometry, microarray analysis, and functional studies. From these a pattern of events in alopecia areata development has emerged. Although the preliminary activation events for the onset of alopecia areata remain unknown, the response of the immune system is characterized by antigen presentation and costimulation of lymphocytes in the lymph nodes and skin, a deficiency of $CD4^+/CD25^+$ regulatory cells, and an action of activated lymphocytes on hair follicles via Fas/FasL signaling and cytokines. Thus, onset of disease may require appropriate (or inappropriate) expression of stimulatory antigens within the hair follicle, the breakdown of the putative hair follicle immune privilege, the presentation of antigens to the immune system, a failure of immune system regulation, and the ability of the activated immune system to disrupt anagen-stage hair follicles. Once the sequence of events is initiated, it may become a self-perpetuating cycle, with epitope spreading leading to a wider range of targets in chronic alopecia areata. Rodent model studies have provided significant insight into alopecia areata, but much more remains to be explained about the mechanisms of disease development. ***Keywords: inflammation/autoimmune disease/mouse model. JID Symposium Proceedings 8:182–187, 2003***

Human alopecia areata (AA) is clinically, pathologically, and genetically a heterogeneous disease defined by a number of criteria (Hoffmann and Happle, 1999). It most likely comprises several disease subsets that may be caused by multiple pathogenic mechanisms. There are now several candidate AA models available, including SCID/human xenograft, spontaneous disease development in a variety of domesticated species, and spontaneous and induced disease in inbred rodent strains (Gilhar *et al*, 1998; McElwee *et al*, 1998a; McElwee *et al*, 1998b; McElwee *et al*, 1999a; Smyth and McNeil, 1999). Each of these models has the potential to provide significant insight into the mechanisms of human AA. As with research on other autoimmune diseases (Griffiths and Remmers, 2001), each model may not reflect the full range of highly variable clinical and pathological presentations observed in humans; however, collectively they may define common, fundamental parameters and individually yield information on particular subforms of the disease. The most extensively characterized model to date is the C3H/HeJ mouse (Sundberg *et al*, 1994). This model meets the basic criteria of AA: The disease is tissue specific, affecting anagen-stage hair follicles; hair follicle morphology is disrupted, but no scar tissue is formed in response to the inflammatory infiltrate; no persisting infectious agents have been found that could explain inflammation; and the disease is chronic but potentially reversible with immunomodulatory treatments or by spontaneous remission. Most recently, this model was extensively examined using flow cytometry and microarray analysis and subsequent functional studies. From these studies an understanding of mouse AA development has emerged (McElwee and Hoffmann, 2002).

Currently, AA is generally regarded as an autoimmune disease, although the antigenic target(s) of attack has not yet been proved (McElwee *et al*, 1999b). Classically, autoimmune diseases are controlled through a complex interaction between multiple genetic and epigenetic factors. The activity of these components working synergistically and antagonistically likely determines the degree of disease susceptibility, actual disease onset, severity, duration, and potential spontaneous or treatment-induced resolution of the condition. Genes and environmental modulation of autoimmunity can operate within the individual at three primary levels: the overall reactivity of the immune system, the specific antigen and its presentation, and the target tissue itself (Marrack *et al*, 2001). For an understanding of AA as an autoimmune disease, each of these areas requires investigation.

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Abbreviations: AA, alopecia areata; HLA, human leukocyte antigen; IFN, interferon; MHC, major histocompatibility complex; TCR, T cell receptor; Th, T helper cell; TNF, tumor necrosis factor.

IMMUNE SYSTEM REACTIVITY

Environmental stressors The events that lead to autoimmune disease in humans remain unresolved. It has been postulated that antigenic determinants of pathogens that cross-react with target organ–expressed antigens may trigger an autoimmune response by molecular mimicry and that this ultimately leads to

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autoimmune disease (Oldstone, 1998; Hiemstra *et al*, 2001). Alternatively, an infectious agent could induce cellular injury and release self antigens, generating cytokine-mediated bystander activation of self-reactive lymphocytes that cross-react with additional, but genetically distinct, self antigens (Horwitz *et al*, 1998; Regner and Lambert, 2001). Similar events have been postulated and discounted for AA over many years, and research on C3H/HeJ mice has failed to define a candidate infectious agent (Sabouraud, 1896; Ikeda, 1967; Skinner *et al*, 1995; Jackow *et al*, 1998; McElwee *et al*, 1998c). It is possible, however, that the inciting agent is only transiently expressed and that analysis of individuals or models downstream of the activating event yields a negative result. The viral or microbial infection that initiates the autoimmune phenomenon may not be present by the time overt disease develops (Oldstone, 1998). Thus, the possibility of exogenous agent involvement in AA onset cannot be ruled out. It is also possible that the general viral and vaccine load on the immune system may raise sensitivity and reactivity and increase the probability of an inappropriate immune response in an immune system with genetically determined instability.

Other exogenous immune system stimuli may be involved in AA susceptibility. In the mouse model, diet, specifically soy oil content, has been identified as one factor capable of increasing resistance to AA development. Mice fed a diet high in soy oil were less likely to develop AA after skin grafting, a known method of transferring AA between affected and unaffected animals (McElwee *et al*, 1998b). In part, this resistance may be due to soy isoflavones such as genistein. The challenge from the skin graft and the subsequent failure of overt AA development lead to an alteration in the immune system with a possible increase in activation-induced cell death in the spleen (McElwee *et al,* 2002). Gonadal hormones have been identified as a further AA susceptibility–modifying factor in the mouse model. While estrogen supplementation may increase disease severity, testosterone supplementation can inhibit disease onset in the skin graft–induced model (McElwee *et al*, 2001). These and other studies demonstrate that, while AA susceptibility in rodent models and humans is genetically determined, significant input from the environment may modulate disease onset, severity, persistence, and remission, in part because of modulating general immune system reactivity.

AA frequency and severity are apparently associated with a greater frequency of allergies as compared to the general population (Muller and Winkelmann, 1963; Ikeda, 1965; Penders, 1968; De Waard-van der Spek *et al*, 1989; Weise *et al*, 1996). It remains to be seen whether there is a direct relationship whereby an allergic response is capable of increasing immune system sensitivity and instability such that AA susceptibility increases, or whether the increased frequency of allergies in AA patients is merely a reflection of genetically determined immune system sensitivity. In rodent models, the incidence of allergies has not been investigated, although the DEBR model has a high degree of sensitivity to anesthesia (McElwee, in press). Stress has been suggested as a potential instigator of autoimmune diseases, possibly through glucocorticoid modulation of inflammatory cytokine expression (Elenkov and Chrousos, 2002). Stress has also been postulated as a potential influence on AA development, but, although the circumstantial evidence is significant, little direct evidence is available in support of the claim (Mehlman and Griesemer, 1968; Colon *et al*, 1991; Liakopoulou *et al*, 1997; Kavak *et al*, 2002). Other promoters of the immune system in autoimmune diseases may include drugs and toxins (Bigazzi, 1994; Elkayam *et al*, 1999; Holsapple, 2002; Lawrence and McCabe, 2002). In principle, one or more of these factors may modulate the immune system in AA, but in practice little is known about the effects of environmental stimuli on rodent models or humans.

Autoreactive leukocytes For hair follicles to be targeted and overt hair loss to occur, relevant autoreactive cell subsets must be present in an activated state and in significant numbers such that the follicles are adversely affected. The primary cell types found around and within human and rodent AA-affected hair follicles are $CD4^+$ and $CD8^+$ (Ranki *et al*, 1984; Zhang and Oliver, 1994). Recent flow cytometry studies defined a significant skin infiltrate of these cells, whereas skin-draining lymph nodes contain an increased percentage and total number of antigen-presenting cells (Zöller *et al*, 2002; McElwee *et al*, 2002). The high numbers of $CD4^+$ and $CD8^+$ lymphocytes circumstantially suggest their involvement in hair follicle dystrophy. $CD4^+$ lymphocytes of the Th1 subset are responsible for transfer of experimental autoimmune encephalomyelitis in rodent models (McDonald and Swanborg, 1988; Karpus and Swanborg, 1989; Mustafa *et al*, 1991). In experimental autoimmune encephalomyelitis, activated pathogenic T cells cross the blood–brain barrier and secrete proinflammatory cytokines and chemokines that attract other leukocytes to the local environment. Similarly in mouse AA, the putative immune privilege of the anagen-stage hair follicle is quickly broached by skin-draining lymph node cells derived from AA-affected mice transferred to non-AA-affected littermates (Carroll *et al*, 2002). Investigations to define the relevant subsets of immune cells capable of transferring AA are in progress. However, preliminary studies in which $CD4^+$ or $CD8^+$ cells were targeted in rodent models demonstrated that removal of one or the other population from AA-affected individuals is associated with hair regrowth. With recovery of the cell population, there is a reinitiation of hair loss (McElwee *et al*, 1996; McElwee *et al*, 1999c; Carroll *et al*, 2002). The SCID/human xenograft model has also provided data supporting a collaboration between $CD4^+$ and $CD8^+$ cells to promote AA (Gilhar *et al*, 2002). These and other studies suggest that $CD4^+$ and $CD8^+$ cell subsets are capable of perpetuating the AA lesion. In mouse and rat models, there is a clear division in distribution between the $CD4^+$ and $CD8^+$ cell populations. $CD4^+$ cells are almost exclusively perifollicular, whereas $CD8^+$ cells readily penetrate the hair follicle to take up intrafollicular residency. As such, these cells are likely to be the population with the greatest pathogenic potential, leaving $CD4^+$ cells possibly providing $CD8^+$ cell support and directly acting on the follicles through intermediaries, including hair growth–inhibiting cytokines, secretory Fas, and granzymes (Carroll *et al*, 2002; Zöller *et al*, 2002; Freyschmidt-Paul *et al*, 2003).

Immune system regulation Subsequent to immune system activation, other mechanisms of regulation must fail for AA to develop and be perpetuated. $CD4^+/CD25^+$ cells have been identified as one of probably several lymphocyte regulatory cell subsets that can restrain pathogenic cell activity. They are essential for maintaining homeostasis, they are able to suppress the induction of autoimmune disease and suppress $CD4^+/CD25^-$ cells, and they inhibit the effector function of autoreactive $CD8^+$ cells (Suri-Payer *et al*, 1998; Gao *et al*, 1999; Annacker *et al*, 2001). Little is currently known about immune regulatory mechanisms in human AA. Recent studies in the skin graft–induced AA mouse model revealed that $CD4^+/CD25^+$ cell levels drop significantly on activation of AA and prior to the onset of overt hair loss (Zöller *et al*, 2002). In contrast, sham-grafted mice are able to maintain $CD4^+/CD25^+$ cell numbers and quickly recover normal inflammatory cell numbers after injury. This apparent active depression of regulatory cells in AA-challenged mice may be a key factor in AA susceptibility. This and other regulatory methods may be defective in AA development, and their investigation may yield further insight into the mechanisms of the disease.

ANTIGEN AND PRESENTATION

MHC restriction For autoimmune disease to occur, a sustained activation of autoreactive lymphocytes is required. The amount

and localization of the antigen presented, along with the background responsiveness and regulation of the immune system and the availability of high-avidity autoreactive lymphocytes, are defining parameters in successful disease induction (Ludewig *et al*, 2001). For the most part, lymphocytes recognize antigenic peptides only in association with self MHC molecules and are initially activated exclusively in lymphoid organs (Karrer *et al*, 1997). Retrieval, processing, and presentation of antigenic peptides are conducted by professional antigen-presenting cells. The number of these cells, the duration of presentation, and the quality of presentation help determine whether autoimmunity occurs. Antigen-presenting cells are equipped with MHC class II for presentation of antigens to T helper, $CD4^+$ cells (Inaba *et al*, 1998) and particular MHC alleles are known to be associated with autoimmune disease susceptibility (Nepom and Erlich, 1991). The structural nature of the MHC molecule can permit preferential binding of specific antigenic peptides and may consequently increase the level of antigen presentation to the degree that autoimmunity may develop (Wucherpfennig *et al*, 1995; Wucherpfennig, 2001). Studies with humans have defined several HLA gene alleles in association with the AA phenotype (de Andrade *et al*, 1999; Colombe *et al*, 1999). In a genome-wide analysis of the C3H/HeJ mouse model, a susceptibility locus for AA was identified within the region of H2, the mouse equivalent of the human HLA region (Sundberg *et al*, 2003). More recently, dendritic cells have been characterized as expressing MHC class I and have the capacity to present antigens derived from apoptotic cells via MHC I direct to $CD8^+$ cells (Albert *et al*, 1998; Larsson *et al*, 2001; Fonteneau *et al*, 2002). Although attempts to associate MHC class I gene alleles with the AA phenotype have so far met with little success, $CD8^+$ cells are fundamentally involved in the development of AA in rodent models (McElwee *et al*, 1996; Carroll *et al*, 2002). Thus, in addition to MHC class II, MHC I restriction may be important, and the presentation of antigens in association with MHC I on dendritic cells may play a role in the onset of AA.

Costimulation For AA to develop, the immune system must be receptive to activation. Assuming that AA is an autoimmune disease, this requires the presence of antigen-presenting cells providing excitatory stimuli for lymphocytes within the individual. For successful lymphocyte stimulation to occur, antigen-presenting cells must express costimulatory molecules, primarily B7.1, B7.2, ICAM-1, and LFA3. These bind ligands CD28, CTLA4, LFA1, and CD2 on naive lymphocytes, and this, in association with MHC- restricted antigen presentation, induces full lymphocyte activation. Recent flow cytometry analysis revealed significant changes in expression of costimulatory molecule expression in both skin-infiltrating lymphocytes and skin-draining lymph node-derived cells (Zöller *et al*, 2002). Other studies have shown that monoclonal antibodies against the costimulatory antigens B7.1 and B7.2 can inhibit the onset of AA in the skin graft-induced mouse model, demonstrating the importance of antigen-presenting cells in AA induction (Carroll *et al*, 2002). Use of the same monoclonal antibodies in mice that already overtly express AA has little effect, however, indicating that chronic AA involves multiple redundant pathogenic mechanisms. Removal of one part of the pathogenic system is not enough to block the perpetuation of the disease. This may have implications for future treatment development and suggests that a multitarget therapeutic approach may be superior to targeting a single aspect of the immune system's activity.

TARGET TISSUE

Loss of immune privilege The hair follicle is regarded as an immune-privileged site (Westgate *et al*, 1991; Paus *et al*, 1999). In human and rodent model AA, disease onset is associated with increased expression of MHC class I and class II in the lower, transient portion of anagen-stage hair follicles (Messenger and Bleehan, 1985; Bröcker *et al*, 1987; Zhang and Oliver, 1994). Previously, it was postulated that a loss of immune privilege through an abnormal increase in hair follicle MHC expression might be the inductive factor for AA (Paus *et al*, 1993). However, no evidence of constitutive MHC expression has been defined in rodent models in the transient portions of anagen-stage hair follicles prior to inflammatory cell infiltration of AA-affected hair follicles. Sham-graft injury that induces a localized expression of MHC class II in hair follicles does not activate AA (Zöller *et al*, 2002; McElwee, personal observations), and AA can be readily transferred from affected mice to normal-haired recipient mice by skin grafting or by lymph node–derived cell transfer (McElwee *et al*, 1998b; Carroll *et al*, 2002). This suggests that hair follicle MHC expression is a consequence of inflammation, rather than a cause, probably because of the high levels of IFN-γ and TNF-α known to be expressed by inflammatory cells (Zöller *et al*, 2002). The expression of MHC molecules by hair follicle keratinocytes is nonetheless likely to be very important for the infiltration and disruption of hair follicles by autoreactive lymphocytes.

Hair follicle immunoprotection is likely transient due to the nature of the hair follicle cycle. Research suggests that the onset of catagen is associated with an infiltration of immune cells; candidate antigen-presenting cells (Parakkal, 1969; Westgate *et al*, 1991; Paus, 1996; Eichmüller *et al*, 1998). Regression of the hair follicle in catagen involves high levels of apoptosis and significant remodeling of the lower, transient portion of the hair follicle (Weedon and Strutton, 1981; Lindner *et al*, 1997). It is thus possible that the immune system is constantly exposed to low levels of hair follicle–derived antigens as the follicles cycle through catagen and given the ability of dendritic cells to present apoptosis-derived antigens. Autoimmune disease is not regarded as an all-or-nothing event. Rather, there are degrees of autoreactivity and a threshold level above which overt autoimmune disease is induced (Ludewig *et al*, 2001; McElwee *et al*, 1999b). A reflection of this may be the low level of hair follicle–specific antibodies found in some humans and rodents in the absence of overt AA (Tobin *et al*, 1994a; Tobin *et al*, 1994b; Tobin *et al*, 1997). If, however, catagen regression became disordered and the immune cell infiltrate associated with catagen inappropriately presented antigenic peptides in association with expression of costimulatory molecules, antigen presentation to the immune system might breach the threshold for stimulation of autoreactive cells. In a genetically susceptible individual, resident in a permissible environment, AA might follow. In the C3H/HeJ mouse model, AA is rapidly induced in a normal-haired mouse by transplantation of AA-affected skin, indicating that what hair follicle immune privilege there may be is little defense against the aggression of an activated immune system (McElwee *et al*, 1998b).

Hair follicle defects The involvement of hair follicle defects in susceptibility to human AA has been postulated and may be involved in rodent model AA (Bystryn and Tamesis, 1991; Goldsmith, 1991). Several studies based on AA models indicate that the primary hair loss–inducing component of AA is the immune system rather than a potential functional hair follicle defect with inflammation developing as a secondary event (McElwee *et al*, 1996; McElwee *et al*, 1998b; McElwee *et al*, 1999c; Carroll *et al*, 2002). However, it is possible that antigens are inappropriately expressed within the hair follicles of AA-affected individuals such that the immune system is challenged. The target antigen may be expressed at a higher density than is appropriate, or the antigen may be expressed transiently at a point within the hair follicle cycle at which it should not normally be expressed. Alternatively, the inciting antigen may be genetically or environmentally modified such that it is more stimulatory to the immune system. Studies indicate that structural modification of peptides can enhance binding to

MHC molecules or alter T cell receptor (TCR) recognition and so increase the degree and nature of antigen presentation (Wucherpfennig, 2001). Thus, hair follicle defects in themselves may not promote the clinical AA phenotype, but it is possible that they may increase the chance of peptide recognition by autoreactive cells and maintain antigen presentation at a high level once autoimmune disease begins.

Hair follicle disruption While high cytokine expression in isolation is not known as a trigger factor for autoimmune disease development, high expression around and within the target tissue in conjunction with other stimuli can enhance disease pathogenesis (von Herrath *et al*, 1995). The inflammatory infiltrate in rodent models expresses high levels of both Th1- and Th2-type cytokines (Zöller *et al*, 2002), some of which may act directly on the hair follicle. TNF-α binding to its receptor is required to promote apoptosis in experimental autoimmune encephalomyelitis and inflammatory bowel disease (Piguet *et al*, 1998; Kassiotis and Kollias, 2001). Similarly, TNF-α activity in AA may have a negative impact on the survival of hair follicle keratinocytes in AA. While the Th2-type cytokine IL-10 is commonly regarded as a potential regulator of autoimmunity, recent studies identified its pathogenic potential in the NOD mouse model for autoimmune diabetes (Balasa *et al*, 2000). One study using the mouse model and histocompatible mice deficient in IL-10 suggests that IL-10 may also contribute to AA susceptibility (Freyschmidt-Paul *et al*, 2002). IFN-γ may promote keratinocyte expression of MHC molecules that aid other inflammatory mechanisms. Other cytokines may negatively regulate keratinocyte cell proliferation and encourage hair follicles to truncate their growth cycle and enter a telogen resting state (Randall, 2001). Overall, cytokines may be significant modulators of hair follicle disruption.

Cytotoxicity may also be mediated through Fas/Fas Ligand (FasL) interaction. Expressed on activated lymphocytes, the binding of Fas expressed on target cells by FasL on activated lymphocytes, or released in a secretory form, results in apoptosis for the target cell. This mechanism of tissue destruction is not MHC restricted and has the potential to damage innocent bystander cells not directly targeted by the pathogenic lymphocyte clones. Fas/FasL signaling is also believed to promote antigen presentation. Thus, Fas has multiple roles (Siegel *et al*, 2000). In the C3H/HeJ mouse model for AA, Fas and FasL are significantly differentially expressed in skin-infiltrating lymphocytes, and Fas is highly expressed on dystrophic hair follicle keratinocytes (McElwee *et al*, 2002; Freyschmidt-Paul *et al*, 2003). Histocompatible mice deficient in Fas or FasL are comparatively resistant to the induction of AA, a result similar to that defined for insulin-dependent diabetes mellitus models (Itoh *et al*, 1997; Su *et al*, 2000). This resistance was in part localized to the skin as Fas- and FasL-deficient skin transplanted to AA-affected mice resisted immune activity and continued to grow hair (Freyschmidt-Paul *et al*, 2003). The possibility of an autocrine and paracrine action of Fas and FasL within and between hair follicle keratinocytes may add to the action of the inflammatory infiltrate and may explain why FasL-deficient hair follicles were also relatively resistant to AA.

Lymphocytes may also effect tissue damage through direct cell-specific mechanisms. Perforin, produced by cytotoxic cells, is a potent mediator of cell lysis (Russell and Ley, 2002). For perforin-induced lysis of target cells to occur, the TCR of an autoreactive cell must ligand with the relevant antigenic peptide presented in conjunction with MHC on the target cell. Whether perforin-mediated cell destruction occurs in AA is unknown, but the presence of highly activated $CD8^+$ cells within dystrophic hair follicles and the high expression of MHC molecules on hair follicle keratinocytes circumstantially suggest that MHC-restricted, perforin-mediated tissue damage can occur in AA. Other cytotoxic cell mechanisms may also be involved. Granzymes, which may gain access to target cells via perforin or induce apoptosis independent of perforin through binding cell surface receptors (Motyka *et al*, 2000), have been identified by microarray analysis as highly expressed in human AA (Carroll *et al*, 2002). Inducible nitric oxide synthase expression is apparent during mouse AA development and may suggest a role for nitric oxide in disease pathogenesis (McElwee, personal observation).

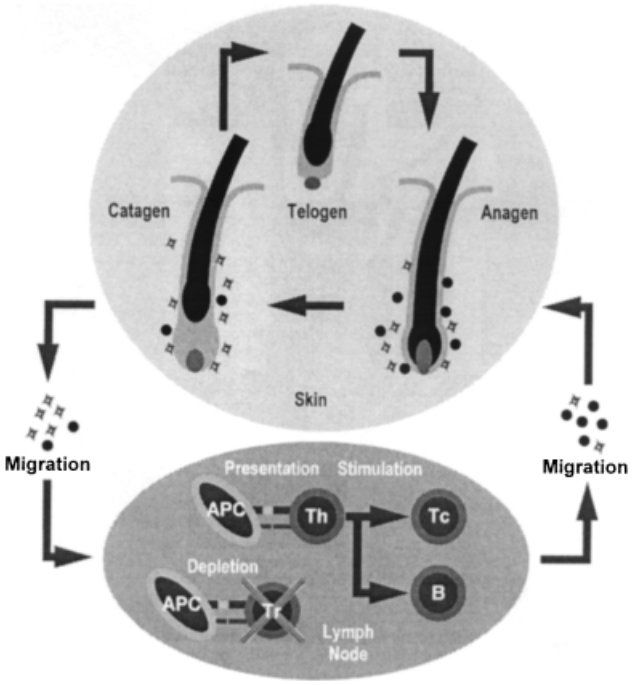

Figure 1. Mechanism of alopecia areata. Unknown events lead to the uptake and processing of antigens by antigen-presenting cells (APC) that migrate to regional lymph nodes. The inciting antigens are offered to a receptive immune system in association with appropriate costimulation to activate autoreactive T helper (Th) and T cytotoxic (Tc) lymphocytes, whereas T regulatory (Tr) cells are depleted. Further stimulus through cytokines may recruit antibody-producing (B) lymphocytes. Activated cells migrate to the skin utilizing adhesion molecules and surround anagen-stage hair follicles. Loss of hair follicle immune privilege permits inflammatory cell infiltration, hair follicle disruption, and uptake of antigen debris by APC that may lead to epitope spreading with disease progression. Hair follicles may avoid irreversible damage by entering prolonged telogen, whereas immune surveillance ensures truncation of any future anagen initiation events.

Resistance to pathogenic mechanisms Hair follicles may have some methods of resistance against these effector mechanisms of tissue damage. They may avoid total destruction by entering a telogen resting state, where antigen expression is much more limited. There is some argument as to whether there is a significantly increased frequency of telogen-stage hair follicles associated with human AA. In rodent models, AA progression is typically characterized by an initial increase in dystrophic anagen follicles, but, as individuals reach the chronic stable phase of AA, most follicles are in a telogen state (McElwee, personal observation). The natural properties of hair follicles and their regenerative ability may also provide resistance to the destructive capacity of cytotoxic lymphocytes. As long as dermal papilla cells survive and remain in close communication with keratinocytes, hair follicles may be reformed if inflammation is removed. In this way, despite the aggressive nature of the peri- and intrafollicular inflammation and the severe disruption of the hair

follicle unit, the possibility of spontaneous or treatment-elicited recovery from AA is possible.

CONCLUSIONS

In our current understanding of rodent models, the development of AA fundamentally involves a general genetic susceptibility with possible supplementation by a variety of minor disease severity–modifying genes. However, the actual onset of the disease along with its extent, persistence, and resistance to treatment in a given individual rodent or human may be modulated by epigenetic factors, including diet, gonadal hormones, pathogens, and other environmental influences. Both genetic and environmental factors will modulate AA at three levels. The responsiveness of the immune system may be influenced through pathogen, allergen, stress, and toxin load, with sensitivity to each defined by genetics. The specific inciting antigens and how they are presented to the immune system may be defined by environment-derived cross-reactive antigens or by inappropriate antigen exposure in the hair follicle cycle, while genes may dictate the threshold level required for stimulating autoreactive cells through the nature of the inciting or cross-reactive self antigens, MHC, TCR, costimulatory molecules, and regulatory cells. Within the skin, the failure of anagen-stage hair follicle immune privilege may be modulated by local resident pathogens or by physical trauma, and the subsequent adhesion molecule expression, cell infiltration, cytokine milieu, and hair follicle disruption may involve a complex gene interaction.

Onset of AA is dependent on a sequence of events **(Fig 1)** involving the presence of autoreactive lymphocytes with high affinity and avidity for hair follicle antigenic epitopes; antigen retrieval, processing, and carriage to lymphoid organs; appropriate antigen presentation in association with costimulation; deficiency of lymphocyte regulation; breakdown of immune privilege in hair follicles and perhaps other hair follicle-specific defects; and failure of the hair follicle unit to resist the onslaught of focal inflammation. The primary mechanism of hair follicle disruption in rodent AA is effected by $CD8^+$ lymphocytes in a manner dependent on Th1 $CD4^+$ lymphocytes. Although a Th1-mediated mechanism is fundamental to AA development, Th2 cell activity is also involved, particularly in the chronic stages of disease. Without knowing the source of the specific antigen epitopes that trigger AA onset, it is not possible to conclusively define AA as fundamentally autoimmune in nature. However, rodent model research has produced significant evidence consistent with this hypothesis.

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