The Potential Role of Cytokines and T Cells in Alopecia Areata

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T cells play an important role in alopecia areata (AA) because AA can be reinduced by the injection of hair follicle-specific $CD8^+$ T cells into AA scalp biopsies, which were grafted onto *scid* mice, and the depletion of $CD8^+$ T cells restores hair growth in the Dundee experimental bald rat. Moreover, AA can be transferred by grafting of alopecic skin from C3H/HeJ mice with AA-like hair loss onto unaffected littermates, but the onset of AA is inhibited by i.p. injection of anti-CD44v10 antibodies. Interestingly, grafted anti-CD44v10-treated mice have decreased numbers of $CD8^+$ T cells within the skin. Beside T cells several clinical and experimental data point towards cytokines that might be crucial inducers of hair loss in AA. An aberrant expression of cytokines of the Th1 type and IL-1β has been detected in scalp areas involved by AA, and polymorphisms of cytokine genes such as IL-1-receptor antagonist, IL-1α, and TNF-α have been shown to determine disease susceptibility and severity. Moreover, IL-1 has been shown to be a potent inhibitor of human hair growth *in vitro*. Such IL-1-incubated hair follicles show ultrastructural changes similar to those observable *in vivo*. On the other hand mice transgenic for IL-1α develop patchy hair loss and during the depilation-induced hair cycle in C57/BL6 mice, members of the IL-1 family are overexpressed with the onset of spontaneous catagen. Taking all of the presently available data together, we may hypothesize that $CD8^+$ T cells are of crucial importance in AA by their interaction with MHC-I restricted autoantigens, and cytolysis of their target cells. Hair loss, however, may occur because proinflammatory cytokines may interfere with the hair cycle leading to premature arrest of hair cycling. ***Key words: autoimmunity/hair/hair cycle/immunology. Journal of Investigative Dermatology Symposium Proceedings 4:235–238, 1999***

Alopecia areata (AA) is a common type of hair loss with a lifetime risk of 1.7% in the general population (Safavi *et al*, 1995). In clinical practice most patients will present with reversible patchy hair loss whereas others may develop complete baldness (AA totalis). Histopathologic features of AA in humans include perifollicular lymphocytic infiltrates involving only anagen hair follicles with subsequent miniaturization of the affected hair follicles (Headington, 1991). Several animal species have been reported to develop hair loss resembling human AA, including dogs, cats, horses, rodents, and nonhuman primates (Conroy, 1979; Michie *et al*, 1991; Sundberg *et al*, 1995; McElwee *et al*, 1998a). In the larger species, however, AA is poorly characterized, the animals are outbred, and they are not readily available for study, which makes them of little practical use as research models. A reversible type of hair loss closely resembling human AA has been described in C3H/HeJ mice (Sundberg *et al*, 1994). Hair loss in these mice is treatable either by steroids or by allergic contact dermatitis. Microscopically, affected mice develop nonscarring alopecia with dystrophic anagen hair follicles surrounded by a mononuclear cell infiltrate with a predominance of $CD8^+$ lymphocytes and rather small numbers of $CD4^+$ T cells. Telogen hair follicles are not affected. Recently, circulating autoantibodies to hair follicle antigens, similar to those present in human patients with AA, have been found in C3H/HeJ mice with AA (Tobin *et al*, 1997).

Although the cause of AA is at present unknown, we are now able to define a common denominator of all clinical forms of AA occurring in all mammalian species. This is the lymphocytic attack to the lower part of the anagen hair follicle and ectopic expression of MHC class I (Bröcker *et al*, 1987; Hamm *et al*, 1988) and II (Khoury *et al*, 1988) molecules on the epithelium of affected hair follicles, suggesting local release of cytokines. Hence, two key pathogenetic factors leading to hair loss in AA can be discriminated in the form of cytokines and T cells. This review summarizes recent results obtained in different fields of research, and analyses the data in view of the potential role of cytokines and T cells in AA.

T CELLS

Animal models for AA Several animal species have been reported to develop hair loss resembling human AA, e.g., dogs and horses; however, in the larger species, the animals are outbred, are not readily available for study, and the high costs of maintaining these animals makes them of little practical use as research models. Rodent models can be used is several ways for testing modes of therapy or disease prevention.

Depletion of $CD8^+$ T cells restores hair growth in DEBR The Dundee experimental bald rat (DEBR) undergoes progressive AA-like hair

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Abbreviations: AA, alopecia areata; ACD, allergic contact dermatitis; DEBR, Dundee experimental bald rat; HF, hair follicle.

loss ranging from small bald patches on the head, shoulders, and flank to virtually complete hair loss (Michie *et al*, 1991). As in human AA, this hair loss is associated with a dense peri- and intrafollicular mononuclear infiltrate consisting of both $CD4^+$ and $CD8^+$ T cells. Interestingly, the depletion of $CD8^+$ T cells by the intraperitoneal injection of monoclonal anti-CD8 antibodies restores hair growth in this animal model for AA, suggesting that $CD8^+$ T cells play a pivotal role in AA (McElwee *et al*, 1996).

Depletion of $CD4^+$ T cells restores hair growth in C3H/HeJ mice Reversible hair loss, closely resembling human AA, has been described in C3H/HeJ mice (Sundberg *et al*, 1994). This disease arises spontaneously with low frequency in mice beginning at around 6 mo of age, and in some colonies the expression frequency can approach 20% by 18 mo of age. Affected mice first develop patchy hair loss on both ventral and dorsal surfaces. About 10% of affected mice develop subtotal or total hair loss. Microscopically, affected mice develop nonscarring alopecia with dystrophic anagen hair follicles surrounded by a mononuclear infiltrate with a predominance of $CD8^+$ lymphocytes and small numbers of $CD4^+$ cells. Similar studies as mentioned above were conducted on C3H/HeJ mice with AA-like hair loss, but so far only $CD4^+$ T cells were depleted by intraperitoneal injection of monoclonal anti-CD4 antibodies. Surprisingly, even with this approach the treated mice show hair regrowth (McElwee *et al*, 1998b). Whether $CD4^+$ T cells are initially involved in AA or whether $CD8^+$ T cells need $CD4^+$ T cell help is presently not known.

The onset of AA in C3H/HeJ mice after grafting of alopecic skin is inhibited by i.p. injection of anti-CD44v10 antibodies As mentioned above, reversible hair loss closely resembling human AA has been described in C3H/HeJ mice (Sundberg *et al*, 1994). This hair loss can be induced in unaffected littermates by autologous transplantation of alopecic skin from affected C3H/HeJ mice, thus providing an appropriate and reproducible animal model for the dissection of the AA immune cascade. Successful grafting of alopecic skin C3H/HeJ mice affected with AA has been reported to induce hair loss distantly from the graft in unaffected recipients in 100% (McElwee *et al*, 1998b). Recently the splice variant v10 of the cell surface receptor CD44 (CD44v10) has been shown to be expressed on T cells in lesional AA skin (Wagner *et al*, 1998). Furthermore, a CD44v10-neutralizing antibody has been reported to inhibit the elicitation phase of allergic contact dermatitis (ACD) (Rösel *et al*, 1997). Because AA runs a similar pathway of Th1-mediated immune response, we addressed the question whether anti-CD44v10-AB influence the onset of AA in grafted C3H/HeJ mice. In our study AA-like hair loss developed in control mice, but not in those treated with anti-CD44v10-AB.[1] Immunohistochemical examination of grafted and alopecic host skin of control mice showed a perifollicular infiltrate of $CD4^+$ and $CD8^+$ T cells, with a predominance of the latter. In grafted skin of anti-CD44v10 treated mice, however, there was a striking decrease of $CD8^+$ T cells and MHC class I expression on hair follicle epithelium was reduced. Therefore, during the onset of murine AA, the interaction between $CD8^+$ T cells and hair follicle keratinocytes expressing MHC class I seems to be an important pathogenetic factor that is inhibited by anti-CD44v10-AB.

Human AA grafted onto *scid* mice

Primed T cells induce hair loss in AA scalp biopsies grafted onto scid *mice* Scalp biopsies obtained from patients with AA reveal hair regrowth after grafting onto *nude* mice (Gilhar and Krueger, 1987); however, AA can be reinduced by injection of T cells, which were primed in the presence of hair follicle homogenates into AA scalp biopsies grafted onto *scid* mice (Gilhar *et al*, 1998). Remarkably, mainly $CD8^+$ T cells are able to reinduce hair loss in AA grafts in *scid* mice (Gilhar *et al*, 1999). This strongly supports the concept that $CD8^+$ T cells are of crucial importance in AA. What kind of TCR these AA-inducing T cells express is unknown; however, *in situ* an oligoclonal T cell pattern expressing mainly TCR Vβ13 has been found by two independent groups (Hoffmann *et al*, 1996b; Dressel *et al*, 1997).

CYTOKINES

Several clinical and experimental data point towards cytokines such as interleukin-1 (IL-1) as being crucial inducers of hair loss in AA. Cytokines play an important role in both physiology and pathophysiology of human skin, and it is possible that they coordinate the cyclical hair growth. In line with this concept, cytokine gene expression has been reported in anagen rat hair follicles and a crucial role for hair cycling has been proposed (Little *et al*, 1994). Here, we summarize recent results obtained in different fields of research that suggest that cytokines might be common mediators leading to reversible hair loss in AA and possibly in a variety of other inflammatory conditions affecting the scalp.

Cytokines and hair growth in mice

In a depilation-induced hair cycle, IL-1 is overexpressed in catagen Changes in the local, cytokine-mediated signaling milieu of hair follicles (HF) have been implicated as major elements of hair cycle control and several lines of clinical and experimental evidence point towards IL-1 as an important inducer of hair loss. To address the question whether the steady state mRNA levels of gene expression of the IL-1 family parallel distinct phases of the murine hair cycle, the high degree of synchrony during depilation-induced HF cycling in mice was exploited to analyse the mRNA levels of IL-1α, IL-1β, IL-1-receptor antagonist, IL-1 receptor (R)-I, and IL-1-R-II by semiquantitative reverse transcriptase-polymerase chain reaction (Hoffmann *et al*, 1998). The results indicated that the induced murine hair cycle is associated with profound fluctuations in the steady state mRNA levels of members of the IL-1 signaling system. Most interestingly, IL-1α and IL-1β transcript levels increased dramatically with the onset of spontaneous catagen (around day 18) and peaked during telogen (day 25). These fluctuations in the IL-1α and IL-1β transcript levels were paralleled by substantial expression changes of the corresponding signal transducing type I IL-1 receptor. Therefore, these findings are consistent with the concept that IL-1α, IL-1β, IL-1-RI, and IL-1-RII might be involved in the control of catagen development.

Transgenic mice overexpressing IL-1α develop patchy hair loss The IL-1 family represents one of the most complex systems in cytokine biology. Two genes encode for the agonist molecules (IL-1α and IL1-β), a third gene encodes the IL-1 receptor antagonist, and two additional genes encode for the two types of the IL-1 receptor. Because many cells express these five genes *in vitro*, the outcome of an IL-1 inducible event is therefore rather complex. To study this problem animal models are needed. Transgenic mice overexpressing IL-1α in the epidermis are smaller in size and show an obvious cutaneous phenotype. Remarkably, these mice have patchy hair loss reminiscent of AA (Groves *et al*, 1994).

Cytokines and human hair growth

Cytokines are overexpressed in AA skin The earliest clinical sign of AA is patchy hair loss that is, in most cases, self-limited and reversible. The disease might progress, eventually leading to total loss of scalp and body hair, but even in such event a complete hair regrowth is still possible. Symptomatic treatment with immunosuppressive drugs such as cyclosporine A (Gupta *et al*, 1990) or corticosteroids tend to diminish the lymphocytic infiltrate, and hair regrowth occurs. Histologically the hair bulb is infiltrated and surrounded by mainly T helper cells. Remarkably HLA-ABC, -DR, and ICAM-1 overexpression can be observed on hair follicle keratinocytes (Bröcker *et al*, 1987; Hamm *et al*, 1988), indicating

[1] Freyschmidt-Paul P, Seiter S, Zöller M, Sundberg JP, König A, Happle R, Hoffmann R: Treatment with anti-CD44v10 monoclonal antibodies inhibits the onset of alopecia areata in C3H/HeJ-mice. *Arch Dermatol Res* 291:170, 1999 (abstr.)

the local release of cytokines. Several groups have detected, both at the protein (Gollnick and Orfanos, 1990), and mRNA level, cytokines in skin biopsies from patients with AA. A consistent feature was the presence of cytokine of the Th1-type (IFN-γ, IL-2) and IL-1β (Hoffmann *et al*, 1994; Hoffmann, 1995).

Cytokine inhibit hair growth in vitro Because cytokines such as IL-1β (Galbraith *et al*, 1999) have been found in early stages of AA, it is conceivable that they negatively influence hair growth. Single hair follicle cultures have been used to investigate the effect of various substances, including cytokines, on hair growth *in vitro* (Harmon and Nevis, 1993; Hoffmann *et al*, 1996c; Philpott *et al*, 1996). The most potent inhibitors of hair shaft elongation so far investigated *in vitro* are IL-1α (Cork *et al*, 1999) and IL-1β. Both cytokines are equally effective, and the inhibitory effect is completely abrogated by a 1000-fold molar excess of the IL-1 receptor antagonist, which is a natural antagonist of the IL-1 action, or by inhibitors of the cAMP pathway (Hoffmann *et al*, 1997).

Ultrastructural studies In AA, electronmicroscopic examination has revealed initial changes within cells of the dermal hair papilla and the outer root sheath keratinocytes of affected hair follicles. These morphologic changes were defined as a lack of structural organization within the dermal papilla and a marked polymorphism of the dermal papilla cells. The shape of the dermal papilla became more diamond-like (Hull *et al*, 1991). Remarkably, all of these morphologic changes observed *in vivo* can be induced by IL-1β stimulation of single human hair follicles *in vitro* (Philpott *et al*, 1995).

Genetic background of AA A hereditary component has been identified in patients with AA. It is most likely a polygenic disease. An increased frequency of autoimmune diseases has been found in patients with AA, most common associations being thyroid disease and vitiligo. The severity of the inflammatory response in AA may be determined by an interplay of pro- and anti-inflammatory cytokines. Recently, an increased frequency of the allele 2 of the IL-1 receptor antagonist gene was detected in patients with AA, especially in those with extensive hair loss (Tarlow *et al*, 1994). Because the IL-1 receptor antagonist is the natural antagonist of IL-1, it has been assumed that patients who cannot secrete sufficient amounts of the IL-1 receptor antagonist, due to the gene polymorphism, may have a more progressive disease. In line with this concept is the recent observation of an additional gene polymorphism for a subtype of the IL-1α and IL-1β gene.[2] Patients with severe AA tend to have an IL-1 gene polymorphism that causes an exaggerated release of IL-1, which in turn may lead to a more progressive disease. A similar scenario has been supposed for TNF-α, because a gene polymorphism for the TNF-α gene has likewise been found in patients with severe AA as well (Galbraith and Pandy, 1995).

Other diseases associated with hair loss Patients suffering from T cell lymphoma such as the syringolymphoid hyperplasia with alopecia (Esche *et al*, 1998) or other lymphomas often develop severe, but reversible circumscribed hair loss. During the leukemic stage of a cutaneous T cell lymphoma the patients may become bald, but hair regrowth starts upon treatment. The pathogenesis of this type of hair loss is unclear but several reports have shown an aberrant cytokine gene expression within both lymph nodes and serum from affected patients (Merz *et al*, 1991; Hsu *et al*, 1993). A remarkable feature is the overexpression of IL-1 in skin areas where the lymphoma cells enter the epidermis (Tron *et al*, 1988).

Another example of an inflammatory scalp disease with hair loss is seborrheic eczema of the scalp (Orfanos and Frost, 1990). This scalp disease typically induces diffuse hair loss that is completely reversible upon treatment with topical steroids or ketoconazol. Furthermore, patchy hair loss due to syphilis is usually reversible and shows both clinical and histopathologic features reminiscent of AA (Lee and Hsu, 1991; Cuozzo *et al*, 1995).

Inflammatory scalp disease may allow hair growth in AA In some of those cases were AA coincides with other inflammatory diseases of the scalp there will be hair growth. To describe this striking response that is the opposite of the Köbner reaction, the anagrammatic term Renbök phenomenon has been proposed (Happle *et al*, 1991). Examples of this phenomenon are psoriasis of the scalp and allergic contact dermatitis (ACD). The induction and periodic elicitation of an ACD is currently the most effective mode of treatment (Happle and Echternacht, 1977; Hoffmann and Happle, 1996). The underlying mechanism is still unexplained, but contrasting cytokine profiles have been found in treated *versus* untreated bald scalp. An increased expression of interferon-γ, IL-2, and IL-1β was found before treatment with the contact sensitizer, whereas IL-10, TNF-α (Hoffmann *et al*, 1994; Hoffmann, 1995), and TGF-β1 (Hoffmann *et al*, 1996a) were the most abundant cytokines present within the scalp after treatment. Remarkably, IL-1β was reduced after successful treatment.

Similar to humans treated with the contact sensitizer SADBE (Happle *et al*, 1980), the C3H/HeJ mice can be sensitized with 2% SADBE and challenged with individually varying concentrations of the substance and they show clinical features of contact dermatitis for several days.[3] After SADBE treatment there is a striking reduction of aberrant MHC class I expression on hair follicle keratinocytes in murine AA, and a less conspicuous, but still distinct, reduction of ectopic MHC class II expression. These findings are consistent with the down-modulation of abnormal HLA-ABC and HLA-DR expression as observed in human AA after treatment with the contact allergen diphenylcyclopropenone (DCP) (Bröcker *et al*, 1987). The pronounced downregulation of aberrant MHC class I expression can be related to the predominance of $CD8^+$ cells in untreated murine AA, especially in the center of the inflammatory infiltrate being in direct contact with the hair follicle, as well as the reduced number of $CD8^+$ cells after therapy, again pointing towards an important role in the pathogenesis of AA, of a specific interaction between cytotoxic $CD8^+$ T lymphocytes and MHC class I positive hair follicle keratinocytes.

Hair growth is a well-orchestrated process in which each hair follicle cycles between periods of active hair production and periods of rest. These cycles involve both epithelial and mesenchymal structures and recent advances have identified a variety of factors that might be encompassed during this process; however, our understanding of mechanisms of hair growth is far from complete. Although the pathogenesis of AA is still poorly understood, a peri- and intrabulbar accumulation of T lymphocytes (Perret *et al*, 1984) and aberrant expression of ICAM-1 and HLA-DR molecules on follicular keratinocytes and dermal papillae (Messenger and Bleehen, 1985; McDonagh *et al*, 1993; Nickoloff and Griffiths, 1991), provide evidence that an immune process is involved (Baadsgaard, 1991), interfering with the hair cycle and leading to reversible hair loss.

It is conceivable to assume that $CD8^+$ T cells are of crucial importance in AA because they are able to recognize MHC-I restricted autoantigens. As a consequence, FAS- or perforin-mediated apoptosis of target cells may occur, thus leading to their destruction of AA. So far, however, no data have been reported in this regard. Hair loss may occur because proinflammatory cytokines such as IL-1 interfere with the hair cycle, leading to premature arrest of hair cycling with cessation of hair growth. This concept may explain typical clinical features of AA such as a progression

[2]Ahini RT, di Giovine FS, McDonagh AJG, *et al*: Interleukin one composite genotypes as determinants for subtypes of alopecia areata. Third International Research Workshop on Alopecia areata. November 5, 1998, Washington. *J Invest Dermatol, Symp Proc* 4(Suppl 3):53, 1999 (abstr.)

[3]Freyschmidt-Paul P, Sundberg JP, Happle R, *et al*: Successful treatment of alopecia areata-like hair loss with the contact sensitizer squaric acid dibutylester (SADBE) in C3H/HeJ mice. *J. Invest Dermatol*, 113:61–68, 1999

pattern in centrifugal waves (Eckert *et al*, 1968) and spontaneous hair regrowth in concentric rings (del Rio, 1998), suggesting the presence of soluble mediators within affected areas of the scalp. Further studies should show whether the proposed concept holds true. If so, the application of specific cytokine inhibitors would be a promising approach for the treatment of AA.

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