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The immunological aspects of alopecia areata

Human hair follicles undergo rythmic and asynchronous changes. The hair bulbs run through three various stages known as anagen (growth phase), catagen (regression phase), and telogen (resting phase). Numerous growth factors and cytokines are involved in hair follices development and cycling, such as epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1), transforming growth factor (TGF)-alfa, TGF-beta, keratinocyte growth factor (KGF), basic fibroblast growth factor (bFGF), interleukin-1 (Il-1), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) (8). Moreover, various receptors and hormones are involved in hair cycle regulation. Even slight changes in this sensitive milieu may cause alteration in physiological hair follicle development, leading to the shortening of anagen phase, initiation of catagen and increase in the number of telogen follicles (5). Nevertheless, all regulatory mechanisms of hair bulb remain unknown. The critical molecules responsible for premature catagen are still to be investigated.

Alopecia areata (AA) is a common dermatologic disorder that affects men and women equally and can occur at any age. The prevalence is 0.1% to 0.2% of the general population and constitute about 2% of all dermatoses (3, 6). Clinically AA can manifest itself with different patterns. Usually patients present round or oval smooth, nonscarring alopecic patches generally on the scalp, but any hair-bearing surface can be affected. The disease can present with various forms including AA vulgaris, totalis or universalis. The 'exclamation-mark' hairs localized at the margin of lesions are a characteristic feature. Hair loss is a consequence of premature termination of hair anagen phase and initiation of catagen, finally resulting in increase of telogen hair follicles (11).

The pathogenesis of AA remains unclear. Among various hypotheses that may explain the development of the disease like: immunologic theory, autoimmune theory, genetic background, neuropeptides role, viruses etiology and others, the immune alterations are believed to play an important role in the pathogenesis of AA (6). Successful therapy methods of AA with the immunomodulatory agents such as contact sensitizers (for example, diphenylcyclopropenone – DPCP), topical or systemic steroids and oral cyclosporine support immune-mediated pathogenesis.

IMMUNOLOGICAL THEORY

Several studies suggest that specifically the T cells may play an important role in the pathogenesis of *alopecia areata*. Massive perifollicular and intrafollicular lymphocytic infiltrates around anagen phase hair follicles are frequently found in histopathological and histochemical examinations (2,

7). The infiltrate is usually located between the bulbar follicular epithelium and the level of the sebaceous glands (7). Nevertheless, a perifollicular infiltrate may be present in all three stages of disease, the highest activation of lymhocyte is characteristic of anagen phase. Simultaneously a number of peripheral blood lymphocytes T is reduced or normal (6). The inflammatory cell infiltrate consists primarily of CD4+, CD8+ and macrophages with predominance of helper T-cells (CD4+). Hair loss in AA is a result of both direct activity of lymphocytes and indirect influence by production of various cytokines (11). Activated T helper cells produce Th1 type cytokines including: interleukin (Il)-2, interferon (IFN)-gamma that may inhibit hair follicle growth (6). A significant pathogenic role of cytokines in AA have been supported in a number of studies.

IFN-gamma can induce expression of human leukocyte antigen HLA-DR, HLA-A,B,C and intercellular adhesion molecule 1 (ICAM-1) in the dermal papilla and keratinocytes of the matrix and outer root sheath (4, 6). The expression of both ICAM-1 and HLA-DR on the keratinocytes and dermal papillar cells of the hair follicle also support the immune-mediated background. It is well known that IFN-gamma released from activated T cells is the only cytokine believed to be able to induce HLA-DR expression (11). Moreover, epidermal keratinocytes produce Il-1alfa, Il-1beta and tumor necrosis factor (TNF)-alfa that are regarded as inhibitors of hair growth and contributors of hair follicle pathophysiology (6).

Il-1 is a highly pro-inflammatory cytokine that attracts T-lymphocytes, neutrophils and macrophages to the local inflammatory area and influences the matrix cells differentation (2). It has been reported that expression of Il-1 is strongly associated with AA severity (2). There are at least two agonist molecules of Il-1: alfa and beta which share common receptors. Il-1alfa is known as a negative hair growth regulator whereas Il-1 beta induces premature apoptosis in hair follicle bulb keratinocytes by causing the morphological changes of the hair follicle outer root sheath (2, 13). Hoffman et al. (13) suggest that Il-1 is a key cytokine in pathogenesis of AA and Il-1beta might be a crucial mediator of the arrest of hair loss in this disease.

The possible role of TNF is also investigated. TNF alfa has proved to be a strong inducer of apoptosis that inhibits keratinocyte proliferation and together with IFN gamma shows a synergistic antiproliferative effect (1, 11).

Literature data indicate that treatment of AA with topical immunomodulatory agents such as diphenylcyclopropenone (DPCP) is able to induce hair regrowth even in the long lasting process. The mechanisms of that action remain unclear. It has been found that local application of contact sensitizer can influence the CD4+/ CD8+ lymphocyte ratio and change the cytokine milieu: from TH1 type cytokines (IFN gamma, Il-2) and Il-1beta to high expression of Il-8, Il-10, TNF-alfa and TGF-beta1 (2, 3). Il-10 seems to be an inducer of Il-1 receptor antagonist (Il-1Ra) in monocytes. Il-1Ra inhibits action of Il-1. Increased expression of Il-10 after DCPC therapy results in decreased expression of Il-1 (2). Moreover, peri- and intrafollicular expression of HLA-DR is reduced after topical immunotherapy (2).

Anthralin is commonly used in treatment of AA. Hair regrowth has been reported in 20%–25% patients (6). It has been demonstrated that topical anthralin modulates the cytokines profile but the underlying mechanism also remains unknown. What is interesting, the drug application can downregulate the expression of TNF-alfa and IFN-gamma, whereas the release of both Il-1 alfa and beta and their receptor antagonist (Il-1Ra) and Il-10 is increased. All that modalities may be responsible for anthralin's therapeutic effects (10).

A lot of other agents including transforming growth factor (TGF) beta, fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and cutaneous lymphocyte-associated antigen (CLA) may play an important role in the mechanisms of hair loss.

It appeared that TGF-beta is capable to induce morphological changes of the lower bulb and stimulate apoptotic cell death by activation of caspase-9 and caspase-3. Thus TGF-beta is responsible for the induction of catagen phase (1).

CLA is a skin-specific lymphocyte antigen that contributes to the development of many immunological cutaneous disorders. CLA positive limphocytes CD4+ and CD8+ are present not only in peripheral blood but also in perifollicular infiltrates. The immunohistochemical study of peribulbar infiltrates showed CLA-positive CD4+ / CD8+ lymphocytes around hair follicles (14). Yano et al. (14) have showed a correlation between the percentage of CLA positive lymphocytes CD4+ and CD8+ in peripheral blood and clinical stages of AA. Additionally, they found a significantly higher CLA-positivity in patients with severe AA whereas there is no statistic difference of CLA positivity between recovering patients and normal control.

VEGF and FGF are essential for angiogenesis and vascular permeability, therefore may be responsible for maintaining proper vasculature around the hair follicles during the anagen phase. Several studies have provided of evidences that VEGF activity in alopecic hair follicles was significantly reduced comparing to healthy scalp tissue (9).

AUTOIMMUNOLOGICAL THEORY

Although immunological theory is widely known, recent studies are focused on autoimmune abnormalities in patients with AA. In the light of current investigations, highly suggestive evidence exists that AA may be a tissue-specific autoimmune disease. The anagen hair follicle has been suggested as a site of immune privilege. There can be observed absence or very low level of MHC class I or class II antigens expression in the normal anagen hair follicle. Increased MHC expression may result in the appearance of anagen and melanogenesis-associated autoantigens that are exposed to the skin immune system. In *alopecia areata* the normal follicular immune privilege collapses with MHC class I and II expression in the proximal hair follicle epithelium (7). Abnormally exposed antigens activate a second immune system response resulting in hair loss. The number of antigen presenting cells including macrophages or Langerhans' cells increase. IFN-gamma seems to be a very potent stimulator of ectopic MCH class I expression. This condition can be a result of endogenous and exogenous antigens: infections with bacterial superantigens, skin microtraumas, psychoemotional stressors, predisposding immunogenetic factors and IFN-gamma secretion. Therefore, high levels of circulating IgG autoantibodies capable of binding hair follicles specific antigens have been found in *alopecia areata* patients (7). By contrast in normal individuals the low level of IgM antifollicular antibodies has been detected with the use of highly sensitive methods (12). It has been showed that the antibodies to hair bulb in the sera are present in 100% of AA patients comparing to 44% in normal controls (6).

In addition, the deposits of IgG immunoglobulin and complement around hair follicles have been identified at the margin of active lesions (7). The deposits are mainly localized around the lower part of the hair follicles. Tobin et al. (12) have suggested that the presence and titers of antibodies can be a marker for clinical disease activity.

The autoantigens that may be responsible for the development of the *alopecia areata* have been widely searched. Different structures of anagen phase hair bulbs may be a target for IgG autoantibody. The most common antigens of hair follicle range between 44–60 kDa and 200–220 kDa and are mostly located in proliferating and differentiating areas including outer root sheath, matrix, inner root sheath and hair shaft (6, 12).

Other indirect evidences for autoimmune-mediated pathogenesis include the association of AA with various autoimmune conditions: thyroid disease, vitiligo, pernicious anemia, diabetes, lupus erythematosus, myastenia gravis, rheumatoid artritis, polymyalgia rheumatica, ulcerative colitis, lichen planus and other (6). Vitiligo and thyroid desease are the most significant accompaning disorders (7). Coexistence of thyroid disease ranges from 8% to 11.8% in AA patients versus 2% in the normal population (6).

Futhermore, the high levels of various organ-specific autoantibodies have been detected in AA patients sera including thyroid autoantibodies and thyroid microsomal antibodies, gastric parietal cell antibodies, antinuclear and antismooth muscle antibodies (6, 12).

Melanocytes and keratinocytes in AA There are other cells that may be a target of the immune response in *alopecia areata*. The pigmentary abnormalies are frequently observed in AA. The melanocyte damage both histological and ultrastructural, with abnormal melanogenesis is commonly observed (6). This finding suggests that bulbar melanocytes can be a target of the immune response. Hair follicle melanocytes express different antigens from epidermal melanocytes, which can explain selective targeting of hair bulb melanocytes (4). Nevertheless, there is no obvious evidence that melanocytes are affected in the development of AA (12).

Additionally, precortical keratinocytes abnormalities have been found in bulbs of active AA lesions (3).

Neuropeptides in AA. The neurologic factors have been found to play an important role in the modulation of immune response and proliferative processes in AA. Neuropeptides produced by cutaneous nerves including calcitonin gene-related peptide (CGRP) and substance P (SP) may modify the immune reactions and influence disease (4, 6). CGRP have the antiinflammatory effect by inducing mast cell degranulation with relase of TNF-alfa and Il-10 (4). Not only the skin expression but also the serum levels of CGRP are decreased in patients with active AA comparing to normal control. Moreover, CGPR increases vasodilatation and endothelial proliferation, which is essential for the normal hair cycle (4). It has been found that SP is able to induce hair growth in the mice (6). There are studies suggesting a low activity of CGRP and SP in the scalp of patients with AA (6).

CONCLUSIONS

Several hypotheses for *alopecia areata* development have been suggested but the mechanisms leading to hair loss are still unclear. Among various widely accepted theories of AA the immune mediated pathogenesis is the most popular. There is strong evidence of a number of immune and autoimmune abnormalities in AA. Various studies concluded that AA can be passively transferred by autoantibodies or T cells in mice (12). Not only the peri- and intrafollicular accumulation of CD4+ lymphocytes and CD8+ lymphocytes are observed but also autoantibodies directed mainly to self-antigens in hair bulbs have been recently detected. The autoantibodies are a heterogenous group because of different patterns of targets in the anagen phase hair follicle structure. AA has associations with other autoimmune diseases such as vitiligo or thyroid disorders. Successful treatment with immunomodulatory agents is another support for immune-mediated pathogenesis. Novel therapies for *alopecia areata* predominantly influence Th1-cell activation, cytokine profiles and antigen presentation. Local immunotherapy with contact sensitizers like DCPC, topical steroids, anthralin, and immunosuppressive doses of systemic steroids, cyclosporine or photochemotherapy (PUVA) are recommended. Nevertheless, many patients do not

respond to the treatment strategies, which indicates that further studies on AA pathogenesis should be conducted.

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SUMMARY

Alopecia areata is a common skin disease with prevalence 0.1–0.2% of general population. Clinically it is characterized by smooth, nonscarring patches involving the scalp and/or any hair-bearing area on the body. Among several etiological theories explaininig the hair loss including immunologic, autoimmune and genetic hypotheses, there is strong evidence indicating that *alopecia areata* is a tissue-specific, autoimmune condition. Peri- and intrabulbar infiltrate of T cells: CD4+ and CD8+ have been observed in histopathological examination. A number of Th1 cytokines produced by activated Th lymphocytes modulate the immune response in AA icluding Il-1alfa and

beta, Il-2, IFN-gamma. Additionally heterogenous autoantibodies to anagen phase hair follicle have been found. Association with other autoimmune conditions including thyroid disease and vitiligo support immune-mediated pathogenesis. Different immunomodulators have been used to treat this disease. Topical, intralesional and systemic steroids, topical immunotherapy (DCPC), anthralin and photochemotherapy are available, however the mechanisms of action of different treatment options are poorly understood.

Zaburzenia immunologiczne w łysieniu plackowatym

Łysienie plackowate jest częstą chorobą skóry, dotyczącą 0,1–0,2% populacji światowej. Klinicznie charakteryzuje się występowaniem niebliznowaciejących ognisk pozbawionych włosów na skórze owłosionej głowy i/lub w każdej innej lokalizacji, gdzie obecne są mieszki włosowe. Proponowanych jest kilka teorii wyjaśniających etiopatogenezę oraz mechanizmy wypadania włosów w tym schorzeniu, należą do nich między innymi teoria immunologiczna, autoimmunologiczna czy genetyczna. Istnieją dowody wskazujące na to, że łysienie plackowate jest narządowo-swoistym schorzeniem auoimmunologicznym. W badaniach histopatologicznych zaobserwowano wokół oraz wewnątrz mieszków włosowych nacieki z limfocytów T CD4+ i CD8+. Istotną rolę w łysieniu plackowatym odgrywają również cytokiny Th1 produkowane przez aktywowane limfocyty Th. Dodatkowo stwierdzono występowanie heterogennych autoprzeciwciał skierowanych przeciwko różnym strukturom mieszka włosowego. Jednoczesne występowanie łysienia plackowatego z innymi chorobami o podłożu autoimmunologicznym, do których należy między innymi bielactwo nabyte czy schorzenia tarczycy, również przemawia za tłem immunologicznym. W terapii łysienia plackowatego stosuje się leki o działaniu immunomodulującym. Powszechnie zaleca się aplikację miejscową, doogniskową lub ogólną kortkosteroidów, miejscowe stosowanie immunomodulatorów (np. DCPC), cygnolinę i fotochemioterapię, chociaż mechanizmy działania tych leków nie są do końca poznane.