Th1 Chemokine CXCL10 and Alopecia Areata: The Possible Target for the Treatment of Alopecia Areata

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Abstract

Alopecia areata (AA) is an organ-specific and cell-mediated autoimmune disease. Recent studies have suggested the most important effector cell of AA is NKG2D+CD8+ T cells, and outer root sheath (ORS) cells highly express NKG2D ligands, such as MICA, in AA lesions. T lymphocytes densely surround lesional hair bulbs, which is histologically referred to as “swarm of bees”. Immunohistochemical and real-time RT-PCR studies reveal that hair follicles of acute-phase AA expressed an up-regulation of Th1-associated chemokine CXCL10. In the skin lesions of acute-phase AA, CXCR3+CD4+ and CXCR3+CD8+ T cells infiltrated in the juxta-follicular area. In chronic-phase AA, CXCR3+CD8+ T cells dominated the infiltrate around hair bulbs, possibly contributing to the prolonged state of hair loss. Lymphocytes obtained from a lesional skin of acute-phase AA contained CXCR3+CD4+ and CXCR3+CD8+ T cells at higher percentages than those of PBMCs, suggesting preferential emigration from the blood. Furthermore, freshly isolated PBMCs from acute-phase AA patients had a strong velocity of chemotaxis toward CXCL10 with increased expression of F-actin. Antithymic drugs have been used in Japan, and these have possibility to down-regulate chemotactic activity in AA. Olopatadine shows suppressive effects of chemotactic activity in the AA patients’ CD4+ and CD8+ T cells towards CXCL10 by reducing CXCR3 expression, F-actin polymerization, and Ca++ influx. In conclusion, the increased production of CXCL10 from hair follicles induces preferential infiltrates of Th1 and Tc1 cells in the acute phase of AA, and Tc1 infiltration remains prolonged in the chronic phase. Therefore, inhibitory treatment of chemotactic activity might be novel target for the treatment of AA.

Keywords: Alopecia areata; Chemokine; Chemotaxis; Swarm of bees; T cell

Introduction

Alopecia areata (AA) has recently been shown to be a tissue-specific autoimmune disease [1,2]. A T-cell-mediated immune reaction may induce hair loss based on autoimmune etiopathogenesis [3]. The hair follicle (HF) autoantigen is not quite completely identified, but melanocyte-related protein has been suggested as a strong candidate [4-6].

In the physiological condition, autoantigens generated by anagen HF s should be protected from recognition by autoreactive T cells. Therefore, anagen hair bulbs may maintain an immunoprivileged milieu at the proximal outer root sheath (ORS) and hair matrix by creating immunotolerated milieu, such as low or absent expression of major histocompatibility complex (MHC) class I, the substantial expression of immunosuppressants, and the rare distribution of immune cells [7-15]. However, as shown in Figure 1, if hair follicle immune privilege (HF-IP) is collapsed by stressors, autoantigens are revealed, resulting in an autoimmune reaction by NKG2D+CD8+ autoreactive T cells that causes the unique pathological feature called “swarm of bees” in the acute phase of AA. Th1 cytokines, as represented by interferon (IFN)-γ, are dominantly detected in AA lesions and may induce the collapse of HF-IP, including the up-regulation of MHC class I [16]. In addition to IFN-γ IL-15 is also another key cytokine. IL-15 is over expressed in keratinocytes of AA lesions, and IL-15 activates and proliferates cytotoxic T cells [17]. JAK inhibitors, such as ruxolitinib, tofacitinib and baricitinib, may down-regulate IFN-γ and IL-15 expression that bring improvement of hair loss lesions in AA [17]. Recent idea of the pathogenesis of AA, especially between hair follicle keratinocyte and CD8+ T cell is suggested in Figure 2.

There have been several studies on the expression of chemokines and their receptors in AA lesions [18,19]. For example, IFN-γ-inducible expression of Th1 chemokines, CXCL9/MIG and CXCL10/IP-10, were detected in the lesions of AA patients [18,20]. CXCL9 was elevated in human AA, and its level correlated with disease activity [21]. Transcriptional profiling was also demonstrated and CXCL10 was highly up-regulated in AA lesion compared to non-lesional skin [22]. CXCR3+ Th1 and Tc1 cells may favorably infiltrate the sites that show high expression of Th1 chemokines, CXCL9/MIG and CXCL10/IP-10. Actually, we have already reported the dense infiltration of CXCR3+CD4+ Th1 cells and CXCR3+CD8+ Tc1 cells. Therefore, inhibition of chemotactic activity might be candidate for the novel treatment of AA.

Expression of Th1/Tc1 chemokine CXCL10/IP-10 in AA lesion

There have been several studies on the expression of chemokines and their receptors in AA lesions. In a mouse model, murine AA-skin grafted mice showed a marked increase of Th1/Tc1 chemokine, CXCL9 and CXCL10, as early as five weeks post-transplant before the development of AA [23]. It can be speculated that upregulation of Th1/Tc1 chemokine induces accumulation of Th1 and Tc1 cells around hair bulbs, so called “swarm of bees” [23]. In a study by Gilhar et al.,
biopsied human AA samples were grafted onto SCID mice that showed hair regrowth because the grafted skin escaped from autoimmune reactions. Subsequently, the patients’ lesional T cells, which had been cultured with follicular homogenate, melanocyte peptides, or human melanoma cell homogenate, were injected into the grafted area [18] that resulted in recurrence of AA. The frequency of SCID mouse with CXCL10+ AA lesions were significantly higher in T cell-injected (13/13) compared to non-injected (2/9) (P < 0.002, two-sided Fisher’s exact test). Other studies have also shown Th1 chemokines, CXCL9/MIG and CXCL10/IP-10, in the lesions of AA patients [19,20]. Our study also revealed that the CXCL10 immunoreactivity was found in the outer and inner root sheath epithelial cells, dermal papilla cells, and juxta-follicular interstitial cells, while normal HFs showed slight CXCL10 staining in the outer root sheath. By RT-PCR, mRNA expression of CXCL10 was observed in the lesional skin of acute-phase AA but not in a normal skin [24].

Upregulation of CXCL10 in AA and juxta-follicular interstitial cells, while normal HFs showed slight CXCL10 staining in the outer root sheath. By RT-PCR, mRNA expression of CXCL10 was observed in the lesional skin of acute-phase AA but not in a normal skin [24]. Upregulation of CXCL10 in AA lesions compared with non-lesional skin was also reported in other transcriptional profiling study [22]. Chemokines (CX3CL1, CXCL1, CCL5 and CXCL10) associated with cellular immunity were overexpressed in AA skin. CX3CL1 and CXCL10 are not only induced by IFN-γ, but also act as an amplifier of polarized Th1 responses [25].

Infiltration of Th1/Tc1 cells around hair bulb in AA lesion

We have reported that CXCR3+ T cells markedly infiltrated in and around the hair bulbs compared with CCR4+ T cells in acute phase of AA. In the chronic phase of AA, CD4+ T cells around the HFs were decreased in number while the infiltration of CD8+ T cells remained constant. In this phase, the accumulation of CXCR3+ T cells was denser than CCR4+ T cells (Figure 1). The number of CXCR3+CD4+ Th1 cells in PBMCs was significantly higher in acute phase AA patients than in chronic phase AA patients or healthy controls [24]. Another study showed dominant infiltration of CCR5+ Th1 cells relative to CCR4+Th2 cells in the C3H/HeJ mouse model [26].

Figure 2: Immune reactions between NKG2D+CD8+ T cells and hair follicle keratinocyte. CD8+NKG2D+ T cells infiltrate localize around the hair bulb, where they form immune reactions with follicular epithelial cells through MHC class I-peptide complexes and NKG2D ligand (NKG2DL). NKG2D binding to NKG2DL stimulates CD8+ T cell that induces cytotoxicity and cellular survival. Activated CD8+ T cells release IFN-γ, which binds the IFN-γR on the surface of the follicular epithelial cell, which in turn signals via JAK1 and JAK2 to promote production of IL-15 and its chaperone IL-15Ra. This binds the IL-15R complex (IL-2Rβ and γc) on the CD8+ T cell surface, causing signaling via JAK1 and JAK3 to enhance the production of IFN-γ and amplify the feedback loop.

Chemotactic activity and F-actin polymerization of Th1/Tc1 cells in AA

Interestingly, real-time horizontal chemotaxis assay showed that the chemotactic velocities of circulating CD4+ and CD8+ T cell towards CXCL10 in acute phase AA were higher than those in chronic phase AA and in healthy controls, suggesting that Th1 and Tc1 are activated in the peripheral blood of acute phase AA patients [24]. Time-lapse images of cell migration during chemotaxis were observed directly with an optically accessible horizontal chemotaxis apparatus EZ-TAXIScan (Effector Cell Institute, Kanagawa, Japan) via a CCD camera (GE Healthcare, Tokyo, Japan) as described previously (Figure 2).
Japanese guideline for AA recommends antihistaminic drug treatment to improve the condition of chronic-phase AA patients, compared to healthy control. Antihistamines, such as fexofenadine and ebastine, have been shown to inhibit chemotactic activity of T cells in AA through different mechanisms.

### Clinical availabilities for the treatment of AA

Present treatments for AA are actually same as a decade ago. U.S., British and Japanese guidelines of AA recommend several therapies including contact immunotherapy, local injection of corticosteroid, topical corticosteroid, oral corticosteroid, UV-irradiation including narrow-band UVB and excimer light, immunosuppressants, and minoxidil [28,33,34]. In these treatments, Japanese guideline strongly recommends contact immunotherapy and local injection of corticosteroid in the hair loss lesions as B level [28]. Although the mechanism of contact immunotherapy is still remained to be elucidated, Th1/Th2 cytokine balance might be affected as down-regulation of IFN-γ and up-regulation of IL-4 [35]. Therefore, it can be speculated that the chemotactic activity of CXCR3+CD4+ T cells and CXCR3+CD8+ T cells might be down-modulated by contact immunotherapy although there is no in vivo / in vitro evidence of suppressive effect in contact immunotherapy. High dose corticosteroid pulse therapy is also another possible treatment for acute phase AA [36,37]. This therapy might be not effective in chronic stage of AA, and should be applied on the patients suffering with AA within 6 months. Narrowband UVB or PUVA therapy is also alternative treatment for AA. However, there have been not enough data on UV-irradiation and chemotactic activity of T cells in AA although UVB irradiation of normal human skin favors the development of type-2 T-cells in vivo [38]. PUVA therapy may have suppressive effects on T cell migration in vitro [39].

### Frontal fibrosing alopecia and chemokines

Frontal fibrosing alopecia (FFA) is one of the cicatricial alopecia. This permanent hair loss disease is preferentially occurs at the front area of scalp, and it is sometimes needed to be distinguished with AA ophiasis which is characterized by the loss of hair in the shape of a wave at the circumference of the head [40]. Detailed pathogenesis of FFA is still unknown but recent study revealed that collapse of bulge area of scalp, and it is sometimes needed to be distinguished with AA [41]. As AA, accumulated T cells and hair follicle keratinocyte express CXCR3 and CXCL10, respectively that mean chemokine release, some of antihistamines, as olopatadine, are capable of directly downmodulating the function of T cells to migrate toward chemokines (Figure 3) [24].

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**Inhibition of chemotactic activity by antihistaminic drug**

Antihistaminic drugs have been widely used in the treatment of AA in Japan. Japanese guideline for AA recommends antihistaminic drug treatment for the treatment of AA as C1 level [28]. For example, Antihistamine fexofenadine enhanced the efficacy of contact immunotherapy for extensive AA in patients with an atopic background [29]. In a murine study, AA was improved in mice by ebastine presumably by inhibiting T cell accumulation around the hair bulbs [30]. In the same paper, authors show the successful hair regrowth achieved after 5-month by oral ebastine and topical corticosteroid treatment.

It is known that some antihistamines suppress keratinocyte chemokines secretion, such as CXCL10 [31], supporting their therapeutic efficacy for AA. For example, olopatadine suppressed chemotactic activity of the AA patients’ CD4+ and CD8+ T cells towards CXCL10 by reducing CXCR3 expression, F-actin polymerization, and Ca++ influx. Antihistamines are known to suppress the production of chemokines [31,32]. In addition to the suppression of chemokine release, some of antihistamines, as represented by olopatadine, are capable of directly downmodulating the function of T cells to migrate toward chemokines (Figure 3) [24].

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pathogenesis of FFA [43]. Under these backgrounds, physicians should distinguish to handle the scattering and non-scarring alopecia.

### Summary and Future Clinical Perspectives

The pathogenesis of AA remains unclear but current studies strongly suggest a T cell-mediated autoimmune process, and effector cell is NKG2D+CD8+ T cells. IFN-γ and IL-15 are the key cytokines, and JAK inhibitors, such as ruxolitinib, tofacitinib and baricitinib, shows improvement of hair loss lesions in AA [17,44]. Even though tofacitinib and ruxolitinib are a promising novel treatment option, transient effect of JAK inhibitor has been reported [45]. The hallmark of pathological change in AA is the accumulation of lymphocytes around hair bulbs. Overexpression of the Th1 chemokine, CXCL10, induces the infiltration of CXCR3+ T cells around the hair bulbs in AA lesions. Therefore, the inhibition of chemotactic or cytokine activity could be novel therapies for AA. Our study has already shown that the antihistamine drug, olopatadine, downregulated CXCR3 expression, F-actin polymerization and calcium influx in patients with AA [46]. Th1 chemokines and cytokines could be new target in solving the puzzle of the pathogenesis of AA.

### Conflict of Interest

The author declares that they have no conflict of interest.

### References


