The role of the Th1 chemokine CXCL10 in vitiligo

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Vitiligo is a disorder that causes the presence of pale patchy areas of depigmented skin on the face, hands and wrists; these patches are initially small, but often grow and change in shape (1). The disorder is classified in segmental vitiligo (SV) and non-SV (NSV): (I) in NSV there is usually a symmetry in the location of depigmentation; (II) SV differs in appearance, and it is not associated with autoimmune diseases (2). There are many therapies for vitiligo, the most used are local steroids and ultraviolet light. Vitiligo is an autoimmune skin disease arising from an aberrant immune responses against melanocytes. The existence of various associations between vitiligo and other autoimmune diseases has been proved. Generalized vitiligo is a component of the APECED (APS1) and Schmidt (APS2) multiple autoimmune disease syndromes, pernicious anemia and Addison's disease (3). Thyroid disease, autoimmune thyroid disease (4) and presence of thyroid specific autoantibodies have a mean prevalence of, respectively, 15%, 14% and 21% in patients with vitiligo (5).

Genetic and environmental factors are important in the pathogenesis of vitiligo. Genetic studies concern mainly patients with NSV. Genomewide linkage studies and genome-wide association studies (GWAS) are the "gold standard" for detecting susceptibility genes (6). Up to now, about 36 NSV susceptibility loci have been identified: 90% encode immunoregulatory proteins; 10% encode melanocyte proteins. In the major histocompatibility complex region, major associations were identified in the class I gene region (between HLA-A and HLA-HGC9) and class II gene region (between HLA-DRB1 and HLA-DQA1). Other associations were identified with *PTPN22*, *LPP*, *IL2RA*, *GZMB*, *UBASH3A* and *C1QTNF6* genes, which are implicated in autoimmune diseases (6). Among melanocyte proteins TYR encodes tyrosinase, which is an enzyme that catalyzes melanin biosynthesis, and it is a major autoantigen that is implicated in generalized vitiligo (7). Another gene associated with vitiligo is the *NALP1* gene, which regulates inflammation and cell death of myeloid and lymphoid cells. NALP1 produces inflammatory products such as caspase1 and caspase7, which activate the cytokine interleukin-1beta which is expressed at high levels in patients with vitiligo (8). *TSLP* gene encodes thymic stromal lymphopoietin that induces naive CD4$^+$ T cells to produce cytokines that induce a T helper (Th)2 response [IL-4, IL-5, IL-13, tumor necrosis factor (TNF)-α] and inhibit production of Th1cytokines [IL-10, interferon (IFN)-γ] (9). The deficiency of *TSLP* gene expression produces the dominance of the Th1 immune response, which is involved in vitiligo development.

IFN-γ is the most important cytokine, that is associated with the Th1 immune response (10). This cytokine acts through chemokines that are small glycoproteins active for a wide variety of cell types. The IFN-γ-inducible protein (IP)-10, also called chemokine (C-X-C motif) ligand (CXCL)10, was identified as a chemokine that is induced by IFN-γ in various cell types, such as neutrophils, lymphocytes, endothelial cells, fibroblasts, thyrocytes and other epithelial cells (11,12). CXCL10 binds to its specific receptor, chemokine (C-X-C motif) receptor (CXCR)3, and regulates immune responses by recruitment and activation of T cells, monocytes, and natural killer cells. CXCR3 is expressed not only by immune cells but also by endothelial cells, mesangial cells, thyrocytes and other epithelial cells. Recently, it has been shown that the tissue expressions of CXCR3 and CXCL10 are increased in various autoimmune diseases, and that they play fundamental parts in leukocyte homing into the inflamed tissues and contribute to the process of tissue damage (12). Determination of high level of CXCL10

in peripheral liquids is a marker of host immune response, especially of a Th1 orientated immune response. In inflamed tissues recruited Th1 lymphocytes are responsible for enhanced IFN-γ and TNF-α production, which in turn stimulate CXCL10 secretion from a variety of the above mentioned cells, therefore creating an amplification feedback loop (13).

It has been recently reported that the serum and/or the tissue CXCL10 expressions are increased in organ specific autoimmune diseases, such as type 1 diabetes, Graves' disease, or Graves' ophthalmopathy, or systemic rheumatological disorders like rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis and HCV-related cryoglobulinemia (13).

A modulatory role of peroxisome proliferator-activated receptor-γ or -α agonists on CXCR3 chemokines in autoimmune disorders has been shown, and further studies are ongoing to explore the use of new molecules that act as antagonists of CXCR3, or block CXCL10, in autoimmune diseases (13-16).

The role of CXCL10 in the immunopathogenesis of vitiligo has been highlited in three new studies (17-19).

It has been shown that intercellular adhesion molecule-1 (ICAM-1) expression has been detected in melanocytes around active vitiligo. Moreover, it has been shown that RNA released from necrotic keratinocytes induced the upregulation of ICAM-1, through activation of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB). This process was associated with a dramatic increase in proinflammatory cytokine and chemokine transcripts (CXCL10, CXCL11, etc.) (17).

More importantly, a recent study reports that both human vitiligo and a mouse model of vitiligo reflect an IFN-γ-specific Th1 immune response in the skin that involves the IFN-γ-dependent chemokines CXCL9, 10, and 11 (18).

To identify the key cytokine pathways in vitiligo, the gene expression profile in lesional skin patches of vitiligo patients was measured, which contained a T cell infiltrate. Compared to healthy controls, a significant loss of melanocyte-specific transcripts in lesional skin was observed, while chemokine expression revealed a predominantly Th-mediated signature (*IFNG*-specific). Th1 chemokines CXCL9, CXCL10, CXCL11, and CCL5 were highly induced in vitiligo, in comparison with adhesion molecules ICAM1 and VCAM1. In serum of vitiligo patients CXCL10 level was high, while CXCL9 and CXCL11 were not significantly different from healthy controls.

Melanocyte-specific CD8+ T cells expressed CXCR3 (the common receptor for CXCL9, CXCL10 and CXCL11, unlike healthy controls (18).

A mouse model of vitiligo was also used (19). Krt14-Kitl* mice have a number of epidermal melanocytes, similar to human skin; following transfer of premelanosome protein-specific CD8+ T cells (PMELs) and *in vivo* activation recombinant vaccinia virus expressing their cognate antigen (PMEL), Krt14-Kitl* mice develop epidermal depigmentation on their foot pads, ears, and tails. Profiling of lesional skin from this mice with vitiligo showed a gene chemokines expression similar to human vitiligo, with a Th1-specific response in the skin, and expression of the IFN-γ-dependent chemokines CXCL9, CXCL10, and CCL5. Autoreactive T cells express CXCR3, similarly to what observed in humans with disease (18). It was also evaluated that if CXCR3 is required for the development of depigmentation, by transferring either wild-type or *Cxcr3*⁻/⁻ PMEL T cells to induce vitiligo, and it was observed that *Cxcr3*⁻/⁻ T cells were unable to induce depigmentation (18). In this mouse model of vitiligo, *Cxcl10*⁻/⁻ hosts developed minimal depigmentation suggesting that vitiligo depends on this chemokine. CXCL10 plays a role in directed migration within the skin of T cells, and it was required for T cell function beyond simple recruitment (18). When mice were treated with either CXCL9 or CXCL10 neutralizing antibodies, neutralization of CXCL10 significantly reduced depigmentation in this model, while neutralization of CXCL9 did not. Surprisingly, CXCL10 neutralization in mice with established, widespread depigmentation induces reversal of disease, evidenced by repigmentation. These data identify a critical role for CXCL10 in both the progression and maintenance of vitiligo and thereby support inhibiting CXCL10 as a targeted treatment strategy (18).

On the base of the results obtained from this important study (18), it was evaluated the effect of inhibition of IFN-γ in vitiligo. STAT1 activation is required for IFN-γ signaling and recent studies revealed that simvastatin, an FDA-approved cholesterol-lowering medication, inhibited STAT1 activation *in vitro* (20). Therefore, it was hypothesized that simvastatin may be an effective treatment for vitiligo. In fact, simvastatin both prevented and reversed depigmentation in the mouse model of vitiligo, and reduced the number of infiltrating autoreactive CD8+ T cells in the skin. Treatment of melanocyte-specific, CD8+ T cells *in vitro* decreased proliferation and IFN-γ production, suggesting additional effects of simvastatin directly on T cells. Based on these data, it has been suggested that simvastatin may be a

safe, targeted treatment option for patients with vitiligo (20).

The above mentioned results (18,20) show the importance of the Th1 immune response in the development of vitiligo, and of CXCR3 receptor and its chemokine CXCL10, suggesting these could be novel targets of future therapeutical approaches. Further studies are needed to explore the use of new molecules that act as antagonists of CXCR3, or block CXCL10, in vitiligo in a clinical setting.

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