Study of Interleukin 17 Immune Response in Vitiligo Patients

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Abstract

**Background**: Vitiligo is an acquired depigmented disorder of the skin and mucosa caused by substantial loss of functioning melanocytes. It is characterized by well-circumscribed whitish macules and/or patches that can occur on any part of the body. Vitiligo affects around 1-2% of the population. Multiple pathogenic factors have been proposed to clarify the etiology of vitiligo, including the neural theory, genetic predisposition, and impaired anti-oxidative defense. Interleukin17 (IL-17) has six family members (IL-17A to IL-17F). Although IL-17A and IL-17F share the highest amino acid sequence homology, they perform distinct functions; IL-17A is involved in the development of autoimmunity, inflammation, tumors, and also plays important roles in the host defenses against bacterial and fungal infections, it was studied that IL17 has strong influence on inflammatory mediation and melanocyte destruction in vitiligo.

**Key words**: vitiligo, interleukin 17 (IL-17).

**Introduction**
Vitiligo is an acquired, common, multifactorial skin depigmenting disorder characterized clinically by the development of milky-white macules on the skin resulting from a selective loss of functional epidermal melanocytes in the affected area (1).

**Epidemiology**
It affects around 0.5% of the world population. Both sexes are equally affected, and there are no apparent differences in rates of occurrence according to phototype or race. Women may be more likely to present for treatment (2).

**Clinical presentation**
Depigmentation of the skin and hair follicles is the clinical hallmark of vitiligo. This results in white macules and patches increasing in number and size over time. In fair-skinned patients, vitiligo is first noted in sun-exposed sites because of tanning of unaffected skin. Several factors have been associated with the onset of disease, including pregnancy, cutaneous trauma and significant psychological stress. Patients may exhibit Koebner phenomenon, in which new lesions of vitiligo appear in areas of trauma (3).

**Role Adaptive immunity**

**CD8+ T cells: Role of progression**
Vitiligo patients have increased numbers of autoreactive melanocyte-specific, cytotoxic CD8+ T cells in their blood and skin. Cytotoxic CD8+ T cells are responsible for the
destruction of melanocytes. Cytokines secreted within the skin help these autoreactive T cells locate stressed melanocytes. Interferons play a central role in the spread of vitiligo lesions by bringing an increased expression of CXCL10, which regulates the invasion of epidermal and follicular tissues by CD8+ T-cells. Interferon-γ and IFN-γ-induced chemokines (CXCL9 and CXCL10) are highly expressed in the skin and blood of patients with vitiligo (4).

The expression of additional cytokines has been reported in patients with vitiligo, such as IL-17 and IL-33. Interleukin 17 and Th17 cells play an important role in autoimmunity. It was found that there is an increase in the levels of IL-17 in the blood as well as the tissue samples of patients with vitiligo. The levels of Th17 cells correlated well with disease activity in generalized vitiligo (5).

**Interleukin-17**

Interleukin-17 is a family of six cytokines that includes IL-17A through IL-17F. Interleukin-17A and IL-17F are the most homologous to one another, as well as their roles in immune modulation. The two cytokines are expressed by the Th17 subset of CD4+ cells. Interleukin-17A and Th17 cells have important roles in the development of allergic, autoimmune diseases and protective mechanisms against bacterial and fungal infections (5).

**Source of IL-17**

Many types of innate and adaptive immune cells produce IL-17. T helper 17 cells are defined as the major cellular source of IL-17. They are generated of Th1 and Th2 cell development, and the differentiation of naive CD4 + T cells to this subset is triggered and tightly regulated by a cytokine network (6).

It was found that IL-17 is characterized as a signature cytokine of the Th17 lineage but it can also be derived from other innate and adaptive immune cell populations. Gamma delta T cells (γδ T cells) express a T cell receptor consisting of a γ-chain and a δ-chain. Similar to CD4 + T cells, γδ T cells can also be subdivided into different populations based on their cytokine production. These IL-17-producing γδ T cells are localized to mucosal tissues including the skin, lung, and intestine. The γδ T cells express recognition receptors like Toll-like receptor 2 (TLR2) and C-type lectin domain family 7 member A (CLEC7A), which allow production of IL-17 in response to invading microbes (7).

The rapid reaction of γδ T cells to antigen provides immediate immune response to eradicate the pathogens by producing pro-inflammatory cytokines and recruitment of immune cells to the site of infection. These cells have important role in response to bacteria and fungi encounter (8).

Interleukin -17 can be produced by NK cells in an IL-23- and IL-6-induced manner. Natural killer T (NKT) cells are a heterogeneous group of T cells that share properties of both T cells and NK cells. These cells are able to secrete IL-17 in the presence of IL-1β, transforming growth factor β (TGF-β), and IL-23, independently of IL-6 (9). Lymphoid tissue inducer (LTi) cells are key components of the lymphoid structures. Lymphoid tissue inducer and LTi-like cells have been defined as cells of lymphotoxin-α,
lymphotoxin-β, the chemokine receptors (CCR7) and CXC receptor 5 (CXCR5) which inhibit fungal and bacterial infection by releasing IL-17 and IFNγ in intestinal mucosa (10).

A subset of CD8+ T cells participates in host defense against foreign microorganisms by releasing IL-17. In addition, B cells were found to be a major source of IL-17 during Trypanosoma cruzi infection and enhance the eradication of this parasite. Neutrophils are the early immune activity responding cells for acute inflammation and adaptive immunity by production of IL-17 (11).

Mast cells are tissue residents which secrete biologically active products including IL-17 that play a protective role in host defense against pathogens, wound healing, and angiogenesis. Macrophage is another important source of IL-17. Lacking of IL-17 signaling affects monocyte recruitment, adherence, homeostasis and prevents macrophage undergoing apoptosis (12). Paneth cells which are specialized epithelial cells located in small intestine secrete cytokines including IL-17 into the lumen of the intestinal gland for maintenance of gastrointestinal barrier (6).

**IL-17 Receptor family**

The IL-17 receptor family is composed of five receptor subunits including IL-17RA, IL-17RB, IL-17RC, IL-17TH, and IL-17RE. They are all containing single transmembrane domain, ranging in size from 499 to 866 amino acids. The IL-17RA is located on human chromosome 22 while human IL-17RB, IL-17RC, IL-17TH, and IL-17RE are clusters on chromosome 3 (13).

All cell types and tissues which express IL-17 receptor family molecules could become the targeting cells of IL-17 cytokine family membranes initiating signaling transduction. It has been reported that IL-17RA is expressed on various tissues and cell types with high levels in hematopoietic tissues (14).

The main responsive cells to IL-17A are epithelial cells, endothelial cells, fibroblasts, and innate immune cells such as macrophages. Interleukin-17RB is detected in a variety of cell types, and the highest expression level is detected in the kidney, liver, and brain. Interleukin-17RD is expressed on many different tissues including the gastrointestinal tract, breast, lung, and kidney. In contrast to IL-17RD, IL-17RE shows a limited expression profile, the tissue which has the highest expression of IL-17RE is the kidney. Interleukin-17RC has a lower expression in hemopoietic tissues and a higher expression in the liver, kidney, and thyroid (15).

**Signaling Pathways**

The IL-17/IL-17R axis is regulated by various kinases and transcriptional factors. The IL-17F shows most resemblance with IL-17A, exhibiting 50% homology at the protein level. However, the strength of IL-17F signaling is 10–30-fold weaker than IL-17A. After activation, negative feedback loops limit exaggerated signaling by recruiting TNF receptor associated factor 3 to the IL-17 receptor. The function of the Th17 pathway is to counter harmful pathogens that require inflammatory responses. These pathogens are not
sufficiently tackled by Th1- or Th2-mediated immunity and IL-17 contributes to the host defense against fungi and both Gram negative and Gram-positive bacteria (16).

Neutrophils produce IL-17A as an early host defense mechanism, which triggers IFN γ production and bacterial clearance. Interleukin-17A has a strong inflammatory capacity and induces cytokine and chemokine release. Interleukin-17E, also termed IL-25, influences type 2 immunity and inhibits Th17-mediated inflammation (17). It was found that IL-23 is a key factor to maintain the Th17 population. Transforming growth factor β is a transcription factor of IL-17+ cells and Tregs whereas Tregs can convert into Th17 cells in the presence of IL-6. This explains the balance between Tregs and Th17 cells during lymphocyte development (18).

Since the IL-17/IL-17R signaling pathway has strong effect on induction of pro-inflammatory cytokines, its activity needs to be controlled to prevent inflammatory disorders (19).

Structure and Function

The IL-17 family members have unique amino acid sequence that shares no similarity with other known cytokines. The highest homology to IL-17A is IL-17F (60 %), and IL-17E has the least (17 %). The gene-coded IL-17A and IL-17F are located at chromosome 6, and gene-coded IL-17B, IL- 17C, IL-17D, and IL-17E are located at chromosomes 5, 16, 13, and 14. Interleukin-17A contains 155 amino acids, they form homodimer which is linked by disulfide bond. Each subunit of the homodimer is approximately 15–20 kDa, with a molecular mass of 35 kDa in total (20).

The IL-17 family of cytokines play essential roles in host defense against microbial pathogens by rapidly induction of pro-inflammatory cascade of chemokines and cytokines that further activate and recruit neutrophils and monocytes required for timely control of the pathogen by the immune system. The induction of IL-6 facilitates Th17 differentiation and suggests a positive feedback circuit induced by IL-17. Furthermore, induction of other cytokines, such as TNF-α and IL-1β, helps to amplify the signal and induce more inflammatory factors to the infection site (21).

Although crucial in host defense against various invading pathogens, dysregulated IL-17 production can lead to acute or chronic inflammation, which further results in tissue damage and autoimmunity. The IL-17 family of cytokines have been linked to many autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus and psoriasis. It also has a role in the pathogenesis of other inflammation-related diseases such as allergy, transplantation, obesity, and malignancy (22).

Besides the activity to induce the cytokines and chemokines, IL-17 has also the ability to induce the production of other genes to fulfill other IL-17-mediated physiological functions. For example, IL-17 not only protects mucosal barrier during pathogen infections but also homeostasis by enhancing its integrity. Interleukin-17 treatment increases the synthesis of tight junction proteins like claudin-1 and claudin-2 to forming tight structures, thereby connecting epithelial cells to form a network. It also plays a pathological role in
tissue damage, bone destruction, and tumorigenesis through induction of tissue-remodeling factors (matrix metalloproteinases 1,3,9 and 13) (23).

Role of IL-17 in skin disease
In the epidermis, IL-17 can be secreted as a homodimer and binds to its receptor IL-17R on keratinocytes, which leads to increased production of the cytokines, IL-1β, IL-6, IL-8, and TNF-α. Stimulation of keratinocytes with IL-17 upregulates gene expression of human β-defensin 2 and CCL20. Melanocytes are located in the basal layer of the epidermis and are surrounded by about 36 keratinocytes. Melanocytes and keratinocytes establish a division of activities, with one cell producing pigment and the other using it (24).

Interleukin -17 receptors are expressed on keratinocytes, and IL-17 stimulated keratinocytes produce a wide range of inflammatory factors. Murine models showed a link between pathogenic antibodies produced by B cells and IL-17. Interleukin-17 induces anti-apoptotic factors. In cutaneous disorders, increased survival of pathological cells may contribute to a continuous low disease activity level (25).

After skin trauma, the NOD-like receptor 3 inflammasome is activated, which releases massive amounts of IL-1β and IL-18. The Th17 cells carry IL-1β receptors on their cell membrane. After ligand binding, production of IL-17 is triggered which is involved in neutrophil mobilization and is an important response factor after injury. This mechanism could explain the Koebner phenomenon in dermatological disorders (18).

It has been approved that IL-17 is overexpressed in infectious, inflammatory, granulomatous, bullous skin diseases and even in cutaneous malignancies. The efficacy of IL-17 inhibitors has been demonstrated in skin disorders such as psoriasis and vitiligo (25).

Elevated levels of IL-17A and IL-17F, along with other pro inflammatory Th17 type cytokines have been found in the sera and skin of psoriatic patients. The predominant source of IL-17 in psoriasis appears to be neutrophils and mast cells. Regarding neutrophils, this is reflected by the histological findings of Kogoj and Munro abscesses. Anti-IL17 therapy in psoriasis results in fast and impressive neutrophil clearance from psoriatic plaques before an obvious decrease in lymphocytes or dendritic cells is detected. In addition, dose-dependent reductions in keratinocyte proliferation, epidermal hyperplasia, and immune cell infiltration were documented with simultaneous improvements in cellular and molecular disease biomarkers (26).

In atopic dermatitis, an increase of IL-17A, IL-17E and IL-17F has been reported. Increased serum IL-17 and IL-23 concentrations were found in children with atopic dermatitis, which correlated with disease severity (27). Other study reported opposite results, with decreased Th17 cells in children with atopic dermatitis compared with controls. Elevated IL-17A and IL-17E levels are observed in the papillary dermis of atopic dermatitis lesions, especially increasing in acute atopic dermatitis. In vitro human experiments demonstrated that the capacity of T cells to produce IL-17 following allergen stimulation is impaired in patients with atopic dermatitis. This has been proposed as a mechanism for the ineffective clearance of S. aureus by patients (28).
In vitiligo, it was reported that the level of IL-17 is increased in the blood and tissue samples of patients. Vitiliginous lesions contain higher numbers of IL-17 secreting Th17 and CD8+ cells as compared to unaffected skin in both healthy controls and patients and increased expression of IL-17 mRNA within vitiliginous lesions. The levels of Th17 cells correlated well with disease activity in generalized vitiligo (5). Helper T17 cells exacerbate autoimmune inflammation in vitiligo mediated by their effector molecule IL-17 which is a potent producer of CCL20; a homing molecule that can attract cytotoxic CD8+ T cells from systemic circulation into peripheral tissues. The CD8+ T cells kill self-cells upon antigen recognition and form a critical component of adaptive immunity. These cells cause destruction of melanocytes in vitiligo models (29).

Additionally, Th17 cells secrete IL-17, IL-6, IL-22, and TNF-α which stimulate the release of IL-1α, IL-6 and TNF-α in keratinocytes. Tumor necrosis factor-α contribute to keratinocyte apoptosis, which result in autoimmune response and melanocyte disappearance. Interleukin-6 causes polyclonal B cell activation and increase in antibody production which results in immunological damage of melanocytes. Interleukin-17 synergizes with these local inflammatory mediators, which may cause further inhibition of melanocyte proliferation (30).

It was found that IL-17 stimulates endothelial expression of adhesion molecules such as E- and P-selectins, the intracellular adhesion molecule (ICAM-1) and vascular cell adhesion protein (VCAM-1), to enhance neutrophil migration. It also stimulates keratinocytes to release several chemokines that result in further T cell, neutrophil, macrophage, and dendritic cell influx. The presence of infiltrating macrophages and T cells has been shown to coincide with loss of melanocytes. Neutrophil influx promotes production of several ROS. Oxidative stress induced by these intermediates has been associated with vitiligo. Accumulation of ROS may be directly toxic to critical cell components, resulting in melanocyte destruction and subsequent skin depigmentation (31).

Tyrosinase activity could be downregulated by several cytokines such as IFN-γ, IL-1β, IL-17A and TGF-β via the intracellular signalling pathways activation. These cytokines are inflammatory cytokines induced by Th1 and Th17 cell differentiation and maintenance. They are important for the development and maintenance of Th17 cells from naive CD4+ T cells (32). Interleukin-17 also induces the production of angiogenic factors such as vascular endothelial growth factor which is critical in generating new blood vessels and increasing permeability of systemic circulation for the passage of immune cells into peripheral tissues. These functions are thought to explain the critical role of vascular endothelial growth factor in inflammation and may also be operative in enhancing migration of melanocyte-reactive T cells to cutaneous melanocytes in vitiligo. Importantly, increased dermal angiogenesis has been documented in vitiligo, primarily in the center of lesions (33).

Narrow band -UVB, which has demonstrated clinical efficacy in treating vitiligo, helps to relieve oxidative stress and restore oxidant-antioxidant balance through downregulation of Th17 cell abundance as well as serum and tissue IL-17 levels. It leads to significant reductions in IL-17 expression and simultaneous clinical improvement of vitiliginous lesions (34).
References


16. **Song X and Qian Y (2013):** The activation and regulation of IL-17 receptor mediated signaling. Cytokine; 62:175–82.


