Role of T Helper 17 Cells in Allergic Contact Dermatitis

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ABSTRACT

Sensitization and elicitation facilitate the development of allergic contact dermatitis (ACD), one of the common types of dermatitis. Exposure to allergens induces sensitization, after which any re-exposure to the same allergens leads to elicitation and the appearance of ACD. Single re-exposure results in the development of acute ACD, while repeated exposure leads chronic ACD (CACD). Marked epidermal hyperplasia and intense dermal cell infiltration are observed in the early stage of CACD. Subsequently, the activity of Th17 increases again in the late stage of CACD, in which Th2 dominant allergic reaction decreases. This relationship between Th17 and Th2 might induce amelioration of allergic reaction in Th2 dominant CACD. Histamine aggravates CACD by suppressing Th17 response and inducing Th2 response.

Key Words: Allergic Contact Dermatitis; Chronic Allergic Contact Dermatitis; Th17; Th2; Histamine

INTRODUCTION

Dermatitis is the most common disease encountered during dermatology practice. It is characterized by itching and soreness of variable intensity, and in variable degrees, as well as signs such as dryness, erythema, excoriation, exudation, fissuring, hyperkeratosis, lichenification, scaling, and vesiculation. Dermatitis can be acute, subacute, or chronic depending on its clinical and pathological features[1]. Contact dermatitis is known as one of the common types of dermatitis with an itch sensation[2]. It is characterized into three subtypes, namely, irritant contact dermatitis, allergic contact dermatitis (ACD), and photocontact dermatitis. Irritant contact dermatitis is triggered by chemical or physical irritant factors. Chemical irritants that induce contact dermatitis include solvents, surfactant, acid, and alkalis. The most common physical irritant is low humidity due to air-conditioning[3]. On the other hand, ACD is clinically problematic, since it is an occupational and environmental-related disease. In metal allergy, one of the most representative forms of ACD, allergens include nickel, chromium, and cobalt. Photocontact dermatitis or photoallergic contact dermatitis resembles ACD on sun-exposed areas, although sometimes it may extend to covered areas as well[4].

Atopic dermatitis (AD) is a common, chronic or chronically relapsing and severely pruritic eczematous skin disease, which (together with ACD) constitutes what is referred to as allergic dermatitis. ACD basically occurs as a type IV allergic reaction and is a T helper (Th)1 dominant disease. Meanwhile in most AD cases,
both type IV and type I allergic reactions (e.g., atopic conditions such as urticaria, allergic rhinitis, and asthma) are involved. AD is predominantly orchestrated by Th2 cells. A widely used mouse model of ACD is the delayed-type hypersensitivity response (type IV allergic reaction) to small organic haptens with potent sensitizing capacity and Th1 dominant[11]. Repeated exposure to the haptens results in antigen-specific hypersensitivity responses and the development of chronic allergic contact dermatitis (CACD). The skin reaction in mice changes from type IV reaction to type I reaction in correlation to the increased number of repeated applications of the allergen. CACD is a Th2 dominant disease with clinical, histological, and immunological similarities to AD[16-19].

**ALLERGIC CONTACT DERMATITIS (ACD)**

ACD progresses in two stages: an initial sensitization phase, followed by an elicitation phase. The following types of cells are related to ACD development.

1. **Dendritic cells**

   ACD is an inflammatory skin disease that appears as a rash on the skin after exposure to xenobiotics or haptens. A hapten, in combination with a protein, generates an allergen causing sensitization, which is followed by elicitation after re-exposure with the same allergen. The first step involves the maturation of dendritic cells (DCs) including Langerhans cells and dermal DCs and their migration to regional lymph nodes, induced by skin exposure to allergens. After disruption of the epidermal barrier, haptens gain access to deeper compartments of the skin. If an allergen is detected in the context of danger, the antigen-loaded DCs leave the skin and migrate to the lymph node where cytotoxic T (Tc) cells and Th cells are stimulated to penetrate and acquire a specific phenotype. DCs could prime naïve T cells in the lymph node[10]. Allergen-specific T cells form an immunological synapse with DCs and recognize their epitopes presented on major histocompatibility complex (MHC) molecules by the cognate T cell receptor (TCR)[10]. Signals are transmitted between DCs and T cells by interaction of MHC:TCR and costimulatory molecules such as CD86/CD80:CD28. In ACD, a crucial inhibitory function is reported for B7 molecules and their ligands CTLA-4[11-13], ICOSL:ICOS[14,15], and PD-L1/L2:PD-1[16-18]. In response to a complete interaction of activating molecules, allergen-stimulated DCs release cytokines such as IL-6, IL-12 and IL-23 that potentially drive T-cell polarization and influence differentiation of Th1, Th2, Th17, and Tc cells, depending on their type. After expression of homing receptors, T cells could migrate into the skin where from the activating DCs originate[19].

2. **Keratinocytes**

   Keratinocytes are essential for the development of ACD, due to their abundance in the epidermis and to their role in the formation of the skin's anatomical barrier function[20]. Keratinocytes secrete IL-1β, IL-6, IL-10, IL-18 and tumor necrosis factor (TNF), of which IL-1β, IL-18 and TNF are necessary for the maturation and migration of the DCs induced by haptens, from the skin to the lymph node[20,22].

3. **Neutrophils**

   Neutrophils play an antimicrobial and pro-inflammatory role through the production of reactive oxygen species, being found in the inflammatory lesions of the skin of patients with ACD[23]. Neutrophil infiltration is detected in the skin just a few hours after sensitization and reaches a peak in approximately 24 hours[24]. The deficiency of neutrophils in sensitization leads to reduced induction and migration of DCs to the lymph nodes and the deficiency in the induction of specific T cells toward the antigen[24]. Neutrophils are involved in the stages of sensitization and elicitation of ACD[24,25].

4. **Mast cells**

   Mast cells immediately release pre-formed granules containing histamine, proteases, proteoglycans, and TNF[26]. They also secrete late pro-inflammatory mediators, such as IL-3, IL-4, IL-5, IL-6, IL-12, and IL-13. Repeated challenge with allergens results in the development of CACD. (C) Marked epidermal hyperplasia with intercellular edema and intense dermal cell infiltration are observed in the skin of early stage of CACD mice. (D) Epidermal hyperplasia and inflammatory cell infiltration are ameliorated in the skin of late stage of CACD mice induced by more repeated challenge.
Th17 cells are capable of stimulating pro-inflammatory cytokines, such as IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-26, TNF-α, and IL-13, promoting ACD of inflammatory cells in the skin during the effector stage, thereby recruiting neutrophils during the sensitization stage of ACD\(^\text{[26,27]}\). The absence of mast cells hinders the migration of CD8+ T cells\(^\text{[26,27]}\) and reduces the appearance of ACD\(^\text{[23-29]}\).

5. Natural Killer (NK) Cells

NK cells are triggered by secreted cytokines due to Th1 and Th17 cell infiltration in ACD\(^\text{[29]}\). They have a strong cytotoxic capacity and lead to a rapid cleavage of the adherence molecules, which induces the loss of cohesion of keratinocytes and spongiosis\(^\text{[28,30]}\). The NK cells remain as hepatic memory cells specific to the antigen\(^\text{[31,32]}\). A new provocation of the skin with the same hapten induces the recruitment of NK memory cells to quickly respond to the hapten for at least 3 to 4 months\(^\text{[27]}\).

6. CD4+ T and CD8+ T cells

CD4+ T cells have pro-inflammatory effector and anti-inflammatory functions, while CD8+ T cells only have pro-inflammatory effector functions\(^\text{[26]}\). The effector cells are predominantly CD8+ T cells. CD4+ Th1 and CD8+ Tc1 cells are effectors in ACD\(^\text{[31]}\). The deletion of CD8+ Tc1 cells has a greater suppressor effect than that of CD4+ Th1 cells\(^\text{[31]}\). Ni\(^2+\)-specific CD8+ Tc1 cells are also decisive for the development of ACD to Ni\(^2+\)\(^\text{[31]}\). However, the inflammatory reaction is mainly controlled by CD4+ T cells\(^\text{[19]}\).

7. Th1, Th2 and Th17 cells

CD4+ and CD8+ T cells are divided into three relevant subtypes for cutaneous immune response: Th1/Th1, Th2/Th2c, and Th17/Tc17 cells. Th1/Th1 cells secrete IFN-γ and IL-2, of which IFN-γ acts in the keratinocytes to produce Th1 cytokines, leading to the infiltration of inflammatory cells in the skin during the effector stage, thereby promoting ACD\(^\text{[26,31-34]}\), Th2/Th2c cells secrete cytokines IL-4, IL-5, and IL-13\(^\text{[26]}\) that negatively regulate ACD\(^\text{[26,33,34]}\). Th17/Tc17 cells secrete IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-26, TNF-α, and granulocyte monocyte-colony stimulating factor (GM-CSF)\(^\text{[35,36]}\). Th17 cells are capable of stimulating pro-inflammatory cytokines, chemokines, and adhesion molecules produced by keratinocytes\(^\text{[37]}\). IL-17 plays a stimulatory role in the sensitization and effector stages, and IL-17 secretion cells are detected in the infiltrations of acute lesions and in all types of eczema\(^\text{[39]}\).

8. Regulatory T cells

Regulatory T (Treg) cells suppress the immune response through the release of anti-inflammatory cytokines or by deactivating the effector T cells through cell-cell contact\(^\text{[41]}\). The number of Treg cells in the skin significantly increases during the cutaneous inflammatory process, suggesting that they contain a suppressive action in the inflamed location\(^\text{[29]}\). Treg cells play an important role in terminating the ACD reaction and in the control of systemic immune response\(^\text{[41]}\).

TH17 CELLS IN ACD

Th17 cells are a distinct lineage of effector CD4 (+) T cells, characterized by their production of IL-17 and IL-22\(^\text{[42]}\). Their function is to defend against extracellular bacteria and fungi\(^\text{[43,44]}\), but they are also involved in tissue destruction in autoimmunity\(^\text{[45,46]}\). Indications for involvement of Th17 in human skin allergy were reported for the first time in nickel allergic patients\(^\text{[47]}\). Skin affected with ACD to nickel and skin-derived, nickel-specific CD4+ T cell lines express IFN-γ, TNF-α, and IL-17 mRNAs. Pleotropic activities of IL-17 include induction of TNF-α, IL-1β, IL-6, IL-8, granulocyte colony-stimulating factor, and monocyte chemotactic protein-1\(^\text{[47-49]}\). Normal human keratinocytes constitutively express the IL-17 receptor gene, which augments IFN-γ-induced ICAM-1 expression on keratinocytes. IL-17 with IFN-γ and/or TNF-α also stimulates synthesis and release of IL-8 by keratinocytes\(^\text{[47]}\). Evidence for the role of CD4+ T cells in contact hypersensitivity is provided from studies with IL-17 deficient mice that demonstrate strongly reduced ear swelling response to contact allergens\(^\text{[49]}\). Discrimination of the total CD3+ T-cell population shows that decreased cell division in IL-17-/- mice is associated with the CD4+ T and not the CD8+ T cells. In cell transfer experiments where CD4+ T cells originating from wild-type mice are transplanted in IL-17-/- mice, ear swelling response in reconstituted mice is recovered comparable to wild-type mice after application of 2,4,6-trinitro-1-chlorobenzene (TNCB)\(^\text{[50]}\). IL-17 plays an important role in activating T cells in allergen-specific T-cell-mediated immune responses\(^\text{[50]}\). IL-17 is required for the sensitization phase of TNCB-induced contact hypersensitivity responses, and Th1- and Tc1-
mediated immune responses[69]. The role of IL-17 is recognized in the elicitation phase of human ACD[31]. It efficiently amplifies the allergic reaction by rendering T cells accessible to recruitment at the site of skin inflammation in ACD environment[69]. Th17 cells produce IL-17 and IL-22, which mediate epithelial innate immunity[62] and inhibit IL-4 production by Th2 cells[63]. Taken together, the role of Th17 is relatively well-documented in acute ACD, partially through the actions of IL-17 and IL-22.

In a study with patients with psoriasis and ACD, cytokine levels were detected in supernatants of CD3/CD28-activated or NISOD-reactive T cells that were isolated from eczematous and psoriatic lesions[64]. High levels of IL-17 and IL-22 were detected in both types of skin lesions after CD3/CD28 stimulation and were obviously different from small amounts detected in T cells from patients with AD. Significantly higher levels of IL-17 and IL-22 in NISOD-reactive T cells were observed in allergic eczema tissue than in psoriasis lesions, although IL-17 levels was relatively low[64]. The participation of Th17 in the total amount of IL-17 secreting T-cells was determined by analyses of subpopulations in human skin[64]. Co-staining for intracellular IL-17 and CD4 and CD8 revealed more than 90% Th cells in the IL-17+(+). A study analyzing primary human T cells from patients with psoriasis, AD, and ACD suggests that ACD can be distinguished from the other skin disorders by specific cytokine profile, including IL-17[65]. IL-17 expression was quantified in the skins from patients with psoriasis (17%), AD (13%), and AD (9%)[65].

Th17 in ACD as effector cells plays a crucial role on target cells such as recruitment of neutrophils[60], activation of fibrocytes[67], and macrophages[68]. Th17 cells themselves must be controlled by feedback mechanisms, restricting excessive tissue damage. High expression is observed in the skin with AD patients after nickel challenge. The expression and binding of PD-L1 to PD-1 mediates the control of Langerhans cells on IL-17 release from Th cells. PD-L1-PD-1 signaling feedback loop represents a mechanism to avoid excessive cytokine release during ACD related progress of eczema[61].

The skin of mice with acute ACD induced by single challenge with TNCD has visible inflammatory cells and mild intercellular edema, compared to that of normal mice (Figure 1A and B)[69]. Repeating the challenge with allergens results in the development of CACD[69]. Marked epidermal hyperplasia with intercellular edema and intense dermal cell infiltration are observed in the skin of early stage of CACD mice (Figure 1C). However, both are ameliorated in the skin of late stage of CACD mice induced by more repeated challenge (Figure 1D). Acute ACD induced in single challenge is Th1-dominant[60, 61]. CACD alters the balance of locally released cytokines with a shift toward Th2-dominant responses, thereby reducing the more deleterious Th1 response to the skin (Figure 2)[60, 61]. Our study demonstrated that the number of Th17 cells and Th17 cytokine levels decreased in the early stage of CACD (Figure 2)[60]. This is explained by the balance mechanism, since the cytokines that are required for Th1 and Th2 differentiation (IFN-γ, IL-12 and IL-4) concurrently inhibit Th17 differentiation[62, 63]. The confirmation that the absence of both IL-17 and IFN-γ leads to further augmentation of Th2 differentiation in graft-versus-host disease[63] is important as it explains the decrease in the IL-17 and IFN-γ levels within chronic lesions following the induction of the Th2-dominant response in the early stage of CACD. In late-stage CACD, Th17 cytokines were observed to maintain an increase while Th2 cytokines were decreased (Figure 2)[60]. In our studies, IL-23 level was the highest in the eczematous lesion associated with early-stage in CACD (Figure 3)[60]. IL-23 activates the expression of Th17 cells[64] and amplifies the activity of Th17 through IL-23 receptor[64] and acts on memory T cells resulting in elevated IL-17 and IL-17F secretion[65]. From these reports, we could assume that the increase of IL-23 in skin tissue induced Th17 differentiation and an increase in the IL-17 level. IL-23 (++) cells at their most abundant date locate to a similar depth to the phase of the IL-17F (+) and IL-22 (+) cell increment. It is true that these cells released IL-23 and acted for the terminal differentiation of Th17 cells at that site. TGF-β level was the highest in the skin lesion on the early-stage of CACD, similarly to IL-23 level (Figure 3)[60]. The TGF-β cytokine is critical for commitment to Th17 development independently from IL-23 cytokine[66, 67]. In our study, it was also proven that Th17 development on the late-stage of CACD was under the influence of high-level TGF-β. IL-6 level was relatively higher in the dorsal skin on early-stage than late-stage of CACD, even though the acute ACD stage recorded the highest levels (Figure 3)[60]. IL-6 and TGF-β together induce the differentiation of pathogenic Th17 cells from naïve T cells[68]. These observations suggest that elevated levels of IL-23, TGF-β and IL-6 cause increased Th17 response in early-stage of CACD while inducing decreases in Th2 response in late-stage of CACD.

Increased Th17 cytokines lead to decreased Th2 response in late-stage of CACD. This assumption was further supported using IL-17 injection, since IL-4 and IgE levels were suppressed by IL-17 injection; this effect is transmitted through IL-17[69]. Collectively, our study shows that Th17 function is one of the important factors regulating chronic allergic diseases, such as CACD and AD, as Th2-dominant diseases[61].

HISTAMINE AND TH 17 RESPONSES IN CACD

In our previous study using histamine-deficient mice generated by disrupting the histidine decarboxylase (HDC) gene[69], repeated application of TNCB resulted in scaly erythema. Biopsies showed that increased epidermal hyperplasia and epidermal thickness of HDC (+/+) mice was significantly larger than that of HDC (-/-) mice[60]. Remarkable dermal fibrosis with dense cell infiltration was observed in HDC (+/+) mice compared to HDC (-/-) mice[60]. Mast cells were more prevalent in the dermis of eczematous lesions in HDC (+/+) mice than in HDC (-/-) mice, while IL-17 (+) cells were less observed in HDC (+/+) mice compared to HDC (-/-) mice[60]. The number of mast cells in HDC (+/+) mice was significantly larger than that in HDC (-/-) mice[60]. On the other hand, the number of IL-17 (+) cells in HDC (+/+) mice was significantly smaller than that in HDC (-/-) mice[60]. IL-4 levels were significantly higher in HDC (+/+) mice compared to HDC (-/-) mice[60]. The levels of IL-17 and IL-22 (cytokines produced by Th17 cells) in the skin were significantly lower in HDC (+/+) mice compared to HDC (-/-) mice[60]. On the other hand, levels of IL-23, a cytokine establishing Th17 cells, did not differ between HDC (+/+) and HDC (-/-) mice[60].

Recombinant mouse TGF-β1 or mouse anti-TGF-β1 antibody was injected into the dorsal dermis just before each daily challenge of TNCB to assess the effects of histamine on early-stage of human ACD. Injection of recombinant mouse TGF-β1 increased IL-17 and IL-22 levels in the eczematous lesions compared to solvent-injected mice. However, the levels were decreased when the mice were injected with anti-TGF-β1 antibody[60].

Olopatadine (histamine H1 receptor antagonist) or JNJ7777120 (histamine H4 receptor antagonist) were administered 30 min before each daily challenge of TNCB to assess the effects of histamine and...
receptors on Th17 in CACD. Both receptor antagonists caused significantly increase in TGF-β1, IL-17, and IL-22 levels in eczematous lesions[60].

In HDC (-/-) mice, no plasma extravasation reaction was observed after a passive anaphylaxis test[60]. In contrast to immediate-type responses, contact hypersensitivity (delayed-type responses) showed no difference between HDC (+/+) and HDC (-/-) mice[60,70]. However, histamine was found to aggravate CACD eczematous lesions using this CACD model in HDC (-/-) mice[71]. The model also shows an increase in skin levels of Th2 cytokines (IL-4 and IL-5), serum levels of IgE, and the numbers of mast cells and eosinophils in the presence of histamine[60,70]. These levels are controlled through histamine H1 and H4 receptors[60,72]. Furthermore, histamine suppresses Tregs mediated by TGF-β1 and develops Th2 responses in CACD lesions[73]. These studies confirm that histamine is an important factor in the development of CACD, Th2 dominant disease.

The number of Th17 cells increases in the peripheral blood of AD patients[60,70]. However, Th17 cells are less prevalent in the chronic than acute lesions of AD patients[70]. IL-17 and IL-22 levels are lower in the chronic eczematous lesions of CACD compared to the lesions of contact hypersensitivity[69]. These observations suggest that the activity of Th17 may be suppressed in skin of AD and CACD with chronic lesions. Recent studies have demonstrated that Th2 responses can antagonize Th17 responses[73]. The topical application of acrylate gel enhances Th2 responses and down-regulates Th17 responses[60,70]. A microRNA-210 inhibits Th2 differentiation but induces Th17 cell differentiation[73]. AD-mesenchymal stem cells show a down-regulation of Th2 cytokines, while Th17 cytokines are upregulated in AD-mesenchymal stem cells[73]. The absence of both IL-4 and IFN-γ results in augmented Th17 differentiation[73]. The development of Th17 cells from naive precursor cells is inhibited by IL-4 and IFN-γ[62,72]. Th2 responses induced by histamine may suppress Th17 responses.

TGF-β1 and IL-6 are both required for the induction of Th17 cells[62,73]. Histamine suppresses TGF-β1 levels of the lesional skin[73], while promoting expression of IL-6 in murine CACD[60], which concurs with observations in our study. Administration of TGF-β1 increased IL-17 and IL-22 levels in lesional skin. Furthermore, histamine H1 and H4 receptor antagonist increased TGF-β1, IL-17 and IL-22 levels in lesional skin. Therefore, histamine may suppress the induction of Th17 mediated by TGF-β1 in murine CACD.

CONCLUSION

Th17 cells play crucial roles in the establishment of acute ACD as effector cells but decrease their activity in the early stage of CACD, which is a Th2 dominant condition. Th17-induced cytokines (IL-6, IL-23, TGF-β1) are simultaneously upregulated in the early stage of CACD. Subsequently, the activity of Th17 increases again in the late stage of CACD, in which Th2 dominant allergic reactions decrease. Th17 cells might suppress Th2 cells and induce amelioration of allergic reactions in Th2 dominant CACD. Histamine aggravates CACD by suppressing Th17 response while inducing Th2 response.

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