REVIEW ARTICLE

Cytokines and T cells in atopic dermatitis

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ABSTRACT. Atopic dermatitis (AD) is an inflammatory disorder of the skin characterized by an impaired immune response. Several effector T cell subsets, such as pro-inflammatory cells like Th9, Th17 and Th22 cells, expressing high levels of IL-9, IL-17 and IL-22, together with the anti-inflammatory, immuno-modulating Treg cells constitutively producing IL-10, seem to play a role in this condition. IL-9 and IL-9 receptors are significantly increased in lesional AD skin compared to normal control skin. In addition, some polymorphisms in IL-9 and IL-9r genes have been associated with AD. The role of IL-17 and IL-17-producing T cells remains under debate and conflicting data are available. IL-22-producing T-cells seem to correlate with the severity of the AD. The number and function of Treg cells, producing IL-10, have been widely investigated in AD with conflicting results. Other studies suggest that high levels of IL-31 or low levels of IL-21 might be involved in the pathogenesis of AD. This review was undertaken in order to provide a better understanding of the relevance of certain cytokines in AD. We have analysed the new insight into the pathogenesis of AD, with special attention to those cytokines produced by the different T cell subpopulations.

Key words: atopic dermatitis, cytokines, T-cell, AMP

Atopic dermatitis (AD) is a chronic, relapsing, inflammatory skin disease, characterized by the presence of eczematous lesions and pruritus [1], frequently associated with high plasma levels of IgE and eosinophilia [2]. AD affects 15–30% of children and 2–10% of the adult population worldwide [3].

Despite the high number of clinical and experimental studies, and subsequent advances in this field, the pathophysiology of AD is still unclear [4]. The most recent hypotheses concerning the pathogenesis of AD involve numerous factors: genetic predisposition, environmental factors, altered immunological function, and skin barrier dysfunction (mainly fillagrin and ceramides [5]) [3, 6].

Innate and adaptive immunity [7] have a definitive role in the development, maintenance and flare-up of AD. This process involves, in a complex interaction, T cells, antimicrobial peptides (AMPs) and keratinocytes (KC).

Abbreviations

AD	atopic dermatitis
Treg	regulatory T cell
CTLA-4	cytotoxic T-lymphocyte antigen 4
CC	chemokines
AMPs	antimicrobial peptides
TIMs	topical immunomodulators
h-BD	human beta defensin
SNP	single nucleotide polymorphism
PBMCs	peripheral blood mononuclear cell

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AD is characterized mainly by a biphasic, T cell polarization: in the acute phase, AD lesions are dominated by infiltrating Th2 cells and cytokines such as IL-4, IL-5 and IL-13, while in chronic AD lesions there is a switch toward a Th1 phenotype [8, 9]. The high numbers and function of the different T cell subpopulations (Th1, Th2, Th17, Th22, Th9 and Treg cells) (*table 1*), and the respective cytokine milieu has often hindered the interpretation of their interplay.

The connection between KC and the T cell subpopulation, which contribute to the development and maintenance of AD, makes the story even more complicated [10].

Of interest, the altered immune system balance and the barrier disruption specific of AD lesions, predispose patients to viral and bacterial skin infections [2].

An AD flare-up can also be induced by psychological factors, such as stress [11, 12], through an immunological shift toward Th2 cells [13]. The role of the Th2 population and the cytokine pattern in AD flare-ups has been recently reviewed by Brandt and Sivaprasad [14].

T CELLS

T cells are key players in several immunomediated diseases such as psoriasis, lupus, rheumatoid arthritis, and in allergic diseases such as rhinitis and AD. Different T cell populations contribute to the development of the disease although their exact mode of action remains under

Table 1
Different T cell populations and their characteristic cytokine production profile [14].

Th1 cells	IFNγ
Th2 cells	IL-4, IL-5, IL-13, IL-31
Treg	TGFβ, IL-10, IL-35
Tr1	IL-10
Th3	TGFβ
Th17 cells	IL-17A, IL-17F (IL-25), IL-21, IL-22
Th9 cells	IL-9, IL-10
Th22 cells	IL-22, IL-31
$CD4^+$ T cells expressing T cell producing IL-31	IL-22 and IL-13

investigation. A number of different, specific subsets of effector T cells have been discovered and investigated in recent years. Among others Treg, Th22, Th17 and Th9 have been demonstrated to play a similar role in AD.

Treg cells

The forkhead/winged helix transcription factor gene FoxP3 is the fingerprint of native Treg cells [19], and is responsible for their development and function [20].
Treg cells can suppress immune responses through direct cell-cell contact signaling, through cytotoxic T-lymphocyte antigen 4 (CTLA-4), as well as secreting TGF-β and IL-10 [22].
Human $CD4^+CD25^+$ Treg cells represent 5% to 15% of peripheral $CD4^+$ T cells [15]. These $CD4^+CD25^+$ Treg cells have been identified in both peripheral blood and thymus [16, 17]. However, only 1–3% of $CD4^+$ T cells express CD25 at high levels ($CD25high^+$), and only these cells have been shown to possess suppressor activity [18]. Beside the classical Treg cells, there exists a group of inducible Tregs classified according to their cytokine secretion pattern: Treg type 1 (Tr1) cells, secreting high levels of IL-10, and Th3 cells secreting high levels of TGF-β [21].
AD patient have been reported to have significantly increased numbers of peripheral blood Treg cells with normal immunosuppressive activity [23, 24]. However, after super antigen (SAg) stimulation, as in AD lesions colonised by SAg-producing *Staphylococcus aureus*, Treg cells lose their immunosuppressive activity, suggesting that Treg cells might not be functioning in AD skin [25]. Furthermore Lin *at al.* [26] demonstrated that Treg cells from *Staphylococcus aureus*-colonised AD skin show Th2-like effector function. The data in the literature however, do not suggest a straightforward role for Tregs in AD; in fact there are studies that report similar numbers of Tregs in AD patients and normal controls.
Caproni and coworkers [27] clarified the role of Tregs in AD, analysing how topical treatments work in this disease. The authors found that topical immunomodulators (TIMs) modulate Treg population, reducing the number of Tr1 cells and increasing their production of IL-10, while the number of Th3 cells was increased, as well as their product TGF-β. These effects seem to restore a normal balance in the T cell subset and in cytokine production in AD patients when compared with normal controls.
The role of Tregs in AD has been further assessed using the evidence that many AD treatments such as: cyclosporine, UV light, methotrexate and steroids [27], influence T cell subsets and modulate Treg number and function [23, 28].

Th17 cells

Human Th17 cells are a specific subset of memory $CD4^+$ T cells that produce high levels of IL-17A and IL-17F [29]. They express the chemokine receptor CCR6 responsible for skin and mucosal homing [30]. Th17 cells are involved in innate immune regulation, in neutrophil recruitment, and may play a role in allergic disorders [14].
The number of Th17 cells in AD is controversial: they have been reported as both increased [31, 32] and decreased [2, 33] compared to normal controls. These differences may arise from the different stages of disease that were considered in the studies.
Koga *et al.* described an increase in IL-17-producing cells in acute AD lesions compared to chronic lesions [31], while a reduced number of Th17 cells in chronic AD lesions has been recently reported [34]. However, the reduced number of circulating Th17 cells may reflect tissue infiltration of these cells in acute AD lesions [31].
Actually, the Th17 cell count could differ in the different phases of AD, with higher levels crucial for the development of the initial Th2-related phase, and reduced levels in the chronic, Th1-mediated phase [31, 35].
According to some authors, a decreased number of Th17 cells in AD correlates negatively with serum CCL17 levels (a marker of AD activity responsible for the recruitment of Th2 cells), eosinophil counts and plasma IgE levels [2]. Toda *et al.* [32] supported this observation describing a reduction in IL-17 concomitant with an increase in the eosinophil count.
However, the role of IL-17, Th17 and the other T cell subsets need to be investigated in order to elucidate how these populations interact in the setting of AD. In some asthma models for example, Th17 cells have been found to produce Th2-related cytokines apart from IL-17 [36]. In turn, a population of IL-9-producing T cells (the so-called Th9 population) has been described as producing IL-17, in the presence of Th2-polarized cytokines and TGF-β [37, 38].

Th9 cells

Th9 cells, a recently described T cell subset, characteristically produce IL-9 and IL-10. It has been shown that Th2 cells skew towards Th9 cells in a milieu rich in TGF-β and IL-4 [41]. Although they produce IL-10, Th9 cells are characteristically pro-inflammatory and their main task in AD seems to be the recruitment of mast cells through the production of IL-9 [42].

Th22 cells

Th22 cells were characterised some years ago as IL-22-producing T cells different from Th1, Th2 and Th17 cells [39], and expressing high levels of CCR10 [40]. IL-22, the prototypical Th22 cytokine, is highly expressed in AD.
Concomitantly, in chronic AD lesions, high levels of CCL27 can be found. The expression of this chemokine and the presence of CCR10 (the natural ligand of CCL27) on Th22 cells induce the homing of these cells in lesional AD skin [40].

CYTOKINES

AD has long been considered to be a two-phase disease: Th2-related during the acute phase and a Th1-driven disease in the chronic phase. Newly discovered cytokines such as IL-22, IL-31, and IL-33, are also involved in this disease. Moreover, advances in the understanding of the effect of traditional cytokines such as IL-9, IL-10 and others, have helped in the interpretation of the pathogenesis of AD.

IL-9

IL-9 acts as a regulator of haematopoietic cells. This cytokine is the prototypical cytokine expressed by a new subset of T helper cells called Th9, but it is also produced by Th2 cells. To date, only few works have been published concerning the possible action of IL-9 in AD. In a genetic study, polymorphisms in the IL-9- and IL-9-receptor genes have been found to correlate with AD [43] while recently IL-9 plasma levels have been shown to be, not only higher in AD patients when compared to normal controls, but to correlate with symptom severity [44].

Nevertheless, more detailed work should address the role of Th9 and IL-9 in AD; their function could be crucial, together with other cytokines and T cell subsets, to the induction of pruritus through mast cell recruitment and activation.

IL-10

IL-10 is an anti-inflammatory cytokine produced mainly by Treg cells. It down-regulates the immune system minimising tissue damage during inflammation [45].

Data regarding the role of IL-10 in AD subjects are conflicting. Fewer IL-10-producing $CD4^+$ T cells have been described in individuals with severe AD as compared to mild AD and normal controls [46]. Plasma levels of IL-10 seem to correlate inversely with the severity of AD [47]. However, other studies have shown increased IL-10 levels in peripheral blood mononuclear cells (PBMCs) [48] and in lesional skin [49] from AD patients.

The importance of this cytokine in the development and/or maintenance of AD has been recently highlighted by the idea that polymorphisms in the IL-10 gene could represent a genetic marker for AD in childhood [50].

These contrasting results could be, in part, explained by the different assay methods used and by the varying clinical condition of the AD patients in the studies.

IL-17

IL-17A is a homodimeric protein belonging to the IL-17 cytokine family [51], characteristically produced by Th17 cells [52]. IL-17 is a key cytokine in host defence infections, as well as in the pathogenesis of autoimmune diseases [53]. In addition, in keratinocytes, IL-17 has been described as master regulator of antimicrobial peptides (AMPs) [54]. AMPs, part of the ancestral immune system, play a central role in host defence against bacteria, viruses and protozoa [55].

This pro-inflammatory cytokine has been described as an essential factor in the pathophysiology of AD [56]. IL-17 is highly expressed in acute AD [57], while being barely detectable in the chronic phase of the disease [40] thus correlating with the reduced AMPs expression and the higher risk of cutaneous super-infection experienced by AD patients.

IL-21

IL-21 is a recently described member of the IL-2 family [58], and is mainly expressed by activated $CD4^+$ T lymphocytes, especially by Th17 cells [59-61]. IL-21 is a potent immunomodulatory cytokine, with pleiotropic effects on the innate and adaptive immune system [62]. It promotes Th17 cell differentiation in an autocrine manner [63]. Apart from its action on different immune cells, IL-21 may also inhibit IgE production [64, 65].

In a recent study by Lin *et al.* [66] IL-21 levels were found to correlate inversely with INF-γ production and the severity of AD. In addition, animal models [67, 68] seem to confirm that reduced levels of IL-21 contribute to the development of allergic and immunological diseases. However, there exist data describing an increase in IL-21 in AD lesions [69].

IL-22

IL-22 is a member of the IL-10 cytokine family [70], expressed at higher levels in AD patients when compared to psoriasis patients and normal controls [40], suggesting a possible role in the pathophysiology of AD. Originally, IL-22 production was attributed to Th17 cells; however, it is now known that a different subpopulation of T cells (namely Th22 cells) is the principal source of this cytokine in AD and other diseases such as psoriasis [40].

IL-22 is able to induce AMPs production although less efficiently as compared to IL-17 [33]. Thus, the high levels of IL-22 are not sufficient to oppose the effect of Th2 cytokines on AMPs production in AD [71]. The weaker action of IL-22 compared to IL-17, together with the reduction in Th17 cells and IL-17, may be the principal cause of the higher incidence of skin infection in chronic AD [33, 55].

IL-22 also mediates acanthosis and hypogranulosis [72, 73], and is thus responsible for the epidermal hyperplasia observed in AD [56]. Moreover, in keratinocytes, IL-22 downregulates filaggrin expression [74], worsening the epidermal barrier dysfunction in AD.

IL-25

IL-25, also known as IL-17F, is an important modulator of Th2-mediated diseases [75], albeit being mainly produced by Th17 cells [29]. Several other cell types including DCs, eosinophils and basophils produce this cytokine [76]. IL-25 and IL-25R, its cognate receptor, are overexpressed in AD skin, especially in lesional skin [77]. Finally, IL-25 has been shown to induce the production of Th2 cytokines in cultured keratinocytes [76].

IL-31

IL-31 is a recently discovered, four-helix bundle cytokine expressed in many human tissues at low levels [78], and at relatively high levels by Th2-committed $CD4^+$ cells [79]. Experimental mouse models have shown that IL-31 transgenic mice develop skin lesions resembling AD with severe

pruritus [78], and demonstrate a scratching attitude [80] that is reduced by the use of an anti-IL-31 monoclonal antibody [81].

Although high levels of IL-31 correlate directly with IL-4 and IL-13 levels in AD patients [81], its role in the human system still has to be fully assessed.

Staphylococcal enterotoxin B (SEB) up-regulates IL-31 in peripheral blood mononuclear cells [82] as well as AMPs in mast cells [79].

An overexpression of IL-31 has been reported in cells infiltrating AD lesions compared to non-lesional skin and controls. However, it is still not clear if this cytokine has a role in the acute or chronic phase of AD [83]. In a recent work, Szegedi *et al.* found two different IL-31-producing T cells populations: the first producing IL-31 and other cytokines, and a second, thought to represent a new T cell subpopulation, producing only IL-31. The former population was found to produce IL-13 and, to a lesser extent, IL-22. These observations suggest that Th2-committed cells may primarily produce IL-31. IL-22 and IL-31 probably orchestrate the development of the reactive epidermal hyperplasia seen in chronic AD lesions [34]. In addition, IL-31 receptors have been widely found in cutaneous nerve fibres and dorsal root ganglia, suggesting that this cytokine may act as a mediator of pruritus in AD [83].

IL-33

A recently discovered cytokine named IL-33, part of the IL-1 family, has been identified as part of the "alarmin" system in innate immunity [84]. IL-33 has been found to be up-regulated in a variety of Th2 inflammatory diseases including AD [85]. Confirmatory data on its role in AD come from a study by Shimizu *et al.* [86] in which a strong association between a genetic polymorphism in the ST2 (membrane bound IL-33–specific receptor [87]) region and AD has been described.

IL-33 production is induced by many different mechanisms including immunological (antigens or a combination of TNF-α and IFN-γ), mechanical, (tape stripping) [88], and microbial stimuli [89]. IL-33, in turn, induces the expression of IL-5 and IL-13 (*in vitro*), increasing serum immunoglobulins and eosinophil recruitment [90]. However, IL-33 does not only display pro-inflammatory capabilities, it has anti-inflammatory abilities that act on different cells and receptors. In recent studies, Cevikba and Steinhof [91], together with Savinko *et al.* [89], postulated that over-production of IL-33 represent one of the first mechanisms responsible of the onset of AD. In addition, the authors also showed that IL-33 is able to reduce the Th1 process in several diseases providing a better characterisation of IL-33 function in the immune system.

Miscellaneous

In chronic AD, decreases in IFN-γ mRNA levels in PBMCs, and in IFN-γ-producing skin-homing T cells have been also described [92, 93]. A reduction in IFN-γ-producing PBMCs has been associated with increased serum IgE levels in AD [94]. However, this has been disputed by Kallstrom *et al* [95].

With regard to chemokines (CC), as reported elsewhere [2], serum CCL17 levels have been proposed as a useful marker of AD activity in several studies.

In addition to the immunological function of the Th2-related cytokines, IL-4, IL-13 and IL-31 can down-regulate filaggrin-gene expression, therefore contributing to the impairment of skin barrier function, which lends support to the idea of a multifactorial pathophysiology in AD [34].

ANTI-MICROBIAL PEPTIDES

AMPs are small peptidic molecules representing an integral part of a primitive [96] mechanism of immunity present in virtually all organisms. All tissues and cells exposed to microbes are able to produce AMPs [96]. In particular, human keratinocytes and sweat glands are prolific producers of a broad range of these molecules, including β-defensins (h-BD), RNases, cathelicidins (LL-37), and members of the S100 protein family [97]. Although AMPs were previously described to act only as endogenous antibiotics, it is now clear that they trigger and coordinate multiple components of the innate and adaptive immune systems [98].

As stated previously, AD is frequently complicated by recurrent skin infections both in lesional and non-lesional skin sites [99]. The increased susceptibility to pathogens seems to depend mainly on two mechanisms: a deficit in skin barrier permeability (fillagrin), and a reduction in AMPs expression [100].

AD patients demonstrate a deficit of h-BD2 and LL-37 and an overexpression of psoriasin when compared with psoriasis patients [101, 102]. While an LL-37 deficit in AD patients predisposes them to frequent, disseminated *Herpes simplex* virus infections [99], the high levels of psoriasin may reduce the possibility of *Escherichia coli* infections [102]. Surprisingly, hBD-2 and LL-37 expression is reduced only in lesional skin and not in the normal skin of AD patients [99]. This is probably due to the high expression of Th2 cytokines [100, 103, 104] and possibly the reduction of Th17 cells and IL-17 production. Indeed, IL-4 and IL-13 were found to suppress TNF-α-induced expression by AMPs (especially hBD-2 and hBD-3) in keratinocytes, further favouring susceptibility to microbial colonisation [98, 105].

For all these reasons, LL-37 and h-BD1, although with different rationales, have been proposed as possible biomarkers of disease activity [102, 106].

CONCLUSION

In this review we have addressed the role of the different T cell populations (with the exception of Th1 and Th2 populations, which have been widely investigated over the years), and of the main cytokines involved in the pathophysiology of AD, as well as the role of small peptidic molecules that form part of the ancestral immunity (AMPs) (*figure 1*).

The upregulation of IL-33 and its receptor, ST2, in lesional AD skin seems to be a key mechanism involved in the development of AD triggered by allergen exposure, irritants, and scratching, as well as of the bacterial and viral infections seen in this condition.

Acute AD lesions are associated with local, low production of AMPs, and by the induced Th2 milieu that, in turn, reduces the Th17 subset, favoring further downregulation

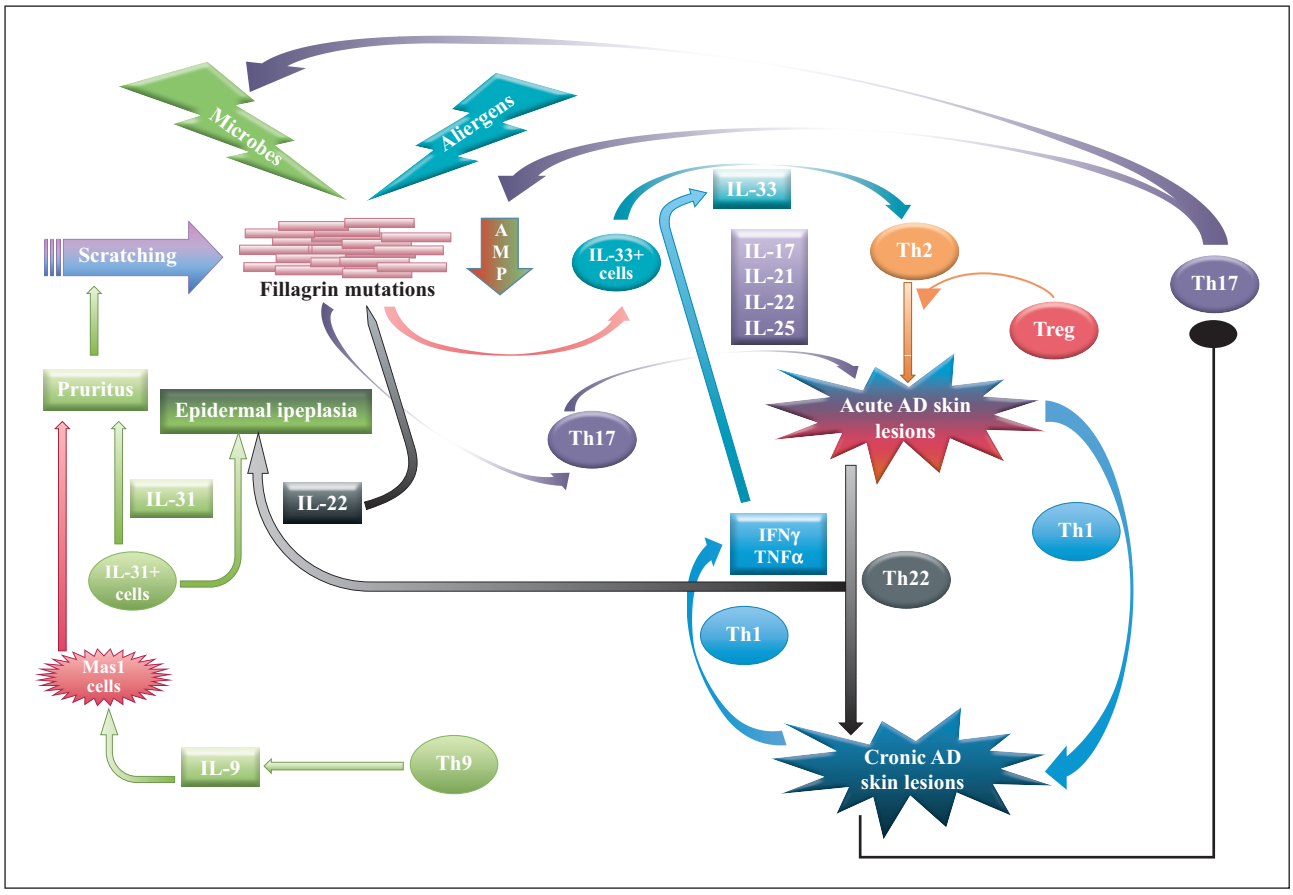

Figure 1
Interplay existing between barrier dysfunctions, external stimuli and immunological deregulations in the acute and chronic phases of atopic dermatitis.

of AMPs production. AMPs downregulation, in its turn, predisposes to further infections. During this phase, Treg cells are not able to modulate the production of Th2 cytokines, increasing Th2 commitment in AD patients. The production of high levels of IL-22 from a distinct subset of T cells (Th22) drives the development of the disease toward the chronic phase. In this latter, Th1-associated phase, the increased amounts of IL-22, together with IL-31, induce epidermal hyperplasia and promote itch. The development of pruritus is also due to Th9 cells producing IL-9, which recruits mast cells that stimulate itch. In this way, an initial stimulus, coupled with barrier defects and a generally predisposing immunological condition (mainly genetically determined) could explain the onset of AD and its progression.

Although more detailed studies should address the complex relationship existing between the different T cell subset, their cytokine production, and keratinocyte involvement, much has already been done to clarify the role of cytokines in this disease.

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