Immune Activity and Vitamin D

5

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Contents

5.1 Introduction

Atopic diseases are characterized by elevated levels of IgE and classically described as Th2 mediated. It is believed that following entry of an allergen into the skin, it is processed by local dendritic cells (DCs) that then migrate to the draining lymph node. Here, the DC presents the allergen for naïve $CD4^+$ T cells, which subsequently differentiate into Th2 cells characterized by their production of IL-4, IL-5, and IL-13 (Fig. 5.1). The Th2 cells activate allergen-specific B cells and promote isotype switch to IgE. The allergen-specific IgE will bind and prime mast cells, which then can be activated following exposure to the allergen (see Fig. 5.1). During the last years it has become clear that several new players are involved in allergen-specific immune responses, among these the epithelial cells, innate lymphoid cells (ILCs), Th17 cells, and vitamin D. This chapter focuses on how filaggrin (or lack of) affects the immune response and vitamin D synthesis in the skin. Both human and mice studies are discussed as much of the knowledge about the effects of filaggrin on the immune system comes from studies using the Flaky tail mice (Flg^{ft} mice) lacking filaggrin [1–3] (Fig. 5.2).

5.2 The T-Cell Response

Atopic dermatitis (AD) is a complex disease dependent on both genetic and environmental factors that induce a complex immune response.

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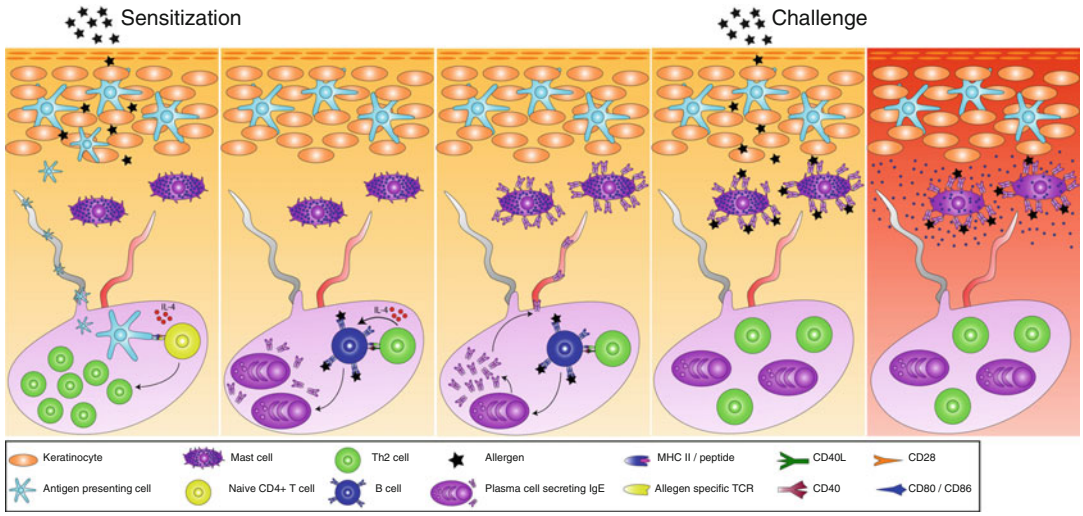

Fig. 5.1 Simple model for immune responses leading to AD. Skin exposure to allergens leads to activation of skin DC that migrates to the draining lymph nodes, where they present allergen for allergen-specific naïve $CD4^+$ T cells. Due to the presence of IL-4, allergen-specific $CD4^+$ T cells differentiate into Th2 cells. These subsequently activate allergen-specific B cells that differentiate into IgE-producing plasma cells. IgE bind to FcεR1 receptors on mast cells in the skin. Upon subsequent exposure to the allergen, IgE on the mast cells bind allergen and induce mast cell activation and thereby skin inflammation

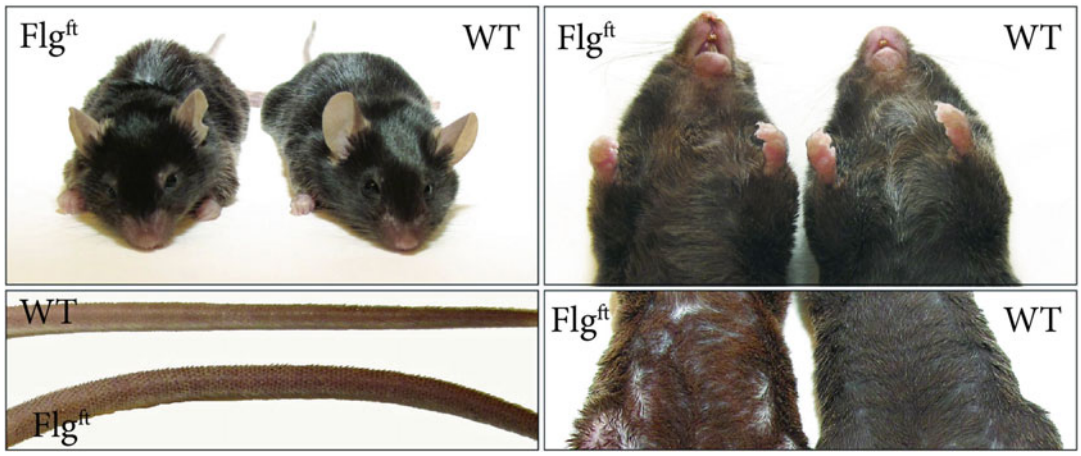

Fig. 5.2 Appearance of age-matched Flg^{ft} and WT mice (C57bl/6)

It is well known that T cells play a central role in the pathogenesis of AD [4, 5]. An AD mouse model showed that αβ T cells were required for skin inflammation, whereas γδ T cells and B cells were not required [5]. In addition, IL-4 expression was upregulated in inflamed skin and found to be produced by αβ T cells [5]. Interestingly, skin inflammation could be induced in mice lacking either B cells or CD40L indicating that IgE is not required for the development of skin inflammation [5].

$CD4^+$ T-cell differentiation is classically divided into Th1 and Th2 responses dominated by IFNγ, IL-2 and IL-4, IL-5 and IL-13, respectively (Fig. 5.3) [6, 7]. Recently, a new $CD4^+$ T-cell subtype has been identified, namely, the

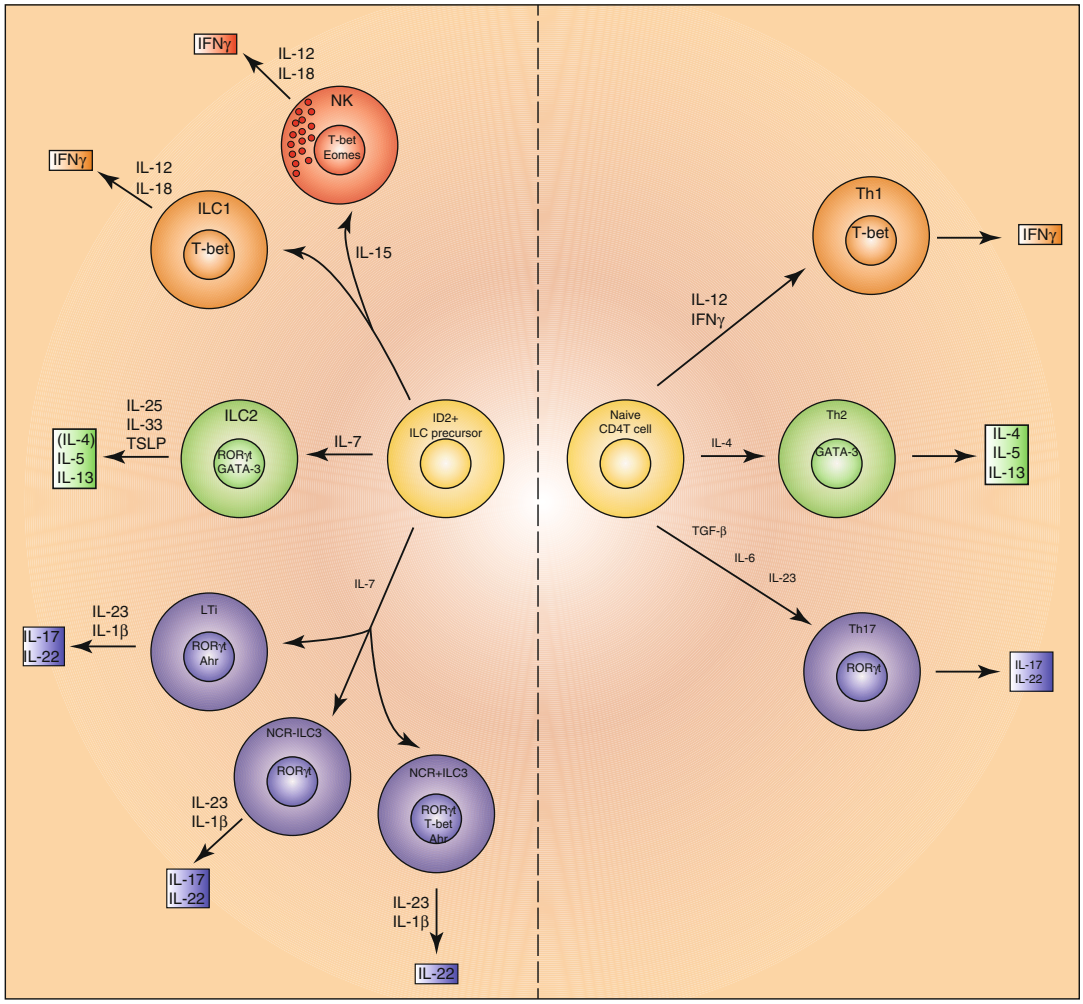

Fig. 5.3 Schematic representation of differentiation of ILC and naïve CD4⁺ T cells showing cytokines and transcription factor involved in the differentiation as well as effector cytokines produced by the cells

Th17 cells (see below). A mouse model for AD-like skin inflammation was used to further investigate the role of Th1 and Th2 cells in AD. Impaired eosinophil recruitment to the skin was seen in mice lacking either IL-4 or IL-5, whereas IFNγ did not seem to be involved in eosinophil recruitment [8]. However, IFNγ seemed to be involved in the response by other mechanisms as reduced skin inflammation was seen in mice lacking either IL-5 or IFNγ [8]. In accordance with this, IL-4, IL-5, IL-13, IFNγ, and IL-12 are upregulated in the skin from AD patients compared to healthy skin [9, 10]. Interestingly, these cytokines appear to be involved at different stages of the disease. The initial inflammation seems to be driven by IL-4 and IL-13, as these are the dominating cytokines in acute lesional skin [9, 10]. In contrast, chronic inflammation seems to be maintained by IL-5, IL-12, and IFNγ [9, 10]. Increased eosinophil infiltration was seen in chronic compared to acute lesions, which correlates with the increased expression of IL-5 in chronic lesions [9]. Thus, the acute inflammation appears to be mediated by Th2 cells and their production of IL-4 and IL-13, whereas the chronic inflammation seems to involve both Th1 and Th2 cells.

Exposure of the skin to protein allergens (ovalbumin (OVA), *Dermatophagoides pteronyssinus* (Derp1)), contact allergens, and irritants induces a more vigorous inflammatory response in Flg^{ft} mice than in control mice [1, 3]. The response to protein antigens seems to involve Th1, Th2, and Th17 cells, which correlates well with the findings in patients with AD (see below [1, 3, 9, 10]). Interestingly, only minor cytokine induction was seen following allergen exposure in control mice having an intact skin barrier [1, 3]. Thus, lack of filaggrin seems to increase the risk of developing allergen-specific T-cell responses mediated by Th1, Th2, and Th17 cells. To date very few studies have investigated how the lack of filaggrin affects T-cell responses in humans. However, by combining Derp1-specific tetramer staining together with IL-4 and filaggrin analysis, one study showed that individuals lacking filaggrin had an increased number of Derp1-specific IL-4-producing $CD4^+$ positive cells in their blood compared to individual with wild-type filaggrin gene (*FLG*) mutation status [11]. Taken together, even though AD classically is described as mediated by Th2 cells, other effector $CD4^+$ T cells seem to be important at different stages of the disease. Lack of filaggrin seems to increase the risk of developing allergen-specific T-cell responses, probably due to increased skin penetration of allergens that elicit a basic inflammatory response and thereby provides a reduced activation threshold for the T cells. In addition, a recent study shows that AD patients with *FLG* loss-of-function mutations had increased levels of IL-1α and IL-1β in the skin compared to both healthy controls and AD patients without *FLG* mutations [12]. Interestingly, the increased IL-1α and IL-1β correlated inversely with the "natural moisturizing factors" (NMFs) that correlated inversely with skin pH [12]. As IL-1 is a pro-inflammatory cytokine known to be involved in the initiation of the immune response in general (e.g., by inducing maturation and migration of DC), increased IL-1 levels are likely to lower the immune activation threshold within the skin of patients lacking filaggrin [13].

5.3 The IL-23/TH17 Axis in AD

Following the discovery that IL-23, and not IL-12, was required for the induction of experimental autoimmune encephalomyelitis (EAE), the mouse model of multiple sclerosis, intensive work was carried out to characterize the effector $CD4^+$ T cells responding to IL-23, which eventually lead to identification of IL-17-producing $CD4^+$ T cells (Th17) in 2005 [14–16]. It is now known that TGF-β and IL-6 are required for initiation of Th17 differentiation, whereas IL-23 is required for the stabilization of the Th17 cells (see Fig. 5.3) [7]. Th17 cells are involved in the pathogenesis of a variety of autoimmune and inflammatory diseases such as EAE, inflammatory bowel diseases, and psoriasis [17]. The primary cytokines produced by Th17 cells are IL-17 and IL-22, both of which stimulate epithelial cells to produce a variety of inflammatory cytokines (e.g., IL-1β, IL-6, TNFα), chemokines (e.g., CXCL8), and antimicrobial peptides (e.g., β-defensin, cathelicidin) [17]. Th17 cells most likely play a role in AD as an increased percentage of Th17 cells are found in the blood and lesional skin of AD patients [18, 19]. Interestingly, Th17 cells seem to serve as an initial cytokine source as they are more prevalent in acute than chronic lesions [18, 19]. Furthermore, Th17 cells are likely associated with the severity of AD as a direct correlation between severity of the inflammation and the percentage of Th17 cells in the blood has been found [18]. The role of Th17 cells in the immune response to protein allergen has been further investigated by using an OVA sensitization mouse model. Here mice were exposed to OVA either epicutaneously (EC) or intraperitoneally (IP) [20]. It was shown that EC OVA sensitization induced both a local and a systemic Th17 response, whereas IP OVA sensitization did not [20]. In contrast, the production of IL-4 and IFNγ following OVA sensitization seemed to be independent on the sensitization route [20]. The Th17 response also appears to drive airway inflammation as neutrophil influx and bronchial hyperactivity induced by OVA inhalation in EC-sensitized mice could be reversed by IL-17 blockade [20].

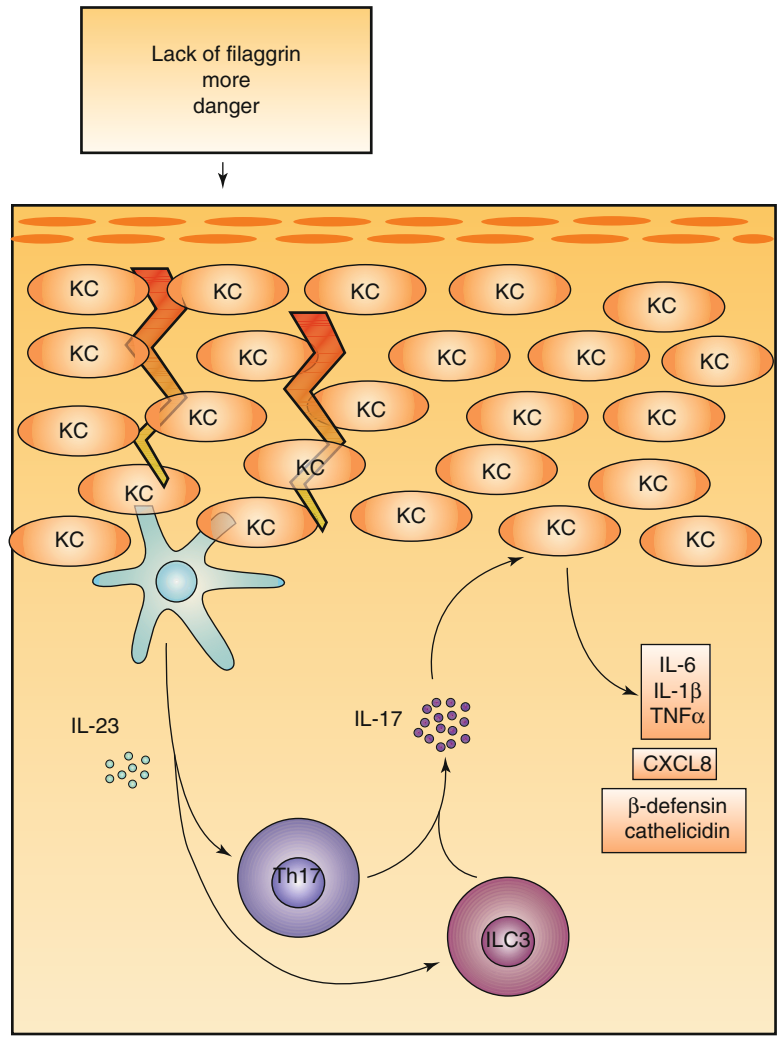

Fig. 5.4 Model for the role of IL-17 in initiation of AD

The reason why EC sensitization, in contrast to IP sensitization, leads to Th17 responses might be explained by the ability of skin-derived DC to produce IL-23, a feature that is not observed in splenic DC [20]. In this model, mice were tape-stripped before OVA exposure of the skin, a procedure that is known to induce disruption of the skin barrier. As EC sensitization with allergens seems to play an important role in allergen sensitization of patients with AD, and as patients lacking filaggrin have an increased risk of developing asthma [21, 22], it was suggested that allergen exposure of skin lacking filaggrin leads to a Th17 response, which upon later allergen exposure of the airways induces a Th17-dependent airway inflammation [20]. In agreement with this, three studies on Flg^{ft} mice have shown an increased IL-17 production in Flg^{ft} mice compared to control mice [1–3]. The increased IL-17 production was found both in the skin at steady state and in OVA-specific $CD4^+$ T cells after EC OVA sensitization [1, 3]. Interestingly, a similar Th17 response was seen in Flg^{ft} mice and control mice following IP sensitization with OVA [3]. Taken together, allergen exposure of the skin seems to favor a Th17 response, and lack of filaggrin increases the risk of developing allergen-specific Th17 responses that again increases the risk of developing severe AD and asthma (Fig. 5.4).

Even though Th17 cells were first described as the IL-17-producing cells, it is now clear that several other types of cells can produce IL-17, i.e., $CD8^+$ T cells, γδ T cells, and ILC [3, 17, 23]. During the last years much focus has been on ILC. The ILC are characterized by lack of expression of markers associated with T cells, B cells, DCs, macrophages, and granulocytes [23]. Interestingly, it seems that ILCs can be subdivided based on transcription factors and cytokine production in a way similar to the $CD4^+$ T effector cells (see Fig. 5.3). Thus, ILC1 are the innate analogs to Th1 cells, ILC2 are the innate analogs to Th2 cells, and, finally, LTi and ILC3 are the innate analogs to Th17 cells [7, 23]. A recent study indicated that cells other than Th17 cells might be responsible of the increased level of IL-17 found in the skin of Flg^{ft} mice at steady state [24]. In this study, Flg^{ft} mice were crossed to RAG2-deficient mice lacking both T and B cells [24]. Lesional skin inflammation characterized by fur loss, erythematous scaly skin, and periocular swelling was seen in 88 % of Flg^{ft} mice after 32 weeks. In contrast, no sign of skin inflammation was seen in mice lacking both filaggrin and RAG2, indicating that the adaptive immune response is required for the skin inflammation in Flg^{ft} mice [24]. However, increased levels of both IL-17A and IL-22 were found in the skin of $RAG2^{-/-}$ Flg^{ft} mice compared to mice only lacking RAG2, indicating that LTi and/or ILC3 might be involved in the increased level of IL-17A found in Flg^{ft} mice at steady state [24]. Thus, it is likely that ILC subtypes are involved in the inflammatory response induced by the lack of filaggrin; however, this needs further investigations (see Fig. 5.4).

Taken together, IL-17 is most likely produced by both Th17 cells and ILC in the inflammatory response observed in AD. Lack of filaggrin leads to an impaired barrier function and thereby probably to danger signals that activate skin DC to produce IL-23. IL-23 subsequently stimulates IL-17 production from ILC and Th17 cells. IL-17 in the skin stimulates keratinocytes to produce pro-inflammatory cytokines and chemokines that eventually lead to increased skin inflammation (see Fig. 5.4).

5.4 The Role of TSLP in the Response

Keratinocytes constitute the majority of the cells in the epidermis and were originally described mechanistically as the cells that form the physical barrier between the environment and the body. However, it has become clear that keratinocytes also play an important immune-modulating role due to their ability to produce a variety of cytokines (e.g., IL-1β, IL-23, TNFα, and IL-10 in response to pathogens, stress, and other environmental triggers) [25–28]. Cytokines produced by keratinocytes can modify the activation and differentiation of skin DC. Thymic stromal lymphopoietin (TSLP) mainly produced by keratinocytes, fibroblasts, and stromal cells can induce a Th2 response [29]. TSLP is highly expressed in the epidermis of patients with AD [30]. Stimulation of $CD11c^+$ DC with TLSP in vitro leads to activation and differentiation of DC that promote Th2 differentiation (Fig. 5.5) [30]. By use of a transgenic mouse model, where TSLP specifically can be induced in the keratinocytes, it was shown that mice developed spontaneous AD characterized by skin inflammation, increased number of skin-homing Th2 cells, and elevated levels of serum IgE 2–3 weeks after TSLP induction [31]. Interestingly, induction of TSLP in TCRβKO mice lacking all $CD4^+$ and $CD8^+$ αβ T cells still lead to skin inflammation, suggesting that T cells are not necessary for the induction of the allergic response [31]. It was suggested that the response could be induced by TSLP acting directly on activated macrophages, eosinophils, mast cells, and other myeloid effector cells [31]. However, ILC2 cells could also be involved. TSLP can induce cytokine production by ILC2 in the skin independent of IL-33 and IL-25 [32]. An increased frequency of ILC2 was found in lesional skin from patients with AD [32]. Furthermore, AD-like skin inflammation could be significantly reduced either by depleting ILC or by using TSLP receptor KO mice [32]. This indicates that TSLP might play an important role in the induction of AD by stimulating ILC2 to produce IL-5 and IL-13. In agreement with studies in human

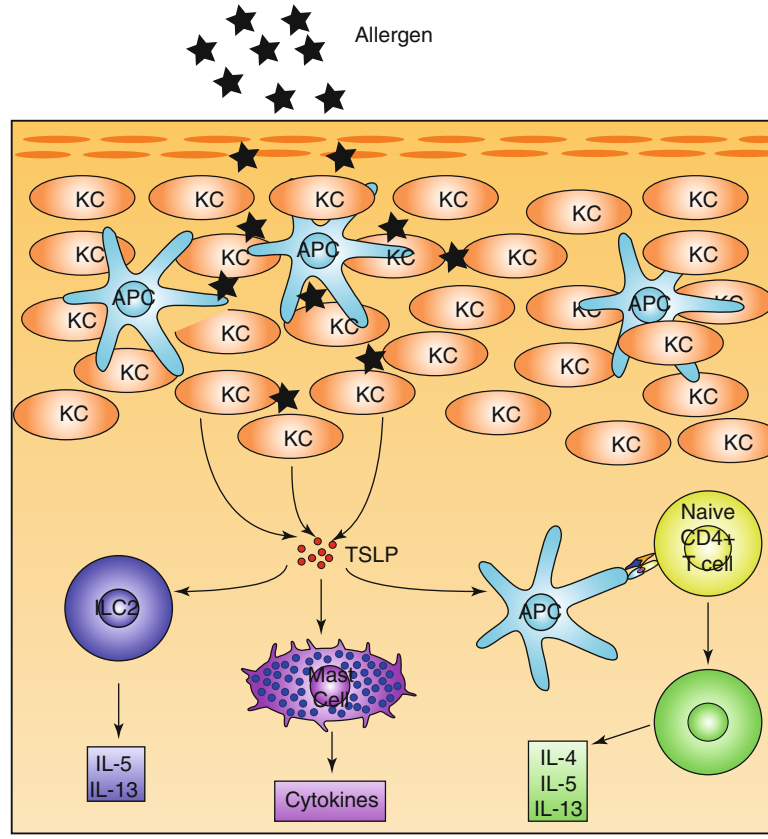

Fig. 5.5 Model for the involvement of TSLP in the immune response during AD

AD patients, TSLP was found to be more expressed in skin from Flg^{ft} mice than in control mice [33]. Furthermore, it was found that the expression and activity of the endogenous proteases kallikrein 5, 7, and 14, which activate TSLP production in keratinocytes, were higher in skin from Flg^{ft} mice compared to control mice in steady state [33]. It can, therefore, be suggested that the increased activity of the endogenous proteases caused by the lack of filaggrin leads to increased production of TSLP via the protease-activated receptor-2 in keratinocytes and that this plays an important role in the induction of both ILC2 and Th2 cells.

5.5 Vitamin D, Filaggrin, and Immune Responses

Several studies have demonstrated that vitamin D regulates keratinocyte growth and differentiation and affects immune responses [34–39]. The major source of vitamin D for most humans is 7-dehydrocholesterol (7-DHC) in the plasma membrane of keratinocyte [40, 41]. The first stage of vitamin D synthesis depends on the UVB (280–320 nm)-mediated photoconversion of 7-DHC to previtamin D_3 in the skin. Once formed, previtamin D_3 is rapidly converted to vitamin D_3 that diffuses to the blood circulation, where it is bound to the vitamin D-binding protein (DBP). Vitamin D_3 is subsequently metabolized in the liver to 25-hydroxyvitamin D_3 ($25(OH)D_3$) and then in the kidney to its biologically active form 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) [40, 42, 43]. $1,25(OH)_2D_3$ is classically considered to function as a endocrine regulator of calcium homeostasis. However, the understanding of vitamin D metabolism and physiological function has evolved dramatically in recent years. Vitamin D is now recognized as a pleiotropic regulator of human physiology with emerging roles in several tissues including the immune system and the skin [44].

The biological actions of $1,25(OH)_2D_3$ are mediated by the vitamin D receptor (VDR) that belongs to the nuclear hormone receptor superfamily [45, 46]. Interaction of $1,25(OH)_2D_3$ with VDR induces heterodimerization with the retinoid X receptor (RXR) and translocation of $1,25(OH)_2D_3$-VDR/RXR complexes into the nucleus [44, 47, 48]. The $1,25(OH)_2D_3$-VDR/RXR complexes bind to specific DNA sequences called vitamin D response elements (VDREs) in target genes, and dependent on the recruited co-regulators either augment or inhibit transcription of the target gene [48–50]. Both keratinocytes and various cells of the immune system express VDR, especially after their activation [38, 51–54].

The normal range of the $1,25(OH)_2D_3$ concentration in serum is 50–175 pM, whereas the concentration of the precursor $25(OH)D_3$ is approximately 1,000-fold higher (50–160 nM). The conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ is mediated by the 1-α hydroxylase CYP27B1 [55]. This conversion was at first believed exclusively to take place in the kidneys; however, it is now clear that CYP27B1 is expressed in various cell types including keratinocytes, macrophages, and activated T cells [56–58], and evidence is rapidly accumulating that local CYP27B1-catalyzed production of $1,25(OH)_2D_3$ is critical for its physiological actions [37, 59]. In this context, the keratinocytes are the only cell type where the complete enzymatic machinery for the synthesis of $1,25(OH)_2D_3$ from 7-DHC has been shown [41, 60–62]. Thus, it can be assumed that UVB-induced production of vitamin D_3 in the skin might result in formation of substantial amounts of local $1,25(OH)_2D_3$, which regulate keratinocyte growth and differentiation and affect the local immune response.

The keratohyalin granules in the stratum granulosum of the epidermis consist primarily of profilaggrin polymers [63] that are proteolytically cleaved into filaggrin monomers. Monomeric filaggrin binds to keratin to form tight bundles facilitating the collapse and flattening of the cells in the stratum corneum [64]. Subsequently, filaggrin is fully degraded to its constituents amino acids dominated by glutamine, arginine, and histidine [65]. Histidine is a substrate for histidase that is highly expressed in the stratum granulosum [66]. Histidase converts histidine to urocanic acid (UCA) in the upper layers of the epidermis. UCA has been suggested to be an important UV photoprotectant as it has a high extinction coefficient in the wavelength range from 260 to 310 nm [67, 68], and it was for several years used as a component of commercial sunscreens [69]. All the prevalent *FLG* mutations are either nonsense or frameshift mutations that result in loss of filaggrin production in the epidermis [70]. Because of the lower levels of filaggrin, individuals with *FLG* mutations have reduced levels of epidermal UCA and thereby reduced UCA-mediated absorption of UVB. This should, in theory, lead to a higher photoconversion of 7-DHC to previtamin D_3 and thereby higher levels of $25(OH)D_3$ and $1,25(OH)_2D_3$. This hypothesis is supported by in vitro experiments demonstrating that knockdown of filaggrin increased UVB sensitivity [71], by in vivo experiments demonstrating that mice with a mutated histidase gene have reduced levels of UCA in the skin and show increased sensitivity to UVB radiation [66], and finally by five general population studies that showed that *FLG* mutation carriers have 10 % higher mean serum $25(OH)D_3$ levels than controls [72]. How *FLG* mutations influence the local concentration of $1,25(OH)_2D_3$ is not known, but it could well be assumed to augment the concentration and thereby have an impact on immune responses. $1,25(OH)_2D_3$ also stimulates keratinocytes and macrophages to increased production of the antimicrobial peptide cathelicidin, which might be of benefit for AD patients [73, 74].

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