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Defining Th-cell subsets in a classical and tissue-specific manner: Examples from the skin

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Th cells are important mediators of adaptive immunity and involved in various diseases. During the past decade, the Th family has expanded from including Th1 and Th2 cells to also encompass Th9, Th17, Th22, and Treg cells; the original classification using the expression of signature cytokines is still the gold standard for definition of subset affiliation. However, the identification of Th cells that do not fit into these tight conceptual boundaries has tumbled the field into an identity crisis. This review gives an overview on different Th-cell classification approaches, their advantages and drawbacks. In addition, this review highlights the functional properties of distinct Th subsets and their effector cytokines in tissues and disease-specific settings with a special focus on inflammatory skin diseases.

Keywords: Cytokine · Microenvironment · Skin · Th cells · Transcription factor

Introduction

Naïve Th cells integrate signals from their T-cell receptor, co-stimulatory molecules and cytokine receptors to polarize into different Th-cell subsets with distinct effector functions. This is a crucial process for the host immune system in order to specialize in the clearance of a diverse array of pathogens. Understanding the function of Th cells requires clear definition and categorization not only of their helper activities but also of their induction and migration programs. Currently, signature cytokine expression, master transcriptional regulators, and cytokine priming requirements are perceived as important (classical) criteria for the classification of Th cells into subset categories. On closer look, however, we need to admit that most of the novel Th-cell subsets do not fulfill classical definition requirements for separate T-cell subsets as they for instance express signature cytokines or transcription factors of two independent subsets at the same time. The emergence of new technologies, as well as the increasing appreciation of epigenetic determination and stabilization of effector T-cell responses, will provide new classification systems for Th-cell heterogeneity and hopefully resolve the current $CD4^+$ T-cell "identity crisis." This review gives an up-to-date overview on the current heterogeneity of Th-cell subsets and the challenges faced with their definition criteria. We will also present novel insights into the function of Th cells in tissues. We will especially focus on Th-cell subsets in the skin as a model organ to investigate the full spectra of functional Th-cell diversity.

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Definition of Th-cell subsets

The historical concept: Subset definition according to cytokine secretion

The first approach to define distinct Th-cell subsets relates to the pioneering work of Mosmann and Coffman, who observed that Th cells could be distinguished according their secreted signature cytokines (reviewed in [1]). They defined two distinct subsets, Th1 cells and Th2 cells, that differed in that Th1 cells

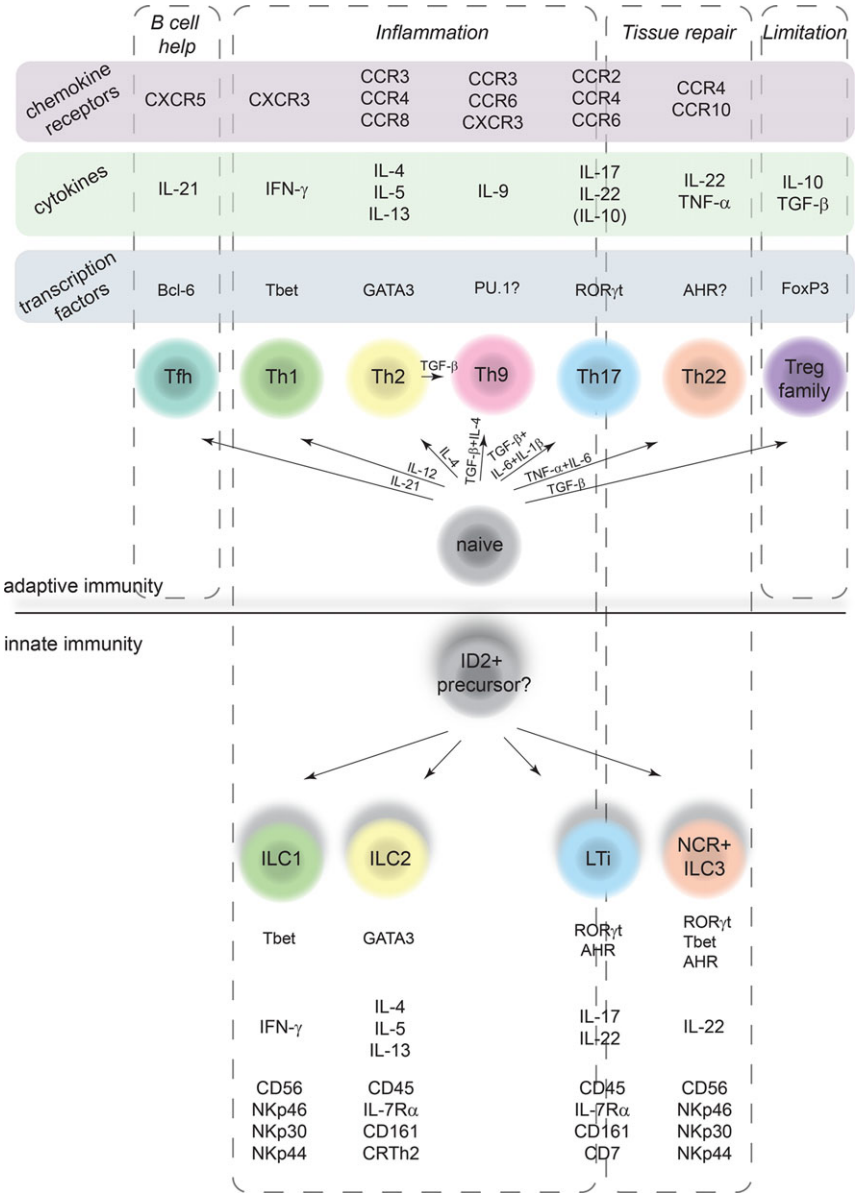

Figure 1. Overview of different T-cell classification approaches. Classically, Th cells are grouped according to their cytokine secretion pattern (cytokine approach, green). Furthermore, expression of lineage-specific transcriptions factors (transcription factor approach, blue) or chemokine receptors (chemokine receptor approach, lavender) is used to assign $CD4^+$ T cells to a certain subset. However, due to the evolving plasticity between subsets, a functional grouping approach seems promising in categorizing Th cells into B-cell, help inflammation, tissue regeneration, and limitation (dashed line boxes). As the Th-cell family is mirrored in innate immunity (bottom), further information and maybe new classification schemes are needed to fully phenotype and group these innate lymphoid cells.

produced IFN-γ and Th2 cells produced IL-4 (Fig. 1). This dichotomous paradigm of Th1 and Th2 subsets persisted for more than 20 years, until about 7 years ago when the emergence of Th17 cells challenged this simplistic dualism of only two Th-cell subsets [2]. The definition of Th17 cells also sparked the concept of a broader heterogeneity in the Th-cell immune compartment (reviewed in [2, 3]). Following the discovery of Th17 cells, which secrete their name-giving cytokine IL-17, other Th-cell subsets emerged on the scene, including Th22 [4–6] and Th9 cells [7], which express the signature cytokines IL-22 and IL-9, respectively. This system of categorization is well-appreciated and immunology textbooks use these terms to distinguish between Th-cell subsets. However, reality is a bit more complex and immunologists are puzzled by the fact that some Th cells are not restricted to these firm lineage boundaries and co-express signature cytokines of distinct subsets in parallel. Th1 or Th2 cells co-secreting IL-17 are two examples of Th-cell subsets that do not fit into the original concept of Th-cell classification. This observation has been attributed to the plasticity of Th-cell subsets. It is still debated how the phenotype of these "plastic" cells is regulated, and if they indeed have to be regarded as distinct subsets [8–10]. This is especially important with respect to the fact that these "hybrid" T cells change their function upon acquisition of additional cytokine secretion properties. That is, IL-17- and IFN-γ-co-expressing cells are considered to be pathogenic in settings of autoimmunity [11], while $IL\text{-}17^+IFN\text{-}\gamma^-$ cells have even been assigned anti-inflammatory functions [12].

In the future, the original Th classification concept will be further challenged by new detection techniques that allow deciphering the full secretome of cells. This overwhelming information will ultimately lead to the question if categorization according to secreted factors is still reasonable.

Subset definition according to lineage-defining transcriptional regulators

Another widely used possibility to classify Th cells is the assignment of lineage-specific transcription factors, which are responsible for the initiation of subset-specific differentiation programs and maintenance of the phenotype (Fig. 1). Tbet, GATA3, and RORC are well-established transcriptional regulators of Th1, Th2, and Th17 cells, respectively. However, this classification concept using master transcriptional activators also has its deficits in delineating Th-cell identity: The aryl hydrocarbon receptor, a ligand-activated transcription factor, has been demonstrated to regulate IL-22 expression, and is also expressed by T cells beyond the Th22 subset (reviewed in [13, 14]). A master transcriptional regulator of human Th9 cells still awaits identification, and even FoxP3, which delineates murine Treg cells, is not exclusively specific for human Treg cells, since it can be upregulated upon polyclonal TCR activation alone [15].

Epigenetics determines the cell-type-specific status of the chromatin landscape. Epigenetic modifications, especially histone modifications and DNA methylation, have been shown to regulate gene accessibility and thus help establish gene expression programs. Inclusion of epigenetics in defining Th subsets allows for better specification of these subsets, and in particular, offers an approximation of their degree of flexibility [16, 17]. Nevertheless, recently a new concept emerged for the specification of Th-cell identity which takes regulatory elements of the genome into consideration. Enhancers are extragenic DNA sequences that mediate the combinatorial recruitment of transcription factors to "enhance" transcription of cognate target genes [18]. They are the accessible part of a cell's genome and are hypersensitive to digestion by DNaseI. New technologies such as genome-wide microarrays and high-throughput sequencing have contributed to establish enhancer landscapes for certain Th-cell subsets (reviewed in [19]). Interestingly, several independent studies demonstrated that these enhancer landscapes determine Th-cell identity irrespective of the putative master transcriptional regulators because the enhancer landscapes of Th1, Th17, and Treg cells were not affected following the deletion of Tbet, ROR-γt, and FoxP3, respectively [20–22]. TCR-dependent signals have been shown to generate the initial phase of the enhancer landscape, which is then followed by modification of cytokine signaling in a STAT-dependent manner. For example, many differentially active enhancers in Th1 and Th2 and Th17 cells have been shown to be STAT4, STAT6, or STAT3 dependent, respectively [20–22]. Master transcriptional regulators therefore rather seem to fine-tune Th-cell functions, while the enhancer landscape sets the tone in response to environmental signals such as microbe-elicited cytokine milieus.

Subset definition according to migration markers

The expression of certain chemokine receptors has significantly contributed to the categorization of Th-cell subsets in humans [23]. The circulating immunological T-cell memory compartment is generally divided into effector memory (T_{EM}) and central memory (T_{CM}) subsets. T_{EM} cells circulate to nonlymphoid tissues whereas T_{CM} cells home to secondary lymphoid organs. They can be distinguished by the expression of CCR7, with T_{EM} cells being $CCR7^-$ and T_{CM} cells being $CCR7^+$ in both humans and mice [24]. Tissue-resident memory T (T_{RM}) cells, which emerged as a novel T-cell subset recently with major functions in first line barrier defense, are also $CCR7^-$ [25] and are retained within peripheral tissues by mechanisms that are not yet fully understood. Here, IL-15 and TGF-β locally produced in the skin [26] and expression of CCR10 [27] combined with lack of KLRG1 [26] expression seem to be important to form and maintain the skin tissue-resident T-cell pool. T_{RM} cells have thus far mainly been studied in mouse models using elegant parabiosis experiments [28], whereas the characterization of human T_{RM} cells has been hampered by low tissue availability.

The differential expression of the chemokine receptor surface antigens CXCR3, CCR4, and CCR6 can be used to distinguish between circulating Th1 ($CXCR3^+CCR4^-CCR6^-$), Th2 ($CXCR3^-CCR4^+CCR6^-$), Th17 cells ($CXCR3^-CCR4^+CCR6^+$) and Th22 ($CXCR3^-CCR4^+CCR10^+$) with high fidelity ex vivo in humans [5, 12, 29]. Recently, we added to this list by introducing a novel population of GM-CSF-only-producing human Th cells, which can be identified by $CXCR3^-CCR4^+CCR6^-CCR10^+$ expression [30]. This elegantly links the cytokine profile of Th cells with specific migration properties, which can be considered correlates of tissue specificity. The co-regulation of chemokine receptor expression and cytokine expression properties during the polarization process can also be induced by certain microbes. *Candida albicans* and *Staphylococcus aureus*, e.g. not only induce IL-17 upregulation on naïve Th-cell precursors but also CCR6 expression [12] in an antigen-specific way in humans. Together, this demonstrates that the differential expression of chemokine receptor surface markers, which marks migration properties, correlates with the functional heterogeneity (cytokine profile) of T-cell subsets.

Th-cell heterogeneity in peripheral tissues

Th cells are generated in secondary lymphoid organs, but mainly fulfill their helper function in peripheral tissues. Therefore, it is of utmost importance to understand not only the phenotype of distinct Th-cell subsets, but also their behavior in a local tissue microenvironment and disease setting. In this section, we highlight the influence of the local tissue on Th-cell homing, antigen specificity, effector function, and differentiation with respect to common skin diseases.

Recirculation versus tissue residency

Another important concept that has recently come to the forefront of immunology is the categorization of Th cells into (re)circulating versus tissue-resident subsets. Although many fundamental findings in human immunology have been made by studying T cells in

the blood, i.e. the discovery of T_{CM} and T_{EM} cells [24], most of the T cells in our body are in fact present in various tissues and not amenable to further analysis by studying the blood immune compartment. In particular, the skin, the biggest human organ, hosts a tremendous number of Th cells (double as much as that in the blood [31], which await further characterization. It is well established that these cells have a substantial impact on host defense and are involved in the pathogenesis of inflammatory diseases such as atopic eczema, allergic contact dermatitis (ACD), and psoriasis.

Recently, long-lived T_{RM} cells have been identified in peripheral tissues, especially the skin (reviewed in [32]). T_{RM} cells do not recirculate as compared to T_{EM} and T_{CM} cells. While the characterization of T_{RM} cells is still in its infancy in humans, mouse studies have recently shed more light on this novel T-cell population, which is best characterized in the $CD8^+$ T-cell compartment. This is due to the preferential use of viral infection models such as models for herpes simplex and human immunodeficiency virus infections and the fact that tissue-resident memory T cells are located in the epidermal skin layer, which in mice is exclusively populated by $CD8^+$ but not $CD4^+$ T cells (reviewed in [33]). In humans, however, $CD4^+$ T cells can reside in the epidermis. Therefore, it can be anticipated that insights gained in mouse models will only reflect the situation in humans with some limitations. Nevertheless, mouse models have so far been crucial for providing evidence of fundamental principles, such as the concept of tissue residency versus tissue recirculation, due to the fact that it is possible to easily perturb the immune system by infections and parabiosis, as well as by virtual unrestricted tissue accessibility for further analysis.

A prerequisite for defining the specific role(s) for Th-cell subsets in tissue is to define how they reach their target organ. In line with a specific chemokine repertoire, distinct Th-cell subsets show characteristic homing abilities. Important chemokine receptors for skin homing are CLA, CCR4, CCR6, and CCR10 (reviewed in [34]). The chemokine receptor CCR10 has been shown to be abundantly present on Th22 cells [5] and reflects a characteristic feature of these cells, namely migration to higher layers of the epithelium according to a CCL27 gradient [35]. In line with this observation, Th22 cells are present in inflammatory skin diseases and predominantly found in the epidermal compartment [4]. This holds also true for other immune cells. For example, Th17 cells induce keratinocytes to secrete CXCL8, which in turn recruits neutrophilic granulocytes into the epidermis and drives the development of neutrophil microabscesses, a hallmark of psoriasis [36]. Thus, not only the differential expression of chemokine receptors but also the chemokine repertoire that distinct Th cells induce in the tissue are critical for their functional abilities. This can have a critical impact on the pathogenesis of tissue-restricted diseases.

Specificity versus bystander activation

Once Th cells reach their target organ, a T-cell activation cascade is necessary to fully activate them. This may happen in different ways. The most common way for activation is a ligation of MHC class II molecules carrying a specific antigen with the T-cell receptor plus co-stimulation during cell–cell contact between Th cells and professional APCs. In the case of differentiated Th cells, the necessity of this co-stimulation is under debate — there are even reports of so-called self-presenting Th cells specific for haptens, such as nickel, that are activated completely independently of APCs [37, 38]. A specific activation of Th cells leads to full activation and secretion of cytokines and chemokines; however, the strength of the stimulus and the point in the cell cycle during which specific activation occurs may influence what cytokines are secreted. Namely, antigen-specific T cells shown, by intracellular cytokine staining, to produce either both IL-4 and IL-17, or IFN-γ and IL-17, were shown to secrete only IL-4 or IFN-γ, respectively, but not IL-17 after stimulation with their cognate antigen and autologous DCs [8]. However, adding staphylococcal-derived enterotoxins induced the co-expression of IL-17 [8]. These enterotoxins — so-called superantigens — are microbial-derived products that activate T cells independently of their receptor specificity by enhancing the binding of TCR/MHC complexes [39], highlighting the necessity of a strong TCR stimulus for induction of IL-17 in T cells. The activation state also seems to be important for the cytokine profile of T cells, since resting Th17-cell clones cannot co-express any IL-10, while prolonged TCR stimulation leads to upregulation of anti-inflammatory IL-10 in a subset of Th17 cells [12]. This highlights that certain functional states of the same cell population, in this case different degrees of activation, can result in different functional outcomes.

However, during an immune response in the skin, only a minority of usually less than 10% of all infiltrating T cells is actually antigen specific. This has been shown in the case of patch test-elicited ACD [36] and atopy patch tests to house dust mite or pollen [8]. This raises the question of the role for these nonspecific bystander cells in the inflammatory reaction. Increasing evidence suggests that such cells may be activated nonspecifically by superantigens. As described before, superantigens are strong inducers for IL-17 and IL-22 in T cells [8, 40]. The skin of about 90% of atopic eczema patients is colonized with *S. aureus*, the source of superantigens, such as staphylococcal enterotoxin B [41]. In contrast, only 25% of the healthy population is colonized with *S. aureus*, but here the nose and not the skin serves as a bacterial reservoir [42]. Applying superantigens to an atopy patch test reaction was shown to lead to aggravation of the developing eczematous lesion, indicating the importance of these factors in an unspecific amplification of inflammation [8].

Beyond bystander activation through superantigens, the role for bystander Th cells during inflammatory processes is still under debate. It might be speculated that all specific immune responses in tissues are accompanied by T cells responsible for preventing an overactivation of the immune response and for regeneration of tissue after the immune response. These tasks are fulfilled by Treg cells and so-called tissue signaling leukocytes, respectively (reviewed in [43]). In addition, the specificity of bystander Th cells is still unclear, but it seems at least in allergen-specific eczema a substantial proportion, in particular of Th17 cells, is specific for

staphylococcal antigens [12, 29] rather than for the eliciting allergen [8, 36]. Furthermore, increasing evidence exists that Th cells recognizing autoantigens may differentiate during the immune reactions in atopic eczema [44], lupus erythematosis [45], or psoriasis [46]. It can be hypothesized that these autoreactive Th cells migrate into the tissue as bystander cells, encounter their antigen and serve as amplifiers of inflammation.

In summary, recruitment of antigen-specific Th cells into tissues initiates a cascade of immune events in the skin that is mediated by the majority of bystander T cells that in parallel migrate to the site of inflammation.

Function of Th-cell subsets in the context of the local microenvironment

Once a Th cell reaches its target organ and is fully activated, it exerts its function via cell contact dependent mechanisms as well as secretion of soluble mediators such as chemokines and cytokines. Roughly, T-cell functions in inflamed tissue are (i) inflammation aimed at clearing the potentially harmful antigen, (ii) limitation of the immune response to prevent a cytokine storm with massive collateral tissue damage, and (iii) regeneration of tissue homeostasis after inflammation. Importantly, all three functional arms have to be in homeostasis, as imbalance of any of these may have negative outcomes (Fig. 2). A simplified view to functionally categorize Th cells would be that IFN-γ-, TNF-α-, and IL-17-producing subtypes are mainly inflammatory, IL-10- and TGF-β-producing T cells are mainly limiting, and IL-22 secretion is mainly associated with coordinating regeneration (Fig. 1). However, most cytokines have overlapping functions and are not exclusively attributable to the aforementioned functions.

Furthermore, the function of a single cytokine critically depends on the context of the local microenvironment. Much progress has been made in understanding T-cell functions in a disease-specific context. This can be exemplified by three model diseases: psoriasis, atopic eczema and ACD that will be discussed separately in the following section.

The pathogenesis of psoriasis is dominated by the Th17 cytokines IL-17, IL-21, IL-22, and TNF-α [30, 47–50]. IL-17 and IL-22 [51] as well as IL-22 and TNF-α [4, 52] co-operatively induce the secretion of antimicrobial peptides by epithelial cells such as human beta defensin 2 and S100 proteins, which prevent microbial colonization. Overrepresentation of IL-22 turns its positive role in tissue regeneration into a pathologic one through the induction of acanthosis, or thickening of the skin [53]. IL-21 has been shown to co-operate with IFN-γ in inducing epidermal hyperplasia [54]. Therefore, the psoriasis-specific cytokine signature results in typical hallmarks of disease, namely rare colonization of skin lesions with microorganisms, the presence of neutrophilic granulocytes in the epidermis, and increased epidermal metabolism resulting in acanthosis and scaling [55]. In addition, the cytokine imbalance of psoriasis is clearly illustrated by therapeutic response to IL-4 [56]. Patients treated with recombinant human IL-4 showed a reduction of clinical scores, lesional Th1 cells, and the IFN-γ/IL-4 ratio, whereas the number of circulating Th2 cells was increased [56]. This study clearly highlights the adjustment of the disease-specific cytokine imbalance as an important therapeutic tool.

In contrast to psoriasis, the skin of atopic eczema patients is frequently colonized by staphylococci, in particular *S. aureus* (reviewed in [57]). This phenomenon is due to a tissue-restricted immune deficiency that relates to the Th2-dominated cytokine microenvironment typically observed in atopic eczema. In vitro, both, IL-4 and IL-13, have been shown to inhibit Th1- [47] and Th17-mediated [8] induction of antimicrobial peptides in epithelial cells via STAT6 and SOCS molecules [58]. The clinical relevance of these two opposing T-cell cytokine signatures has been shown in vivo in a rare population of patients suffering from both psoriasis and atopic eczema in parallel [50]. In such patients, only eczema lesions, but not psoriasis plaques, were colonized by *S. aureus* [50]. Beyond insufficient epithelial immunity, a second hallmark of atopic eczema is an impaired epidermal barrier with consequent transepidermal water loss and dryness of the skin (reviewed in [59]). While mutations in genes of the epidermal differentiation complex, such as filaggrin, are strongly associated with atopic eczema, a Th2-dominated microenvironment also damages the epidermal barrier by downregulating filaggrin and other genes of the epidermal differentiation complex [60–62]. Thus, Th2 cytokines antagonize Th1 and Th17 immunity in the skin and largely explain the phenotype of atopic eczema [57].

A third cutaneous model disease is ACD. Here, small and harmless molecules (haptens) such as nickel elicit an acute eczematous immune response characterized by T-cell cytotoxicity and keratinocyte apoptosis [63, 64]. The clinical phenotype of ACD is largely explained by the cytokine content of the local microenvironment. Depending on the eliciting hapten, a mixed T-cell infiltrate is observed with dominating Th1 cytokines. In such a microenvironment, IL-17 functions as an amplifier of non-specific T-cell apoptosis mediated by IFN-γ [36] and enhances the cytotoxic immune response typical for ACD.

In summary, the function of T-cell cytokines strongly varies depending on the cytokine content of the local microenvironment. Therefore, the function of Th-cell subsets has to be interpreted within the context of the microenvironment and disease setting.

Interplay of environment and Th-cell differentiation: The question of hen and egg

The differentiation requirements for Th cells have been a major focus of research over the past few years. For example, Th17-cell priming requirements have elicited disputes, primarily due to inconsistencies between mouse and human cytokine requirements and in particular due to the controversial role of TGF-β in Th17-cell differentiation [65]. Although Th-cell polarization is a multilayered process that is dependent on signal strength and the engagement of different co-stimulatory molecules following antigen processing, and the establishment of a complex immunological synapse, the focus of interest has been on cytokine requirements.

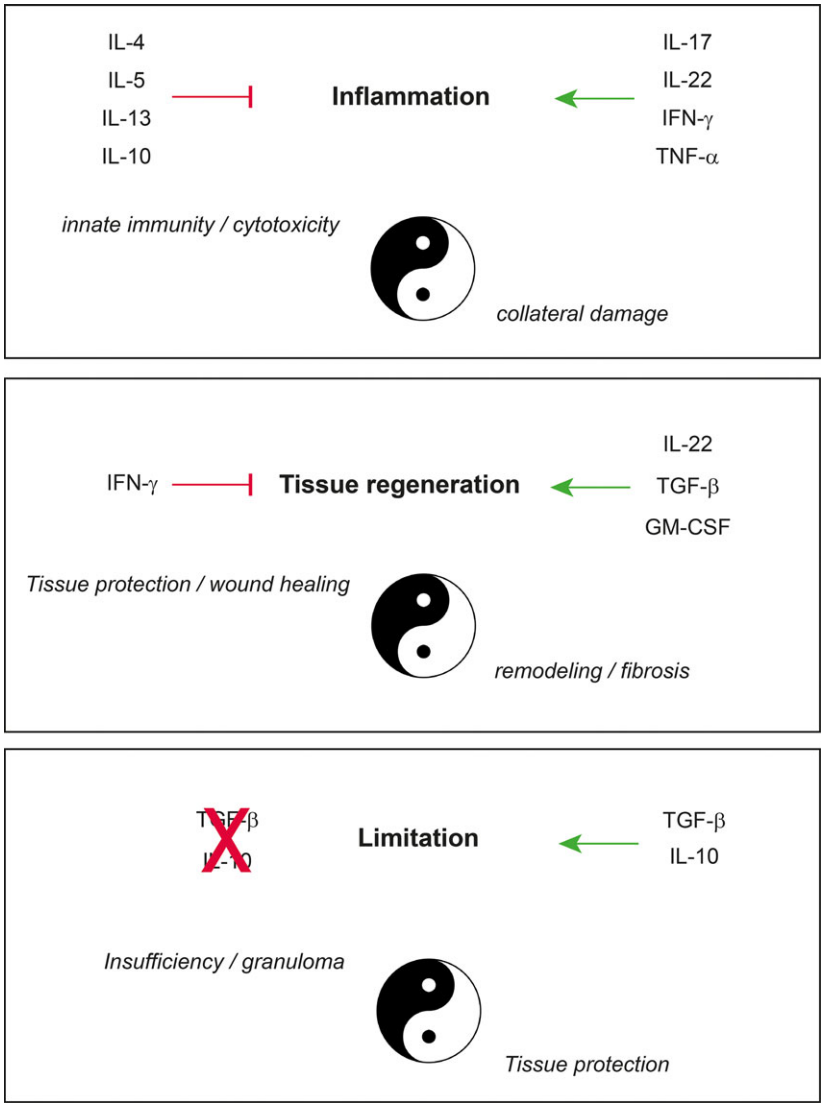

Figure 2. The Yin and Yang of Th cytokine function in tissue inflammation. Protection of life from external and internal harm is granted if tissue regeneration (top), inflammation (middle), and limitation of immune responses (bottom) are well balanced. Imbalances in one or the other process will lead to dysregulated immune responses with various outcomes. These imbalances may occur due to changes in Th-cell presence and subsequent changes in cytokine composition of the local microenvironment. In this case, a positive aspect of inflammation, namely enhanced innate immunity or cytotoxicity after microbial invasion, can turn pathologic if the collateral tissue damage exceeds an acceptable threshold (middle). Furthermore, tissue regeneration, which is mediated by wound-healing processes, can lead to tissue remodeling and fibrosis in the context of overexpression of IL-22, TGF-β, and GM-CSF in the tissue (top). Limitation of immune responses is necessary to prevent excessive tissue damage, but cannot take place if the mediating cytokines, TGF-β and IL-10, are limited in tissue (bottom). Therefore, Th-cell function always has to be considered in the context of a local tissue/disease microenvironment.

Most of the approaches to dissect Th17 priming conditions have therefore used polyclonal stimulation of naïve T cells with anti-CD3 and anti-CD28 antibodies in the presence of well-defined cytokine combinations in vitro. However, human Th17-cell polarization following antigen-specific stimulation with microbes has recently revealed that priming requirements differ, depending on microbial antigen specificity even within the same class of Th cells [12]. Microbial ligands that generate Th17-cell responses through TLR and CLR signaling have primarily, although not exclusively, been defined for *C. albicans* [66, 67]. Fungal components have been shown to bind to Dectin1, Dectin2, and Mincle expressed on APCs, which leads to the recruitment of the tyrosine kinase Syk, activation of the adaptor CARD9, and finally to secretion of IL-23, IL-1, IL-6 [66, 67], which are involved in the generation of human Th17 cells. Interestingly, the generation of *C. albicans*-specific human Th17 cells has been shown to be highly dependent on IL-1β, while *S. aureus*-specific Th17 cells can be primed in its absence [12]. This not only indicates different pathways for the generation of human Th17 cells but also a strong link between microbial antigen specificities of Th cells with their respective priming requirements. This has important consequences for the functionality of Th17 cells, since *C. albicans*-specific, and thus IL-1β-dependent Th17 cells have been shown to co-express IL-17 and IFN-γ but not IL-10, while *S. aureus*-specific Th17 cells have been shown to be IFN-γ negative but IL-10 positive [12]. IL-1β therefore acts as a molecular switch factor for the generation of pro- versus anti-inflammatory Th17-cell properties [3, 68].

A model disease to exemplify the two-sided interactions of environment and Th cells is chronic mucocutaneous candidiasis, a rare disease characterized by chronic and persistent infection of skin and mucosa with *Candida* species [69]. Numerous mutations affecting the differentiation and function of Th17 cells have been described for chronic mucocutaneous candidiasis. Namely, humans with loss-of-function mutations in CARD9 and STAT3 or gain-of-function mutations in STAT1 have reduced Th17 cells [70–72]. In other families, IL-17 or its receptor is

mutated, or autoantibodies against IL-17 are secreted [73, 74]. Beyond the Th17-cell deficiencies associated with inherent genetic defects, immune perturbation with biologics such as anti-IL-17 (Sekukinumab) or anti-p40 (Ustekinumab) for the treatment of chronic inflammatory diseases such as psoriasis could mimic deficiency in Th17-cell numbers or effector functions, and may likewise cause infection [75, 76].

Together, this exemplifies the difficulties in answering the hen and egg question. However, it also highlights the close interaction of the environment and T cells with the impact of microbes on Th-cell differentiation, on the one hand, and, on the other hand, the impact of specific Th-cell subsets on microbial colonization and infection risks [77]. Dysbiosis of the human skin or mucosal surfaces is therefore prone to result in alterations in Th subset composition and thus potentially in immune mediated skin diseases.

Concluding remarks

The increasing diversity of Th cells has introduced difficulties in the assignment of observed phenotypes to a certain subset. Approaches to grouping Th cells according to cytokine secretion, master transcriptional regulators, or chemokine receptor profiles are widely used but still not sufficient to explain heterogeneous phenotypes. Furthermore, Th cells exert their function in a complex, tissue- and disease-specific microenvironment influencing the migratory capacity, activation, and behavior of T cells. Further investigation is needed to elucidate these complex interactions leading to a comprehensive understanding on T-cell function and to new and sophisticated classification approaches for Th cells.

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Abbreviation: **ACD**: allergic contact dermatitis

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