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Regulation of Human Helper T Cell Subset Differentiation by Cytokines

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Abstract

Since the discovery of Th1 and Th2 cells in the late 80's, the family of effector CD4⁺ helper T (Th) cell subsets has expanded. The differentiation of naïve CD4⁺ T cells is largely determined when they interact with dendritic cells in lymphoid organs, and cytokines play a major role in the regulation of Th differentiation in the early stages. Recent studies show that the developmental mechanism of certain Th subsets is not fully shared between mice and humans. Here we will review recent discoveries on the roles of cytokines in the regulation of Th differentiation in humans, and discuss the differences between mice and humans in the developmental mechanisms of several Th subsets, including Th17 cells and T follicular helper (Tfh) cells. We propose that the differentiation of human Th subsets is largely regulated by the three cytokines, IL-12, IL-23, and TGF- β .

Introduction

The family of Th subsets has expanded during the past two decades, and currently includes regulatory T cells (Treg), Th17, Tfh, Th9, and Th22 cells. These subsets are largely defined by the cytokines that each subset expresses, except FoxP3⁺ thymus-derived Tregs and T follicular helper (Tfh) cells. Thymus-derived Tregs are defined by the expression of the transcription factor FoxP3 and their suppressive functions. Tfh cells are often defined by the combination of markers (such as CXCR5, ICOS, PD-1, and Bcl-6) and their follicular localization in vivo, although they function by secreting IL-21 and IL-4 (and IL-10 in some cases) [1].

Studies with in vivo mouse models have significantly contributed to understand the developmental mechanism of each Th subsets. However, significant differences have been introduced in the immune system of humans and mice during more than 60 million years of independent evolution, and conclusions demonstrated in mouse studies are sometimes not fully translated to humans [2]. In the context of Th differentiation, it is becoming clear that the developmental mechanism is not fully shared between mice and humans in certain

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subsets. In this review, we will summarize the current knowledge on the cytokine conditions promoting the development of each Th subset in humans. We classify the Th subsets into two groups according to the similarities in the developmental mechanism between mice and humans: one with large similarities (Th1, Th2, Th9, and Th22) and the other with some differences (induced Treg, Th17, and Tfh). Then we will discuss how cytokines regulate Th differentiation programs in humans.

Th subsets with similar developmental mechanisms between mice and humans

Th1

IL-12 was discovered in the early 90's to play the major role for the generation of Th1 cells in both mice and humans [3,4]. In 1995, STAT4 was identified as the major transcription factor mediating the IL-12 signals, and in 2000, the transcription factor T-bet was discovered to be essential for Th1 development [5]. IFN- γ also contributes to the expression of IFN- γ and T-bet via STAT1 activation [5]. These major pathways associated with the generation of Th1 cells are largely shared between mice and humans. For example, Th1 generation is severely impaired in subjects who lack the expression of functional IL-12 and/or IL-12 receptor, due to mutations of *IL12B* (encoding IL-12p40 subunit common to IL-12 and IL-23), *IL12RB1* (encoding the β 1 chain for the receptors of IL-12 and IL-23), *IRF8* (associated with the development of IL-12-producing dendritic cells (DCs)), and *ISG15* (a molecule that acts in synergy with IL-12) [6].

Th2

In early 90's, IL-4 was discovered as critical cytokines for the generation of Th2 cells in vitro in mice. STAT6 was identified as the main transcription factors downstream of IL-4 signals in 1996, and the transcription factor Gata3 was discovered to be essential for in vivo Th2 development in 1997 [7]. In addition to the IL-4-STAT6, low signals via T cell receptor (TCR) were found to play an important role for the initial expression of Gata3 in activated CD4⁺ T cells [8]. These mechanisms associated with Th2 development are also largely shared between mice and humans [9]. A recent study identified a set of candidate transcription factors associated with the generation of human Th2 cells through genome-wide profiling of histone modifications in human blood CCR4⁺ CD4⁺ T cells (that are enriched with Th2 cells) [10]. The set of the identified transcription factors contains Gata3 and Stat5, but also includes many transcription factors previously not implicated in Th2 cell differentiation. Whether and how these newly identified transcription factors contribute to Th2 cell differentiation in humans and/or mice remain to be determined. It is still possible that eventually the transcriptional network regulating Th2 cell differentiation turns out to be somewhat different between mice and humans.

Th9

Early studies performed in the 90's demonstrated that IL-9 secretion was largely associated with Th2 cells [11]. However, it was also shown that the cytokine combination of IL-4, TGF- β , and IL-2 can induce naïve CD4⁺ T cells in vitro to become producers of IL-9, but no

other Th2 cytokines [12]. The in vivo presence of CD4⁺ T cells producing primarily IL-9 was eventually demonstrated in 2008 (termed as Th9 cells), and Th9 cells are found to be associated with antitumor immunity, allergy, and autoimmune diseases [13]. Current evidence shows that both in mice and humans, IL-4-STAT6 and TGF- β play a key role for the generation of Th9 cells as well as IL-9 expression by other Th subsets [14,15]. Furthermore, the transcription factors Gata3, IRF-4, and PU.1. induced by the combination of IL-4-STAT6 and TGF- β signals were shown to be important for IL-9 expression in both mouse and human CD4⁺ T cells [11]. However, it is of note that recent studies show differences in the regulation of IL-9 expression between mice and humans. For example, in humans, IL-21 promotes IL-9 expression by naïve CD4⁺ T cells primed in the presence of IL-4 and TGF- β [16]. However, in mice, IL-21 strongly inhibits IL-4 and TGF- β -driven IL-9 expression by promoting the expression of transcriptional repressor Bcl-6 [17]. Thus, the magnitude and the quality of Th9 response is likely regulated in a different fashion between mice and humans due to differences in the biological activity of certain cytokines on CD4⁺ T cells.

Th22

There is ample evidence that CD4⁺ T cells that primarily secrete IL-22 but no IL-17 or IFN- γ constitute a subset distinct from Th17 and Th1 cells (termed Th22 cells), in particular in humans. First, Th22 cells express only low amounts of ROR γ t [18], the transcription factor essential for Th17 cell generation [19], and conversely transfection of ROR γ t does not promote human CD4⁺ T cells to express IL-22 [20]. Second, while TGF- β contributes to the generation of Th17 cells (as discussed later), IL-22 expression is inhibited by TGF- β in both humans and mice. This effect (at least in mice) is attributed to the repressor function of the transcription factor c-Maf activated by TGF- β [21]. Third, Th22 clones established from patients with psoriasis were remarkably stable and demonstrated gene profiles distinct from Th1, Th2, and Th17 clones [22]. Yet, a recent mouse study using an IL-22 fate reporter questioned the existence of stable Th22 cells, in contrast to the presence of $\gamma\delta$ cells and innate immune lymphocytes that constitutively produce IL-22 [23]. Thus, the stability of Th22 cells might be different between mice and humans.

In humans, IL-22 expression is promoted by IL-23, IL-12, and IL-6+TNF α and inhibited by TGF- β [18,20,24]. However, IL-22⁺ cells induced in vitro by culturing human naïve CD4⁺ T cells contain cells co-expressing IFN- γ and/or IL-17A, and the precise mechanism that induces bona fide Th22 cells remains to be established. Given that IL-22 expression is promoted by agonists of aryl hydrocarbon receptor (AhR) and AhR expression is associated with IL-22 expression in both mice and humans [25], AhR signals are likely necessary in addition to cytokine signals for the generation of Th22 cells.

Th subsets that show different developmental mechanisms in humans

Induced Treg

While mouse naïve CD4⁺ T cells primed in the presence of IL-2 and TGF- β express high levels of Foxp3, and acquire the capacity to suppress T cell response (termed induced Tregs), human naïve CD4⁺ T cells primed in the same condition do not suppress T cell

response despite high expression of Foxp3 [26]. While there is evidence that the addition of retinoic acid or rapamycin to the combination of IL-2 and TGF- β renders human naive CD4⁺ T cells to become FoxP3⁺ suppressors [27,28], their stability remains unknown. Whether the conversion of naïve CD4⁺ T cells into FoxP3⁺ Tregs can happen in vivo in humans also remains unclear. Human blood FoxP3⁺ Tregs contain CD45RA⁺CD25⁺ “resting” subset and CD45RA⁻ CD25⁺⁺ “activated” subset [29]. Transcriptional analysis of CD45RA⁻ CD25⁺⁺ “activated” Tregs at a single cell level might reveal heterogeneous subpopulations which might contain converted Tregs at periphery.

Th17

The observation that mouse CD4⁺ T cells express IL-17 in response to the combination of IL-6 and TGF- β was first reported in 2006 [19]. Shortly after, however, it was found that this cytokine combination does not induce human CD4⁺ T cells to express IL-17 [30]. Early studies demonstrated that IL-1 β and IL-23 were important to drive the differentiation of human naïve CD4⁺ T cells into IL-17-producers, and that TGF- β inhibited their generation [31,32]. In contrast, later studies showed that TGF- β is indeed essential for the development of human Th17 cells, and acts together with other cytokines such as IL-1 β , IL-6, IL-21, and IL-23, but not IL-12 [20,33]. Indeed, among the human Th17-promoting cytokines, TGF- β is the most potent to induce human cord-blood derived naïve CD4⁺ T cells to express ROR γ t (the transcription factor essential for Th17 development [19]) and CCR6 (the chemokine receptor expressed by human Th17 cells [31]) [20,34]. The discrepancy regarding the impact of TGF- β in human Th17 cell generation seem to be derived from the differences in the purity of truly naïve CD4⁺ T cells and the differences in the culture media used in the experiments. In particular, some serum components including TGF- β and AhR ligand were found to largely affect the expression of IL-17 in cultured human CD4⁺ T cells [20,35]. Importantly, similar to observations in mice [19], stimulation with TGF- β by itself is insufficient to induce human naïve CD4⁺ T cells to express IL-17, and needs to co-operate with STAT3-activating cytokines such as IL-21 and IL-23 [20,34,36]. This is further supported by the observation that patients with hyper IgE syndrome who lack the expression of functional STAT3 are completely devoid of Th17 cells [37].

Another line of evidence that TGF- β is important for the generation of human Th17 cells was obtained from studies on CD161⁺ CD4⁺ T cells, a cell population proposed to be the direct precursor of Th17 cells in humans [38]. CD161⁺ CD4⁺ T cells are already found in newborn thymus and cord blood, and these cells isolated from cord blood constructively express ROR γ t, IL23R, CCR6, but not IL17. The expression of IL-17 by these cells requires stimulation with a combination of IL-1 β and IL-23 together with anti-CD3 and CD28, but not TGF- β [38]. However, after stimulation with IL-1 β and IL-23, a majority of CD161⁺ CD4⁺ T cells become Th1 cells expressing T-bet and IFN- γ . Supplementation of TGF- β into the culture strongly increases the generation of IL-17⁺IFN- γ ⁻ cells by inhibiting the generation of IFN- γ [39]. Thus, TGF- β plays an important role in skewing the differentiation of human naïve CD4⁺ T cells towards the Th17 lineage and away from the Th1 lineage [34], even of cells pre-committed to the Th17 lineage.

There are several differences in the type of cytokines regulating the early differentiation stage of Th17 cells between mice and humans. Among STAT3-activating cytokines, while IL-6 plays a dominant role in mice, IL-6 seems to play only a supportive role in humans [36]. In contrast, while IL-23 plays the major role in the differentiation of Th17 cells in humans, IL-23 is more associated in mice with the maturation of generated Th17 cells into inflammatory cells [19]. The difference of the importance of IL-23 in the early generation of Th17 cells is due to the differences in the regulation of IL-23 receptor expression by naïve CD4⁺ T cells. While mouse naïve CD4⁺ T cells express IL-23 receptor only after priming in the presence of IL-6 and IL-21 [19], human naïve CD4⁺ T cells can immediately respond to IL-23 (even those in cord blood), and the IL-23 receptor expression is further enhanced by IL-23 signals [20,30].

Another puzzling difference between humans and mice is the role of IL-2. While IL-2 suppresses IL-17 expression by mouse CD4⁺ T cells both in vitro and in vivo [40], IL-2 promotes the generation of human Th17 cells in vitro [20] and IL-2 treatment did not affect the number of blood Th17 cells in HIV-infected subjects [41]. Multiple mechanisms are involved in the suppression of mouse Th17 cell generation by IL-2-STAT5: 1) STAT5 competes with STAT3 for binding to the sites in the *IL17a* locus, 2) IL-2 inhibits the expression of receptors for IL-6, and 3) IL-2 promotes the expression of T-bet, which suppresses the expression of ROR γ t [42]. Why IL-2 does not inhibit the generation of human Th17 cells remains unknown.

Tfh

In the early 2000s, studies on CD4⁺ T cells in human tonsils demonstrated that the cells expressing CXCR5 display a superior capacity to induce B cells to produce immunoglobulins in vitro as compared to CD4⁺ T cells lacking CXCR5 expression. Based on their localization and functions, tonsillar CD4⁺ T cells were designated Tfh cells [1]. In 2009, the transcription repressor Bcl-6 was discovered as an essential factor for Tfh cell generation in vivo in mice, and TFH cells were established as an independent Th lineage [1].

The differentiation and maturation process of Tfh cells is more complicated than that of other subsets, and composed of three phases: 1. Initial differentiation towards the Tfh-lineage, 2. Migration into B cell follicles and interaction with B cells, and 3. Maturation in germinal centers. Therefore, as compared to mouse models where the generation of Tfh cells can be assessed in vivo, determining the developmental mechanism of Tfh cells in humans has been a challenge. Importantly, however, multiple mouse studies have demonstrated that the differentiation process of Tfh cells initiates when naïve CD4⁺ T cells interact with DCs loaded with antigens in secondary lymphoid organs [43]. Programming towards the Tfh cell lineage occurs as early as the first few divisions [44–47]. Therefore, to determine the type of cytokines promoting human Tfh cell generation, it is a reasonable strategy to analyze the expression of multiple Tfh molecules (such as CXCR5, PD-1, ICOS, CD40L, Bcl-6, and IL-21) by naïve CD4⁺ T cells stimulated for a few days in vitro (by anti-CD3 and anti-CD28) in the presence of different cytokines.

Recent studies show that the dominant cytokines associated with the development of Tfh cells differ between mice and humans. In mice, IL-6 and IL-21 (which activate STAT3)

have dominant roles [48,49], while IL-12 (which mainly activates STAT4) can also participate in the early phase [50]. In contrast, in humans, IL-12 induces higher expression of IL-21, ICOS, CXCR5 and Bcl-6 on activated naive helper T cells than IL-6, IL-21, and IL-27 [34,51,52]. Consistently, the subjects that lack the expression of functional IL-12 receptor β 1 chain (IL-12R β 1), a common receptor for IL-12 and IL-23, display reduced blood memory Tfh cells and memory B cells, and altered GC formation in lymph nodes [51]. Furthermore, the affinity of IgG against tetanus toxoid in these subjects was significantly lower than that in controls, supporting altered GC responses [51]. Our recent study further identified that TGF- β plays an important role for Tfh cell generation in humans [34]. In the presence of TGF- β , IL-12 and in particular IL-23 promote the expression of multiple Tfh molecules including CXCR5, IL-21 and Bcl-6 on activated naïve CD4⁺ T cells [34]. TGF- β also contributes to the generation of Tfh cells by strongly suppressing the expression of Blimp-1 [34], a transcription repressor that inhibits the function of Bcl-6 [48]. These cytokine combinations also induce human naïve CD4⁺ T cells to express multiple other transcription factors such as c-Maf and Batf that are essential for Tfh development. This is in stark contrast to mouse CD4⁺ T cells, in which TGF- β signals suppress expression of Tfh molecules including IL-21, ICOS and Bcl-6 [34,53–55].

Furthermore, the role of IL-2 might be different between mice and humans. In mice, IL-2-STAT5 strongly inhibits the generation of Tfh cells by promoting the expression of Blimp-1 [56,57] and activating mammalian target of rapamycin (mTOR) [58], while inhibiting the expression of Bcl-6 [59]. Consistently, while CXCR5⁺Bcl-6⁺ CD4⁺ Tfh precursors generated shortly after the interactions with DCs express IL-2R α only at low amounts, CXCR5⁻Bcl-6⁻ CD4⁺ T cells highly express IL-2R α [45]. Intriguingly, a recent study showed that TGF- β contributes to the generation of Tfh cells in mice infected with influenza virus. Yet, such positive regulation by TGF- β seems dependent on the suppression of the expression of IL-2R α by primed CD4⁺ T cells, providing further evidence demonstrating the significance of IL-2 signals in Tfh cell generation in mice [58]. However, in humans at least in vitro, there is no difference in the expression of IL-2R α between CXCR5⁺ and CXCR5⁻ cells developed from naïve CD4⁺ T cells (Schmitt et al, unpublished observations). Whether and to which extent IL-2-STAT5 regulates human Tfh cell generation remains to be determined.

Regulation of the differentiation of human Th subsets

We propose that the development of distinct Th subsets in humans is largely regulated by the three cytokines: IL-12, IL-23, and TGF- β . In this context, the differentiation of Th subsets can be viewed as vectors starting from the origin in a three dimensional model (Figure 1). The three cytokines IL-12, IL-23, and TGF- β define the three axes (herein defined as X, Y, Z axis respectively). First, human Th subsets can be largely split into two groups according to the role of TGF- β in their differentiation: one group promoted by TGF- β (Tfh, Th17, and Th9) and another inhibited by TGF- β (Th1, Th2, and Th22). Second, another two groups can be defined by the role of IL-12 and IL-23: one promoted by IL-12 (Th1, Tfh, and Th22), another promoted by IL-23 (Th17, Tfh), and the other inhibited by IL-12 or IL-23 (Th2 and Th9). In this 3D model, many human Th subsets can be placed on the vertex of the cube.

This model provides several important concepts. First, the developmental vector is similar between Tfh cells ($X^+Y^+Z^+$) and Th17 ($X^-Y^+Z^+$) cells. With this regard, we recently demonstrated that human Th17 cells generated by culturing naïve $CD4^+$ T cells (by the combination of TGF- β , IL-1 β , IL-6, and IL-23) were found to express multiple Tfh molecules including CXCR5, ICOS, PD-1, IL-21, and Bcl-6. Furthermore, in human tonsillar germinal centers, some Tfh cells were found to co-express Bcl-6 and ROR γ t [34]. These observations suggest that the developmental mechanism is largely shared between Tfh and Th17 cells in humans. Whether the common precursors differentiate into mature Th17 cells and Tfh cells might be dependent on the amount of IL-12 exposed during the priming and/or whether the common precursors subsequently interact with B cells or not.

Second, the development of Tfh cells ($X^+Y^+Z^+$) and Th2 cells ($X^-Y^-Z^-$) is regulated in totally opposite directions. This is ironic as both subsets are/had been considered as the major Th subsets promoting antibody response. While both subsets can produce IL-4, the molecular mechanism associated with IL-4 production is different at least in mice: The conserved noncoding sequence 2 is an essential enhancer element for IL-4 expression in Tfh cells, but not in Th2 cells [60]. In germinal centers of human tonsils, Tfh cells do not express GATA3, while some co-express ROR γ t or T-bet together with Bcl-6 [34]. These observations suggest that Th1 and Th17-rich environment inhibits the generation of Tfh cells expressing GATA3. Despite totally different developmental mechanisms, there is evidence that some Th cells display properties of both Tfh and Th2 cells. First, in mice infected with *N. brasiliensis*, GATA3 $^+$ Tfh cells are present in the draining lymph nodes [61]. Second, blood memory Tfh cells in humans contain a subpopulation expressing Th2 cytokines (IL-4, IL-5, and IL-13) and GATA3 [62], although the origin of blood memory Tfh cells remains to be established [63]. However, how Th2-type Tfh cells develop in humans remains unknown. Given a Th2-prone cytokine IL-4 strongly inhibits the expression of IL-21 by human $CD4^+$ T cells [62], probably their development is regulated by other factors, such as the strength and the duration of signals via T cell receptor during the priming [64], and/or various co-stimulatory molecules expressed by DCs [65].

Conclusions

Some differences have been discovered in the regulation of Th differentiation between mice and humans. There is little doubt that it will be increasingly important to analyze human samples (blood, tissues, urine, body fluids etc) and assess whether the findings in mouse models can be translated to humans. Nonetheless, many mechanisms in Th differentiation are remarkably shared between mice and humans, and thus mouse studies will continue to provide important templates to test the pathways in humans. The differences in the Th development between the species seem due in part to the differences in the regulation of cytokine receptor expression and the differences in the biological impact of cytokines on T cell biology. These differences might be also associated with distinct epigenetic modifications. Yet, the molecular mechanisms that regulate the Th differentiation programs in humans have just started getting revealed. Thorough re-evaluation of the cytokine effect on human Th cells including epigenetic modifications will be useful to understand the regulatory mechanisms of human Th differentiation in depth and beneficial to develop novel therapeutic approaches in diseases.

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Highlights

- Development of Th1, Th2, Th9, and Th22 is largely similar between mice and humans.
- TGF- β and STAT3 signals promote Th17 differentiation in both species.
- Some differences exist in the development of induced Tregs, Th17, and Tfh cells.
- Human Th differentiation is largely regulated by IL-12, IL-23, and TGF- β .

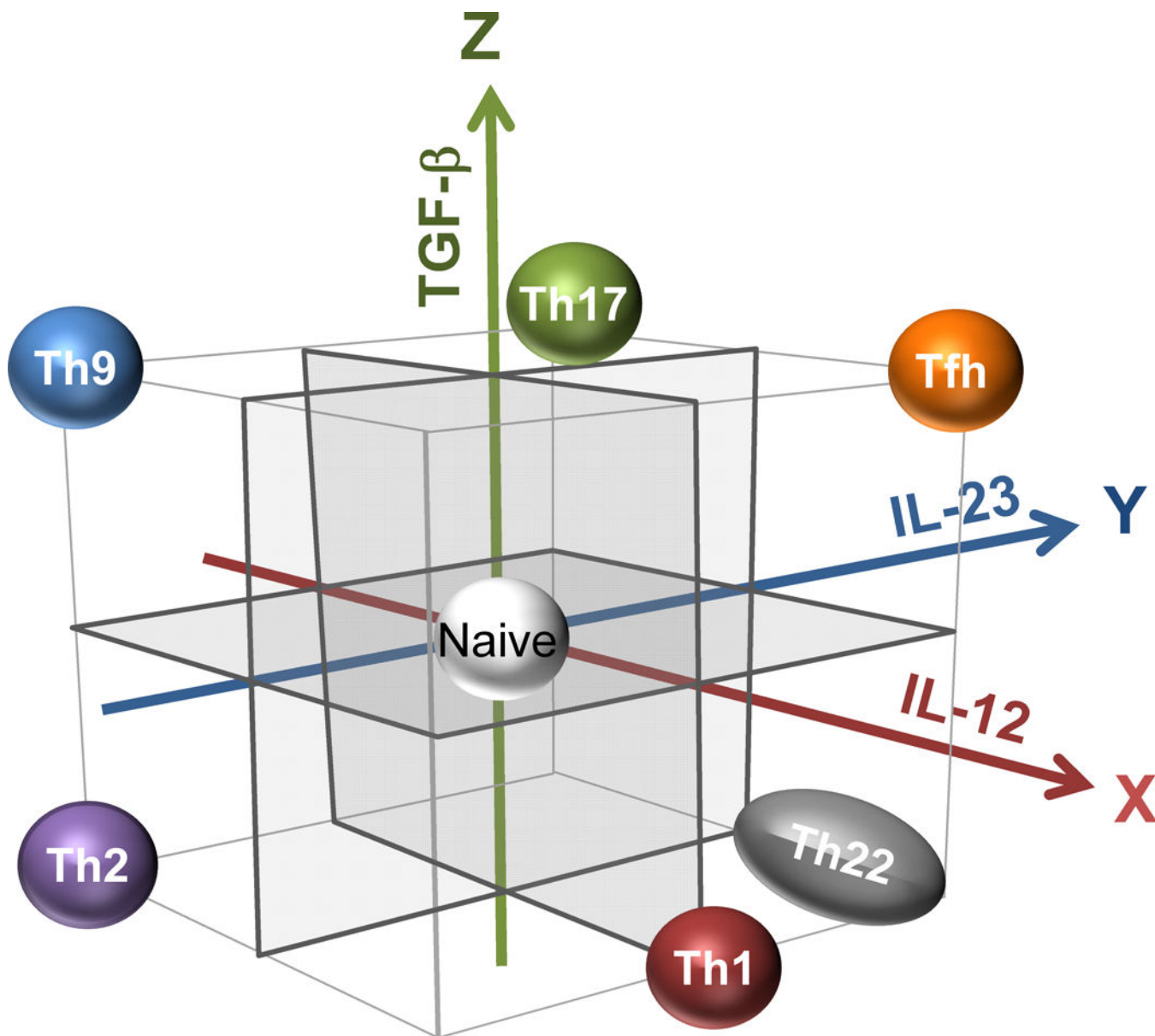


Figure 1. Regulation of human Th differentiation by IL-12, IL-23, and TGF- β

The differentiation of Th subsets can be viewed as vectors starting from the origin in a three dimensional model. The three cytokines IL-12, IL-23, and TGF- β define the three axes.

With this model, many Th subsets are placed on the vertex of the cube. Human Th subsets can be largely split into two groups according to the role of TGF- β in their differentiation: one group promoted by TGF- β (Tfh, Th17, and Th9) and another inhibited by TGF- β (Th1, Th2, and Th22). Second, other groups can be defined by the role of IL-12 and IL-23: one promoted by IL-12 (Th1, Tfh, and Th22) or by IL-23 (Th17, Tfh, and Th22), and another inhibited by IL-12 or IL-23 (Th2 and Th9).