The Effects of Cytokines on T Cell Differentiation

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Abstract

The T cell is an important member of the adaptive branch of the immune system, with many varied, sometimes overlapping, roles. One mechanism by which the immune system is able to provide a defense against the wide range of pathogens and conditions affecting the body is by differentiation of this key cell type into many distinct lineages and subsets, each with a characteristic profile of effector molecules with distinct functions. The complex nature of immunity to the range of potential pathogens is mirrored by the complexity of T cell lineages and subsets which have evolved to defend against them. The many potential combinations of antigen-stimulation and co-stimulatory molecules, cell-cell interactions, and the cytokine milieu in which T cells are activated all contribute to the cell's fate, and therefore the cell's function in the immune system as a whole.

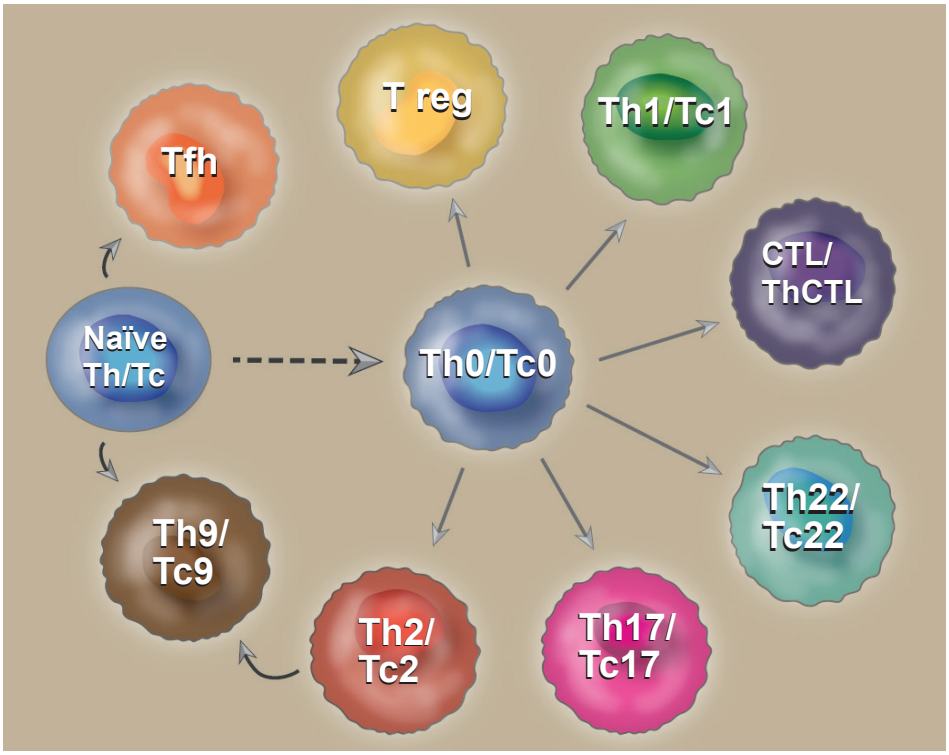

Fig. 1: *T cell differentiation into effector cells expressing a phenotype matching one of several subsets.*

Introduction

T cells are a critical component of adaptive immunity, responsible for cytokine production, direct cytotoxicity, regulation and memory, among other activities. These varied functions are made possible by the differentiation of T cells into a variety of lineages, or subsets, each responsible for specific, sometimes overlapping, functions in the immune system.

T cells arise from T cell precursors which differentiate from hematopoietic stem cells in the bone marrow, then migrate to the thymus. In the thymus, these cells undergo positive and negative selection, concomitant with development of the alpha and beta chains of the antigen-specific T cell receptor (TCR). The T cells emerge from the thymus as single-positive (either $CD4^+$ or $CD8^+$) naïve T cells, capable of recognizing a specific cognate antigen.

These functional, but immature, T cells exit the thymus to the periphery in the body, and await stimulation through their TCRs. T cells, unlike many other immune cells, recognize antigen only when it is presented to them by an antigen presenting cell (APC), such as a dendritic cell (DC), a macrophage, or a B cell. Thus, in the periphery, naïve T cells must encounter an APC presenting the appropriate cognate antigen to become activated. This interaction typically occurs in a secondary lymphoid organ, such as a lymph node or the spleen, where the structure of the organ is optimized to expose T cells to many APCs bearing a variety of antigens collected

in the periphery.

Upon a T cell's recognition of its cognate antigen, presented by an APC, several stimulation events may happen that lead to activation of the T cell. The first signal, TCR stimulation (which can be mimicked *in vitro* by binding of a stimulatory anti-CD3 antibody) is insufficient to fully activate the cell, and can lead to T cell anergy (or hyporesponsiveness to its cognate antigen). Simultaneous engagement of a co-stimulatory molecule on the T cell, such as CD28 interacting with one of its ligands (CD80/CD86) on the APC (or simulation of this interaction by *in vitro* use of antibodies or soluble ligands), further stimulates the cell and can result in downstream signaling sufficient to activate it. The combination of TCR and co-stimulatory signals stimulates autocrine production of IL-2 (1), which aids in survival and proliferation of T cells during priming (2), and differentiation into effector cells expressing a phenotype matching one of several described subsets (Figure 1).

For many of the T cell subsets described herein, there is evidence that both $CD4^+$ (Th) and $CD8^+$ (Tc) naïve cells are capable of exhibiting the phenotype and producing effector molecules associated with those subsets (3,4). As such, the terminology "Th/c" will indicate those phenotypes which apply to both $CD4^+$ and $CD8^+$ T cells.

Subsets

Differentiation from an un-skewed "Th/c0" cell into a T cell expressing the markers and characteristics of one of the described T cell subsets requires various signaling events to occur. After "signal 1" through the TCR, and "signal 2" co-stimulation, additional input is required by the T cell, often in the form of cytokines. Cytokine signaling through cell surface receptors leads to activation of particular intracellular pathways which enable lineage commitment (5). This results in expression of transcription factors particular to the lineage, and subsequent differentiation by the T cell into an effector cell with the phenotype matching one of the described subsets of Th or Tc.

The differentiation of naïve T cells into effector cells is the matter of much study, in humans, mice and other animal models. The process *in vivo* is, like the immune system itself, complex. Stimulation of cells by pathogens or tissue damage involves multiple antigens, receptor-ligand interactions, and a complex milieu of chemokine and cytokine signaling. As a result, a spectrum of T cell phenotypes is possible. These phenotypes were traditionally classified into Th1 and Th2 lineages. Later recognition of Th17 and regulatory T cells expanded the lineage scheme (6). It was subsequently acknowledged that T cells were capable of producing a range of cytokines outside of or in addition to their lineage requirements, allowing for the spectrum of observed T cell phenotypes. More recently these effector cytokines, and observed T cell functions, have been used to further group effector T cells into additional subsets, some of which are described below. The subsets described herein are based on observations made by many groups *in vivo*, but also on *in vitro* differentiation of naïve T cells in culture.

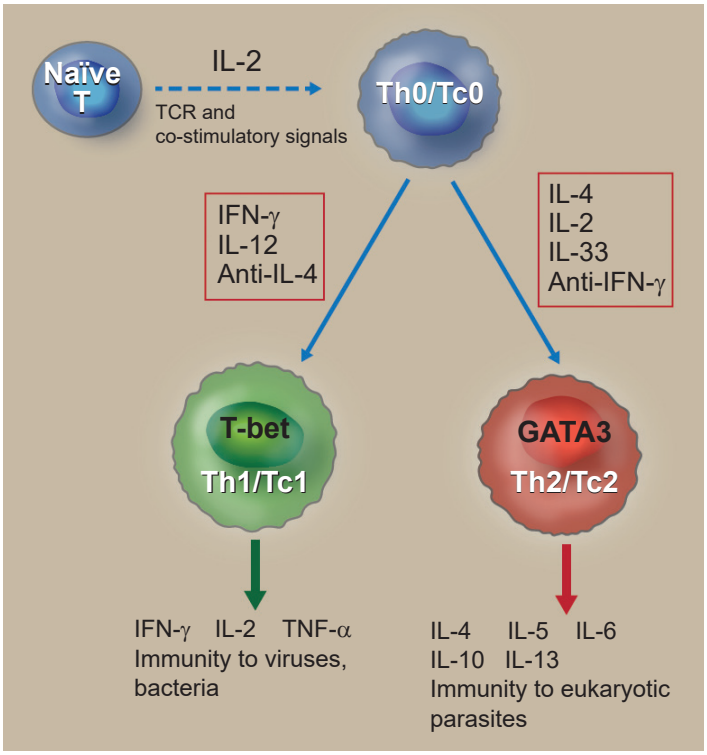

Fig. 2: *Th1/Tc1 and Th2/Tc2 lineages.*

Th/c1

As mentioned, the historical description of T cell lineages was based on a Th1/Th2 cell dichotomy (Figure 2) (7), which eventually resolved into a difference often described as "inflammation" (Th1) vs. "allergy" (Th2) (8). Th1 cells (and to an extent, Tc1 cells [3]) contribute to immunity against intracellular viral and bacterial pathogens, by methods including activation of macrophages, "help" for B cells' antibody production, and release of other pro-inflammatory mediators (9). Differentiation of Th/c1 from naïve T cells is achieved *in vitro* by addition of IL-12, IL-2, IFN-γ, and by neutralization of IL-4 with anti-IL-4 antibodies (3, 6, 10).

IL-12 signals through the IL-12 receptor via the JAK/STAT pathway, particularly Signaling Transducer and Activator of Transcription (STAT) 4 (6), resulting in IFN-γ production (11). IFN-γ activates the STAT1 pathway, which is important for the induction of the transcription factor T-bet during differentiation (6). T-bet is the so-called "master regulator," which is considered the hallmark transcription factor of the Th1/Tc1 lineage. The resulting upregulation of genes associated with the Th1/Tc1 phenotype includes production by the cell of IFN-γ, TNF-α and IL-2 (6).

Th/c2

Th/c2 cells evolved to target extracellular, eukaryotic parasites, such as helminths (12), and are important effector cells in allergic responses (13). Th/c2 effector roles include providing "help" necessary for B cells to switch isotypes of antibody, particularly to IgE, and activation of eosinophils (8). Typical *in vitro* differentiation of Th/c2 cells includes use of IL-4, IL-2, IL-33, and anti-IFN-γ

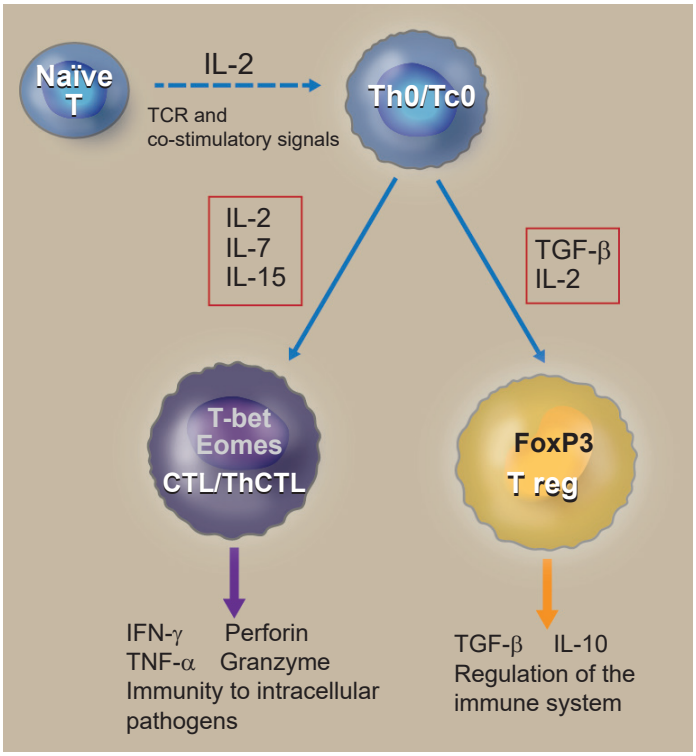

Fig. 3: *Cytotoxic and regulatory T cell subsets.*

neutralizing antibody (1, 3, 4, 6). Transmission of signal through the cytokine receptors involves the JAK/STAT pathway, particularly STAT5 and STAT6. It has been shown that STAT6 signaling leads to induction of GATA3, the "master regulator" associated with the Th2 lineage of T cells (6). Hallmark cytokines produced by Th/c2 cells include IL-4, IL-5, IL-6, IL-10, and IL-13.

Cytotoxic T Cells

Traditionally, the roles of T cells were broadly defined as: $CD4^+$ T cells providing help to the immune system by interacting with B cells and by making cytokines, and $CD8^+$ T cells acting as the cytolytic T lymphocytes (CTL), or "killer T cells" (3, 4). $CD8^+$ CTL identify target cells via antigens expressed in the context of MHC Class I on the target's surface. Typically, antigens presented by MHC Class I derive from intracellular proteins. As such $CD8^+$ T cells are suited to killing cells harboring intracellular pathogens (such as viruses) and cells in which protein production is abnormal (cancer). More recently, there has been acknowledgement in the literature of overlapping roles for $CD4^+$ and $CD8^+$ T cells. As mentioned above, $CD8^+$ T cells are capable of producing effector cytokines (a role traditionally reserved for $CD4^+$ T cells); and a subset of $CD4^+$ T cells (ThCTL or cytolytic T helper cells) are able to directly kill target cells expressing the T cell's cognate antigen in the context of MHC Class II, by way of perforin and granzyme, as do $CD8^+$ CTL (4). (Figure 3)

$CD8^+$ CTL are typically activated *in vitro* by stimulation via the TCR and co-stimulatory surface molecules, and addition of IL-2 (14) and Th1-like conditions. Other cytokines which may be used to support expansion of CTL *in vitro* include IL-7, 12 and 15 (15, 16). It has been shown, however, that $CD8^+$ T cells skewed to various subsets (Tc1, Tc2, Tc17 [3, 17]) also have cytolytic activity, and as such, cytotoxicity may be an inherent role of the $CD8^+$ T cell, no matter the subset. Transcription factors upregulated in CTL include T-bet, induced by TCR signaling, which leads to upregulation of IFN-γ, granzyme B, and perforin, and Eomes, which has been shown to induce expression of IFN-γ and cytotoxic granules (18, 19). (Figure 3)

$CD4^+$ CTL (ThCTL) have also been identified within various subsets (e.g., Th0, Th2 and regulatory T cells [4, 20]) and *in vivo* in a number of chronic viral infections (19). They are often differentiated *in vitro* using Th1-like conditions. However, ThCTL differentiated under "Th0" conditions, with only the addition of IL-2, have been shown to express higher levels of perforin and granzymes, and result in more cytotoxic activity (4, 6, 21). IL-2 leads to upregulation of Eomes, which in turn upregulates cytotoxic granules.

Both $CD8^+$ and $CD4^+$ CTL express perforin and granzymes, which induce apoptosis in the target cell, as well various cytokines. Typically, CTL are described as secreting IFN-γ and TNF-α, both of which are key actors in the immune regulation of pathogens.

Regulatory T cells

Regulatory T cells (Treg) are generally considered to be a lineage or subset of $CD4^+$ T cells, though there is evidence for $CD8^+$ suppressor T cells as well (22, 23). Treg control immune responses, particularly by other subsets of T cells, and their dysfunction can result in various diseases, from autoimmunity (when they do not prevent the immune system from attacking 'self') to cancer (when the immune system is prevented from attacking 'self' cells or tissues growing out of control). Treg which originate in the thymus (and thus are considered their own lineage) are typically referred to as natural or thymic Treg (nTreg/ tTreg), whereas those that differentiate from naïve Th0 cells in the periphery are referred to as inducible (iTreg [13]). Treg depend upon TCR stimulation, co-stimulatory molecules, and IL-2 (Figure 3). They express the high-affinity IL-2 receptor α chain, CD25, and require continued IL-2 signaling to function (13, 24). Inducible Treg can be differentiated *in vitro* by stimulating naïve T cells via TCR and co-stimulatory molecules, plus addition of IL-2 and TGF-β (4, 23). TGF-β signaling leads to the induction of the transcription factor FoxP3, often called the "master regulator" of Treg, which upregulates genes resulting in further TGF-β production and a paracrine feedback loop (13, 23). nTreg express FoxP3, as do a subset of iTreg. There are also iTreg that do not express FoxP3, but still function as regulatory cells (13).

The regulatory function of Treg is mediated through multiple mechanisms, including expression of inhibitory receptors, depriving other cells of cytokine support, and production of anti-inflammatory cytokines. The cytokines most associated with regulatory function by Treg are IL-10 and TGF-β. IL-10 functions in a regulatory capacity primarily by affecting stimulatory capabilities of antigen presenting cells (APC), and by suppressing activation and cytokine production of mast cells and macrophages (13). The role of TGF-β in immune regulation is complex, but functions include

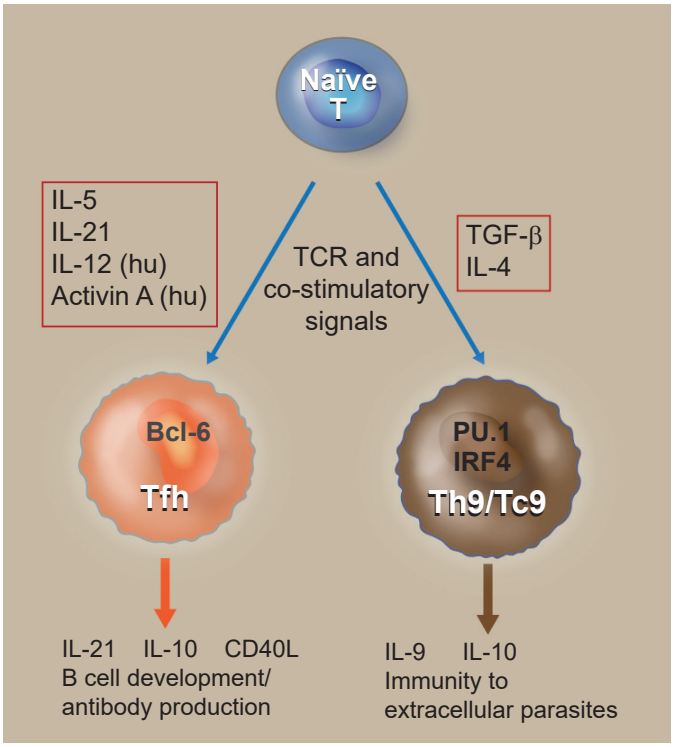

Fig. 4: *Follicular Helper T cells and Th9/Tc9 cells.*

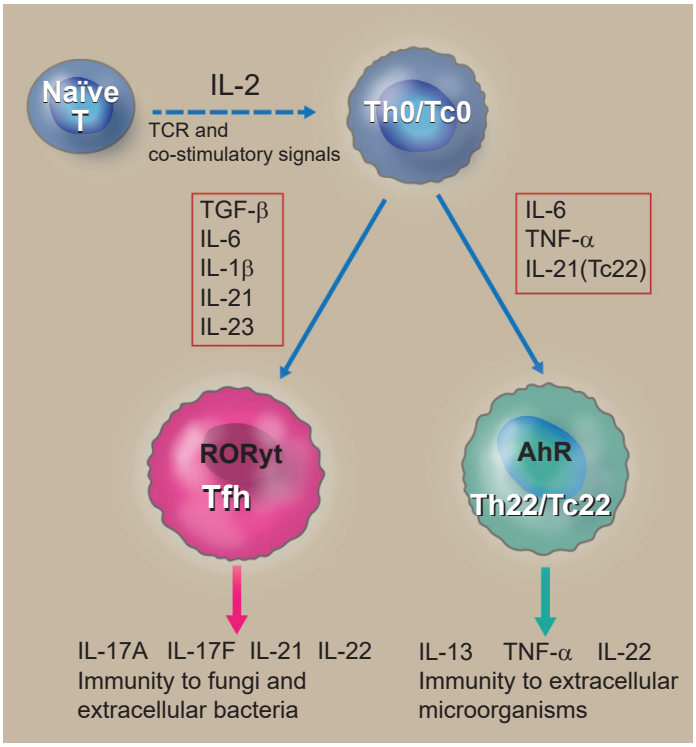

Fig. 5: *Th17/Tc17 and Th22/Tc22 cells.*

direct effects blocking the differentiation of various T cell subsets and effects on APC such as dendritic cells (23).

Follicular Helper T Cells

Follicular helper T cells (Tfh) are as yet only identified among $CD4^+$ T cells. This subset of helper T cells is a critical component of germinal center formation in secondary lymphoid organs, which is important for B cell antibody production and maturation (25). Dysregulation of Tfh has been shown to lead to auto-antibody dependent autoimmune diseases (25, 26). Initiation of the Tfh phenotype is dependent upon several factors, including expression of the chemokine receptor CXCR5, which allows the Tfh to traffic to the B cell follicle in the secondary lymphoid organs (25, 26). Tfh also express other immune checkpoint molecules that are important to their differentiation and continued function, such as ICOS and PD-1 (25, 26). Critical cytokines for differentiation of Tfh are IL-6 and IL-21 (4, 6, 25). In humans, IL-12 and Activin A appear to be important (25). (Figure 4)

Tfh are dependent upon the expression of the transcription factor B-cell lymphoma 6 (Bcl-6), which maintains their phenotype and function (6, 25, 26). Bcl-6 is actually a transcriptional repressor, whose function is to prevent development of phenotypes associated with alternate T cell subsets. For example, ThCTL, mentioned above, express the transcription factor Blimp-1, which can be suppressed by Bcl-6, preventing ThCTL formation (20). Further, it has been shown that Bcl-6 binds and represses transcription factors T-bet (important for IFN-γ production and Th/c1 cells) and RORγt (important for IL-17 production in Th/c17 cells).

The result of Bcl-6 expression and the Tfh phenotype is cytokine support for B cells, particularly via production of IL-21 and IL-10 (4, 25), as well as expression of CD40 ligand (26), which stimulates the CD40 receptor molecule on B cells and is important for T-cell-dependent B cell help (27).

Th/c9

Th9 T cells (producing IL-9) were identified within the past 10 years (28) as a subtype separate from Th2 cells, which were originally ascribed their function. Th9 (and Tc9 [29]) are involved in the immune response to eukaryotic parasites, and are also implicated in various allergic responses (13, 29, 30, 31), like Th2 cells.

Th/c9 cells are differentiated *in vitro* from either naïve T cells, or from Th2 cells, by addition of TGF-β and IL-4 (30, 31). While TGF-β typically leads to the upregulation of FoxP3, and a Treg phenotype, the presence of IL-4 inhibits FoxP3 induction in a STAT6/GATA3-dependent manner (30). The transcription factors most associated with the Th9 phenotype are PU.1 (induced by TGF-β) (12, 23) and IRF4 (29, 31). (Figure 4)

Th/c9 cells produce IL-9, IL-10, and are able to enhance IgE production by B cells, and regulate mast cells and eosinophils (12, 29, 30). IL-9 itself was described as a T cell and mast cell growth factor (28). There is evidence that IL-9-producing Th9 cells are important in combatting *Candida albicans* and helminths (12), but also in contributing to autoimmune skin disorders such as psoriasis (31), and respiratory diseases such as asthma (28).

Th/c17

Both $CD4^+$ and $CD8^+$ T cells are capable of expressing IL-17 (Th17 and Tc17), a potent inflammatory cytokine important for

the immune response to bacteria, fungi, and other extracellular microorganisms. IL-17 is also implicated in many autoimmune disorders and pathogenic inflammatory conditions such as psoriasis, atopic dermatitis, multiple sclerosis, rheumatoid arthritis, and experimental autoimmune encephalitis (12, 13, 31).

Th/c17 cells are differentiated *in vitro* by stimulation of TCR and co-stimulatory molecules, as well as addition of various cytokines including IL-1β, IL-6, TGF-β, IL-21, IL-23, and blocking of IL-4 and IFN-γ with neutralizing antibodies (3, 6, 13, 32). There are differing reports on the timing of IL-23 exposure (6, 33), but it is generally considered to be critical for the Th/c17 phenotype. IL-6 and 21 are known to activate STAT3 (6), which along with TGF-β signaling, leads to induction of RORγt, the "master regulator" of Th/c17 cells (3, 6). (Figure 5)

Effector molecules produced by Th/c17 cells include IL-17A, IL-17F, IL-22, and IL-21 (6, 13). Many of the functions attributed to Th/c17 cells center around secretion of IL-17, including recruitment of neutrophils, activation of innate immune cells, support for B cell antibody production, and release of pro-inflammatory cytokines (13, 31). IL-17 is also a contributor to various types of organ-specific autoimmunity (13).

Th/c22

IL-22 producing $CD4^+$ and $CD8^+$ T cells, or Th/c22, are closely related to the Th/c17 subset. Like Th/c17 cells, Th/c22 cells produce IL-22, often express chemokine receptors that promote migration to the skin (CCR4 and CCR10), and are implicated in tissue inflammation and autoimmunity (34, 35, 36). Th/c22, which do not produce IL-17, are important actors in skin homeostasis, immune-responses and pathologies (36).

Th/c22 cells differentiate upon exposure to TNF and IL-6 (31). Liu, et al. also showed that IL-21 is important for naïve human CD8+ T cells to differentiate *in vitro* into Tc22 (37). The transcription factor AhR (the aryl hydrocarbon receptor) has been identified as important for IL-22 production (Figure 5), though there is evidence that Tc22 cells exist *in vivo* which do not express AhR (31, 34, 36).

Th/c22 cells produce IL-22, the hallmark cytokine of this subset of T cells, as well as additional cytokines, including TNF-α, and IL-13 (34). Th/c22 have been implicated in several autoimmune disorders and inflammatory conditions, primarily due to IL-22. The receptors for IL-22 are expressed on epithelial cells and some fibroblast cells in the gastrointestinal and respiratory systems, and in skin (13). Th/c22 are shown to contribute to inflammatory skin diseases, for example, psoriasis, which involves abnormal proliferation of keratinocytes, a cell type that responds directly to IL-22 (36). IL-22 is also noted to affect many other epithelial and stromal cell types, and to have a role in many inflammatory and autoimmune pathologies, including atopic dermatitis, rheumatoid arthritis, systemic lupus erythematosus and type 1 and 2 diabetes, to name a few (13, 34, 36). As a mediator of host defense, IL-22 has been shown effective for mucosal immunity against bacterial infections, and potentially fungal infections (37).

Summary

The complex nature of the immune system is illustrated perfectly in the variety of lineages and subsets which may ultimately arise from naïve T cells exiting the thymus. The intricate combinations of antigen-stimulation and co-stimulatory molecules, APCs, and of course, the location and cytokine milieu in which the cells find themselves, all contribute to the cell's fate, and therefore the cell's function in the immune system as a whole.

In vitro differentiation of T cells requires high quality, active cytokines and chemokines with low endotoxin levels and high purity, which are available at www.cellsciences.com (Table 1). In addition, Cell Sciences offers thousands of biologically relevant recombinant proteins and corresponding affinity-purified antibodies. To view our full catalog listing, please visit http://www.cellsciences.com.

Table 1. Stimuli necessary for activation and differentiation into T cell subsets (and protein and antibody reagents available).

T Cell Subset	Cell Stimuli		Human (Catalog No)	Mouse (Catalog No)
Th0/Tc0		Anti-CD3	CDM127 / M1654	--
		Anti-CD28	CDM171 / M1650	--
		IL-2	CRI100	CRI145
Th1/Tc1	Th/c0 +	IL-12	CS529	CS357
		IFN-γ	CRI000	CRI001
		Anti-IL-4	CDM264 / PA0482	CMI243
Th2/Tc2	Th/c0 +	IL-4	CRI104	CRI129
		IL-33	CRI225	CSI20129
		Anti-IFN-γ	CDM256 / CMI015	CM2030
CTL/ThCTL	Th/c0 +	IL-2	CRI100	CRI145
		(IL-7)	CRI108	CRI131
		(IL-15)	CRI137	CRI160
Treg	Th/c0 +	TGF-β	CS327	--
		IL-2	CRI100	CRI145
Tfh	IL-6		CRI106	CRI130
	IL-21		CRI172	CS322
	IL-12 (hu)		CS529	n/a
	Activin A (hu)		CS362	n/a
Th9	IL-4		CRI104	CRI129
	TGF-β		CS327	--
Th17/Tc17	Th/c0 +	IL-1β	CRI133	CRI139
		IL-6	CRI106	CRI130
		TGF-β	CS327	--
		IL-21	CRI172	CS322
		IL-23	--	--
		Anti-IL-4	CDM264 / PA0482	CMI243
		Anti-IFN-γ	CDM256 / CMI015	CM2030
Th22/Tc22	Th/c0 +	TNF-α	CRT100	CRT192
		IL-6	CRI106	CRI130
		IL-21	CRI172	CS322

Note: Cytokines, chemokines, and blocking antibodies also available for other species. Please visit www.cellsciences.com for a complete listing.

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