



New T helper cell lineages: true or false?

With the discovery of B and T cells in the sixties by Miller and colleagues and the translation of their respective lineage markers into CD nomenclature in the early eighties it became clear that CD4+ T cells play important roles during the adaptive immune response. Mosmann and colleagues revolutionized immunology in 1986 by dividing mouse CD4+ T helper cells into two distinct T cell populations, Th1 and Th2 cells based on different and mutually exclusive cytokine

populations have been described, some of which are clearly established including Th17 cells, IL-17 producing $\gamma\delta$ T cells and T follicular helper (Tfh) cells. Others like Tr1, Th9, Th22 and iTreg35 cells need more confirmation to be recognized as bona fide T cell populations whereas Th3 cells have disappeared from the current literature.

The cytokine production profile and protein expression at the cell surface, together with

Table 1: T helper cell lineage Cytokine Profiles

	Cell Lineage					
	Th1	Th2	Th17	Treg	Tfh	$\gamma\delta$ T
Lineage Induction & maintenance	IFN γ , IL-12 & IL-18	IL-4	TGF β , IL-1 β , IL-6, IL-21 & IL-23	TGF β & IL-2 & /or IL-15 (murine)	IL-6, IL-12 & IL-21	IL-1 β , IL-23
Lineage Inhibition	TGF β , IL-4, IL-10 & IL-21	IFN γ & TGF β	IL-2, IL-4, IL-10, IL-12, IL-13, IL-25, IL-27 & IFN γ	IL-6 & IL-21	Roquin & CXCR5+ T-reg cells	?
Effector Cytokines	IFN γ & TNF β	IL-4, IL-5, IL-9, IL-13 & IL-6 (murine only)	IL-6, IL-17A, IL-17F, IL-21, IL-22, TNF α & IL-26 & IFN γ (human only)	IL-10, IL-35 TGF β & via cell contact	IL-4, IL-6, IL-10, IL-17, IL-21 & IFN γ	IL-17A, IL-17F, IL-21 & IL-22

production patterns. This division is also true for human CD4+ T helper cells although their cytokine profiles seem to be less strict as compared to those described for their mouse counterparts. Th1 cells are principally involved in immune responses associated with inflammation and tissue injury and the principal Th1+ cytokines IFN γ and TNF β recruit and activate inflammatory leukocytes. Th2 cells inhibit acute and chronic inflammation to limit the injurious consequences of Th1 cell-mediated protective immunity. In addition, the typical Th2 cytokines IL-4, IL-5, and IL-13 are involved in the protection against parasites, as well as in the pathogenesis of allergic diseases.

In the following decade several suggested new T cell populations surfaced but were quickly discarded and it was not until 1995 that Sakaguchi and colleagues clearly demonstrated the existence of T regulatory (Treg) cells. Since then at least 8 new T cell

signal and transcription factor(s) have become the generally accepted signature of a particular T cell population. This view has matured with the discovery of novel cytokines and transcription factors. However, there are differences between man and mouse with respect to the factors that determine the differentiation of the different T cell lineages, as well as to the rigidity of their cytokine production profile. In addition, terminal lineage polarization has become under pressure and requires further investigation.

This mini-review aims to provide an overview of what is currently known about the human and mouse T cell subsets, to highlight the differences between these species if they exist and to describe the plasticity of certain T cell populations.

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► Th1 and Th2 cells

IFN γ , IL-12 and IL-18 promote the differentiation of the Th1 cell lineage under the control of the signaling molecules STAT1, STAT4 and the transcription factor T-bet. Th1 cells produce IFN γ and TNF β which are implicated in cell-mediated immune responses, macrophage activation and clearance of intracellular pathogens. Th1 cells express the chemokine receptors CXCR3, CXCR4, CXCR6, CCR1, CCR2 and CCR5 (Table 2), as well as TIM3 which is involved in Th1 differentiation and its expression on Th1 correlates with IFN γ / TNF α production in MS patients.

IL-4 promotes the differentiation of naïve T cells into Th2 cells which is controlled by STAT5, the transcription factor GATA3 and by the transcription repressor Gfi-1, which stabilizes the Th2 cell phenotype. The ST2 receptor is selectively expressed by Th2 but not Th1 cells and constitutes together with IL-1RAP the

heterodimer receptor for IL-33. Th2 cells produce IL-4, IL-5 and IL-13 while for the mouse IL-6 and IL-9 production is also reported. Th2 cells are CXCR4, CXCR6, CCR2, CCR3, CCR4 and CCR8 positive (Table 2) and express TIM1, an important regulatory molecule for the differentiation and activity of Th2 cells. Th2 cells are involved in humoral immune responses, including IgE production, recruitment of eosinophils and mast cells as well as clearance of extracellular pathogens.

Both Th1 and Th2 cells are involved in the pathogenesis of various autoimmune diseases, as are Th17 cells, but the respective role of each population is not always clear. The Th1 / Th2 balance in an immune response is regulated by positive and negative feedback within and between both cell types and there is a growing body of evidence that the outcome of many diseases critically depends on this Th1 / Th2 balance.

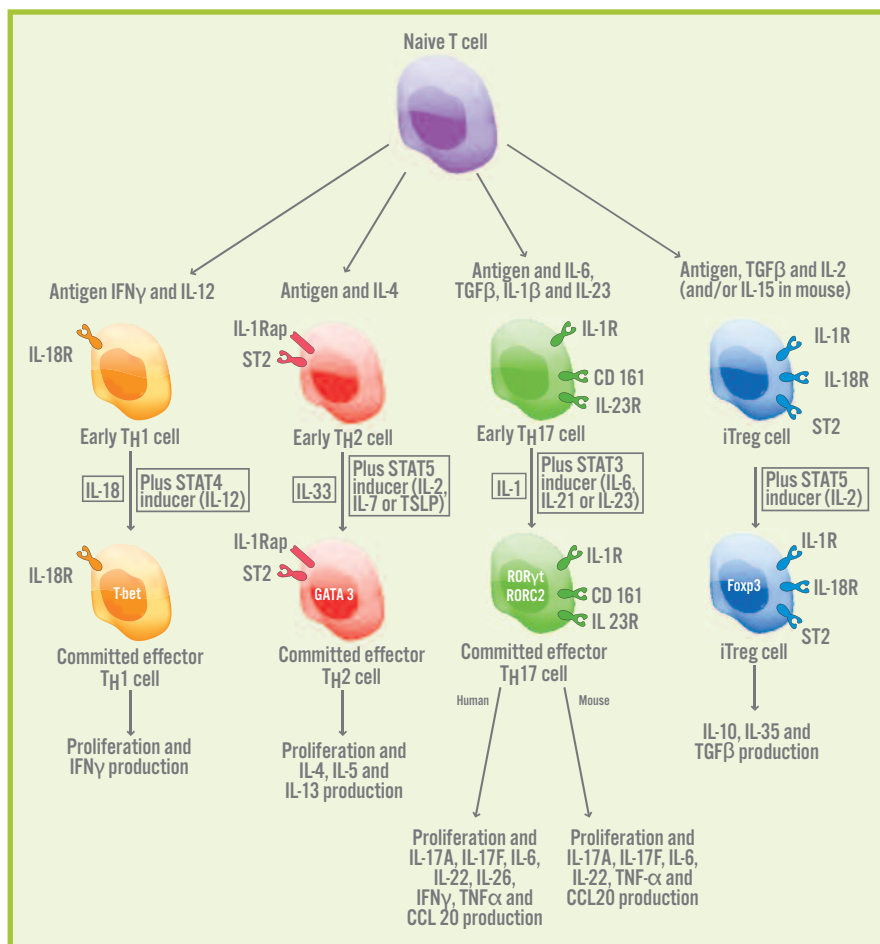
► Th-17 and IL-22 producing cells

The description of the role of Th1 cells in autoimmune diseases has been revised with the discovery of IL-23. IL-23 shares the p40 subunit with IL-12 and diseases earlier attributed to Th1 cell effector function are now also linked to the activity of Th cells producing IL-17 referred to as Th17 cells.

The differentiation of the Th17 cell lineage in the mouse is driven by TGF β and IL-6 whereas its pathogenic phenotype is stabilized by IL-23. In the absence of IL-6, Th17 cell differentiation can be induced by IL-21. Together, these cytokines modulate IL-17 production by inducing the expression of a key lineage-specific transcription factor, the retinoid-related orphan receptor ROR γ t which is dependent on signaling via STAT3. Two other transcription factors, ROR α and AHR, are also involved in Th17 differentiation. ROR α is induced by TGF β and IL-6 and synergizes with ROR γ t to promote the differentiation of the Th17 lineage in a STAT3 dependent manner. The AHR is a transcription factor that interacts with many ligands including environmental toxins and is specifically expressed in mouse and human Th17 cells. Finally, the transcription factor IRF4 has been shown to be essential for mouse Th17 cell differentiation although its role in human is currently unknown.

Although the precise mechanism remains controversial, human Th17 cell differentiation from naive cells is induced by the combined activity of IL-1, IL-6 and / or IL-23. IL-21 is reportedly involved in this process as well (Figure 1), through its effects on naive T cells. TGF β has been reported to inhibit the induction of Th17 cells, however, others show the indirect requirement for TGF β in differentiation of Th17 through suppression of Th1 development. Human Th17 lineage differentiation is dependent on the expression of RORC2, the orthologue of the ROR γ t transcription factor in the mouse. In both mouse and man Th17 cells produce high levels of IL-17A, IL-17F, IL-22, IL-6, CCL20 and TNF α . In addition, IL-26 (a member of the IL-10 cytokine family) and IFN γ are produced from human derived Th17 cells. Although Th17 cells also produce IL-21, the production of this cytokine is not characteristic of this cell lineage. Human Th17 cells express IL-23R, CXCR4, CXCR6, CCR4, CCR6, CCR9 (Table 2) and can be identified by the selective expression of CD161. Populations of Th17 cells can be defined by their specific expression of chemokine receptors. CCR4 and CCR6 expression defines a population of Th17 cells expressing IL-17 but not IFN γ , whereas cells

Figure 1: Differentiation, Expansion and Proliferation of T helper cell lineages



expressing CCR6 and CXCR3 produce either IL-17 and IFN γ (Th17/Th1) or IFN γ only (Th1). IL-23 is essential for the maintenance of the Th17 cells while IL-27 down regulates IL-17 production. Th17 cells play an important role in the defense against extracellular bacteria and fungi but are also involved in autoimmune diseases.

Recently, the identification of another human Th lymphocyte population producing IL-22 but no or little IL-17 has been reported. However, it is not clear at present whether Th17 and Th22 cells display functional differences. These "Th22" cells are involved in cutaneous inflammatory diseases like psoriasis and are also known to produce IL-13 and like Th17 cells secrete very high levels of TNF α . Although no specific transcription factors have been identified for this T cell population the AHR plays a dominant role in the pathway leading to IL-22 production. Both differentiated Th17 and "Th22" cells express CCR6 a chemokine receptor that plays a pivotal role in the migration of these T cell populations into inflamed tissue. In addition, "Th22" cells display a selective expression of CCR10, the ligands of which, CCL27 and CCL28, are expressed in skin and gut tissue respectively and are involved in the tissue-specific migration of these cells.

► T regulatory cells (Treg)

The CD4+ Treg cell lineage belongs to the larger family of T regulatory cells (Treg) (Sakaguchi and colleagues). Natural Treg (nTreg) cells are derived from the thymus under the influence of IL-2 or in the case of IL-2 deficient mice under the influence of IL-15 (Figure 1).

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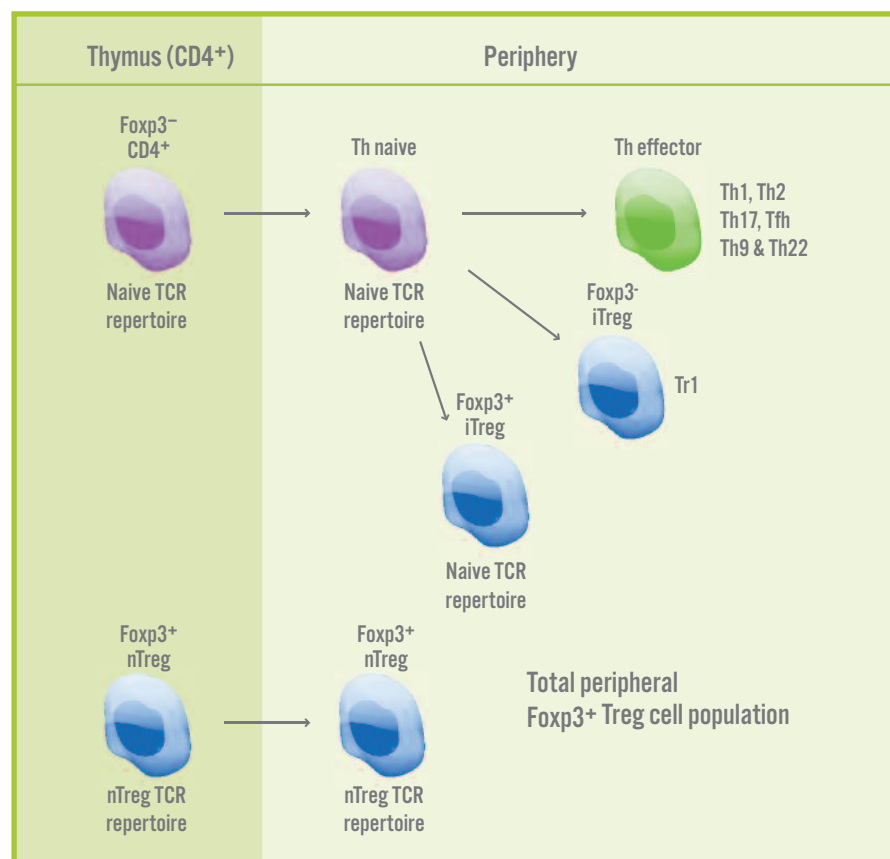
These cells are inert, do not proliferate and do not secrete any cytokine. They express CD4, CD25^{high}, CD28, CD45R^{low} (mouse), CD127^{low}, GITR, Folate receptor 4 (mouse) and CTLA4. Following activation by the TCR these cells also express latent TGF β and CD103. They exert a strong inhibition on CD4 effector cells by cell contact, IL-10, IL-35 and TGF β and have the ability to suppress autoimmune diseases. This is true for activated nTreg cells migrated to the periphery but also for induced

peripheral Treg cells (iTreg). iTreg cell differentiation is promoted by low dose antigen bound to the TCR, TGF β / IL-2 and CD28 under the guidance of the transcription factors Foxp3 / STAT5. iTreg cells can proliferate while the inhibition mechanisms are similar as those of activated naïve Treg cells and both populations are found together *in vivo* (Figure 2). Like nTregs, iTregs are involved in self tolerance and immune modulation. Both active naïve and induced Treg cells down regulate the activities of Th1, Th2, Th17, CTL and DC, depending on the environment.

How the function of Treg cells is down regulated is still poorly understood, IL-6, activation of the Toll-like receptors 2 and 8 expressed on Treg cells and recently the signaling of sphingosine-1-phosphate (S1P) via its five receptors (S1P1 – S1P5) has been described to result in the inhibition of Treg activity. Treg cells consist of nTregs developed

in the thymus and peripheral induced iTregs and although Foxp3 is still considered as the most useful marker for nTregs and iTregs no markers are known to distinguish between both cell types. The transcription factors T-bet, GATA3 and ROR γ t are also expressed in subsets of Treg cells, reflecting a more complex lineage than originally described. Moreover, in addition to genuine Foxp3 expressing Treg cells several T cell populations with immune regulatory activity have been described with transient or even absent Foxp3 expression. This heterogeneous family includes Th3, Tr1, and iTr35 cells, characterized by the production of TGF β , IL-10 and IL-35, respectively. As neither specific transcription factors, nor selectively expressed cell surface markers have been reported for each of these populations, more research is needed before these cells can be considered as separate lineages.

Figure 2: Thymic and Peripheral generation of Treg Cells



Natural Treg (nTreg) cells differentiate in the thymus and migrate to peripheral tissues. Induced peripheral (iTreg) cells differentiate in secondary lymphoid organs and tissue. The peripheral population of Treg cells comprises both nTreg and iTreg cells.

Table 2: T helper cell lineage Chemokine Receptor Expression

Chemokine receptor expression	Cell Lineage					
	Th1	Th2	Th17	Treg	Tfh	$\gamma\delta$ T
CCR1	+					
CCR2	++	+				
CCR3	-	++				
CCR4	+	+++	+	+		
CCR5	++	-	-			
CCR6			+++	+		+++
CCR7				+	-/+	
CCR8		+++				
CCR9			+			
CCR10						
CXCR3	+++		+			
CXCR4	+	+	+		-	
CXCR5					+++	
CXCR6	++	+	+		-	

Chemokine receptors are present on many cell populations, some of them "selectively". The combination of the constitutively expressed chemokine receptors and the lack of expression of others may be the most appropriate indicator for a specific cell lineage. Particularly when there are many variations in cell lineage chemokine receptor expression reported in the literature. (-/+ denotes loss of expression in secondary lymphoid tissues).

► Th9 cells

Th9 cells can be induced by exposing differentiated Th2 cells to TGF β which leads to a loss of the characteristic Th2 profile and a switch to IL-9 secretion while IL-4 is suppressed (Figure 4). In addition the exposure of naïve CD4+ Th cells to TGF β and IL-4 can lead directly to the Th9 cell profile characterised by IL-9 secretion. In spite of producing IL-10 these Th2 cells lack suppressive functions. However, Tregs and Th17 cells also produce IL-9 and this has been shown to be dependent on the transcription factor GATA3. It is possible therefore that these IL-9 producing Th9 cells may well represent subpopulations within Th2, iTreg and Th17 cell populations.

► T follicular helper cells (Tfh)

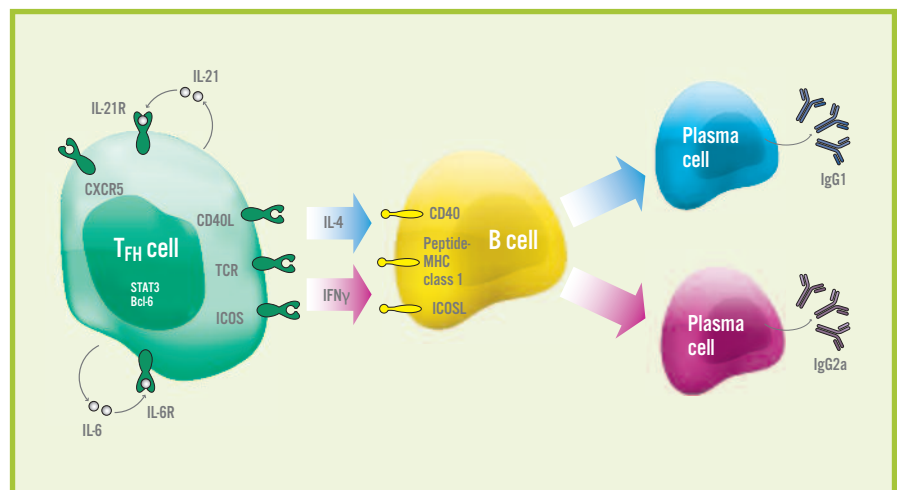
Another subset of CD4+ T cells, T follicular helper cells are present in germinal centers and Peyer's patches. While Tfh cells in the Peyer's patches seem to generate from Treg cells, those in the germinal rings may have multiple origins. Tfh cells express CXCR5, ICOS, IL-21, the transcription factors STAT3 and Bcl-6 and require autocrine production of IL-6 and IL-21. In mice the transcription factor Bcl-6 enhances the differentiation of Tfh while it suppresses both Th1 and Th17 pathways.

For this reason BCL-6 is only expressed during a defined period to avoid counter productivity. In mice, B cells have been shown to produce IgG2a antibodies under the influence of Tfh cell derived IFN γ . If Tfh cells secrete IL-4, B cells switch to IgG1 (Figure 3).

The involvement of specific transcription factors, including T-bet and GATA-3 in such cytokine secreting Tfh cells has been reported. Recently it was shown that IL-12 can induce naïve human CD4+ cells into Tfh cells (Ma and colleagues). CXCR5+ cells are a heterogeneous

population and the same author defined human Tfh cells as CD45RA-CXCR5hi expressing ICOS, PD-1, CD40L, CD57, CD69, CD95, CD200 and SAP while they do not express CXCR4 and CXCR6, chemokine receptors associated with Th1 and Th17 respectively (Table 2). Expression of some of the surface antigens is still controversial and may change with the definition of true Tfh cells. The cells produce IL-21 with diminished expression of Th17 (IL-17, IL-22, ROR γ t), Th1 (T-bet, IFN γ) and Treg (Foxp3) specific genes. It is the maintenance of CXCR5 expression, in conjunction with the loss of expression of CCR7, which allows them to migrate in the CXCL13-rich B cell follicles of the secondary lymphoid tissues. Interestingly, in Tfh cells in contrast to other T-cell subsets the sphingolipid G-protein-coupled receptor 1 (also called S1P1 receptor or Edg-1) is down-regulated 5- to 7-fold. This receptor is required for lymphocytes to exit from secondary lymphoid organs and the thymus. It has also been reported that human Tfh cells express IL-10 induced by nearby B cells and in addition produce high levels of CXCL13.

It remains to be seen if Tfh selecting high affinity B cells are the same as those that induce class switching. In addition, the hallmark Th1, Th2 and Th17 cytokines (INF γ , IL-4, IL-10 and IL-17) as well as the transcription factors T-Bet and GATA-3 have been shown to be produced by certain Tfh cells. The question of whether or not Tfh cells represent a particular state of the Th1, Th2 and Th17 cell types therefore remains. However, in

Figure 3: Antibody class switching driven by Tfh cells in a mouse model


contrast to Th17 cells, Tfh cells produce IL-17 without expressing ROR γ t and the production is strictly depending on ICOS signaling and the related transcription factor c-MAF. This observation argues for a separate lineage but it is also possible that co-expression of c-MAF and ROR γ t results in a mixed-lineage intermediate Tfh / Th17 as exists for ROR γ t and Foxp3.

► $\gamma\delta$ T cells

The $\gamma\delta$ T cells are mainly present in the peripheral blood population (1-5% of the T cells) but also as the predominant intra-epidermal T-cell population in murine and human skin during an allergic response. $\gamma\delta$ T cells exhibit several characteristics that place them at the border between the more evolutionarily primitive innate immune system that permits a rapid beneficial response to a variety of foreign agents and the adaptive immune system where B and T cells coordinate a slower but highly antigen-specific immune response leading to long-lasting memory against subsequent challenges by the same antigen.

NKT cells and $\gamma\delta$ T cells have been shown to be important sources for IL-17. $\gamma\delta$ T cells share characteristics with Th17 cells including the expression of CCR6, IL-23R, the transcription factor ROR γ t and Aryl Hydrocarbon Receptors. Only CCR6+IL-17 producing $\gamma\delta$ T cells express Toll-like receptors like TLR-1, TLR-2 (not TLR4) and dectin-1 allowing direct interaction with certain pathogens. This is in contrast to other $\gamma\delta$ T cells and Th17 cells which do not show expression of these receptors. In contrast to Th17 cells which need IL-6, TGF β and TCR engagement, $\gamma\delta$ T cells need IL-1 β and IL-23 and no TCR engagement to produce IL-17A, IL-17F, IL-21 and IL-22. $\gamma\delta$ T cells are an important source of innate IL-17 and IL-21, they orchestrate an inflammatory response against bacteria and environmental signals long before the adaptive response of Th17 cells come into action. In the EAE mouse model $\gamma\delta$ T cells were found at a high frequency in the brain together with Th17 cells. $\gamma\delta$ T cells may even amplify the IL-17 production from Th17 cells, either indirectly through stimulation of IL-23 production by APC or by direct stimulation of Th17 cells through the IL-17R.

► Heterogeneity of T helper cell lineages

Every Th lineage can produce a certain number of cytokines but it is difficult to discriminate between one cell secreting different cytokines or subpopulations within the same Th lineage individually secreting one cytokine. The Th1 lineage can express IL-2, INF γ , TNF α , TNF β and on occasion IL-10 at the same time as INF γ . Th2 cells produce IL-4, IL-5, IL-9, IL-10, IL-13 and possibly IL-25. However, most of these Th1 and Th2 cells do not express all these cytokines at the single cell level. The Th17 cells secrete IL-17A, IL-17F, IL-22 and under certain conditions IL-21. Recently it was shown that Th17 and Th9 cells can secrete IL-10 but this production is conditional rather than constitutive. These observations make all the Th-lineages rather heterogeneous with Tfh cells possibly even more heterogeneous in respect to their origin as they can develop from naive CD4+ cells but can also be derived from Th1, Th2, Th17 and Treg cells. CXCR5 represents the hallmark of Tfh cells and expression of this chemokine receptor on T cell subsets may change the homing behavior of Th1, Th2, Th17 towards B cells in the germinal ring ultimately defining them as Tfh cells.

► IL-10 activity in T helper cells

IL-6 induces IL-10 production in Th17 cells and this is dependent on the transcription promoter c-MAF. IL-12, the major Th1 promoter, stimulates IL-10 production in Th1 cells. In the presence of high dose antigen, IL-12 triggers Th1 lymphocytes to produce both INF γ and IL-4 with activation of Erk. IL-27, a member of the IL-12 family, can also induce IL-10 production in Th1 and Th17 cells. IL-12 and IL-27, like IL-6, use the transcription promoter c-MAF to induce IL-10. An active binding site for c-MAF on the IL-10 promoter has been identified indicating c-MAF as a central transcription factor for IL-10 regulation. IL-21, produced by Th1, Th2, Th17 and Tfh cells, amplifies the production of IL-10 in Th1 cells, Tr1 cells and Th17 cells through the signaling factors STAT1 and STAT3.

IL-10 was thought to cross-regulate Th cell responses but there is growing evidence that IL-10 can also function in an autocrine loop in those Th cells which temporarily express

IL-10 such as Th1, Th2, Tr9 and Th17 cells. In these cells IL-10 may serve as a negative feedback to dampen the magnitude of immune responses and thereby help prevent tissue damage.

► Plasticity of the T helper cell lineages

Th2 cells can produce INF γ when stimulated with IL-12 and Th1 cells can become INF γ +IL-4+ cells when cultured under Th2 conditions although this flexibility may depend on the degree of differentiation. As a general rule Th1 and Th2 cells cannot be converted to Treg or Th17 cells but Th17 cells can convert to Th1 cells. TGF β stimulated Th2 cells can change into IL-9 producing T cells while IL-4 is suppressed and these cells are still associated with Th2 type responses such as allergy. Th17 cells are easily converted by IL-12 or IL-4 to become INF γ or IL-4 producing cells respectively. In the case of INF γ producing cells the IL-17 expression is maintained. T cells that co-express transitionally Foxp3 and the Th17 cell associated transcription factor ROR γ t have been described. nTreg cells stimulated by IL-6 express ROR γ t and IL-17 and reflects the role of this cytokine in the inhibition of Treg cells while stimulating Th17 development. Treg cells can under certain conditions express the Th1 related transcription factor T-bet and produce INF γ .

When considering the plasticity of Th1, Th2, Th17 or Treg lineages together with the subpopulations of Th cells like Tr1, Th3, Th9 and Th22 it becomes evident that fully definitive differentiated cells associated with specific disease states is not the reality. While clear cut models of cell lineage differentiation and associated cytokine profile are highly desirable these do not necessarily correspond to how the immune system in man is organized.

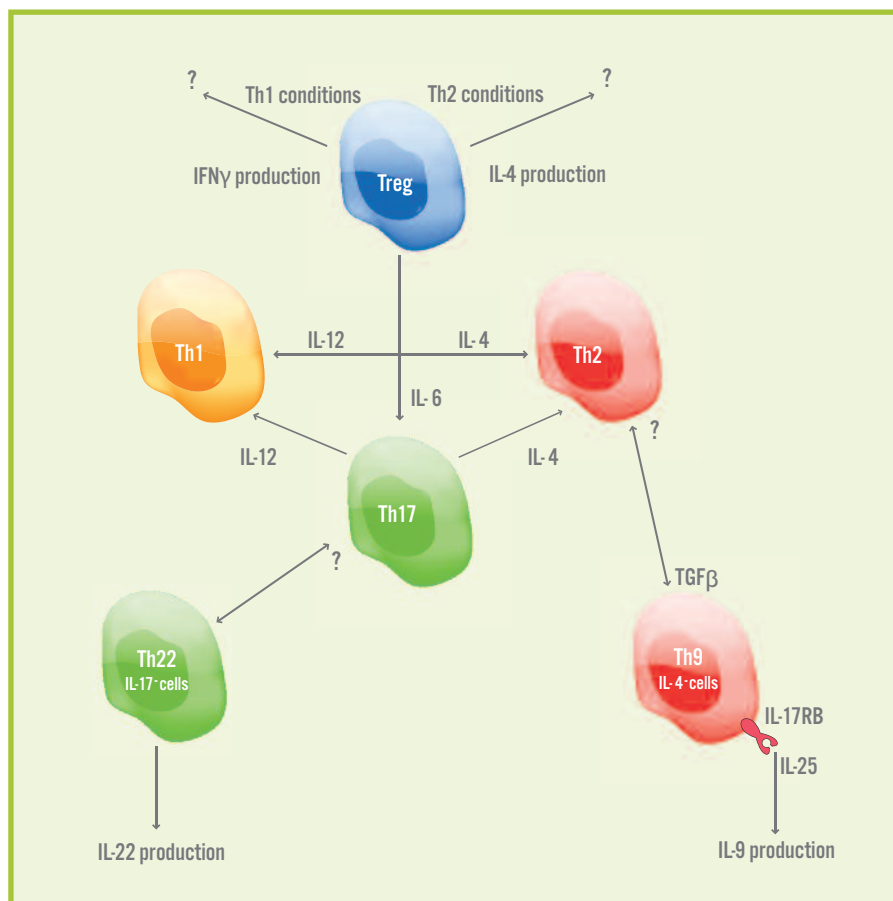
At least two reasons are likely for this discrepancy: 1) many concepts are based on mouse models but it seems that in mice T cell lineages are less flexible in terms of cytokine production and plasticity than in man and may reflect the highly inbred origin of the mice strains and 2) although not all, but many of the studies which led to the notion of T cell lineages are *in vitro* studies with cytokine concentrations and mixtures not reflecting the *in vivo* microenvironment.

As the body of research grows and the reliability of cytokine signature and cell subset related transcription factor profiles as hallmarks for cell lineage identification are further questioned, new tools will be increasingly required to aid lineage classification. Such tools include microRNAs and epigenetics. microRNAs are thought to stabilize the phenotype of cells and preserve the identity of the cell lineage, however one microRNA often has many targets making it difficult to attribute it to one activity, i.e. one lineage.

When considering the plasticity of Th1, Th2, Th17 or Treg lineages together with the subpopulations of Th cells like Tr1, Th3, Th9 and Th22 it becomes evident that fully definitive differentiated cells associated with specific disease states is not the reality.

The other hope is to look into subsets with epigenetic marks of genes encoding transcription factors like T-bet and STAT3. However, consistent with plasticity is the finding that these master regulators are not uniformly repressed in opposing lineages and that such lineages have bivalent epigenetic marks indicating that both permissive and repressive are present and therefore can alter the fate of the lineages. With these last findings it is time to come to terms and to accept that plasticity of cells in the immune system is a sine qua non to regulating immune responses in different microenvironments.

Figure 4: T helper cell plasticity



The classification of Th cell populations should be used as a tool to understand differentiation of Th cells, but should not be used in isolation. The information gained should be utilized in

conjunction with an understanding of how the Th cell plasticity is regulated to gain a fuller understanding of the cellular function in a specific disease state.

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T-cell lineage related Diaclone Products

Diaclone Monoclonal Antibodies

Specificity (Anti-Human)	Clone	Purified	Azide Free	FITC	PE	Biotin	Histology
CD4	B-A1	●	●	●	●		●
CD25 / IL-2R α Chain	B-B10		●		●	●	
CD25 / IL-2R α Chain	B-F2	●	●	●			●
CD25 / IL-2R α Chain	B-G3			●			
CD28	B-T3	●	●	●	●		
CD40	B-B20	●	●	●	●	●	
CD45	B-A11	●	●	●	●		
CD45	B-B3						●
CD45RA	B-C15	●	●	●	●		●
CD45RO	UCHL1	●		●			
CD57	NC1	●					
CD95	B-G27	●	●	●	●		
CD95	B-D29	●	●				
CD154 / CD40 Ligand	B-B29	●	●	●	●		
IFNγ	B-B1		●	●	●		
IL-1β	B-A15		●	●	●		
IL-2	B-G5		●	●	●		
IL-4	B-S4		●				
IL-4	B-G28				●		
IL-6	B-E8		●		●		
IL-6	B-F6		●				
IL-10	B-S10		●				
IL-12p35+p70	B-T21		●	●			
IL-12p40+p70	B-P24		●	●			
IL-12p40+p70	B-P40		●				
IL-13	B-B13		●	●			
IL-15	B-E29		●				
IL-17A	B-B51		●				
IL-17A	B-A50	●					
IL-17F	B-F60		●				
IL-23	B-Z23		●				
IL-27	B-G49		●	●	●		
TNFα	B-D9			●	●		
TNFα	B-C7		●				

Diaclone Kits

Analyte	Species	ELISA	ELI-Pair	ELISpot	ELISpot-PC	ELISpot-pair
CD25 / IL-2R	Human	●	●			
CD40L	Human	●				
CD95 / FAS	Human	●				
IFNγ	Human	●	●	●	●	●
IFNγ	Murine	●	●	●	●	●
IFNγ	Rat	●	●	●	●	●
IFNγ High Sensitivity	Human	●				
IL-1α	Human	●	●			
IL-1α	Murine	●				
IL-1β	Human	●	●	●	●	●
IL-1β	Rat	●				
IL-2	Human	●	●	●	●	●
IL-2	Murine	●	●	●	●	●
IL-2 High Sensitivity	Human	●				
IL-4	Human	●	●	●	●	●
IL-4	Murine	●				
IL-4	Rat	●	●			
IL-4 High Sensitivity	Human	●				
IL-5	Human	●	●	●	●	●
IL-5	Murine	●				
IL-6	Human	●	●	●	●	●
IL-6	Murine	●	●			
IL-6	Rat	●				
IL-6 High Sensitivity	Human	●				
IL-10	Human	●	●	●	●	●
IL-10	Murine	●	●			
IL-10 High Sensitivity	Human	●				
IL-12p70	Human	●	●	●	●	●
IL-12p70	Murine	●				
IL-12 p70 High Sensitivity	Human	●				
IL-12p40+p70	Human	●				
IL-13	Human	●	●	●	●	●
IL-15	Human	●				
IL-17A	Human	●	●	●	●	●
IL-17F	Human	●	●	●	●	●
IL-17A/F	Human	●	●	●	●	●
IL-23	Human	●				
IL-27	Human	●				
TNFα	Human	●	●	●	●	●
TNFα	Murine	●				
TNFα	Rat	●	●	●	●	●
TGFβ1	Human	●				
TGFβ1	Murine	●				
TGFβ1	Rat	●				

Diaclone Dual ELISpot / Dual Fluorospot

Analyte 1	Analyte 2	Species	Dual ELISpot	Dual Fluorospot
IFNγ	IL-2	Human	●	●
IFNγ	IL-4	Human	●	●
IFNγ	IL-5	Human	●	●
IFNγ	IL-10	Human	●	●
IFNγ	IL-17A	Human	●	●
IL-10	IL-2	Human	●	●
IL-10	IL-4	Human	●	●

Diaclone DIAplex: Multiple analyte detection by flow cytometry, Flexible by design

Design	Analyte	Species	Complete Panels				
			Th1 Complete	Th2 Complete	Th1/Th2 Complete	Inflammation Complete	Complete
IL-1β	Human					●	●
IL-2	Human	●			●		●
IL-4	Human			●	●		●
IL-6	Human			●	●		●
IL-8	Human					●	●
IL-10	Human			●	●		●
IL-12p70	Human	●			●		●
IL-17A	Human					●	●
IFNγ	Human	●			●		●
TNFα	Human	●			●	●	●

