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Memory CD4⁺ T Cells: Fate Determination, Positive Feedback and Plasticity

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Abstract

Naïve CD4⁺ T cells undergo massive cell proliferation upon encountering their cognate ligand. This proliferation depends upon appropriate cues from the antigen-presenting cells that have processed and present the peptide to the T cells, and requires the establishment of a cytokine environment that can support such proliferation. Expansion of antigen-specific CD4⁺ T cells needs to be coupled with differentiation into one of several effector/regulatory phenotypes if the priming event is to result in cells that can initially act to control the particular pathogen that elicited the response and later to serve as memory cells to insure an appropriate response upon reintroduction of the pathogen. Here, we discuss the initiation of T helper lineage commitment, the positive feedback regulation by the cytokine environment to enhance and stabilize the differentiation into distinct T helper subsets, and the biological significance of CD4⁺ T cell plasticity and long-term CD4⁺ T cell memory.

Keywords

T helper cell differentiation; Effector CD4⁺ T cells; Memory CD4⁺ T cells; T cell plasticity; T helper lineage-specific transcription factors; Positive feedback regulation by cytokines

Multiple CD4⁺ T Cell Fates

While it has been known since the early 1980s and even before, that CD4⁺ T cells can produce extremely potent factors that would regulate the immune/inflammatory response, it was only with the pathbreaking work of Mosmann and Coffman (1) and slightly later Bottomly and her colleagues (2) that it was recognized that production of distinct sets of effector and regulatory molecules is a property of distinct types of memory phenotype CD4⁺ T cells. This initial work established a paradigm that has dominated the field for more a decade and a half, the Th1/Th2 concept. What was demonstrated was that long-term CD4⁺ T cell clones can be separated into two groups, that produce IFN γ and IL-2 as their dominant cytokines or alternatively IL-4. The former cells are designated Th1 and the latter Th2 cells (1).

The cytokine-producing properties of these cell types are very stable in tissue culture. Long-term T cell lines retain their pattern of cytokine secretion apparently indefinitely. Indeed, later, when it was learned how to differentiate naïve CD4⁺ T cells into Th1 or Th2 cells *in vitro*, these differentiated cells could be transferred into recipient mice where they would

retain their properties for the life of the recipient (3; Jane Hu-Li, unpublished observations), implying that a key element of the CD4⁺ differentiated state is stability.

Quite soon after the demonstration that long-term T cell clones largely fall into the Th1 or Th2 differentiated states, several groups came to the conclusion that *in vivo* CD4⁺ T cells can be polarized into one of these two alternative fates during particular types of infections and that choosing the “wrong” fate could have disastrous consequences in animals infected with particular pathogens. Perhaps the most striking results came from the analysis of immune responses to infection with parasites. It was shown by Locksley and his colleagues that most inbred mouse strains mount protective immune responses to *Leishmania major*. In these animals, the antigen-specific CD4⁺ T cells are dominated by those that produce IFN γ (4). However, some mouse strains, most notably BALB/c, succumb to *L. major* infection. These animals have made a vigorous immune response, but the responding CD4⁺ T cells were largely IL-4-producers (5). Indeed, IL-4 or IL-4 receptor knockout BALB/c mice control *L. major* infection substantially better than do wild-type BALB/c mice, establishing that an IL-4-dominated CD4⁺ T cell response is “inappropriate” for *L. major* infection (6). Further, it showed that naïve CD4⁺ T cells can differentiate *in vivo* into cells that dominantly produce IFN γ (Th1 cells) or cells that dominantly produce IL-4 (Th2 cells), that the host generally chooses a response that is most appropriate to the threat, and that failing to choose the most appropriate fate could have disastrous consequences (6).

Molecular Basis of CD4⁺ T Cell Fate Determination

These results implied that the conditions established at the time of antigen encounter would have a major impact on the differentiated state that the expanding naïve CD4⁺ T cells would choose and the properties they would later display as effector and subsequently as memory cells. Accordingly, to understand the molecular basis of the differentiation process became an important goal. The first successes in determining the conditions important to the adoption of one of the alternative differentiated states was for *in vitro* polarization of naïve CD4⁺ T cells into Th2 cells (7, 8). This work showed that naïve CD4⁺ T cells stimulated with anti-CD3 and anti-CD28, and somewhat later, with their cognate antigen (9, 10), would acquire the capacity to produce IL-4 and other Th2 cytokines if their initial T cell receptor (TCR) stimulation occurs in the presence of IL-4 and IL-2. The initial striking finding that the principal product of the differentiated cell is also a key factor in its differentiation is very provocative and raises the possibility that CD4⁺ T cell differentiation may have an important positive feedback component.

When the analysis of Th1 differentiation *in vitro* was carried out sometime later (10), it was found that the key factor that needed to be added to the culture to foster such differentiation was IL-12, a product of dendritic cells (DC) and macrophages but not of CD4⁺ T cells. Indeed, it was first shown that the addition of heat-killed *Listeria monocytogenes* (HKLM) to the differentiation culture led to Th1 differentiation. This was then shown to be due to the capacity of HKLM to cause macrophages in the culture to produce IL-12 (11).

The dominant role played by IL-12 appeared at odds with the positive feedback concept. Rather, it argued that different pathways of differentiation are determined by the particular STAT family member that is activated at the time of priming, with STAT6 activated by IL-4 favoring Th2 differentiation, and STAT4 activated by IL-12 favoring Th1 differentiation. Indeed, CD4⁺ T cells from STAT4 KO mice are deficient in Th1 differentiation *in vitro* and these mice are susceptible to *Listeria monocytogenes* (12, 13). STAT6 KO mice fail to differentiate to Th2 cells *in vitro* (14, 15, 16), although they can, under certain conditions, undergo such differentiation both *in vitro* and *in vivo* (17, 18).

It was only somewhat later, with further analysis of the Th1 differentiation process, that such differentiation was recognized to be both more complex and more analogous to the Th2 process. Thus, a careful study revealed that neutralization of IFN γ during IL-12-driven Th1 differentiation partially diminishes polarization to the Th1 phenotype (19), and analysis of Th1 differentiation by T cells from IFN γ receptor 2 KO mice reveals major defects (20). Further analysis indicated that there is a complex process underlying Th1 differentiation, in which both STAT4 and IFN γ , acting through STAT1, promote activation of the “master regulator transcription factor” of Th1 cells, T-bet, whose activity feeds back to cause additional IFN γ production and more T-bet expression thus promoting Th1 differentiation and the acquisition of the capacity for high-rate IFN γ production (21, 22, 23).

In parallel with the recognition of the greater complexity of Th1 differentiation involving a requirement for two cytokines, IL-12 and IFN γ , at the time of antigen-stimulation for optimal differentiation, came a reexamination of the role of IL-2 in Th2 priming. The very first paper describing *in vitro* Th2 priming had indicated a need for IL-2 (7), but the importance of IL-2 in Th2 differentiation was not fully appreciated because of its central role in T cell survival and proliferation. Careful reexamination revealed that IL-2 acting through STAT5 collaborates with the Th2 master regulatory transcription factor, GATA3, in the activation and transcription of the *Il4* gene (24, 25). Indeed, mutations in STAT5 result in defective Th2 differentiation both *in vitro* and *in vivo* (26, 27).

The lessons learned from *in vitro* differentiation of Th1 and Th2 cells were relearned when the existence of additional fates was recognized and when *in vitro* differentiation of the naïve cells to these “new” fates was studied. Thus, Th17 cells can be induced *in vitro* from naïve cells when stimulated through their TCR in the presence of TGF β and IL-6, a STAT3 activator (28, 29, 30). The key master regulator in this case is ROR γ t (31). Although the differentiating Th17 cells fail to produce IL-6, they produce another potent STAT3 activator, IL-21, which in turn acts on activated CD4⁺ T cells to further upregulate STAT3-driven IL-23 receptor expression (32, 33, 34). Indeed, IL-23, also a STAT3 activator, produced by DC plays a crucial role in the maintenance of Th17 phenotype (32, 33, 34). Thus, the possibility of a major feedback loop in Th17 differentiation and maintenance as an indirect process, through the intermediary first of IL-21 and later of IL-23, becomes a real possibility.

Having established that positive feedback is a possibility, in that differentiating cells can make one of the key cytokines that drives their differentiation, the regulation and dynamics of this process needs to be established. In a later section of this review, we consider in detail the molecular basis of endogenous IL-4 production in differentiating Th2 cells and how such “early” T cell-derived IL-4 contributes to Th2 differentiation.

In vivo Differentiation

However, it is also important to determine to what degree the proliferating/differentiating T cell itself is the source of the “biasing” cytokine that can begin the fate determination process and how physiologically important self-feedback is in this process.

There are many potential sources of IFN γ and IL-12 that could be important in Th1 differentiation. Perhaps most notable are DCs, in particular the subset of DCs expressing CD8 α , which produce the Th1-polarizing cytokines in response to encounter with and detection of microbial and viral components (35, 36). NK cells may also contribute to establishing the cytokine environment that influences the differentiation of CD4⁺ T cells as they respond to their cognate antigen. NK cells are normally excluded from lymph nodes (LN) but are recruited to the draining LN upon immunization with antigen plus adjuvant. NK cells produce large amounts of IFN γ in response to IL-12 that has been produced by

DC. Thus, NK cells may play an important role as an exogenous source of IFN γ driving toward Th1 differentiation (37).

The issue of what cell makes the IL-4 that induces Th2 differentiation has been grappled with ever since the importance of IL-4 in *in vitro* Th2 differentiation was established. In addition to CD4⁺ T cells (as discussed later), IL-4 can be made in large amounts by NKT cells (38), basophils (39), and some γ/δ T cells (40). Since these cells are generally not in the immediate priming environment of naïve CD4⁺ T cells, their role has been a matter of uncertainty. However, this may need to be rethought in view of a report that the effect of IL-4 produced during a priming event may be detected throughout the whole lymph node and is not simply confined to the immediate vicinity of the IL-4-producer (41).

Even more provocative is the question of how important IL-4 is *in vivo* Th2 differentiation. Indeed, Th2 differentiation in response to infection with some, but not all, helminths can be achieved in the absence of IL-4, IL-4 receptor (IL-4R) or STAT6 (42). However, GATA3 is still indispensable for Th2 differentiation elicited by helminth infection (43). This may be either accounted for by an IL-4-independent pathways leading to GATA3 upregulation during *in vivo* Th2 induction or because GATA3 upregulation above the level found in naïve CD4⁺ T cells may not be essential to initiate and sustain the Th2 state, although GATA3 is essential for Th2 differentiation. Therefore, understanding the regulation of GATA3 expression and its mechanism of action in *in vivo* Th2 differentiation is an issue of considerable importance.

Pathways Regulating IL-4-Independent GATA3 expression

Naïve CD4⁺ T cells express a measurable amount of GATA3, presumably a relic of the important function GATA3 plays in CD4⁺ T cell differentiation in the thymus (43, 44). Its expression is upregulated when stimulated under Th2-polarizing conditions *in vitro*; fundamentally, such conditions are the provision of exogenous IL-4 and the neutralization of IFN γ and IL-12 (45). Upregulation of GATA3 in this setting requires both the provision of IL-4 and then engagement of the TCR, implying the involvement of the pathways downstream of TCR in GATA3 upregulation. Indeed, several pathways have been proposed to mediate TCR-induced GATA3 upregulation independent of IL-4/STAT6. These include the NF- κ B1/Bcl3 complex (46, 47), the Notch1/CSL pathway (48, 49), the Wnt/ β -catenin/TCF1 pathway (50). In each of these situations, CD4⁺ T cells from gene-targeted mice are deficient in expressing GATA3 in response to TCR. A potential difficulty with these experiments is that the deleted genes play an important role in thymocyte development as well as in peripheral T cell activation. Therefore, the cells being studied may bear developmental abnormalities and/or activation defects that result in their failure to upregulate GATA3 expression and subsequent IL-4 production. Thus, more detailed study will be needed to determine whether these pathways can directly mediate GATA3 upregulation in naïve peripheral CD4 T cells, whether they do so independently of IL-4/STAT6 and whether they are indispensable for such upregulation.

In order to clarify the role of TCR signaling in IL-4-independent GATA3 upregulation, we made use of a TCR signal strength model in which weak and strong TCR signals have been reported to lead to Th2 and Th1 differentiation, respectively (51). When naïve CD4⁺ T cells from 5C.C7 TCR-transgenic (Tg) mice are stimulated *in vitro* with low concentrations of their cognate antigen, a pigeon cytochrome c (PCC) peptide, they undergo substantial Th2 differentiation. In these weakly stimulated T cells, TCR-driven activation of extracellular signal-regulated kinases (ERK) is weak and transient, and GATA3 is upregulated beginning at 12 hours of activation in an IL-4-independent manner, by a yet-unknown mechanism. The upregulated GATA3 collaborates with STAT5 activated by endogenously produced IL-2 to

mediate IL-4-independent production of IL-4. We refer to this phase of the Th2 differentiation process as the “induction phase”. It is generally completed within the first 24 hours of stimulation of naïve CD4⁺ T cells with low concentrations of PCC peptide. This limited amount of TCR-induced endogenous IL-4 in turn acts through the IL-4 receptor/STAT6 pathway to further enhance GATA3 expression in activated CD4⁺ T cells. This further enhanced GATA3 and the continued IL-2-driven STAT5 activation jointly lead to production of a far larger amount of IL-4, providing a positive feedback loop that results in the completion and stabilization of Th2 phenotype. We refer to this IL-4-dependent phase of the Th2 differentiation process as the “polarization phase” (25).

By contrast, 5C.C7 TCR-Tg naïve CD4⁺ T cells stimulated with high concentrations of PCC peptide fail to differentiate into Th2 cells. In these cells, TCR-dependent, IL-4-independent GATA3 upregulation does not occur and IL-2-driven STAT5 activation is transiently blocked despite the production of a large amount of IL-2 by the “high dose-stimulated” T cells. It appears that the failure to upregulate GATA3 by cells stimulated with high concentrations of peptide is mediated by ERK. Indeed, stimulation with high concentrations of PCC peptide induces strong and prolonged TCR-driven ERK activation. Blockade of the ERK pathway with a pharmacological inhibitor allows T cells receiving a strong TCR signal to upregulate GATA3 and to activate STAT5 in response to endogenously produced IL-2, leading to the restoration of IL-4 production during the induction phase and the subsequent completion of the Th2 polarization process (25).

Controversy on the Role of ERK Pathway in Regulating GATA3 Expression

The role of ERK as the major negative regulator of GATA3 upregulation and IL-4 production has been called into question by studies using mice in which dominant-negative (dn) Lck or dn H-Ras is selectively overexpressed in T cells. Naïve CD4⁺ T cells from these mice show diminished Th2 differentiation *in vitro* (52). This result has been interpreted to indicate that TCR-driven Ras/ERK activation is *required* for Th2 differentiation, possibly through enhancement of IL-4-stimulated STAT6 activation (53) and/or through prevention of ubiquitin/proteasome-mediated degradation of GATA3 in the developing Th2 cells as a result of inhibiting the activity of Mdm2, an E3 ubiquitin ligase specific for GATA3 (54).

The opposing views on the role of ERK in Th2 differentiation need to be considered in terms of our two-phase model of Th2 polarization. Indeed, naïve CD4⁺ T cells from dn Lck-Tg mice actually produce significantly more IL-4 than do those from the littermate controls over the first 24 hours after stimulation with a cognate peptide (52), implying that the Lck/Ras/ERK pathway actually blocks IL-4 production during the induction phase. However, IL-2 production by stimulated CD4⁺ T cells from dn Lck-Tg mice is significantly decreased in comparison to that by control cells (52). Diminished IL-2 production could account for impaired Th2 differentiation, since STAT5 activation is essential for both the induction and polarization phases of Th2 differentiation and the degree of STAT5 activation may have fallen below a threshold level during the polarization phase. Indeed, we observed that blockade of the ERK pathway impairs Th2 differentiation of 5C.C7 TCR-Tg naïve CD4⁺ T cells stimulated with low concentrations of PCC peptide because of a substantial diminution in IL-2 production and that exogenous IL-2 fully rescues Th2 differentiation in cells in which the ERK pathway is blocked (25).

Recently, Leonard and his colleagues showed that IL-2 suppresses Mdm2 mRNA expression in CD4⁺ T cells in a STAT5-dependent manner (27). Therefore, it is conceivable that the Ras/ERK cascade may indirectly stabilize GATA3 expression through its action on IL-2 production by activated CD4⁺ T cells, which downregulates Mdm2 expression. Thus, the degree of ERK activation in response to TCR stimulation may coordinately control Th2

differentiation by suppressing GATA3 upregulation at a transcriptional level during the induction phase and by preventing GATA3 from proteasome-mediated degradation during the polarization phase.

Significance of CD4⁺ T Cell Fate to Memory

While early events in CD4⁺ T cell differentiation have been analyzed in considerable detail and much is known about the timing and nature of the process, we are only now coming to understand their significance for long-term T cell memory. Early in this review, it was pointed out that long-term Th1 and Th2 CD4⁺ T cell lines were very stable. It seemed reasonable that this should be the case. In general, the fate adopted during a primary CD4⁺ T cell response to a given pathogen is optimized to eliminate or control the pathogen. The consequences of developing a non-optimized response are often quite disastrous as illustrated by the inappropriate Th2 response mounted by BALB/c mice infected with *L. major* (5, 6). It seemed reasonable therefore that CD4⁺ T cells that had adopted a given fate as a result of priming by infection with a particular microbial pathogen would retain that differentiated state so that when challenged by a second experience with the same pathogen, they would surely make a response of the correct phenotype. However, an analysis of chromatin accessibility of T-bet and GATA3, the master regulatory factors of Th1 and Th2 cells, respectively, indicated that these genes show association with both trimethylated lysine 4 histone H3 (H3K4me3) and H3K27me3 in the lineages other than those in which they are expressed (55). This implies that these master regulatory factors are in a poised state, often associated with plastic genes. Indeed, an increasing number of instances of change in phenotypic state have now been observed, indicating that plasticity in gene expression and function may be important physiologic properties of differentiated T helper cells. Striking examples of plasticity include the capacity of Th17 cells to adopt Th1 characteristics (56) or of iTregs to become Th17 cells (57). Lohning and colleagues have reported a particularly impressive example of Th2 cells changing their properties when confronting an infection with lymphocytic choriomeningitis virus (58). In this case, rather than adopting a “full” Th1 phenotype, they acquired the capacity to produce IFN γ and expressed T-bet while retaining, although at lower levels, potential for IL-4 production and expression of GATA3 (58).

Plasticity can certainly occur *in vitro* and under experimental conditions *in vivo*. It is still far from clear how often such plasticity occurs in the course of normal responses to pathogens and what its importance is in protective immunity. However, it may be argued that plasticity could be of considerable importance when memory cells respond to pathogens different from those that elicited their priming. There is considerable evidence of T cell cross-reactivity. This has been shown most persuasively for virus-specific CD8 responses (59) but is probably a general phenomenon. Indeed, in aging individuals, the proportion of naïve CD4⁺ T cells falls quite precipitously. It can be expected that in such individuals, responses to newly introduced pathogens may depend to a greater or lesser degree on cross-reactivity of memory T cells that had been primed to different pathogens. Under these circumstances, the capacity of the differentiated CD4⁺ T cells to alter the cytokines they produce and their phenotypic state could be of great importance since there is no assurance that the polarized phenotype adopted by cells responding to the priming pathogen would be appropriate to a response to the cross-reactive pathogen.

Concluding Remarks

The differentiation of CD4⁺ T cells has been studied in greater detail than any other inducible polarization process in the immune system and represents one of the most tractable systems in which to study differentiation in higher vertebrates. Here we have shown that this

process has, as a general rule, the potential for a potent feedback regulatory control. The positive feedback nature of this control should make possible a strong phenotype polarization even under conditions in which the initial biasing stimulation is relatively weak and thus should strongly aid in the development of a CD4⁺ T cell response that is most appropriate for the nature of the infectious agent that elicited the response. Indeed, positive feedback should serve to ensure that cells adopt predictable differentiation patterns and that when mixtures of stimulants are present, the outcome will be that some cells adopt one polarized phenotype and others an alternative polarized phenotype rather than all the precursors developing into cells with intermediate properties. Much work is nonetheless needed to ascertain not only whether one can demonstrate such striking positive feedback regulation *in vivo* but whether this mechanism is critical to the development of polarized CD4⁺ T cells under conditions of *in vivo* infections and inflammatory responses.

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References

1. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol.* 1986; 136:2348–2357. [PubMed: 2419430]
2. Killar L, MacDonald G, West J, Woods A, Bottomly K. Cloned, Ia-restricted T cells that do not produce interleukin 4 (IL4)/B cell stimulatory factor 1 (BSF-1) fail to help antigen-specific B cells. *J Immunol.* 1987; 138:1674–1679. [PubMed: 2950166]
3. Hu-Li J, Huang H, Ryan J, Paul WE. In differentiated CD4⁺ T cells, interleukin 4 production is cytokine-autonomous, whereas interferon γ production is cytokine-dependent. *Proc Natl Acad Sci USA.* 1997; 94:3189–3194. [PubMed: 9096368]
4. Sadick MD, Locksley RM, Tubbs C, Raff HV. Murine cutaneous leishmaniasis: resistance correlates with the capacity to generate interferon- γ in response to *Leishmania* antigens in vitro. *J Immunol.* 1986; 136:655–661. [PubMed: 3079789]
5. Heinzel FP, Sadick MD, Holaday BJ, Coffman RL, Locksley RM. Reciprocal expression of interferon γ or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct T cell subsets. *J Exp Med.* 1989; 169:59–72. [PubMed: 2521244]
6. Noben-Trauth N, Paul WE, Sacks DL. IL-4- and IL-4 receptor-deficient BALB/c mice reveal differences in susceptibility to *Leishmania major* parasite substrains. *J Immunol.* 1999; 162:6132–6140. [PubMed: 10229856]
7. Le Gros G, Ben-Sasson SZ, Seder R, Finkelman FD, Paul WE. Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. *J Exp Med.* 1990; 172:921–929. [PubMed: 2117636]
8. Swain SL, Weinberg AD, English M, Huston G. IL-4 directs the development of Th2-like helper effectors. *J Immunol.* 1990; 145:3796–3806. [PubMed: 2147202]
9. Seder RA, Paul WE, Davis MM, Fazekas de St Groth B. The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4⁺ T cells from T cell receptor transgenic mice. *J Exp Med.* 1992; 176:1091–1098. [PubMed: 1328464]
10. Hsieh CS, Heimberger AB, Gold JS, O'Garra A, Murphy KM. Differential regulation of T helper phenotype development by interleukins 4 and 10 in an $\alpha\beta$ T-cell-receptor transgenic system. *Proc Natl Acad Sci USA.* 1992; 89:6065–6069. [PubMed: 1385868]
11. Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 CD4⁺ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science.* 1993; 260:547–549. [PubMed: 8097338]

12. Thierfelder WE, van Deursen JM, Yamamoto K, Tripp RA, Sarawar SR, Carson RT, Sangster MY, Vignali DA, Doherty PC, Grosveld GC, Ihle JN. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. *Nature*. 1996; 382:171–174. [PubMed: 8700208]
13. Kaplan MH, Sun YL, Hoey T, Grusby MJ. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. *Nature*. 1996; 382:174–177. [PubMed: 8700209]
14. Kaplan MH, Schindler U, Smiley ST, Grusby MJ. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. *Immunity*. 1996; 4:313–319. [PubMed: 8624821]
15. Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, Nakanishi K, Yoshida N, Kishimoto T, Akira S. Essential role of Stat6 in IL-4 signalling. *Nature*. 1996; 380:627–630. [PubMed: 8602263]
16. Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, Chu C, Quelle FW, Nosaka T, Vignali DA, Doherty PC, Grosveld G, Paul WE, Ihle JN. Lack of IL-4-induced Th2 responses and IgE class switching in mice with disrupted *Stat6* gene. *Nature*. 1996; 380:630–633. [PubMed: 8602264]
17. Dent AL, Hu-Li J, Paul WE, Staudt LM. T helper type 2 inflammatory disease in the absence of interleukin 4 and transcription factor STAT6. *Proc Natl Acad Sci USA*. 1998; 95:13823–13828. [PubMed: 9811885]
18. Jankovic D, Kullberg MC, Noben-Trauth N, Caspar P, Paul WE, Sher A. Single cell analysis reveals that IL-4 receptor/Stat6 signaling is not required for the in vivo or in vitro development of CD4⁺ lymphocytes with a Th2 cytokine profile. *J Immunol*. 2000; 164:3047–3055. [PubMed: 10706693]
19. Bradley LM, Dalton DK, Croft M. A direct role for IFN- γ in regulation of Th1 cell development. *J Immunol*. 1996; 157:1350–1358. [PubMed: 8759714]
20. Lu B, Ebensperger C, Dembic Z, Wang Y, Kvatyuk M, Lu T, Coffman RL, Pestka S, Rothman PB. Targeted disruption of the interferon- γ receptor 2 gene results in severe immune defects in mice. *Proc Natl Acad Sci USA*. 1998; 95:8233–8238. [PubMed: 9653170]
21. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell*. 2000; 100:655–669. [PubMed: 10761931]
22. Lighvani AA, Frucht DM, Jankovic D, Yamane H, Aliberti J, Hissong BD, Nguyen BV, Gadina M, Sher A, Paul WE, O'Shea JJ. T-bet is rapidly induced by interferon- γ in lymphoid and myeloid cells. *Proc Natl Acad Sci USA*. 2001; 98:15137–15142. [PubMed: 11752460]
23. Afkarian M, Sedy JR, Yang J, Jacobson NG, Cereb N, Yang SY, Murphy TL, Murphy KM. T-bet is a STAT1-induced regulator of IL-12R expression in naïve CD4⁺ T cells. *Nat Immunol*. 2002; 3:549–557. [PubMed: 12006974]
24. Cote-Sierra J, Foucras G, Guo L, Chiodetti L, Young HA, Hu-Li J, Zhu J, Paul WE. Interleukin 2 plays a central role in Th2 differentiation. *Proc Natl Acad Sci USA*. 2004; 101:3880–3885. [PubMed: 15004274]
25. Yamane H, Zhu J, Paul WE. Independent roles for IL-2 and GATA-3 in stimulating naïve CD4⁺ T cells to generate a Th2-inducing cytokine environment. *J Exp Med*. 2005; 202:793–804. [PubMed: 16172258]
26. Kagami S, Nakajima H, Kumano K, Suzuki K, Suto A, Imado K, Davey HW, Saito Y, Takatsu K, Leonard WJ, Iwamoto I. Both Stat5a and Stat5b are required for antigen-induced eosinophil and T-cell recruitment into the tissue. *Blood*. 2000; 95:1370–1377. [PubMed: 10666213]
27. Liao W, Schones DE, Oh J, Cui Y, Cui K, Roh TY, Zhao K, Leonard WJ. Priming for T helper type 2 differentiation by interleukin 2-mediated induction of interleukin 4 receptor α -chain expression. *Nat Immunol*. 2008; 9:1288–1296. [PubMed: 18820682]
28. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGF β in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing cells. *Immunity*. 2006; 24:179–189. [PubMed: 16473830]
29. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT. Transforming growth factor- β induces development of the T_H17 lineage. *Nature*. 2006; 441:231–234. [PubMed: 16648837]

30. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector T_H17 and regulatory T cells. *Nature*. 2006; 441:235–238. [PubMed: 16648838]
31. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. The orphan nuclear receptor ROR γ t directs the differentiation program of proinflammatory IL-17⁺ T helper cells. *Cell*. 2006; 126:1121–1133. [PubMed: 16990136]
32. Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, Schluns K, Tian Q, Watowich SS, Jetten AM, Dong C. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature*. 2007; 448:480–483. [PubMed: 17581589]
33. Korn T, Bettelli E, Gao W, Awasthi A, Jäger A, Strom TB, Oukka M, Kuchroo VK. IL-21 initiates an alternative pathway to induce proinflammatory T_H17 cells. *Nature*. 2007; 448:484–487. [PubMed: 17581588]
34. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR. IL-6 programs T_H-17 differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol*. 2007; 8:967–974. [PubMed: 17581537]
35. Maldonado-López R, De Smedt T, Michel P, Godfroid J, Pajak B, Heirman C, Thielemans K, Leo O, Urbain J, Moser M. CD8 α ⁺ and CD8 α [−] subclass of dendritic cells direct the development of distinct T helper cells in vivo. *J Exp Med*. 1999; 189:587–592. [PubMed: 9927520]
36. Pulendran B, Smith JL, Caspary G, Brasel K, Pettit D, Maraskovsky E, Maliszewski CR. Distinct dendritic cell subsets differentially regulate the class of immune response *in vivo*. *Proc Natl Acad Sci USA*. 1999; 96:1036–1041. [PubMed: 9927689]
37. Martín-Fontecha A, Thomsen LL, Brett S, Gerard C, Lipp M, Lanzavecchia A, Sallusto F. Induced recruitment of NK cells to lymph nodes provides IFN- γ for T_H1 priming. *Nat Immunol*. 2004; 5:1260–1265. [PubMed: 15531883]
38. Yoshimoto T, Paul WE. CD4^{POS}, NK1.1^{POS} T cells promptly produce interleukin 4 in response to in vivo challenge with anti-CD3. *J Exp Med*. 1994; 179:1285–1295. [PubMed: 7908323]
39. Seder RA, Paul WE, Dvorak AM, Sharkis SJ, Kagey-Sobotka A, Niv Y, Finkelman FD, Barbieri SA, Galli SJ, Plaut M. Mouse splenic and bone marrow cell populations that express high-affinity Fc ϵ receptors and produce interleukin 4 are highly enriched in basophils. *Proc Natl Acad Sci USA*. 1991; 88:2835–2839. [PubMed: 1826367]
40. Ferrick DA, Schrenzel MD, Mulvania T, Hsieh B, Ferlin WG, Lepper H. Differential production of interferon- γ and interleukin-4 in response to Th1- and Th2-stimulating pathogens by $\gamma\delta$ T cells *in vivo*. *Nature*. 1995; 373:255–257. [PubMed: 7816142]
41. Perona-Wright G, Mohrs K, Mohrs M. Sustained signaling by canonical helper T cell cytokines throughout the reactive lymph node. *Nat Immunol*. 2010; 11:520–526. [PubMed: 20418876]
42. van Panhuys N, Tang SC, Prout M, Camberis M, Scarlett D, Roberts J, Hu-Li J, Paul WE, Le Gros G. *In vivo* studies fail to reveal a role for IL-4 or STAT6 signaling in Th2 lymphocyte differentiation. *Proc Natl Acad Sci USA*. 2008; 105:12423–12428. [PubMed: 18719110]
43. Zhu J, Min B, Hu-Li J, Watson CJ, Grinberg A, Wang Q, Killeen N, Urban JF Jr, Guo L, Paul WE. Conditional deletion of *Gata3* shows its essential function in T_H1-T_H2 responses. *Nat Immunol*. 2004; 5:1157–1165. [PubMed: 15475959]
44. Pai SY, Truitt ML, Ho IC. GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. *Proc Natl Acad Sci USA*. 2004; 101:1993–1998. [PubMed: 14769923]
45. Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell*. 1997; 89:587–596. [PubMed: 9160750]
46. Das J, Chen CH, Yang L, Cohn L, Ray P, Ray A. A critical role for NF- κ B in *Gata3* expression and T_H2 differentiation in allergic airway inflammation. *Nat Immunol*. 2001; 2:45–50. [PubMed: 11135577]
47. Corn RA, Hunter C, Liou HC, Siebenlist U, Boothby MR. Opposing roles for RelB and Bcl-3 in regulation of T-box expressed in T cells, GATA-3, and Th effector differentiation. *J Immunol*. 2005; 175:2102–2010. [PubMed: 16081776]
48. Amsen D, Antov A, Jankovic D, Sher A, Radtke F, Souabni A, Busslinger M, McCright B, Gridley T, Flavell RA. Direct regulation of *Gata3* expression determines the T helper differentiation potential of Notch. *Immunity*. 2007; 27:89–99. [PubMed: 17658279]

49. Fang TC, Yashiro-Ohtani Y, Del Bianco C, Knoblock DM, Blacklow SC, Pear WS. Notch directly regulates *Gata3* expression during T helper 2 cell differentiation. *Immunity*. 2007; 27:100–110. [PubMed: 17658278]
50. Yu Q, Sharma A, Oh SY, Moon HG, Hossain MZ, Salay TM, Leeds KE, Du H, Wu B, Waterman ML, Zhu Z, Sen JM. T cell factor 1 initiates the T helper type 2 fate by inducing the transcription factor GATA-3 and repressing interferon- γ . *Nat Immunol*. 2009; 10:992–999. [PubMed: 19648923]
51. Constant S, Bottomly K. Induction of TH1 and TH2 CD4⁺ T cell responses: The alternative approaches. *Annu Rev Immunol*. 1997; 15:297–322. [PubMed: 9143690]
52. Yamashita M, Hashimoto K, Kimura M, Kubo M, Tada T, Nakayama T. Requirement for p56^{lck} tyrosine kinase activation in T_H subset differentiation. *Int Immunol*. 1998; 10:577–591. [PubMed: 9645606]
53. Yamashita M, Kimura M, Kubo M, Shimizu C, Tada T, Perlmutter RM, Nakayama T. T cell antigen receptor-mediated activation of the Ras/mitogen-activated protein kinase pathway controls interleukin 4 receptor function and type-2 helper T cell differentiation. *Proc Natl Acad Sci USA*. 1999; 96:1024–1029. [PubMed: 9927687]
54. Yamashita M, Shinnakasu R, Asou H, Kimura M, Hasegawa A, Hashimoto K, Hatano N, Ogata M, Nakayama T. Ras-ERK MAPK cascade regulates GATA3 stability and Th2 differentiation through ubiquitin-proteasome pathway. *J Biol Chem*. 2005; 280:29409–29419. [PubMed: 15975924]
55. Wei G, Wei L, Zhu J, Zang C, Hu-Li J, Yao Z, Cui K, Kanno Y, Roh TY, Watford WT, Schones DE, Peng W, Sun HW, Paul WE, O'Shea JJ, Zhao K. Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4⁺ T cells. *Immunity*. 2009; 30:155–167. [PubMed: 19144320]
56. Lee YK, Turner H, Maynard CL, Oliver JR, Chen D, Elson CO, Weaver CT. Late developmental plasticity in the T helper 17 lineage. *Immunity*. 2009; 30:92–107. [PubMed: 19119024]
57. Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP, Shah B, Chang SH, Schluns KS, Watowich SS, Feng XH, Jetten AM, Dong C. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. *Immunity*. 2008; 29:44–56. [PubMed: 18585065]
58. Hegazy AN, Peine M, Helmstetter C, Panse I, Fröhlich A, Bergthaler A, Flatz L, Pinschewer DD, Radbruch A, Löhning M. Interferons direct Th2 cell reprogramming to generate a stable GATA-3⁺T-bet⁺ cell subset with combined Th2 and Th1 cell functions. *Immunity*. 2010; 32:116–128. [PubMed: 20079668]
59. Selin LK, Nahill SR, Welsh RM. Cross-reactivities in memory cytotoxic T lymphocyte recognition of heterologous viruses. *J Exp Med*. 1994; 179:1933–1943. [PubMed: 8195718]