THE LINEAGE DECISIONS OF HELPER T CELLS

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After encountering antigen, helper T (T_H) cells undergo differentiation to effector cells, which can secrete high levels of interferon-γ, interleukin-4 (IL-4), IL-10 and other immunomodulators. How T_H cells acquire, and remember, new patterns of gene expression is an area of intensive investigation. The process is remarkably plastic, with cytokines being key regulators. Extrinsic signals seem to be integrated into cell-intrinsic programming, in what is becoming an intriguing story of regulated development. We summarize the latest insights into mechanisms that govern the lineage choices that are made during T_H-cell responses to foreign pathogens.

DECISION MAKING IN THE IMMUNE SYSTEM

After engagement of the T-cell receptor (TCR) by the appropriate peptide–MHC complex, which triggers clonal expansion, helper T (T_H) cells rapidly undergo programmed differentiation (reviewed in REFS 1,2). This differentiation process can result in highly polarized immune responses in the case of chronic infections, such as parasitic infections. A more heterogeneous response is often shown by analyses of acute immune responses.

Naive T_H cells can differentiate to at least two functional classes of cell during an immune response[3] — T_H1 cells, which secrete interferon-γ (IFN-γ), and T_H2 cells, which secrete interleukin-4 (IL-4) (reviewed in REFS 1,2). Hereafter, we refer to IFN-γ and IL-4 as effector cytokines. T_H1 cells are responsible for cell-mediated immunity, whereas T_H2 cells are responsible for extracellular immunity. In terms of the control of parasites, T_H1 immunity provides protection against intracellular protozoa, such as *Leishmania* species and *Toxoplasma gondii*, whereas T_H2 immunity is associated with protection against intestinal helminths. As well as their protective roles in host defence, both subsets of T_H cell have been implicated in pathological responses. T_H1 cells can mediate organ-specific autoimmunity and T_H2 cells have been implicated in the pathogenesis of asthma and allergy. The final composition of the T_H-cell response to antigen can, therefore, determine whether the outcome of infectious, inflammatory and autoimmune responses is favourable or unfavourable.

The process by which an uncommitted T_H cell develops into a mature T_H1 or T_H2 cell is a useful model of developmentally regulated gene expression (FIG. 1). There is good evidence to indicate that this differentiation process is highly plastic. Many factors influence the decision to become a T_H1 or T_H2 cell. The cytokines IL-12 and IL-4, acting through signal transducer and activator of transcription 4 (STAT4) and STAT6, respectively, are key determinants of the outcome (reviewed in REFS 1,2). It has been proposed also that antigen dose, co-stimulators, genetic modifiers and other non-cytokine factors have crucial roles in determining the dominance of a T_H-cell

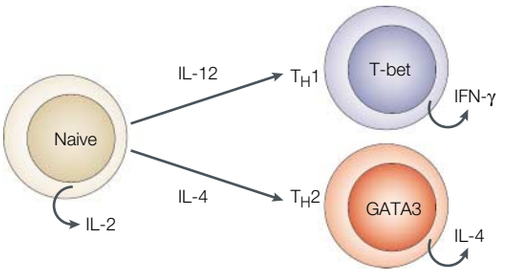

Figure 1 | **The original instructive model of helper T-cell differentiation.** An uncommitted, naive helper T (T_H)-cell precursor can become either a T_H1 or T_H2 cell under the instructive influence of interleukin-12 (IL-12) or IL-4, respectively. T_H1 cells express T-bet and secrete interferon-γ (IFN-γ). T_H2 cells express GATA3 and secrete IL-4.

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EPIGENETIC
Refers to the heritable, but potentially reversible, states of gene activity that are imposed by the structure of chromatin or covalent modifications of DNA and histones.

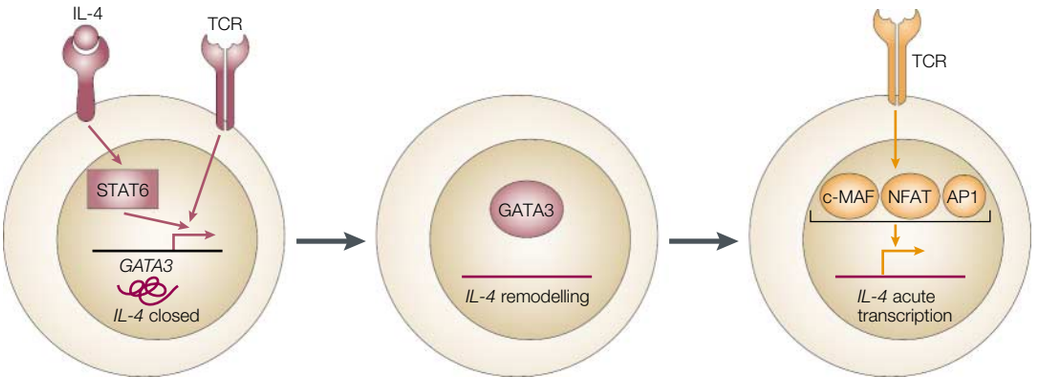

Figure 2 | **Pathways for the induction of expression of IL-4.** Naive helper T (T_H) cells stimulated with interleukin-4 (IL-4) and antigen, through the T-cell receptor (TCR), upregulate *GATA3* transcription. The GATA3 protein induces heritable remodelling of the *IL-4* locus, which is characteristic of fully differentiated T_H2 cells. Experimental evidence indicates that GATA3 might not have a crucial role in the acute transcription of the *IL-4* gene. Instead, a second step, mediated by signals downstream of the TCR, causes rapid acute transcription of *IL-4*. The mediators of this effect include lineage-specific (such as c-MAF) and non-specific (such as NFAT and AP1) transcription factors. NFAT, nuclear factor of activated T cells.

response. How each signal influences the differentiation process is an area of active investigation and, often, lively controversy.

Many data indicate that certain crucial transcription factors have causal roles in the gene-expression programmes of T_H1 and T_H2 cells (FIG. 1). For example, the T-box transcription factor T-bet has been shown to have a central role in T_H1-cell development[4,5], inducing both transcriptional competence of the locus encoding IFN-γ and selective responsiveness to the growth factor IL-12 (REFS 6,7). By contrast, the zinc-finger transcription factor GATA3 seems to be crucial for inducing some, but perhaps not all, key attributes of T_H2 cells — in particular, transcriptional competence of the T_H2 cytokine cluster, which includes the genes encoding IL-13, IL-4 and IL-5 (REFS 8–14).

Box 1 | **Chromatin remodelling**

There is an emerging consensus that the subset-specific expression of genes can be attributed to two types of effect of factors — general locus-activating effects or acute transcriptional effects. The first type of effect is associated with changes in gene structure, such as the repositioning of nucleosomes or decompaction of condensed chromatin fibres, and it is known as epigenetic or (imperfectly) as chromatin remodelling. The term epigenetic implies that the state is transferred from parent to daughter cell — in other words, some of the changes in gene activity that accompany helper T-cell differentiation are heritable. By contrast, the term chromatin remodelling can be used also in reference to acute, reversible, time-dependent changes in the chromatin structure of a promoter. Beyond chromatin remodelling, the acute transcriptional induction of subset-specific genes can be mediated by transcription factors that are either activated or expressed selectively in one subset, but that can induce transcription of remodelled gene targets only.

Chromatin remodelling might be required to induce competence for gene activity or transcription, and it is mediated by histone modifications, of which the best studied are acetylation, methylation and phosphorylation. In addition, histones can be ADP-ribosylated and ubiquitylated. The role of these modifications in gene expression is uncertain. The roles of acetylation, methylation and phosphorylation are complex and might vary depending on the specific histone residue that is being modified. Chromatin remodelling can be indicated by various technical parameters, including increased DNase-I hypersensitivity and histone acetylation.

In this article, we highlight some recent advances in our understanding of this model of developmentally regulated alterations in gene activity. We do not attempt to be encyclopaedic, but we try primarily to discuss and unite the transcriptional and EPIGENETIC mechanisms that act during T_H1- and T_H2-cell development, as well as mentioning a few topical cytokine-related issues.

How T_H2 cells arise

In terms of effector T-cell differentiation, T_H2-cell development was the first process to be linked to the actions of a cytokine. IL-4 was recognized early on to promote the development of the T_H2-cell subset[15–18]. Later, this activity was shown to operate through the actions of STAT6 (FIG. 2). The observation that T cells from Stat6-deficient mice have a severe block in T_H2-cell development led to the conclusion that STAT6 is necessary for T_H2-cell development[19–21].

Some transcription factors are expressed selectively by T_H2 cells. c-MAF, a member of the b-ZIP (basic-region leucine-zipper) protein family, was identified initially as a T_H2-cell-specific transcription factor that regulates the expression of IL-4 (REF. 22). c-Maf promotes skewing towards T_H2-cell development when it is expressed in transgenic mice[23], and c-Maf-deficient mice have a selective defect in IL-4 production[24]. A second, and perhaps further upstream, T_H2-cell-specific transcription factor, GATA3, was identified originally as regulating the expression of a broad array of T_H2 cytokines[10,11]. The expression of GATA3 is induced rapidly by IL-4, through STAT6, increasing from a low level in naive T cells to a high level in T_H2 cells[9] (FIG. 2). Whereas the requirement for STAT6 and c-MAF in T_H2-cell development has been confirmed using gene-targeting, this has not been reported so far for GATA3, because this seems also to be an essential factor for normal thymocyte development and embryonic survival[25–27]. GATA3 can transactivate the *IL-5* promoter[11,12,28], whereas c-MAF can transactivate the *IL-4* promoter[12,22]. As we discuss further later, the role of GATA3 in establishing competence of *IL-4* gene activity

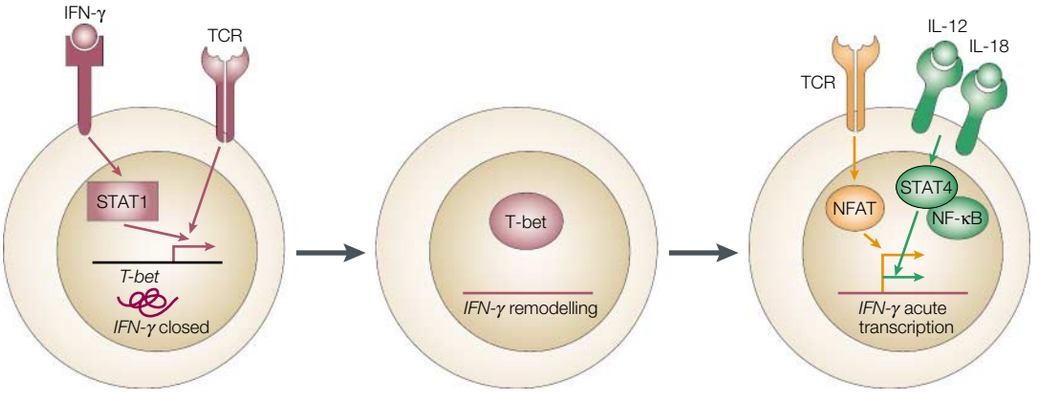

Figure 3 | **Pathways for the induction of expression of IFN-γ.** Naive helper T (T_H) cells activated under T_H1-inducing conditions are exposed to interferon-γ (IFN-γ) signalling during T-cell receptor (TCR) engagement, leading to the activation of signal transducer and activator of transcription 1 (STAT1). Downstream of STAT1, the expression of T-bet is induced, and T-bet acts in this model to induce remodelling of the repressed *IFN-γ* locus (shown) and induce expression of the interleukin-12 receptor β2-subunit (IL-12Rβ2) (not shown). Subsequently, the committed T_H1 cell, which expresses receptors for IL-12 and IL-18, has at least two pathways available to induce the acute transcription of IFN-γ — the TCR signalling pathway or a cytokine signalling pathway that can include combined IL-12 and IL-18 signalling. TCR-induced transcription of the gene encoding IFN-γ is distinguishable pharmacologically from cytokine-induced transcription — the former is sensitive to cyclosporin A (CsA), whereas the latter is CsA resistant. Different nuclear factors might mediate each pathway. NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB.

seems to be related to its ability to induce the remodelling of CHROMATIN[8,12,29] (BOX 1 and FIG. 2).

GATA3 has been shown to promote T_H2-cell development strongly, when expressed by a transgene[10] or by a retrovirus[9,28]. Furthermore, GATA3 induces T_H2-cell development of *STAT6*$^{-/-}$ T cells[8], including the production of T_H2-cell-specific cytokines and expression of c-MAF, which depend normally on STAT6. A putative dominant-negative mutant of GATA3 decreases T_H2-cell-mediated pulmonary allergic responses when expressed by a transgene in mice[30]. In addition, antisense oligonucleotide treatment directed against GATA3 can repress T_H2-cell responses *in vivo*, which further implies a requirement for GATA3 for T_H2-cell development[31].

Several non-T_H2-cell-specific transcription factors contribute to the regulation of expression of T_H2 cytokines, particularly members of the nuclear factor of activated T cells (NFAT) family (reviewed in REF. 2). Interactions between the different NFAT-family members on T_H2-cytokine genes are complex, and the transcription factors can have both positive and negative effects. In general, the role of these factors in T_H2-cytokine gene expression seems to be in mediating the acute transcription of inducible cytokines after triggering of a differentiated T_H2 cell through the TCR (FIG. 2).

As well as STAT6, MEL18, a Polycomb-group protein, also seems to regulate the expression of GATA3 (REF. 32). Although Polycomb-group proteins are associated generally with the heritable silencing of loci[33], MEL18 seems to act as a positive regulator of *GATA3* transcription. So, in the absence of MEL18, the levels of GATA3 and IL-4 are reduced, and reintroduction of GATA3 using a retroviral vector can correct the defect in IL-4 expression partially[32]. There are at least two potential post-transcriptional regulators of GATA3 — the zinc-finger proteins FOG1 (friend of GATA1) and ROG (repressor of GATA). Overexpression of FOG1 repressed T_H2-cell development induced by ectopic GATA3 in *STAT6*$^{-/-}$ T cells and decreased the efficiency of *GATA3* transcriptional autoactivation[34,35]. ROG was isolated as a GATA3-interacting protein; its overexpression decreases GATA3 activity and the production of T_H2 cytokines[36].

How T_H1 cells arise

T-bet was identified recently as a T_H1-cell-specific factor that can induce the production of IFN-γ by developing T_H2 cells[5]. A member of the T-box family of transcription factors, T-bet seems to be expressed in developing and committed T_H1 cells. In addition to its role in inducing the expression of IFN-γ, T-bet seems to be involved in chromatin remodelling of the gene that encodes IFN-γ[6] (FIG. 3), induction of expression of the IL-12 receptor β2-subunit (IL-12Rβ2)[6,37] and stabilizing its own expression, either through an intrinsic autocatalytic loop or the autocrine effects of IFN-γ signalling[6,37]. Although both $CD4^+$ and $CD8^+$ T cells, as well as natural killer (NK) cells, express T-bet, there seems to be less dependence on T-bet for high-level expression of IFN-γ in $CD8^+$ T cells than in $CD4^+$ T cells or NK cells[4]. Nevertheless, the crucial importance of T-bet for the development of T_H1 responses *in vivo* is underscored by the susceptibility of *T-bet*-knockout mice to challenge with *Leishmania major*[4] and their predisposition to allergic airway disease[38].

How T-bet induces expression of IFN-γ is still an area of active investigation. Recently, a genetic interaction was reported between *T-bet* and *HLX* (H2.0-like homeobox 1)[7]. The *HLX* gene seems to be expressed in developing T_H1 cells, although its expression is induced at a slower rate than for *T-bet*. Transcription of *HLX* seems to be downstream of T-bet activity, and HLX protein seems to interact with T-bet protein, which mediates the synergistic induction of IFN-γ expression[7].

CHROMATIN
Composed of nucleosomes, which are the basic repeating units of eukaryotic genomes. Nucleosomes consist of 146 base pairs of DNA wound around an octamer of histone proteins.

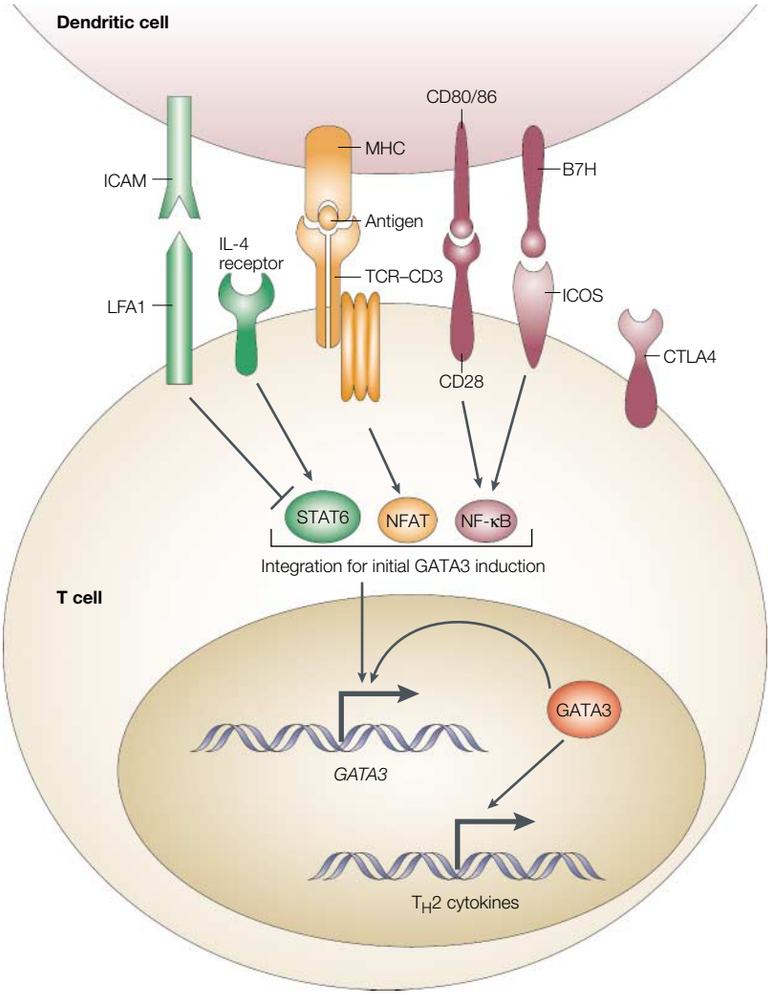

Figure 4 | **Redundant pathways of T_H2-cell development — integration at the level of *GATA3* transcription.** The observation that T helper 2 (T_H2) cells can develop in the absence of signal transducer and activator of transcription 6 (STAT6), both *in vivo* and *in vitro* (see text), might indicate that there are alternative pathways for the induction of expression of GATA3. As GATA3 expression undergoes positive feedback by transcriptional autoactivation, signalling through nuclear factor-κB (NF-κB) by CD28, and perhaps other receptors, might reach the threshold that is necessary for sustainable GATA3 autoactivation and T_H2-cell commitment. Strong co-stimulation (in the absence of inhibition by IL-12, IFN-γ or LFA1) might, therefore, provide a STAT6-independent pathway of T_H2-cell development. CTLA4, cytotoxic T-lymphocyte antigen 4; ICAM1, intercellular adhesion molecule 1; ICOS, inducible co-stimulator; IFN-γ, interferon-γ; IL-12, interleukin-12; LFA1, leukocyte function-associated antigen 1; NFAT, nuclear factor of activated T cells.

The role of HLX *in vivo* has not been examined fully yet because knockout mice die during embryogenesis[39].

The expression of T-bet seems to be induced readily in naive T cells by IFN-γ signalling, mediated by STAT1 (REFS 37,40) (FIG. 3). Although STAT1 seems to be a crucial factor for the induction of expression of T-bet, type I IFNs, which activate STAT1 also, do not seem to be able to induce *T-bet* transcription[40]. Whether T-bet protein can autoactivate transcription of the *T-bet* gene is less clear. Endogenous expression of T-bet was shown to be induced by ectopic T-bet, which implies that autoactivation does occur[6,7], but a reduction in the level of endogenous *T-bet* transcription in the absence of autocrine IFN-γ signalling[37,40] was observed also, which indicates that there is an exocrine mechanism. Because only early time points in development have been examined in each of these studies, it is possible that a shift from a STAT-dependent pathway to an autoactivation pathway could occur at some later time. Finally, as STAT1-deficient cells can be driven to T_H1-cell polarization, it is possible either that expression of a low level of T-bet might be independent of IFN-γ signalling, allowing T_H1-cell development, or that the requirement for T-bet for T_H1-cell development is conditional. The latter possibility is raised by the observation that T-bet-deficient $CD4^+$ T cells from the autoimmune *Lpr* mouse background produce abundant IFN-γ[41], which indicates that there is some flexibility in the requirement for T-bet. So, it is uncertain whether STAT1-independent T_H1-cell development arises from an alternative extrinsic signal or an autonomous property of the activated T cell.

Acute transcription of the gene encoding IFN-γ

In terminally differentiated T_H1 cells, reiteration of IFN-γ expression can occur through two experimentally distinct pathways — TCR ligation or cytokine (IL-12 and IL-18) stimulation. IL-18 augments the production of IFN-γ by differentiated T_H1 cells, despite its inability to drive the development of T_H1 cells on its own[42]. The combination of IL-12 and IL-18 can induce the production of IFN-γ by differentiated T cells in the absence of signalling through the TCR[42,43] (FIG. 3). The IFN-γ production that is induced is more prolonged and more resistant to treatment with cyclosporin A (CsA) (an inhibitor of NF-AT activation) than that induced by TCR crosslinking[43], which indicates that cytokine-driven IFN-γ production is not coupled to the NFAT pathway. *In vivo*, it seems that IL-12 and IL-18 act synergistically to induce maximum IFN-γ production[44,45].

IL-12- and IL-18-induced IFN-γ production correlates with induction of expression of the GADD-FAMILY PROTEINS GADD45β and GADD45γ. Overexpression of GADD45β augments IFN-γ production[46], and absence of GADD45γ decreases IFN-γ production, thereby inhibiting T_H1-cell development[47]. The transcriptional details of IL-12- and IL-18-induced IFN-γ production are uncertain still. It is clear that IL-12- and IL-18-induced IFN-γ production depends strongly on STAT4, whereas TCR signalling can induce IFN-γ production, although at a reduced level, in *STAT4*$^{-/-}$ T_H1 cells[37]. The other factors that mediate cytokine-induced IFN-γ production — in contrast to CsA-sensitive, anti-CD3 antibody-induced IFN-γ production — are uncertain still, although some evidence indicates that these might be p38 mitogen-activated protein kinase (p38 MAPK)-sensitive factors[46,48], which is consistent with previous suggestions (reviewed in REF. 49).

The secondary, cytokine-dependent pathway of IFN-γ expression that has been described for $CD4^+$ T_H1 cells might operate in other cell types. IL-12 and IL-18 have an active role in inducing the production of IFN-γ by $CD8^+$ T cells and NK cells in response to bacterial pathogens[50]. Similarly, there is some indirect evidence that such a bystander pathway might operate in $CD4^+$ T cells *in vivo*. Even after a T_H1 response has been initiated, it has been

GADD-FAMILY PROTEINS (Growth-arrest and DNA-damage inducible proteins). In response to environmental stresses, these proteins mediate activation of the p38 mitogen-activated protein kinase pathway and are involved in the regulation of growth and apoptosis.

shown that continuous IL-12 production is required for protection in some experimental infections, including with *T. gondii*[51] and *L. major*[52,53]. It will be of interest, therefore, to determine whether continuous production of IL-12 is required to stabilize the T_H1-cell phenotype or the magnitude of the effector response — that is, IFN-γ production. Perhaps consistent with the latter possibility, deletion of the gene encoding IL-18 results in reduced resistance to some pathogens[44,54], despite the non-essential role of this cytokine in T_H1-cell development[42]. So, the extent to which the TCR and cytokine-driven pathways act to induce IFN-γ production *in vivo* during the effector phase of T_H1 responses will require additional investigation.

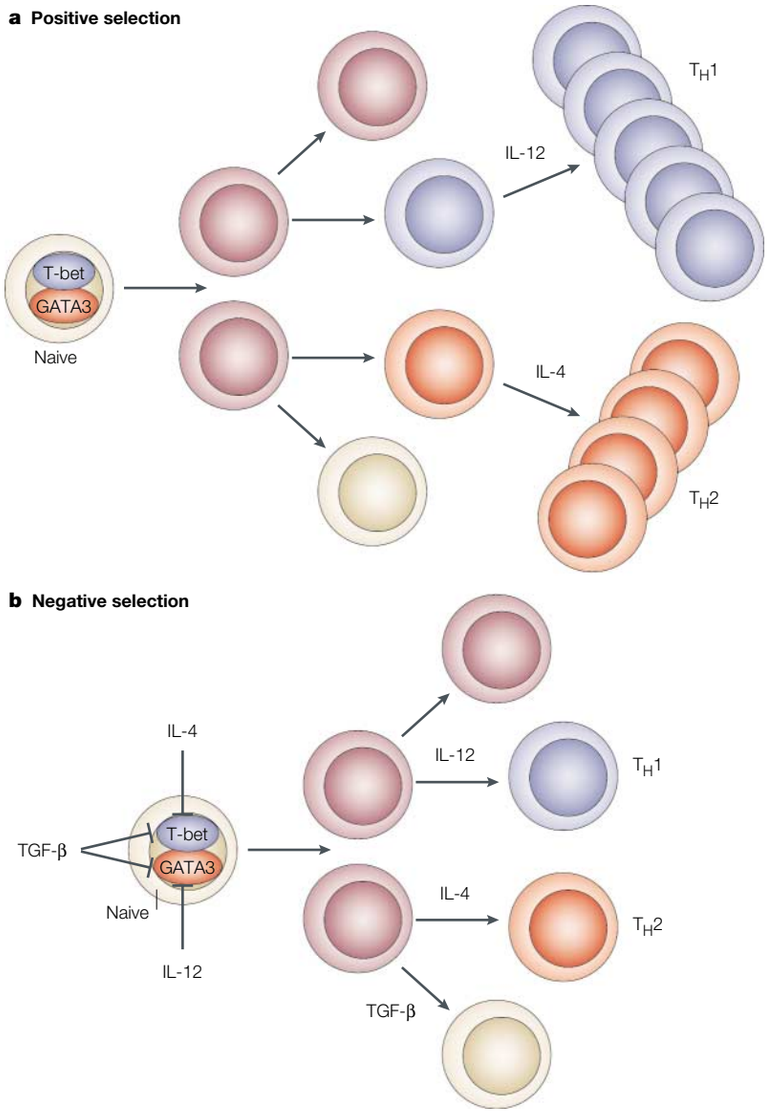

Figure 5 | **Models of secondary roles for cytokines in differentiation. a** | Positive selection. After activation, the expression of T-bet and GATA3 is induced and T cells divide, acquiring a mixed T helper 1 (T_H1)- and T_H2-lineage profile (indicated as burgandy shading). With further division, lineage commitment progresses, including the selective responsiveness to growth-factor signals. The presence of interleukin-12 (IL-12) will favour the growth of cells that express the IL-12 receptor β2-subunit (IL-12Rβ2) (committed T_H1 cells; blue), whereas the presence of IL-4 will favour the growth of cells that express GATA3 (committed T_H2 cells; red), which seems to synergize with growth-factor-independent 1 (GFI1), a downstream mediator of signal transducer and activator of transcription 6 (STAT6) signals. **b** | Negative instruction. As cells acquire competence for growth-factor signal transduction, IL-12 silences *GATA3*, IL-4 silences *T-bet*, and transforming growth factor-β (TGF-β) works to repress both transactivators. This instructive effect of cytokines helps to 'lock-in' patterns of differentiation, but it still relies on some prior degree of cytokine-independent lineage commitment. It is speculated that T-bet and GATA3 are active during the initial cell division(s), because their silencing by cytokines might require passage through the cell cycle.

Recently discovered T_H1-cell-promoting factors

The recently discovered cytokine IL-23 is composed of the p40 subunit of IL-12 paired with a unique chain, p19 (IL-23α), that is related distantly to IL-12 p35 (REF. 55). IL-23 binds IL-12Rβ1, but not IL-12Rβ2; it interacts instead with a unique receptor subunit, IL-23R[56]. IL-23 is reported to activate STAT4 (REF. 55), and it might act during both the induction of T_H1-cell development and the acute reiteration of IFN-γ production induced by cytokines, in cooperation with IL-18 (as described for IL-12 and IL-18; REF. 46). However, a role for IL-23 in initiating early T_H1-cell development has not been established yet. In addition to its effects on T cells, IL-23 might influence dendritic cells (DCs), by enhancing the stimulatory capacity of these antigen-presenting cells (APCs)[57].

Another heterodimeric cytokine, IL-27 (REF. 58), was reported recently, which is composed of EBI3 (Epstein–Barr virus-induced gene 3)[59] and p28, a subunit related to IL-12 p35. IL-27 is produced by APCs; it induces the proliferation of naive T cells selectively, acts with IL-12 to promote IFN-γ production and is the ligand for TCCR (T-cell cytokine receptor, also known as WSX1), a receptor on T cells that is involved in early T_H1-cell development[60,61]. The precise mechanisms by which IL-27 and TCCR act in early T_H1-cell development are unclear, but IL-27 seems to interact synergistically with IL-12 at an early checkpoint in T_H1-cell commitment[62].

Instruction, selection and parental controls

Most previous studies have been interpreted to support an instructive model of T_H-cell differentiation (FIG. 1). In this model, IL-4 carries instructive signals to the T-cell nucleus through a series of intermediate molecules. First, the IL-4 receptor activates STAT6 in the naive T cell. Then, phosphorylated, dimeric STAT6, in the context of a fully activated T cell, efficiently increases the expression of GATA3 from a low to high level. At some point, a feedback of transcriptional autoactivation occurs, which stabilizes GATA3 expression[8,12]. GATA3 acts directly on certain cytokine promoters, as in the case of *IL-5* (REFS 11,12), or indirectly through important *cis* elements, as for the IL-4- and IL-13-encoding cytokine loci[29], to transactivate the promoter or mediate the reversal of chromatin-based repression. In this model, the IL-4 signalling pathway does not provide signals for cell growth or proliferation, but simply instructs the cell to 'open' the T_H2-cytokine loci for expression.

Cytokines might select rather than instruct. Several questions have, however, arisen concerning models that indicate that the lineage-defining transcription factors T-bet and GATA3 are absent in naive cells, and that the

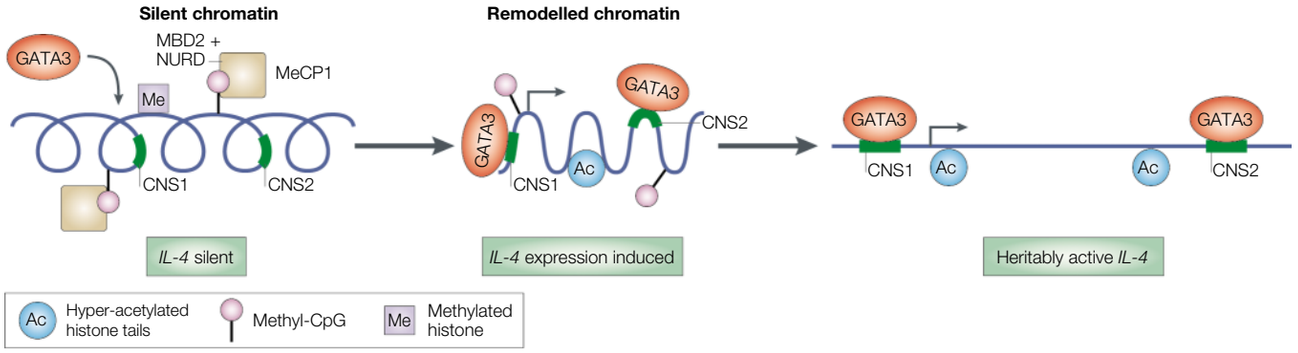

Figure 6 | **Model of orderly derepression of the *IL-4* gene.** The interleukin-4 (*IL-4*) locus is actively repressed in naive CD4+ T cells by molecules that link methylated DNA (MBD2) to repressive chromatin (NURD). Together, MBD2 (methyl-CpG-binding domain protein 2) and NURD (nucleosome remodelling and histone deacetylase) form the MeCP1 complex. The sites of action of this repression probably overlap with crucial *cis*-acting elements in the locus, such as the *IL-4–IL-13* intergenic region (CNS1), the second intron of *IL-4*, and the 3′ enhancer (CNS2). Activation of the locus occurs in a stepwise fashion. First, GATA3 mediates displacement of MBD2, leading to chromatin changes such as histone acetylation, an effect that is concurrent with the induction of *IL-4* transcription. Later, the acquisition of additional epigenetic changes, such as stable CpG demethylation, occurs along the cytokine gene. CNS, conserved non-coding sequence.

induction of their expression is caused by exposure to IL-12 and IL-4, respectively (FIG. 1). One problem lies in the observation that an effector T-cell lineage might have the unique ability to respond to a specific cytokine. Indeed, there is evidence that T_H2 cells transduce signals more efficiently through the IL-4 receptor[63] and that T_H1 cells selectively express a signalling chain of the IL-12 receptor[64]. So, the ability of a cytokine to stimulate an increased level of transactivator messenger RNA in a T-cell population[65] might be an indirect effect of favouring the growth of T cells that express such mRNA.

Recently, expression of growth-factor independent 1 (GFI1) was described to be induced selectively by STAT6 signalling in T cells and to mediate the clonal expansion of T_H2 cells[66]. This result provides a potential explanation for a selective, rather than instructive, component to T_H2-cell development. Previously, GATA3 was found to influence mainly T_H2-cytokine gene expression rather than cell growth[67], which indicates that there is an instructive effect. However, GFI1 seems to drive clonal expansion of only those T_H cells that co-express GATA3, which indicates a cooperative model that incorporates both instructive and selective components. It is uncertain precisely how the interaction between GATA3 and GFI1 occurs in T_H2-cell development. Despite the known requirement of STAT6 for T_H2-cell development, *Stat6*$^{-/-}$ T_H2 cells have been observed in several systems[8,68,69]. In two systems of pathogen-induced responses, reduced, but detectable, T_H2 responses were seen in *Stat6*$^{-/-}$ backgrounds[68,69]. In an *in vitro* system, the ectopic expression of GATA3, introduced by a retrovirus, in *Stat6*$^{-/-}$ T cells induced endogenous expression of GATA3 and triggered full T_H2-cell development[8]. However, in none of these systems has the expression of GFI1 been examined.

Instructions from signals other than cytokines. As the previous discussion indicates, it is not entirely clear whether instructive or selective models can explain T_H2-cell development. The interesting, albeit reduced, development of T_H2 cells in *Stat6*$^{-/-}$ mice could be evidence of an intrinsic mechanism that generates a diversity of committed T_H-cell fates in the progeny of a single cell without requiring instructive signals. There could, however, be additional instructive signals, other than IL-4 and STAT6, that give rise to the T_H2-cell fate. Redundant signalling pathways, for example, might induce the transcription of *GATA3* directly, independently of STAT6 (FIG. 4). CD28 co-stimulation has been reported to augment the expression of GATA3 (REF. 70), and a role for nuclear factor-κB (NF-κB) has been proposed also[71]. Although these studies did not examine the efficiency of these effects in the absence of STAT6, it is conceivable that strong CD28 co-stimulation, which can activate NF-κB[72], could be a physiological, STAT6-independent pathway to promote T_H2-cell development. In fact biasing T_H1- or T_H2-cell development, caused by some DC subsets[73], has been proposed to be cytokine independent in some cases (reviewed in REF. 74).

As GATA3 has a transcriptional autoactivating property — which leads to the massive upregulation of *GATA3* transcription by GATA3 protein[8,12] — it is possible that this pathway could be effective as an initial source of IL-4 (FIG. 4). It can be speculated that inducible co-stimulator (ICOS), another CD28-family co-stimulatory receptor present on T cells, could regulate the expression of GATA3, because ICOS signalling might overlap partially with CD28 signalling and is known to promote T_H2 responses (reviewed in REF. 75). Finally, the LFA1 (leukocyte function-associated antigen 1)–ICAM1 (intercellular adhesion molecule 1) pathway has been reported to regulate the T_H1–T_H2 balance, such that LFA1 signalling favours T_H1-cell development, although this has not been examined in the setting of STAT6 deficiency[76–79]. Regulation of the expression of ICAM1 by DCs could modify the strength of LFA1 ligation, thereby tuning the T_H1–T_H2 balance (FIG. 4). It is uncertain how LFA1 signalling is integrated with the other T_H1 signalling pathways , but it might act in a STAT6-independent manner.

In summary, there are a few candidate proteins that could regulate the expression of GATA3 directly and possibly trigger transcriptional autoactivation of *GATA3*, independently of STAT6. These proteins would also be considered to be instructive signals (FIG. 4).

Extrinsic meets intrinsic during proliferation. Despite the existence of these potentially instructive mechanisms, it is still possible that a programmed diversification mechanism operates during early T-cell priming (FIG. 5). One unresolved issue concerning the nature of the T_H-cell-fate decision is whether naive T cells themselves adopt new fates or whether they proliferate and produce progeny that acquire new fates[80–85]. The importance of this distinction lies in whether an antigen-specific clonotype must make an exclusive decision to become only T_H1 cells or only T_H2 cells. If differentiation occurs before proliferation, then naive parental T cells might be forced to make exclusive, binary choices (FIG. 1). By contrast, differentiation during or after proliferation would be consistent with an ability of the naive parental T cell to have many progeny, each with potentially distinct, or multi-lineage, fates (FIG. 5). In this case, achieving polarized differentiation might depend more on the selective growth and survival of particular cell fates (FIG. 5a; positive selection) or the selective repression of particular transcription factors (FIG. 5b; negative instruction) than on signals that give rise to the new cell fate (FIG. 1; conventional, positive instruction).

Just as there seem to be STAT6-independent T_H2 cells[8,68,69], STAT4-independent T_H1-cell commitment has been described[6,37,40]. A further problem in invoking STAT4 as the most upstream factor of T_H1-cell commitment arises from the surprising result that ectopic IL-12 signalling in developing T_H2 cells cannot induce transcriptional competence of the gene encoding IFN-γ[86,87]. Consistent with the notion of positive selection by cytokines (FIG. 5a), STAT4 and STAT6 help mediate cell division and the migration of lymph-node effector cells to peripheral tissues[6,88], perhaps by antagonizing proliferative arrest[89] and counter-regulating lymph-node homing molecules[90,91].

Consistent with the concept of negative instruction (FIG. 5b), the cytokines IL-12 and IL-4 have a prominent role in the silencing of *GATA3* and *T-bet*, respectively[6,8,9,82]. Transforming growth factor-β (TGF-β) seems to be able to silence transcription of both *GATA3* and *T-bet*[82,92–95]. It could be speculated that the ground state (or default) for transcription of *T-bet* and *GATA3* loci is one of activity (FIG. 5) in naive T cells. Evidence for this comes from the finding that silencing of *T-bet* or *GATA3* loci by prohibitive cytokine signals seems to require passage through S-phase of the cell cycle[82], and that cells that do not divide remain multi-potent[82,96]. One potential benefit of such a system of gene regulation would be to provide a default state of diversification at the outset of T_H-cell differentiation.

The requirement for cell-cycle progression[81–83] for gene silencing, and its corollary, the ability of clonally related T_H cells to adopt many fates, might a provide a basis for the various extracellular cues that have been described to shape highly pleiotropic outcomes. Indeed, the existence of STAT6-independent T_H2-cell commitment[8,68,69] and STAT4-independent T_H1-cell commitment[6,37,40], the non-selective transcription of cytokines[96,97] and transactivators[82], and the acetylation of histones[98,99] are all consistent with a degree of intrinsic commitment that might precede cytokine-driven selection or instruction.

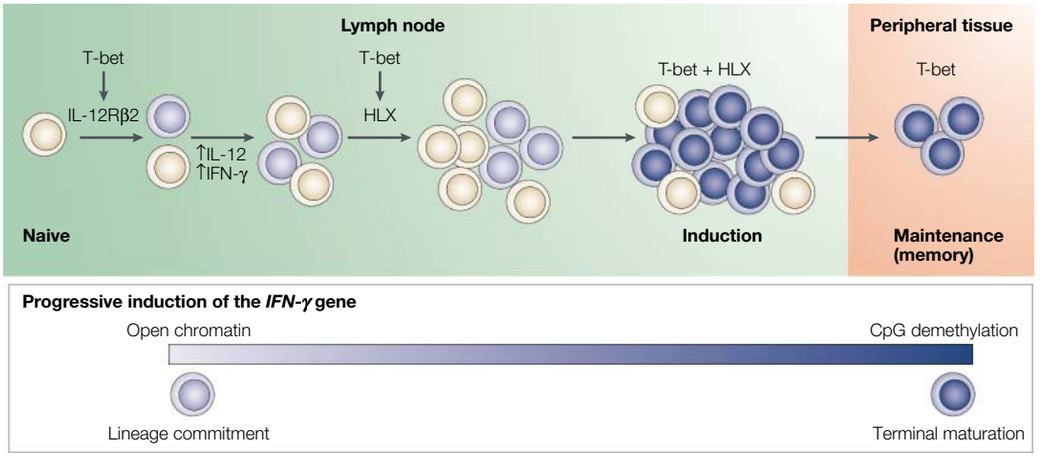

Figure 7 | **Model of stages of T_H1-cell induction and maturation.** Hypothetical schema in which the intrinsic regulation of T helper 1 (T_H1)-cell development is triggered by T-bet. T-bet induces expression of the interleukin-12 receptor β2-subunit (IL-12Rβ2) and chromatin remodelling of the locus encoding interferon-γ (IFN-γ). Later, T-bet induces expression of HLX (H2.0-like homeobox 1), which cooperates synergistically with T-bet to mediate high-level induction of transcription of the gene encoding IFN-γ. After this stage, the gene encoding IFN-γ undergoes demethylation, probably corresponding to the point at which its transcriptional competence no longer depends strictly on T-bet activity. This might be considered the memory of a differentiated effector cell. It is speculated that the initial cell divisions occur in the lymph nodes, whereas the later stages occur in sites of tissue inflammation. Cell-extrinsic input is provided by IFN-γ signalling, which helps induce expression of T-bet, and IL-12 signalling, which helps T_H1 cells to divide and augments transcription of the gene encoding IFN-γ.

Epigenetic effects — silence is golden

In the past few years, several lines of evidence have indicated that chromatin structure has an integral role in T_H-cell differentiation. The induction of competence for effector-cytokine gene expression seems to involve the derepression of silent chromatin contexts[81,100–102]. In mammals, silencing might involve the concerted actions of epigenetic mechanisms (including DNA methylation), condensation of chromatin and transposition of loci near to heterochromatic nuclear subdomains[103]. Some of these mechanisms are highly interdependent. Proteins that bind methylated CpG, for example, have been shown to recruit histone deacetylases and repressive chromatin-remodelling complexes[104–106]. Conversely, some repressive chromatin- and histone-modifying proteins have been shown recently to influence patterns of DNA methylation[107–109]. The HISTONE CODE has received a great deal of attention as a determinant of transcriptional competence[110]. This is owing to the association of gene activity with specific post-translational modifications of histone tails that differ from the modifications that are associated with gene silencing. How chromatin states (active or silent) are established, maintained, altered and inherited are now fertile areas of investigation.

It is accepted generally that there are various chromatin modifications, lacking in naive T cells, that accompany the acquisition of effector-cytokine gene activity[81,101]. The *IL-4* locus acquires DNASE-I HYPERSENSITIVITY (BOX 1) in T_H2 cells, but not in naive T_H or T_H1 cells[101]. This acquisition of DNase-I hypersensitivity is genetically downstream of GATA3 expression[8]. Also, the *IL-4* locus becomes demethylated in certain regulatory regions (such as the second intron enhancer)[81], but this has not been shown yet to be a direct effect of GATA3 (REF. 100). Similarly, the gene encoding IFN-γ acquires nuclease hypersensitivity in some of its introns in T_H1 cells, but not T_H2 cells[6,7,101]. The formation of hypersensitivity site I is genetically downstream of the expression of T-bet[6,7]. Later in differentiation, this region of the gene undergoes demethylation[7]. It is not clear, however, what mediates this effect. Moreover, it has been shown recently that heritably permissive cytokine loci are marked by acetylated histones[98,99], which are a feature of decondensed chromatin.

The role of gene silencing in T_H cells is complicated further by the recent observation that the inactive cytokine loci of naive T cells might undergo a deeper state of silencing in lineages that finally 'forbid' their expression. Using highly sensitive techniques, rapid transcriptional activation of the effector-cytokine genes and localization of these genes to non-centromeric subdomains of the nucleus can be detected in naive $CD4^+$ T cells[96]. In differentiated T_H1 and T_H2 cells, however, the 'forbidden' cytokine loci seem to become repositioned to centromeres[96] and they can also show evidence of *de novo* CpG methylation[111]. The studies showing non-centromeric location of effector-cytokine loci during their early activity[96,97] might seem to be at odds with the absence of DNase-I hypersensitivity sites[6,7,101,102], hyperacetylated histones[98,99] or demethylation of CpG dinucleotides[81,111], which characterize effector-cytokine loci in naive T cells. However, this ground state of incomplete gene silencing might indicate simply that there are many levels of chromatin-based repression in the regulation of these lineage-restricted genes.

How loci are derepressed

Functional evidence that silencing elements in naive T cells are rate-limiting for the induction of cytokine gene expression was obtained using small-molecule inhibitors of DNA methylation and histone deacetylases[81]. Genetic evidence for silencing effects was obtained recently in mice with a conditional deletion of the maintenance methyltransferase *Dnmt1* in T cells[112]. In addition, mice with a deletion of *Mbd2*, which encodes a methyl-CpG-binding protein that recruits repressive complexes, also have ectopic expression of effector-cytokine genes[100] (FIG. 6). MBD2 links gene silencing to DNA methylation and seems to set the threshold of GATA3 function. Absence of MBD2 markedly increases the efficiency of GATA3-dependent and -independent induction of expression of IL-4 (REF. 100). Indeed, one role of GATA3 as an inducer of expression of IL-4 might be to displace MBD2 from chromatin at the *IL-4* locus, bringing the two opposing regulators — GATA3 the activator and MBD2 the silencer — into genetic and biochemical competition. So, chromatin structure and organization are emerging as important regulatory principles, but it is still controversial how T_H cells develop heritable states of gene activity or silencing.

Several crucial regulatory *cis* elements have been discovered in the *IL-4* locus. The conserved non-coding sequence (CNS) that lies between the *IL-4* and *IL-13* loci, CNS1, has been deleted from transgenic mice harbouring a human T_H2-cytokine gene cluster[113], and the endogenous sequence has been deleted also from the mouse loci[114]. Both situations result in a reduction in the level of expression of IL-4. The hypersensitive sites at the 3′ end of the *IL-4* gene, known as V/VA, have been deleted also, leading to a defect in the expression of IL-4 by T cells and mast cells[115]. By contrast, deletion of CNS1 perturbs T-cell production of IL-4 only[114]. So, the chromatin organization of a single locus might be cell-type specific. It seems that the elements contained in CNS1 and the second intron of *IL-4* are sufficient for tissue-specific, chromatin-dependent, GATA3-dependent gene induction[13]. Sites at the 3′ end of the *IL-4* gene might also have a redundant role in promoting T_H2-cell-specific activation[13]. Binding of MBD2 has been detected at CNS1 and the second intron of the *IL-4* locus[100].

GATA3 induces changes in chromatin structure of the *IL-4* gene[8,12] that have been associated previously with T_H2-cell-specific expression[101]. In addition to altering chromatin structure, GATA3 has been placed genetically upstream of the selective pattern of histone acetylation that is acquired at the *IL-4* locus in T_H2 cells[99]. Also, GATA3 seems to influence chromatin activation of the *IL-4* locus by displacing MBD2 (REF. 100) (FIG. 6). This seems to occur at the time of transcriptional induction and before demethylation. Later during differentiation, it seems that the *IL-4* locus undergoes progressive

HISTONE CODE
Refers to various post-translational modifications of histones that might impose states of gene activity or silence.

DNASE-I HYPERSENSITIVITY
Refers to sites of nuclease sensitivity when nuclei from cells are exposed to limiting concentrations of DNase I. The digested regions of DNA correspond to sites of open DNA, which might be factor-binding sites or areas of altered nucleosome conformation.

demethylation in T_H2 cells[81,111], although this does not seem to be a proximate effect mediated by GATA3 (REF. 100).

These described actions of GATA3 are reminiscent of the roles of GATA4 and HNF3β (hepatocyte nuclear factor-3β), transcription factors that decompact target genes during liver differentiation[116]. The essential role of GATA3 in the induction of *IL-4* gene expression contrasts with its non-essential role as a conventional transcription factor for the *IL-4* promoter, but a strong transactivator of the proximal *IL-5* promoter[12,117,118]. In addition to the lack of evidence of a role for GATA3 as a

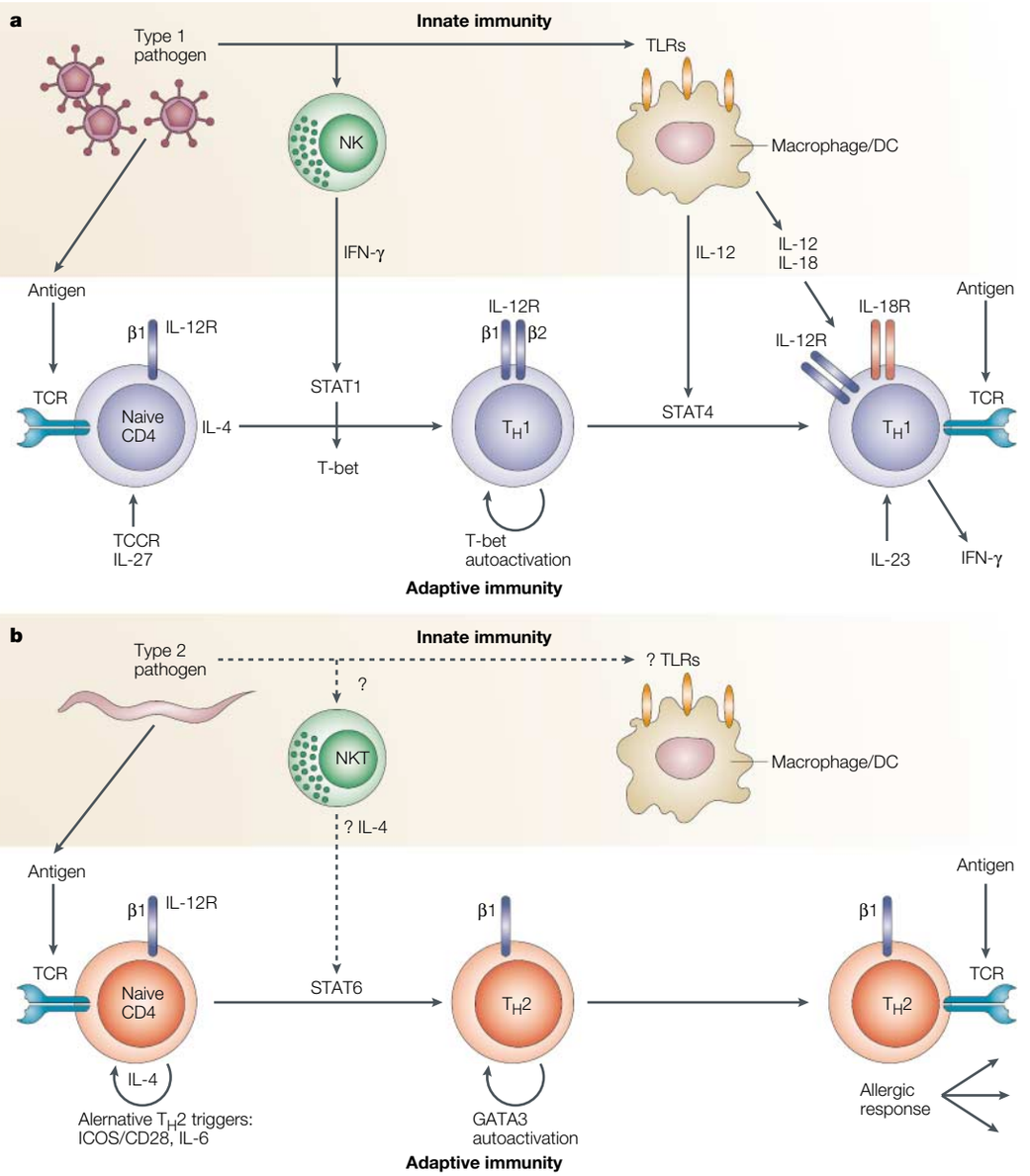

Figure 8 | **Relationships between pathogens, the innate immune system and T_H-cell development. a** | T helper 1 (T_H1)-cell development is augmented by signals from innate immune responses. The first commitment step results from interferon-γ (IFN-γ) acting through signal transducer and activator of transcription 1 (STAT1), together with T-cell receptor (TCR) signalling, to increase markedly the expression of T-bet by naive T cells. This results in remodelling of the gene encoding IFN-γ to an active status and induces expression of the interleukin-12 receptor β2-subunit (IL-12Rβ2). Next, IL-12 signalling can amplify T_H1 responses in two ways. IL-12 can augment the production of IFN-γ directly and can promote expression of the IL-18 receptor (IL-18R), opening up an alternative pathway for IFN-γ production, which might operate for as long as the inflammatory cytokines are produced. DC, dendritic cell; NK cell, natural killer cell. **b** | T_H2-cell development could occur in response to an extrinsic source of IL-4 or as a default pathway in the absence of inhibition by innate immune signals. In naive T cells, basal expression of GATA3 is low, but might be sufficient for low-level production of IL-4 by some naive T cells, leading to a cascade of T_H2-cell development if no inhibition is encountered. Furthermore, the expression of GATA3 might be under positive regulation by CD28 and inducible co-stimulator (ICOS) signalling, and repression through engagement of leukocyte function-associated antigen 1 (LFA1). So, the priming dendritic cell (DC) might influence T_H2-cell commitment through expression of CD80/CD86, PD1 and intercellular adhesion molecule 1 (ICAM1). NKT cell, natural killer T cell. TLR, Toll-like receptor.

classical transactivator of the *IL-4* gene, some evidence indicates that T-bet might also be a remodeller of condensed chromatin structure at the gene encoding IFN-γ, rather than a crucial transactivator of the proximal promoter of this gene. A dominant-negative form of T-bet reduced T_H1-cell commitment of naive T cells, but had little effect on the production of IFN-γ by fully differentiated T_H1 cells[7]. This indicates that T-bet might not be a classical transcription factor for the *IFN-γ* promoter, a finding that is in line with the non-essential role of T-bet in the expression of IFN-γ by $CD8^+$ T cells[4]. This finding indicates also that a key inducer of competence to express a gene might be different from both the factors that maintain heritable competence of gene expression (FIG. 7) and those that mediate acute transcription from that gene (FIG. 3).

Remembrance of things past

DNA methylation seems to have a pleiotropic role in regulation of the loci encoding IL-4 and IFN-γ. T cells from *Dnmt1*-knockout mice produce excessive amounts of effector cytokines[112]. DNA methylation might have an important role at the start of T_H-cell differentiation as a direct inhibitor of factor binding or as a scaffold for silencing complexes[100]. The remodelling of chromatin at the *IL-4* locus and activation of transcription seem to precede the demethylation of this gene[100,111] (FIG. 6). This is true also for the locus encoding IFN-γ[7] (FIG. 7). Together, these data imply that the methyl-mediated repression of gene expression can be separated from the methylation modification itself.

Demethylation seems to correlate with maturation of transcriptional output from a locus[111]. Whether demethylation causes the elevated transcriptional output or is, instead, a modification that 'locks-in' this state is an area of active controversy[119]. The programmed elimination of methyl groups, as arises from interference with maintenance methylation, might simply be a convenient way to imprint the activity of a locus in lymphocytes. The ability to clonally inherit competence for activity of the genes encoding IFN-γ and IL-4, which characterizes terminally differentiated T_H1 and T_H2 cells, respectively, might be owing to the loss of CpG methylation[81]. Indeed, transcriptional maturation of the gene encoding IFN-γ, which is mediated by the combined actions of T-bet and HLX, seems to precede the onset of demethylation[7] (FIG. 7). Instead, demethylation of the locus encoding IFN-γ seems to correlate most closely with the stage of differentiation at which a dominant-negative form of T-bet no longer antagonizes transcription and chromatin remodelling of the gene encoding IFN-γ[7]. It is uncertain still which factors are responsible ultimately for demethylation of the cytokine loci.

Where are we now?

Much detail has been added to our picture of T_H1- and T_H2-cell commitment. To a large extent, the transcriptional and epigenetic changes in cytokine genes that are associated with their selective activation or repression during T-cell activation are the same processes that govern gene expression controlled by tissue- or lineage-specific factors in general. Previous debates about instructive versus selective models have tended to dissipate as examples of both processes in T_H2-cell development have been shown to be valid. The original model shown in FIG. 1 has now been modified and extended to include many points of control. FIGURE 8 presents newer models of T_H1- and T_H2-cell development that incorporate much of the recent transcriptional and signalling advances.

For T_H1-cell development, we continue to emphasize the important contribution of the innate immune system, which was recognized initially in 1993 (REF. 120). However, now we understand that both IL-12 and IFN-γ provide important signals for T_H1 responses. IFN-γ, derived perhaps from pathogen-activated NK cells, can strongly augment the expression of T-bet during primary T-cell activation through STAT1 signalling, and increased expression of T-bet acts in primary T_H1-cell commitment by remodelling at the IFN-γ locus and by driving expression of the IL-12 receptor. IL-12, derived perhaps from pathogen-activated macrophages (or from appropriately activated DCs) amplifies the production of IFN-γ by T cells and drives increased expression of the IL-18 receptor, which opens the door to an alternative pathway for inducing IFN-γ production. Issues that are not completely resolved yet include the mechanisms of action of IL-27 and TCCR in early T_H1-cell priming, whether expression of T-bet involves transcriptional autoactivation in a similar manner to that ascribed to GATA3, and the role of IL-23 in pathogen-mediated versus autoimmune responses. In summary, T_H1 responses seem to be driven primarily by active signals derived from pathogen-activated innate immune sources, and a strong T_H1 response might be maintained only in the presence of such signals. In this manner, when the pathogen has been cleared, signals driving the high-level production of IFN-γ (and its potentially dangerous consequences) should abate.

For T_H2-cell development, it is still uncertain whether any active innate signals drive this process. Although certain possible sources, such as NKT cells and basophils, have been considered (reviewed in REF. 121), these do not seem to be necessary. Potentially, it might be that the absence of activation of the innate immune system removes inhibition of T_H2-cell development by IFN-γ and IL-12, which allows T_H2-cell development to be driven by positive feedback through IL-4 and GATA3. As T_H2-lineage commitment in some cells can occur independently of STAT6 activation, it is possible that some alternative signal, such as IL-6 (IFN-β2) or B7H expressed by DCs, might trigger the initial production of IL-4. However, the identity of such alternative stimuli remains an unresolved issue. Importantly, *GATA3* transcription itself seems to be responsive to GATA3 protein, providing an intrinsic signal that maintains T_H2-cell commitment at the cellular level. After T_H2-cell commitment, GATA3 has downstream effects on several genes, and it is probable that in the near future we will make progress in understanding precisely how these effects occur.

Concluding remarks

The study of T_H-cell differentiation *in vitro* is anticipated to continue to yield insights into the problem of how cells give rise to alternative fates. This *in vitro* system provides robust conditions to drive polarized differentiation and exciting tools to analyse the developmental genetics and biochemical foundations of heritable states of gene expression. However, we should keep in mind that ultimately these T_H-cell subsets provide protection against pathogens, which require different types of effector mechanism. Future studies will need to analyse T_H1- and T_H2-cell commitment *in vivo*. The first reagents for such studies have now been reported in the form of cytokine-reporter-knockout mice, in which cytokine gene expression is not perturbed[88,122]. Although *in vitro* analyses have provided information about the signals and factors that act in lineage commitment, we might soon know more about the physiological context — when and where various steps in commitment take place in response to pathogens, and from which APCs T cells receive instructive or selective signals.

1. Abbas, A. K., Murphy, K. M. & Sher, A. Functional diversity of helper T lymphocytes. *Nature* **383**, 787–793 (1996).
2. Ho, I. C. & Glimcher, L. H. Transcription: tantalizing times for T cells. *Cell* **109**, S109–S120 (2002).
3. Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A. & Coffman, R. L. Two types of murine helper T-cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* **136**, 2348–2357 (1986).
4. Szabo, S. J. *et al*. Distinct effects of T-bet in T_H1-lineage commitment and IFN-γ production in CD4 and CD8 T cells. *Science* **295**, 338–342 (2002).
5. Szabo, S. J. *et al*. A novel transcription factor, T-bet, directs T_H1-lineage commitment. *Cell* **100**, 655–669 (2000).
This study reported the cloning and characterization of T-bet, which is a key regulator of T helper 1 (T_H1)-lineage commitment.
6. Mullen, A. C. *et al*. Role of T-bet in commitment of T_H1 cells before IL-12-dependent selection. *Science* **292**, 1907–1910 (2001).
7. Mullen, A. C. *et al*. Hlx is induced by and genetically interacts with T-bet to promote heritable T_H1 gene induction. *Nature Immunol.* **3**, 652–658 (2002).
8. Ouyang, W. *et al*. Stat6-independent GATA-3 autoactivation directs IL-4-independent T_H2 development and commitment. *Immunity* **12**, 27–37 (2000).
This study showed that GATA3 might act independently of STAT6 and that GATA3 induces chromatin remodelling of the *IL-4* locus.
9. Ouyang, W. *et al*. Inhibition of T_H1 development mediated by GATA-3 through an IL-4-independent mechanism. *Immunity* **9**, 745–755 (1998).
10. Zheng, W. & Flavell, R. A. The transcription factor GATA-3 is necessary and sufficient for T_H2 cytokine gene expression in CD4 T cells. *Cell* **89**, 587–596 (1997).
11. Zhang, D. H., Cohn, L., Ray, P., Bottomly, K. & Ray, A. Transcription factor GATA-3 is differentially expressed in murine T_H1 and T_H2 cells and controls T_H2-specific expression of the interleukin-5 gene. *J. Biol. Chem.* **272**, 21597–21603 (1997).
References 10 and 11 were the first studies to implicate GATA3 as a T_H2-cell-specific transcription factor.
12. Lee, H. J. *et al*. GATA-3 induces T helper cell type 2 (T_H2) cytokine expression and chromatin remodeling in committed T_H1 cells. *J. Exp. Med.* **192**, 105–115 (2000).
13. Lee, G. R., Fields, P. E. & Flavell, R. A. Regulation of IL-4 gene expression by distal regulatory elements and GATA-3 at the chromatin level. *Immunity* **14**, 447–459 (2001).
This study defined the regulatory elements of the *IL-4* locus that mediate GATA3-dependent chromatin induction.
14. Ferber, I. A. *et al*. GATA-3 significantly downregulates IFN-γ production from developing T_H1 cells in addition to inducing IL-4 and IL-5 levels. *Clin. Immunol.* **91**, 134–144 (1999).
15. Hsieh, C. S., Heimberger, A. B., Gold, J. S., O'Garra, A. & Murphy, K. M. Differential regulation of T helper phenotype development by interleukins 4 and 10 in an αβ T-cell-receptor transgenic system. *Proc. Natl Acad. Sci. USA* **89**, 6065–6069 (1992).
16. Seder, R. A., Paul, W. E., Davis, M. M. & 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.* **176**, 1091–1098 (1992).
17. Le Gros, G., Ben-Sasson, S. Z., Seder, R., Finkelman, F. D. & Paul, W. E. 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.* **172**, 921–929 (1990).
18. Swain, S. L., Weinberg, A. D., English, M. & Huston, G. IL-4 directs the development of T_H2-like helper effectors. *J. Immunol.* **145**, 3796–3806 (1990).
19. Kaplan, M. H., Schindler, U., Smiley, S. T. & Grusby, M. J. Stat6 is required for mediating responses to IL-4 and for development of T_H2 cells. *Immunity* **4**, 313–319 (1996).
20. Shimoda, K. *et al*. Lack of IL-4-induced T_H2 response and IgE class switching in mice with disrupted *Stat6* gene. *Nature* **380**, 630–633 (1996).
21. Takeda, K. *et al*. Essential role of Stat6 in IL-4 signalling. *Nature* **380**, 627–630 (1996).
22. Ho, I. C., Hodge, M. R., Rooney, J. W. & Glimcher, L. H. The proto-oncogene *c-maf* is responsible for tissue-specific expression of interleukin-4. *Cell* **85**, 973–983 (1996).
This study was the first to identify c-MAF as a T_H2-cell-specific transcription factor.
23. Ho, I. C., Lo, D. & Glimcher, L. H. c-maf promotes T helper cell type 2 (T_H2) and attenuates T_H1 differentiation by both interleukin-4-dependent and -independent mechanisms. *J. Exp. Med.* **188**, 1859–1866 (1998).
24. Kim, J. I., Ho, I. C., Grusby, M. J. & Glimcher, L. H. The transcription factor c-Maf controls the production of interleukin-4 but not other T_H2 cytokines. *Immunity* **10**, 745–751 (1999).
25. Hendriks, R. W. *et al*. Expression of the transcription factor GATA-3 is required for the development of the earliest T-cell progenitors and correlates with stages of cellular proliferation in the thymus. *Eur. J. Immunol.* **29**, 1912–1918 (1999).
26. Pandolfi, P. P. *et al*. Targeted disruption of the *GATA3* gene causes severe abnormalities in the nervous system and in fetal-liver haematopoiesis. *Nature Genet.* **11**, 40–44 (1995).
27. Ting, C. N., Olson, M. C., Barton, K. P. & Leiden, J. M. Transcription factor GATA-3 is required for development of the T-cell lineage. *Nature* **384**, 474–478 (1996).
28. Ranganath, S. *et al*. GATA-3-dependent enhancer activity in *IL-4* gene regulation. *J. Immunol.* **161**, 3822–3826 (1998).
29. Takemoto, N. *et al*. Cutting edge: chromatin remodeling at the IL-4/IL-13 intergenic regulatory region for T_H2-specific cytokine gene cluster. *J. Immunol.* **165**, 6687–6691 (2000).
30. Zhang, D. H. *et al*. Inhibition of allergic inflammation in a murine model of asthma by expression of a dominant-negative mutant of GATA-3. *Immunity* **11**, 473–482 (1999).
31. Finotto, S. *et al*. Treatment of allergic airway inflammation and hyperresponsiveness by antisense-induced local blockade of GATA-3 expression. *J. Exp. Med.* **193**, 1247–1260 (2001).
32. Kimura, M. *et al*. Regulation of T_H2-cell differentiation by *mel-18*, a mammalian polycomb-group gene. *Immunity* **15**, 275–287 (2001).
33. Francis, N. J. & Kingston, R. E. Mechanisms of transcriptional memory. *Nature Rev. Mol. Cell. Biol.* **2**, 409–421 (2001).
34. Zhou, M. *et al*. Friend of GATA-1 represses GATA-3-dependent activity in $CD4^+$ T cells. *J. Exp. Med.* **194**, 1461–1471 (2001).
35. Kurata, H. *et al*. Friend of GATA is expressed in naive T_H cells and functions as a repressor of GATA-3-mediated T_H2-cell development. *J. Immunol.* **168**, 4538–4545 (2002).
36. Miaw, S. C., Choi, A., Yu, E., Kishikawa, H. & Ho, I. C. ROG, repressor of GATA, regulates the expression of cytokine genes. *Immunity* **12**, 323–333 (2000).
37. Afkarian, M. *et al*. T-bet is a STAT1-induced regulator of IL-12R expression in naive $CD4^+$ T cells. *Nature Immunol.* **3**, 549–557 (2002).
38. Finotto, S. *et al*. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. *Science* **295**, 336–338 (2002).
39. Hentsch, B. *et al*. *Hlx* homeobox gene is essential for an inductive tissue interaction that drives expansion of embryonic liver and gut. *Genes Dev.* **10**, 70–79 (1996).
40. Lighvani, A. A. *et al*. T-bet is rapidly induced by interferon-γ in lymphoid and myeloid cells. *Proc. Natl Acad. Sci. USA* **98**, 15137–15142 (2001).
41. Peng, S. L., Gerth, A. J., Ranger, A. M. & Glimcher, L. H. NFATc1 and NFATc2 together control both T- and B-cell activation and differentiation. *Immunity* **14**, 13–20 (2001).
42. Robinson, D. *et al*. IGIF does not drive T_H1 development but synergizes with IL-12 for interferon-γ production and activates IRAK and NF-κB. *Immunity* **7**, 571–581 (1997).
43. Yang, J., Murphy, T. L., Ouyang, W. & Murphy, K. M. Induction of interferon-γ production in T_H1 $CD4^+$ T cells: evidence for two distinct pathways for promoter activation. *Eur. J. Immunol.* **29**, 548–555 (1999).
44. Neighbors, M. *et al*. A critical role for interleukin-18 in primary and memory effector responses to *Listeria monocytogenes* that extends beyond its effects on interferon-γ production. *J. Exp. Med.* **194**, 343–354 (2001).
45. Takeda, K. *et al*. Defective NK-cell activity and T_H1 response in IL-18-deficient mice. *Immunity* **8**, 383–390 (1998).
46. Yang, J., Zhu, H., Murphy, T. L., Ouyang, W. & Murphy, K. M. IL-18-stimulated GADD45β required in cytokine-induced, but not TCR-induced, IFN-γ production. *Nature Immunol.* **2**, 157–164 (2001).
47. Lu, B. *et al*. GADD45γ mediates the activation of the p38 and JNK MAP kinase pathways and cytokine production in effector T_H1 cells. *Immunity* **14**, 583–590 (2001).
48. Rincon, M. *et al*. Interferon-γ expression by T_H1 effector T cells mediated by the p38 MAP kinase signaling pathway. *EMBO J.* **17**, 2817–2829 (1998).
49. Dong, C., Davis, R. J. & Flavell, R. A. MAP kinases in the immune response. *Annu. Rev. Immunol.* **20**, 55–72 (2002).
50. Lertmemongkolchai, G., Cai, G., Hunter, C. A. & Bancroft, G. J. Bystander activation of $CD8^+$ T cells contributes to the rapid production of IFN-γ in response to bacterial pathogens. *J. Immunol.* **166**, 1097–1105 (2001).
51. Yap, G., Pesin, M. & Sher, A. Cutting edge: IL-12 is required for the maintenance of IFN-γ production in T cells mediating chronic resistance to the intracellular pathogen, *Toxoplasma gondii*. *J. Immunol.* **165**, 628–631 (2000).
52. Park, A. Y., Hondowicz, B. D. & Scott, P. IL-12 is required to maintain a T_H1 response during *Leishmania major* infection. *J. Immunol.* **165**, 896–902 (2000).
53. Stobie, L. *et al*. The role of antigen and IL-12 in sustaining T_H1 memory cells *in vivo*: IL-12 is required to maintain memory/effector T_H1 cells sufficient to mediate protection to an infectious parasite challenge. *Proc. Natl Acad. Sci. USA* **97**, 8427–8432 (2000).
54. Kinjo, Y. *et al*. Contribution of IL-18 to T_H1 response and host defense against infection by *Mycobacterium tuberculosis*: a comparative study with IL-12 p40. *J. Immunol.* **169**, 323–329 (2002).
55. Oppmann, B. *et al*. Novel p19 protein engages IL-12 p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* **13**, 715–725 (2000).
56. Parham, C. *et al*. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rβ1 and a novel cytokine receptor subunit, IL-23R. *J. Immunol.* **168**, 5699–5708 (2002).
57. Belladonna, M. L. *et al*. IL-23 and IL-12 have overlapping, but distinct, effects on murine dendritic cells. *J. Immunol.* **168**, 5448–5454 (2002).
58. Pflanz, S. *et al*. IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive $CD4^+$ T cells. *Immunity* **16**, 779–790 (2002).
59. Devergne, O. *et al*. A novel interleukin-12 p40-related protein induced by latent Epstein–Barr virus infection in B lymphocytes. *J. Virol.* **70**, 1143–1153 (1996).

60. Chen, Q. *et al*. Development of T_H1-type immune responses requires the type I cytokine receptor TCCR. *Nature* **407**, 916–920 (2000).
61. Yoshida, H. *et al*. WSX-1 is required for the initiation of T_H1 responses and resistance to *L. major* infection. *Immunity* **15**, 569–578 (2001).
62. Robinson, D. S. & O'Garra, A. Further checkpoints in T_H1 development. *Immunity* **16**, 755–758 (2002).
63. Huang, H. & Paul, W. E. Impaired interleukin-4 signaling in T helper type 1 cells. *J. Exp. Med.* **187**, 1305–1313 (1998).
64. Szabo, S. J., Dighe, A. S., Gubler, U. & Murphy, K. M. Regulation of the interleukin (IL)-12R β2-subunit expression in developing T helper 1 (T_H1) and T_H2 cells. *J. Exp. Med.* **185**, 817–824 (1997).
65. Kurata, H., Lee, H. J., O'Garra, A. & Arai, N. Ectopic expression of activated Stat6 induces the expression of T_H2-specific cytokines and transcription factors in developing T_H1 cells. *Immunity* **11**, 677–688 (1999).
66. Zhu, J. *et al*. Growth factor independent-1 induced by IL-4 regulates T_H2-cell proliferation. *Immunity* **16**, 733–744 (2002). **This study identified GFI1 as a downstream mediator of STAT6 signal transduction. It provides a basis for how IL-4 can induce the proliferation of T_H2 cells selectively.**
67. Farrar, J. D. *et al*. An instructive component in T helper cell type 2 (T_H2) development mediated by GATA-3. *J. Exp. Med.* **193**, 643–650 (2001).
68. Finkelman, F. D. *et al*. Stat6 regulation of *in vivo* IL-4 responses. *J. Immunol.* **164**, 2303–2310 (2000).
69. Jankovic, D. *et al*. 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 T_H2 cytokine profile. *J. Immunol.* **164**, 3047–3055 (2000).
70. Rodriguez-Palmero, M., Hara, T., Thumbs, A. & Hunig, T. Triggering of T-cell proliferation through CD28 induces GATA-3 and promotes T helper type 2 differentiation *in vitro* and *in vivo*. *Eur. J. Immunol.* **29**, 3914–3924 (1999).
71. Das, J. *et al*. A critical role for NF-κB in GATA3 expression and T_H2 differentiation in allergic airway inflammation. *Nature Immunol.* **2**, 45–50 (2001).
72. Ghosh, P., Tan, T. H., Rice, N. R., Sica, A. & Young, H. A. The interleukin-2 CD28-responsive complex contains at least three members of the NF-κB family: c-Rel, p50 and p65. *Proc. Natl Acad. Sci. USA* **90**, 1696–1700 (1993).
73. Rissoan, M. C. *et al*. Reciprocal control of T-helper-cell and dendritic-cell differentiation. *Science* **283**, 1183–1186 (1999).
74. Liu, Y. J., Kanzler, H., Soumelis, V. & Gilliet, M. Dendritic-cell lineage, plasticity and cross-regulation. *Nature Immunol.* **2**, 585–589 (2001).
75. Greenwald, R. J., Latchman, Y. E. & Sharpe, A. H. Negative co-receptors on lymphocytes. *Curr. Opin. Immunol.* **14**, 391–396 (2002).
76. Salomon, B. & Bluestone, J. A. LFA-1 interaction with ICAM-1 and ICAM-2 regulates T_H2 cytokine production. *J. Immunol.* **161**, 5138–5142 (1998).
77. Camacho, S. A. *et al*. A key role for ICAM-1 in generating effector cells mediating inflammatory responses. *Nature Immunol.* **2**, 523–529 (2001).
78. Luksch, C. R. *et al*. Intercellular adhesion molecule-1 inhibits interleukin-4 production by naive T cells. *Proc. Natl Acad. Sci. USA* **96**, 3023–3028 (1999).
79. Smits, H. H. *et al*. Intercellular adhesion molecule-1/LFA-1 ligation favors human T_H1 development. *J. Immunol.* **168**, 1710–1716 (2002).
80. Ben-Sasson, S. Z., Gerstel, R., Hu-Li, J. & Paul, W. E. Cell division is not a 'clock' measuring acquisition of competence to produce IFN-γ or IL-4. *J. Immunol.* **166**, 112–120 (2001).
81. Bird, J. J. *et al*. Helper T-cell differentiation is controlled by the cell cycle. *Immunity* **9**, 229–237 (1998). **This study was one of the first to propose a role for cell-cycle and epigenetic effects in T_H-cell differentiation.**
82. Mullen, A. C. *et al*. Cell cycle controlling the silencing and functioning of mammalian activators. *Curr. Biol.* **11**, 1695–1699 (2001).
83. Richter, A., Lohning, M. & Radbruch, A. Instruction for cytokine expression in T helper lymphocytes in relation to proliferation and cell-cycle progression. *J. Exp. Med.* **190**, 1439–1450 (1999).
84. Laouar, Y. & Crispe, I. N. Functional flexibility in T cells: independent regulation of $CD4^+$ T-cell proliferation and effector function *in vivo*. *Immunity* **13**, 291–301 (2000).
85. Sad, S. & Mosmann, T. R. Single IL-2-secreting precursor CD4 T cell can develop into either T_H1 or T_H2 cytokine secretion phenotype. *J. Immunol.* **153**, 3514–3522 (1994).
86. Heath, V. L. *et al*. Cutting edge: ectopic expression of the IL-12 receptor-β2 in developing and committed T_H2 cells does not affect the production of IL-4 or induce the production of IFN-γ. *J. Immunol.* **164**, 2861–2865 (2000).
87. Nishikomori, R. *et al*. Activated STAT4 has an essential role in T_H1 differentiation and proliferation that is independent of its role in the maintenance of IL-12Rβ2 chain expression and signaling. *J. Immunol.* **169**, 4388–4398 (2002).
88. Mohrs, M., Shinkai, K., Mohrs, K. & Locksley, R. M. Analysis of type-2 immunity *in vivo* with a bicistronic IL-4 reporter. *Immunity* **15**, 303–311 (2001).
89. Doyle, A. M. *et al*. Induction of cytotoxic T lymphocyte antigen 4 (CTLA-4) restricts clonal expansion of helper T cells. *J. Exp. Med.* **194**, 893–902 (2001).
90. Langenkamp, A., Messi, M., Lanzavecchia, A. & Sallusto, F. Kinetics of dendritic-cell activation: impact on priming of T_H1, T_H2 and nonpolarized T cells. *Nature Immunol.* **1**, 311–316 (2000).
91. Sallusto, F., Lenig, D., Forster, R., Lipp, M. & Lanzavecchia, A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* **401**, 708–712 (1999).
92. Gorelik, L., Constant, S. & Flavell, R. A. Mechanism of transforming growth factor β-induced inhibition of T helper type 1 differentiation. *J. Exp. Med.* **195**, 1499–1505 (2002).
93. Gorelik, L., Fields, P. E. & Flavell, R. A. Cutting edge: TGF-β inhibits T_H-type 2 development through inhibition of GATA-3 expression. *J. Immunol.* **165**, 4773–4777 (2000).
94. Heath, V. L., Murphy, E. E., Crain, C., Tomlinson, M. G. & O'Garra, A. TGF-β1 down-regulates T_H2 development and results in decreased IL-4-induced STAT6 activation and GATA-3 expression. *Eur. J. Immunol.* **30**, 2639–2649 (2000).
95. Neurath, M. F. *et al*. The transcription factor T-bet regulates mucosal T-cell activation in experimental colitis and Crohn's disease. *J. Exp. Med.* **195**, 1129–1143 (2002).
96. Grogan, J. L. *et al*. Early transcription and silencing of cytokine genes underlie polarization of T-helper cell subsets. *Immunity* **14**, 205–215 (2001). **This study was one of the first to show that gene silencing mediated by *trans*-postioning of loci to heterochromatin might 'lock-in' states of T_H-cell differentiation.**
97. Kamogawa, Y., Minasi, L. A., Carding, S. R., Bottomly, K. & Flavell, R. A. The relationship of IL-4- and IFN-γ-producing T cells studied by lineage ablation of IL-4-producing cells. *Cell* **75**, 985–995 (1993).
98. Avni, O. *et al*. T_H-cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. *Nature Immunol.* **3**, 643–651 (2002).
99. Fields, P. E., Kim, S. T. & Flavell, R. A. Cutting edge: changes in histone acetylation at the IL-4 and IFN-γ loci accompany T_H1/T_H2 differentiation. *J. Immunol.* **169**, 647–650 (2002).
100. Hutchins, A. S. *et al*. Gene silencing quantitatively controls the function of a developmental *trans*-activator. *Mol. Cell* **10**, 81–91 (2002).
101. Agarwal, S. & Rao, A. Modulation of chromatin structure regulates cytokine gene expression during T-cell differentiation. *Immunity* **9**, 765–775 (1998).
102. Agarwal, S., Avni, O. & Rao, A. Cell-type-restricted binding of the transcription factor NFAT to a distal IL-4 enhancer *in vivo*. *Immunity* **12**, 643–652 (2000).
103. Richards, E. J. & Elgin, S. C. R. Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. *Cell* **108**, 489–500 (2002).
104. Ng, H. H. *et al*. MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex. *Nature Genet.* **23**, 58–61 (1999).
105. Nan, X. *et al*. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* **393**, 386–389 (1998).
106. Jones, P. L. *et al*. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nature Genet.* **19**, 187–191 (1998).
107. Jackson, J. P., Lindroth, A. M., Cao, X. & Jacobsen, S. E. Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* **416**, 556–560 (2002).
108. Jeddeloh, J. A., Stokes, T. L. & Richards, E. J. Maintenance of genomic methylation requires a SWI2/SNF2-like protein. *Nature Genet.* **22**, 94–97 (1999).
109. Dennis, K., Fan, T., Geiman, T., Yan, Q. & Muegge, K. Lsh, a member of the SNF2 family, is required for genome-wide methylation. *Genes Dev.* **15**, 2940–2944 (2001).
110. Jenuwein, T. & Allis, C. D. Translating the histone code. *Science* **293**, 1074–1080 (2001).
111. Lee, D. U., Agarwal, S. & Rao, A. T_H2-lineage commitment and efficient IL-4 production involves extended demethylation of the *IL-4* gene. *Immunity* **16**, 649–660 (2002).
112. Lee, P. P. *et al*. A critical role for Dnmt1 and DNA methylation in T-cell development, function and survival. *Immunity* **15**, 763–774 (2001).
113. Loots, G. G. *et al*. Identification of a coordinate regulator of interleukins 4, 13 and 5 by cross-species sequence comparisons. *Science* **288**, 136–140 (2000).
114. Mohrs, M. *et al*. Deletion of a coordinate regulator of type-2 cytokine expression in mice. *Nature Immunol.* **2**, 842–847 (2001).
115. Solymar, D. C., Agarwal, S., Bassing, C. H., Alt, F. W. & Rao, A. A 3′ enhancer in the *IL-4* gene regulates cytokine production by T_H2 cells and mast cells. *Immunity* **17**, 41–50 (2002).
116. Cirillo, L. A. *et al*. Opening of compacted chromatin by early developmental transcription factors HNF3 (FoxA) and GATA-4. *Mol. Cell* **9**, 279–289 (2002).
117. Zhang, D. H., Yang, L. & Ray, A. Differential responsiveness of the *IL-5* and *IL-4* genes to transcription factor GATA-3. *J. Immunol.* **161**, 3817–3821 (1998).
118. Takemoto, N., Arai, K. & Miyatake, S. Cutting edge: the differential involvement of the N-finger of GATA-3 in chromatin remodeling and transactivation during T_H2 development. *J. Immunol.* **169**, 4103–4107 (2002).
119. Bird, A. DNA methylation patterns and epigenetic memory. *Genes Dev.* **16**, 6–21 (2002).
120. Hsieh, C. S. *et al*. Development of T_H1 $CD4^+$ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science* **260**, 547–549 (1993).
121. Murphy, K. M. *et al*. Signaling and transcription in T-helper development. *Annu. Rev. Immunol.* **18**, 451–494 (2000).
122. Stetson, D. B., Mohrs, M., Mallet-Designe, V., Teyton, L. & Locksley, R. M. Rapid expansion and IL-4 expression by *Leishmania*-specific naive helper T cells *in vivo*. *Immunity* **17**, 191–200 (2002).

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