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T-bet: a bridge between innate and adaptive immunity

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Abstract

Originally described over a decade ago as a T cell transcription factor regulating T helper 1 cell lineage commitment, T-bet is now recognized as having an important role in many cells of the adaptive and innate immune system. T-bet has a fundamental role in coordinating type 1 immune responses by controlling a network of genetic programmes that regulate the development of certain immune cells and the effector functions of others. Many of these transcriptional networks are conserved across innate and adaptive immune cells and these shared mechanisms highlight the biological functions that are regulated by T-bet.

T-bet (encoded by *Tbx21*) is an immune cell-specific member of the T-box family of transcription factors (FIG. 1). The adaptive immune system arose approximately 500 million years ago in jawed fish probably as a result of the emergence of the recombination-activating gene (*RAG*) transposon[1]. Prior to this event, primitive B cell-like and T cell-like cells could be identified in jawless fish, such as lampreys[2]. Intriguingly, a comparative evolutionary analysis indicates that the eomesodermin (*EOMES*), T-box brain protein 1 (*TBR1*) and T-bet subfamily of T-box genes arose very early in evolution before the appearance of classical adaptive immunity and can be identified in both lampreys (see ensembl database) and amphioxus[3]. Many of our current ideas about the key functions of T-bet derive from studies in T cells. The initial description of T-bet as a master regulator of commitment to the T helper 1 (T_H1) cell lineage as well as the elucidation of intersecting T cell transcriptional pathways has enabled rapid progress to be made in defining the genetic modules that control T cell polarity. However, it is possible that these regulatory pathways may have been coopted from more ancient cell types, especially as it is now appreciated that innate immune cells also express T-bet.

The effects of T-bet on the regulation of adaptive immune functions are well recognized and the molecular mechanisms have mostly been elucidated by analysing the genomic transcriptional targets in different cell types. Transcription factors activate or repress their target genes by binding to accessible promoter and enhancer elements. The development of

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methodologies to identify the direct genomic targets of T-bet has transformed our ability to dissect the intracellular pathways that regulate T cell differentiation. Chromatin immunoprecipitation (ChIP) coupled with array-based proximal promoter analysis (ChIP-chip) provided insight into the genes that are directly regulated by T-bet. The advent of massively parallel DNA sequencing (also known as ChIP-sequencing (ChIP-seq)) has more recently facilitated both greater resolution of promoter targets and insight into the distal enhancer elements that T-bet regulates[4,5]. ChIP-seq data sets from other transcription factors, such as GATA-binding protein 3 (GATA3), retinoic acid receptor-related orphan receptor-γt (RORγt; encoded by *Rorc*) and signal transducer and activator of transcription 4 (STAT4), combined with knowledge of the chromatin architecture at specific loci, has increased the understanding of how these molecules interact to coordinate T cell function[4–9]. Interestingly, it has recently been shown that T-bet binds to large domains that have clusters of enhancers associated with important T cell genes — known as 'super enhancers' — which are characteristics of master regulatory genes that define cell identity[10]. However, in most cases, it remains to be formally proven that these specific interactions take place at a single-cell level and that they do not reflect the heterogeneity within the cell populations studied. By contrast, much less is known about the range of T-bet target genes in primary innate immune cells because of the relatively large cell numbers that are required for these types of studies. However, many of the transcriptional pathways that have been defined in T cells are also relevant in innate immune cells, which indicates that considerable insights can be gained by investigating the parallel roles of T-bet in innate and adaptive immunity.

In this Review, we discuss the shared transcriptional mechanisms that regulate the development and/or the effector functions of innate and adaptive immune cells. We review the role of T-bet in maintaining mucosal homeostasis through its action in dendritic cells (DCs) and innate lymphoid cells. Special emphasis is given to the role of T-bet in the biology of $CD4^+$ T_H cells. We discuss the intricate molecular mechanisms that are regulated by T-bet to promote T_H1 cell differentiation and to suppress the development of opposing T_H cell lineages. In addition, we describe how T-bet expression 'tips the balance' in favour of terminal differentiation of $CD4^+$ and $CD8^+$ effector T cells at the expense of memory cell formation.

T-bet in the innate immune system

T-bet in DCs.

Much less is known about the function of T-bet in the innate immune system than is known about its function in T cells. T-bet was initially shown to be expressed in human monocytes and myeloid DCs after stimulation with interferon-γ (IFNγ)[11]. Loss-of-function studies indicated that, in the absence of T-bet, DCs were unable to efficiently prime T_H1 cell responses and they did not respond to the protective adjuvant activity of CpG DNA *in vivo* during *Listeria* spp. infection[12,13] (FIG. 2). T-bet was also shown to directly regulate inflammatory arthritis via its expression in DCs and by regulating T cell activation[14].

T-bet has more recently been recognized as being an important regulator of intestinal homeostasis[15]. T-bet expression in DCs was shown to regulate the homing of mast cell progenitors to mucosal tissue through the control of expression of mucosal addressin cell

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adhesion molecule 1 (MADCAM1) and vascular cell adhesion molecule 1 (VCAM1)[16]. However, despite a marked reduction in the number of mucosal mast cells, *Tbx21*$^{-/-}$ mice were still able to vigorously respond to intestinal infection with *Trichinella spiralis*.

In order to focus solely on the role of T-bet in innate immunity, T-bet-deficient mice were crossed onto a *Rag2*$^{-/-}$ background. Remarkably, mice deficient in both T-bet and RAG2 (*Tbx21*$^{-/-}$*Rag2*$^{-/-}$ mice) developed a spontaneous intestinal inflammation that resembled ulcerative colitis in humans[17]. Inflammation in this model was transmissible to T-bet-sufficient mice and was driven by a dysbiotic microbiota[17,18]. The model became known as the TRUC (*Tbx21*$^{-/-}$*Rag2*$^{-/-}$ ulcerative colitis) mouse model and has proved useful in uncovering mechanisms of inflammation-associated colorectal carcinoma and in providing a novel conceptual framework for innate immune dysregulation in ulcerative colitis[19,20]. Dysregulated tumour necrosis factor (TNF) production from colonic DCs underlies disease in TRUC mice as it causes epithelial cell apoptosis (FIG. 2). In CD4$^+$ T cells, T-bet transactivates *Tnf*, whereas in DCs, the *Tnf* locus is repressed by T-bet[21]. The generation of an isogenic colony of mice that did not develop colitis (TRnUC mice (*Tbx21*$^{-/-}$*Rag2*$^{-/-}$ no ulcerative colitis)) enabled the identification of a crucial pathobiont, *Helicobacter typhlonius*. Metagenomic sequencing of the colonic microbiome from TRUC and TRnUC mice, combined with complementation and transmissibility studies, confirmed that *H. typhlonius* was responsible for the colitic phenotype of TRUC mice[22].

T-bet in ILCs.

Innate lymphoid cells (ILCs) are a newly described type of cell that share many functional attributes with effector T cell subsets[23]. ILCs are important at mucosal sites, where they regulate epithelial homeostasis in relation to the intestinal microbiome, and they are dysregulated in inflammatory disease in both mice and humans[24,25]. It is increasingly recognized that shared transcriptional mechanisms are conserved between ILCs and CD4$^+$ T cells and that ILC subsets mirror their T_H cell counterparts in terms of their cytokine-producing capabilities. Thus, group 1 ILCs express T-bet, group 2 ILCs express GATA3 and group 3 ILCs express RORγt[26]. Natural killer (NK) cells have also been classified as group 1 ILCs, but given that they have substantial differences to this cell type, they will be considered separately. ILCs express the IL-7 receptor (IL-7R) and CD90 (also known as THY1) and are negative for all lineage markers and antigen (B cell and T cell) receptors. ILCs are dependent on IL-7R signalling through the common cytokine receptor γ-chain (γ_c). ILC1s, which are another subset of group 1 ILCs, are positive for NK cell p46-related protein (NKp46; also known as NCR1), as are a subset of group 3 ILCs (NKp46$^+$ ILC3s), which also express IL-23R[26].

The first description of a functional role for T-bet in ILC biology came from studies in the TRUC mouse model (FIG. 3). Colitis in TRUC mice was abrogated by genetic or antibody-mediated depletion of ILCs. Furthermore, T-bet seemed to control the plasticity of RORγt$^+$ ILCs, by inducing IFNγ expression and by repressing IL-17A production (FIG. 2). These effects were partly mediated through direct repression of IL-7R expression by T-bet[22]. As IL-7 has been shown to stabilize RORγt expression in ILCs[27], this provides a potential mechanism for the reciprocal expression pattern of T-bet and RORγt (FIG. 2). DC-derived

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TNF functioned with IL-23 to drive IL-17 production by ILCs; this indicates that there is a newly identified level of innate cellular crosstalk at the inflamed mucosal surface (FIG. 3).

Other studies have shown that T-bet has a crucial role in the development and function of group 1 and 3 ILCs. The absence of T-bet was shown to result in a loss of NKp46$^+$ ILC3s in the small intestine[28]. T-bet was found to be expressed in CC-chemokine receptor 6 (CCR6)$^-$ ILC3s but not in CCR6$^+$ ILC3s. Within the T-bet$^+$CCR6$^-$ ILC3 subset, both NKp46$^+$ and NKp46$^-$ ILC3s were identified, and T-bet expression was found to be increased in proportion to NKp46 expression, which also correlated with increasing IFNγ expression[29]. Other canonical T-bet target genes, such as CXC-chemokine receptor 3 (*Cxcr3*) and CD95 ligand (*Cd95l*; also known as *Fasl*) are also expressed in T-bet$^+$CCR6$^-$ ILC3s. The signals that regulate T-bet expression in ILC3s are incompletely defined; IL-12 was shown not to be required but IL-23 induced T-bet expression[29]. IFNγ-producing T-bet$^+$ ILC1 subsets have recently been reported in mice and humans and these cells were shown to accumulate in inflamed mucosal tissues of patients with Crohn's disease[24,25]. Whether ILC1s represent a separate lineage or whether they are related to NK cells or ILC3s remains to be determined.

T-bet in NK cells.

In combination with the T-box transcription factor EOMES, T-bet regulates the development and the terminal maturation of NK cells[30,31] (FIG. 2). In the absence of T-bet, susceptibility to metastatic cancer, including melanoma, was shown to be increased because of impaired NK cell function and survival *in vivo*[32]. T-bet deficiency alone only induces a partial defect in NK cell numbers as a result of a compensatory increase in EOMES, whereas deletion of both T-bet and EOMES results in a complete absence of NK cells[30]. The development of immature NK cells that express TNF-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10) was shown to be dependent on T-bet, whereas maturation and loss of TRAIL expression was shown to be more strongly influenced by EOMES[30].

T-bet in non-conventional T cells.

T-bet is also expressed in invariant NKT (iNKT) and γδ T cells. These thymus-derived cells share features of both innate and adaptive immune cells and require T-bet either for their development (in the case of iNKT cells) or for their production of effector cytokines (in the case of γδ T cells). In the absence of T-bet, the number of Vα14i NKT cells is significantly reduced, possibly because of impaired survival that results from reduced IL-15R signalling secondary to low expression of the T-bet target gene *Cd122* (also known as *Il15rb*)[31,33]. Despite the marked reduction in numbers of iNKT cells in the absence of T-bet, the remaining iNKT cells were able to induce airway hyper-reactivity through their augmented IL-4 and IL-13 production[34].

T-bet is not expressed in naive γδ T cells but its expression is induced in γδ T cells following T cell receptor (TCR) signalling[35] (FIG. 2). A high percentage of γδ T cells will produce IFNγ after TCR stimulation, and this is regulated by inducible T-bet and constitutive EOMES expression[36]. T-bet deficiency results in a 50% reduction in the frequency of IFNγ-producing γδ T cells, which suggests that constitutive EOMES

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expression and the hypomethylation of intron 1 at the *Ifng* locus contribute to the remaining IFNγ production by T-bet-deficient γδ T cells[36].

T-bet in T_H cells

T-bet: the master regulator of T_H1 cell differentiation.

Selective expression of T-bet accounts for T_H1 cell development and for the T_H1 cell-specific expression of IFNγ[37] (BOX 1; FIG. 4). Ectopic expression of T-bet in differentiated T_H2 or T_H17 cells results in their conversion into IFNγ-producing T_H1-like cells[37–40]. Conversely, T-bet-deficient CD4+ T cells only produce small amounts of IFNγ under T_H1 cell-polarizing conditions and fail to mount effective T_H1 cell responses during infections with *Leishmania major*, *Mycobacterium tuberculosis* and *Salmonella enterica* subsp. *enterica* serovar Typhimurium[41–43].

T-bet is not expressed in naive CD4+ T cells, but is readily induced in response to TCR, IFNγ–STAT1 and IL-12R–STAT4 signalling pathways[37,44–47]. TCR and IFNγ receptor (IFNγR) signalling induce the first wave of T-bet expression, which is independent of IL-12R signalling that is caused by the TCR-mediated inhibition of expression of IL-12Rβ2 subunit[44–46] (FIG. 4). Cessation of both TCR stimulation and IL-2R–STAT5 signalling induces IL-12Rβ2 subunit expression, which thus enables IL-12R signalling[46,48] (FIG. 4). IL-12 functions via STAT4 to induce a second wave of T-bet expression, which is required for the stabilization of the T_H1 cell phenotype[8,45–47]. It is unclear whether imprinting of the T_H1 cell developmental programme involves autoactivation of the *Tbx21* gene by T-bet. Ectopic expression of T-bet induces endogenous expression of *Tbx21*, which suggests that autoactivation occurs[4,44,45]. However, in one reported study, endogenous *Tbx21* induction was dependent on autocrine IFNγR signalling[44]. The question of whether T-bet autoregulation occurs in developing T_H1 cells has been recently re-examined using T-bet–ZsGreen-reporter mice. In this study, it was shown that T-bet was not required for its own induction when IL-12 or IFNγ were present during differentiation; however, T-bet may be involved in directly promoting its own expression when induced by IL-12and IFNγ-independent pathways[8]. Another level of complexity with regard to the regulation of T-bet expression in T_H1 cells has been added by the observations that T-bet expression in T_H1 cells is fine-tuned post-transcriptionally by the microRNA-29 cluster and post-translationally by the ubiquitin-proteasomal degradation pathway[49–51].

Together with STAT4, T-bet has a central role in the generation of transcriptionally competent T_H1 cell-specific genes in CD4+ T cells. The molecular mechanisms by which T-bet modifies chromatin state have recently been reviewed[52] and will be summarized here in general terms. T-bet-mediated chromatin changes are primarily dependent on the ability of T-bet to recruit enzymes that generate chromatin modifications associated with either gene activation (histone H3 or H4 acetylation, and H3 lysine 4 (H3K4) dimethylation) or gene repression (H3K27 trimethylation)[53,54]. CD4+ T cells that have been polarized under T_H1 cell-associated conditions have increased expression of permissive marks at gene loci that are known to be positively regulated by T-bet. Conversely, genes that are normally repressed by T-bet in T_H1 cells are characterized by the presence of repressive H3K27 trimethylation marks[8,47,55–58] (FIG. 4). High levels of *Ifng* transcription are subject to change in T_H1 cells

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depending on epigenetic modifications that remodel the *Ifng* locus; T-bet and STAT4 cooperate in this process[8,47,56]. T-bet directly activates the *Ifng* gene by binding to the *Ifng* promoter and to multiple distal regulatory elements located −54, −34, −22 and −6 kilobases (kb) upstream and +18–20 kb downstream of the *Ifng* gene, almost all of which serve as T-bet-dependent enhancers[56–58]. T-bet-mediated transactivation of the *Ifng* gene is further enhanced by H2.0-like homeobox protein (HLX) and runt-related transcription factor 3 (RUNX3) transcription factors, the expression of both of which is induced by T-bet in differentiating T_H1 cells[59,60].

Another key regulatory role of T-bet is to organize the three-dimensional architecture of the *Ifng* locus by enhancing occupancy of the transcriptional repressor CCCTC-binding factor (CTCF) between the boundaries of the *Ifng* locus (−70 kb and +66 kb) and a +1 kb site. T-bet binding at these locations promotes CTCF-dependent chromatin looping, which brings T-bet-binding enhancers and CTCF-binding sites in close proximity at the *Ifng* promoter. This configuration promotes robust *Ifng* expression in T_H1 cells[61]. T-bet directly activates approximately 50% of T_H1 cell-specific genes including cytokines (for example, *Ifng*, lymphotoxin-α (*Lta*) and *Tnf*), chemokines (for example, XC-chemokine ligand 1 (*Xcl1*), CC-chemokine ligand 3 (*Ccl3*) and *Ccl4*) and chemokine receptors (*Cxcr3* and *Ccr5*), which are required for the proper effector function and the migration of T_H1 cells, and for the recruitment of other immune cells to sites of inflammation[8,21,47,62] (FIG. 4). Although most of the T_H1 cell-specific gene expression is T-bet dependent, T-bet has limited effects in establishing global enhancer competence in T_H1 cells. This function has been attributed to STAT4, which has a major effect on the activation of T_H1 cell lineage-specific enhancers[5].

Reinforcement of the T_H1 cell differentiation programme relies not only on the T-bet-regulated activation of T_H1 cell-specific genes but also on the concomitant inhibition of alternative T_H cell differentiation pathways. T-bet regulates this either by suppressing the induction of other lineage-specifying transcription factors in T_H pre-cursor cells or by interfering with their transcriptional activity (FIG. 4). Thus, T-bet deficiency is often accompanied by increased production of T_H2 cell-specific or T_H17 cell-specific cytokines[38,43].

Inhibition of T_H2 cell lineage commitment.

In contrast to T-bet, GATA3, which is the master regulator of the T_H2 cell differentiation programme, is already expressed in naive $CD4^+$ T cells[63]. Furthermore, activation of naive $CD4^+$ T cells results in a transient, non-selective histone acetylation at both T_H1 cell-specific and T_H2 cell-specific cytokine genes, which persists for 17 to 20 hours after TCR stimulation[55]. It is at these early stages of T_H cell lineage commitment that T-bet must compete with GATA3 to establish a T_H1 cell-specific gene expression programme. Early TCR signalling induces T-bet expression, and IL-2-inducible T cell kinase (ITK)-mediated phosphorylation of T-bet at Tyr525 promotes the interaction of T-bet with GATA3 (REF. 64). This interaction redistributes GATA3 away from T_H2 cell-specific genes to T-bet-binding sites at T_H1 cell-specific loci[4,64]. Sequestration of GATA3 away from T_H2 cell-specific genes (primarily the *Il4–Il5–Il13* locus) and the binding of a T-bet–RUNX3 complex to the *Il4* silencer (DNase I hypersensitivity site 4 (HS4)) prevents the expression of T_H2 cell-

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specifc cytokine genes in developing T_H1 cells[4,60,64] (FIG. 4). Furthermore, T-bet binds to and promotes repressive chromatin modifications at the *Gata3* locus, which thus inhibits the *de novo* expression of *Gata3* in T_H1 cells[8]. In addition to GATA3, nuclear factor of activated T cells 1 (NFAT1; also known as NFATC2) promotes T_H2 cell differentiation through the activation of *Il2*, *Il4*, *Il5* and *Il13*. T-bet phosphorylation at Thr302 is crucial for the inter action of T-bet with NFAT1 and loss of this interaction through mutation of the Thr302 residue abrogates the ability of T-bet to suppress NFAT1-dependent IL-2 and T_H2-type cytokine expression[51]. T-bet can also limit IL-2 production by suppressing RELA activity through serine phosphorylation. T-bet phosphorylation at Ser508 by casein kinase I and glycogen synthase kinase 3 (GSK3) kinases promotes the interaction of T-bet with RELA, which impairs RELA binding to the *Il2* promoter and the subsequent transcriptional activation of the *Il2* gene by RELA[65]. Since IL-2 is required for upregulation of the IL-4Rα subunit, the negative regulatory effects of T-bet on *Il2* gene expression efficiently limit IL-4 signalling and T_H2 cell differentiation.

T-bet can also override a previously programmed T_H2 cell differentiation state. Lymphocytic choriomeningitis virus (LCMV) infection, as well as a combination of type I or type II interferons and IL-12 *in vitro*, has been shown to reprogramme committed T_H2 cells into T_H1-like T_H2 cells. These cells co-express GATA3 and T-bet and they produce T_H1- and T_H2-type cytokines following reactivation[66]. The conversion of T_H2 cells into T_H1-like cells was shown to be entirely dependent on T-bet both *in vitro* and *in vivo*. Sequential acquisition of T-bet and its downstream differentiation programme without extinguishing the previously acquired T_H2 cell differentiation programme produced a population of optimally adapted T_H cells, which were highly effective against LCMV but which protected tissues against immune-mediated pathology[66].

Inhibition of T_H17 cell lineage commitment.

T-bet expression in T_H precursor cells inhibits commitment to the T_H17 cell lineage by blocking RUNX1-mediated induction of the T_H17 cell-specific transcription factor RORγt[38]. Thus, differentiation of *Tbx21*$^{-/-}$ $CD4^+$ T cells under T_H17 cell-skewing conditions produces a higher frequency of T_H17 cells with a high expression of IL-17A, IL-17F and IL-21 (REFS 38,39). T-bet expression in mature T_H17 cells results in their conversion into IFNγ-producing T_H17 cells, which seems to be an important step in the pathophysiology of experimental autoimmune encephalomyelitis (EAE), as *Tbx21*$^{-/-}$ T_H17 cells are not pathogenic in the EAE model[67–70]. Although, little is known about the downstream targets of T-bet that are responsible for the pathogenicity of T_H17 cells, it has recently been shown that *Tbx21*$^{-/-}$ T_H17 cells express lower levels of transforming growth factor-β3 (TGFβ3) than wild-type T_H17 cells, and exogenous addition of TGFβ3 could restore the pathogenic potential of *Tbx21*$^{-/-}$ T_H17 cells in the passive EAE model[68].

A switch to IFNγ production is induced by the exposure of T_H17 cells to IL-12 or IL-23 (REFS 67,71,72). The *Tbx21* promoter has bivalent histone methylation marks in T_H17 cells, which suggests that the *Tbx21* locus is maintained in a poised state and is responsive to changes in environmental cues[67,71–73]. In addition to T-bet upregulation, the conversion of T_H17 cells into T_H1-like cells is accompanied by the downregulation of RORγt and T_H17

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cell-specific cytokines[38,72] (FIG. 4). Silencing of *Rorc* in committed T_H17 cells following IL-12R signalling has been linked with STAT4-induced and T-bet-induced epigenetic repression of the *Rorc* gene[72]. This is in contrast to what has been observed in T_H2 cells, in which T-bet induction did not result in silencing of GATA3 expression. Although it is possible for opposing transcription factors to be present in the same cell during transitions, termination of *Rorc* expression in T_H17 cells during conversion into T_H1-like cells could reflect a greater susceptibility of the *Rorc* locus to epigenetic remodelling by T-bet and STAT4 transcription factors[72].

Inhibition of T_{FH} cell lineage commitment.

The early steps in T_H1 cell commitment have many features in common with the T follicular helper (T_{FH}) cell differentiation pathway[74]. IL-12 signals through STAT4 to induce a transitional stage of T_{FH} cell-like T_H1 cells, which express IL-21 and B cell lymphoma 6 (BCL-6). However, these T_{FH} cell-like features are not sustained, as STAT4 also promotes T-bet expression, which inhibits the induction of T_{FH} cell-specific genes by limiting the expression of BCL-6. Interestingly, the *Bcl6* locus is not fully repressed in differentiated T_H1 cells[74]. In fact, the low concentrations of BCL-6 that are present in T_H1 cells are necessary for T-bet to effectively repress alternative T_H cell gene programmes. T-bet interacts with BCL-6 and recruits it to the promoters of genes that are repressed by T-bet in T_H1 cells[75]. When the concentrations of BCL-6 are low, T-bet sequesters BCL-6 away from its target genes, which thereby effectively blocks T_{FH} cell lineage commitment (FIG. 4). The primary determinant of the relative ratio of T-bet to BCL-6 in T_H1 cells is the concentration of IL-2 (REF. 76). In the presence of high levels of IL-2, the levels of BCL-6 are kept low. However, in low IL-2 concentrations, forkhead box O (FOXO) transcription factors induce BCL-6 and tip the balance in favour of expression of BCL-6 and the T_{FH} cell gene expression profile. Thus, low doses of IL-2R signalling can change polarized T_H1 cells into T_{FH} cells, which suggests that T_H1 cells retain a great degree of flexibility and have a T_{FH} cell-like gene profile[76]. Similarly, T_{FH} cells can acquire the properties of T_H1 cells after exposure to T_H1 cell-polarizing cytokines[77]. T_{FH} cells have positive chromatin modifications at the *Tbx21* locus, which maintains this gene in a state that is primed for re-expression. T-bet expression endows T_{FH} cells with the ability to produce IFNγ without affecting their ability to produce IL-21 and to express T_{FH} cell-specific markers[77].

Peripherally derived T_{Reg} cells.

Similarly to what was observed in other T_H cell subsets, in peripherally derived regulatory T (pT_{Reg}) cells (also known as induced or adaptive T_{Reg} cells) the *Tbx21* locus is maintained in an open conformation and T-bet is upregulated in response to STAT1-mediated IFNγR and IL-27R signalling[73,78–80]. T-bet-deficient pT_{Reg} cells do not survive well after adoptive transfer into scurfy mice and fail to protect the mice from T_H1 cell-mediated inflammatory responses. This suggests that T-bet is required for the functional fitness of pT_{Reg} cells[80]. It is incompletely understood how T-bet controls pT_{Reg} cell function and it is equally intriguing how pT_{Reg} cells maintain a FOXP3-regulated gene expression profile in the presence of T-bet. As adoptive transfers of pT_{Reg} cells have been considered as a potential therapy for inflammatory and autoimmune diseases, the plasticity of the pT_{Reg} cell transcriptional programme has gained increasing attention. There is the possibility that a pT_{Reg} cell could

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gain the effector profile of a T_H1 cell in a dominating T_H1 cell-associated environment. A recent study showed that T-bet$^+$ pT_{Reg} cells express 10–20-fold less T-bet than conventional T_H1 cells even in T_H1-type inflammatory conditions[80]. The low level of T-bet expression in pT_{Reg} cells is maintained by the silencing of the *Il12rb2* locus, which prevents T-bet$^+$ pT_{Reg} cells from completing IL-12R–STAT4-dependent T_H1 cell differentiation. This maintains the suppressive function of these cells without the production of pro-inflammatory cytokines[79]. However, T-bet expression in pT_{Reg} is absolutely essential for their proper migration. T-bet induces the expression of CXCR3 — a T_H1 cell-associated chemokine receptor — which enables T-bet$^+$ pT_{Reg} cells to migrate to sites of T_H1 cell-mediated inflammation and to suppress local T_H1 cell responses.

Although *Tbx21* encodes the T_H1 cell lineage-specifying transcription factor, the promoter of the *Tbx21* gene is marked by permissive and repressive genetic marks in T_H2, T_H17, T_{FH} and pT_{Reg} cells, and it is poised for subsequent activation or silencing[71,73,77]. Maintenance of the master transcription factor loci in a poised, bivalent epigenetic state in the opposing T_H cell subsets is the basis for functional plasticity of CD4$^+$ T_H cells, which respond to changes in the environment by adopting the gene expression profiles of other T_H cell lineages[73]. T-bet has a dominant role, often tipping the balance towards a T_H1 cell phenotype. As described in the aforementioned examples, sometimes the acquisition of T_H1 cell properties is accompanied by the repression of the existing gene expression profile (in the case of T_H17 cells). In other cases, the responding T_H cell subset gains features of the T_H1 cell lineage, such as IFNγ and CXCR3 expression, but keeps its genetic profile (in the case of T_H2, T_{FH} and pT_{Reg} cells). Functional plasticity of CD4$^+$ T_H cells could be one mechanism to provide maximum host protection by generating large numbers of T_H1 cells from pre-existing T_H cell subsets following infection with intracellular pathogens. However, in the case of pT_{Reg} cells, increased T-bet expression is required for proper migration of pT_{Reg} cells to sites of T_H1-cell dominated responses to minimize the amount of immune-mediated pathology.

Generation of B cell and T cell memory

T-bet in CD8$^+$ T cell memory.

The acquisition of CD8$^+$ T cell effector functions and the development of CD8$^+$ T cell memory (BOX 2) are crucially dependent on transcriptional events that are regulated by T-bet and EOMES. CD8$^+$ T cells that lack either T-bet or EOMES show a minor defect in their effector functions, which suggests that these two transcription factors have overlapping and partially redundant roles in establishing the differentiation programme of CD8$^+$ T cells[81,82]. However, CD8$^+$ T cells that are deficient in both T-bet and EOMES lose their cytotoxicity and aberrantly produce large amounts of IL-17A in response to infection with LCMV[83]. T-bet expression is rapidly induced by TCR and IL-12R signalling, and is required for the early production of IFNγ and granzyme B (encoded by *Gzmb*) by antigen-specific CD8$^+$ T cells[84,85] (FIG. 5). EOMES, which is induced later in a RUNX3-dependent manner, can substitute for T-bet to promote IFNγ and granzyme B expression and, together with STAT5, regulates perforin gene expression in CD8$^+$ T cells[84,86] (FIG. 5a). The concerted action of T-bet and EOMES results in the development of fully differentiated effector CD8$^+$ T cells that

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migrate to inflamed tissues and that, following antigen recognition, secrete cytokines (for example, IFNγ and TNF) or lyse infected cells by releasing cytotoxic granules that contain granzymes and perforin.

T-bet and EOMES have essential but reciprocal roles in regulating effector and memory T cell differentiation pathways[87–89] (BOX 2; FIG. 5b). Inflammatory signals that are present during $CD8^+$ T cell priming define a gradient of T-bet and EOMES expression and, consequently, influence the fate of effector $CD8^+$ T cells[87]. Strong IL-12R signalling provides the instructive signal for the terminal differentiation of short-lived effector cells (SLECs) by enhancing and maintaining STAT4-mediated mammalian target of rapamycin (mTOR) kinase activity. This functions as a molecular switch to concomitantly induce T-bet expression and to repress EOMES expression through the inhibition of FOXO1 transcriptional activity[85,88,89]. Thus, T-bet deficiency, IL-12 deficiency or inhibition of mTOR activity results in a profound absence of SLECs and in the accumulation of long-lived memory precursor effector cells (MPECs)[87,89] (FIG. 5b). In addition to cytokine signalling, asymmetric partitioning of key transcriptional regulators, such as T-bet, between the daughter cells during cell division may regulate the SLEC versus MPEC fate decision[90]. The cell that acquires more T-bet during cell division becomes a terminally differentiated effector cell, whereas the cell that has low T-bet levels has a higher memory cell developmental potential[90].

Once the pathogen is cleared, the vast majority of antigen-specific $CD8^+$ T cells that are generated during clonal expansion die. A small fraction of $CD8^+$ T cells that survive the contraction phase, the MPECs, generate a reservoir of memory $CD8^+$ T cells, which depend on the cytokines IL-7 and IL-15 for their long-term persistence (BOX 2). As $CD8^+$ T cells acquire a memory phenotype, EOMES expression increases whereas T-bet levels decrease[91–93] (FIG. 5c). Nevertheless, low levels of T-bet are maintained in memory $CD8^+$ T cells, and T-bet and EOMES cooperate to sustain proper expression of CD122 (the β-subunit of IL-2R and IL-15R) to promote the longevity and the homeostatic proliferation of memory $CD8^+$ T cells[94]. Effector memory T (T_{EM}) cells express higher levels of T-bet and seem to be more differentiated than central memory T (T_{CM}) cells[92]. By contrast, the expression of EOMES is higher in T_{CM} cells than in T_{EM} cells[91,92] (FIG. 5c). EOMES deficient memory $CD8^+$ T cells are less efficient at competing to populate the bone marrow niche and are defective in long-term persistence and secondary expansion after antigen re-challenge[92]. These findings suggest that EOMES expression is needed for the competitive fitness and the persistence of T_{CM} cells, whereas T-bet expression is associated with a more differentiated phenotype of T_{EM} cells.

Subsequent antigen encounters or the exposure of memory $CD8^+$ T cells to inflammatory cytokines leads to a rapid upregulation of T-bet and to the terminal differentiation of memory $CD8^+$ T cells during secondary and tertiary infections[91] (FIG. 5c). Limited T-bet expression in *Tbx21*$^{+/-}$ $CD8^+$ T cells enables memory $CD8^+$ T cells to retain a less differentiated state during multiple rounds of antigen re-challenge, which thereby prevents the senescence of memory $CD8^+$ T cells[91]. Furthermore, memory $CD8^+$ T cells that develop in the absence of $CD4^+$ T cell help seem to be more T_{EM} cell-like, as they are characterized by elevated T-bet levels. In these cases, T-bet deletion has been found to prevent the aberrant

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differentiation of 'unhelped' memory $CD8^+$ T cells[93]. Therefore, it may be possible to minimize terminal differentiation of secondary and tertiary memory $CD8^+$ T cells and to correct the dysfunctional programming of $CD8^+$ T cells that develop in the absence of $CD4^+$ T cell help simply by limiting T-bet expression[91,93].

During chronic infections, $CD8^+$ T cell responses are quite different from those observed during acute infections. When they are exposed to persistent antigenic stimulation, $CD8^+$ T cells become dysfunctional, exhausted and fail to differentiate into effective memory $CD8^+$ T cells. The exhaustion of $CD8^+$ T cells is accompanied by the upregulation of inhibitory molecules (such as programmed cell death protein 1 (PD1; encoded by *Pdcd1*), lymphocyte activation gene 3 protein (LAG3) and Band T-lymphocyte attenuator (BTLA)), as well as defective proliferation, cytokine production and cytotoxicity in these cells[95,96]. In this context, elevated T-bet levels improve the functionality and the long-term durability of antigen-specific $CD8^+$ T cells, whereas EOMES expression enhances $CD8^+$ T cell exhaustion[97,98]. T-bet represses the expression of inhibitory receptors, including PD1, and improves the fitness of exhausted $CD8^+$ T cells[97]. Thus, therapeutic strategies that aim to increase T-bet expression in $CD8^+$ cells could minimize $CD8^+$ T cell exhaustion and improve $CD8^+$ T cell responses during chronic infections[97].

Collectively, these studies show the complex regulation of T-bet and EOMES expression that takes place during acutely resolved versus chronic infections. Our understanding of the differentiation pathways that are regulated by T-bet and EOMES will provide novel ideas for therapeutic interventions and will improve the efficacy of memory $CD8^+$ T cell responses in the context of acute and chronic infections.

T-bet in $CD4^+$ T cell memory.

Unlike the formation of $CD8^+$ T cell memory, which has been intensely studied over many years, very little is known about the requirements for the formation, the proper function and the maintenance of $CD4^+$ T cell memory. Similarly to $CD8^+$ T cell responses, there is marked phenotypic heterogeneity in the antigen-specific $CD4^+$ T cells at the peak of the response. However, the particular features that distinguish a population of committed memory precursor cells from the larger pool of diverse effector cells remain a matter of debate. Infection with intracellular bacteria or viruses induces the formation of both T_H1 cells and T_{FH} cells, and these effector $CD4^+$ T cell subsets can be readily distinguished by their differential expression of CXCR5, P-selectin glycoprotein ligand 1 (PSGL1) and LY6C (FIG. 5d). T_H1 cells do not express CXCR5 and are positive for PSGL1 and LY6C.

Among T_H1 cells, two major subsets can be identified on the basis of LY6C expression: $LY6C^{hi}$ and $LY6C^{low}$ cells. $PSGL1^{hi}LY6C^{hi}$ cells have features of terminally differentiated effector cells, such as increased expression of T-bet, CXCR3, IFNγ and granzyme B. By contrast, $PSGL1^{hi}LY6C^{low}$ cells express intermediate levels of T-bet and are less differentiated than their $PSGL1^{hi}LY6C^{hi}$ counterparts[99]. High levels of expression of CD62L (also known as L-selectin) and CCR7 are associated with $CD4^+$ T_{CM} cells, and these cells are preferentially enriched in the $PSGL1^{hi}LY6C^{low}$ memory compartment, which persists longer and proliferates better than the $LY6C^{hi}$ subset following antigen rechallenge[99]. Similarly to antigen-specific $CD8^+$ T cells, elevated T-bet expression is

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required for maximal clonal expansion and for the formation of terminally differentiated $PSGL1^{hi}LY6C^{hi}CD4^{+}$ T cells. Loss of one or two copies of T-bet results in a proportionally increased frequency of $PSGL1^{hi}LY6C^{low}$ T_H1 memory precursor cells[99]. Thus, T-bet balances terminal differentiation and memory cell potential in both $CD4^{+}$ and $CD8^{+}$ T cells[99]. Following antigen re-challenge, T_H1 memory cells efficiently produce secondary T_H1 effector cells. These cells have high T-bet, IFNγ and granzyme B expression, which suggests that memory T_H1 cells maintain a default T_H1 cell lineage differentiation programme in the absence of antigens[100] (FIG. 5d).

In contrast to T_H1 effector and T_H1 memory cells, T-bet is expressed at very low levels in T_{FH} cells ($CXCR5^{+}PSGL1^{low}LY6C^{low}$ or $CXCR5^{+}PSGL1^{low}LY6C^{int}$) and T_{FH} memory cells[99–101]. Furthermore, T_{FH} cells maintain repressive methylation of the granzyme B locus throughout effector and memory cell differentiation. They also produce limited amounts of IFNγ following reinfection with LCMV[99–101]. Silencing of the *Gzmb* locus and other T_H1 cell-specific loci in T_{FH} memory cells reinforces the T_{FH} cell-lineage programme and prevents the expression of T_H1 cell-specific genes following acute LCMV infection[100]. In contrast to these findings, $CXCR5^{+}CCR7^{+}$ memory $CD4^{+}$ T cells described by Pepper *et al.*[101] resemble T_{CM} cells rather than T_{FH} cells, as they have down-regulated T_{FH} cell markers (such as high BCL-6 and PD1) and they have a potential to equally reconstitute T_H1 and T_{FH} effector cells following reinfection with *Listeria monocytogenes*[101]. It remains to be determined whether primary T_H1 and T_{FH} effector cells give rise to T_H1 and T_{FH} memory cells in a linear fashion and lineage-specific effector functions are maintained in these cells even in the absence of antigens by their master regulators T-bet and BCL-6, respectively; or, alternatively, whether $PSGL1^{hi}LY6C^{low}$T-bet^{int} cells represent more terminally differentiated T_{EM} cells than $CXCR5^{+}PSGL1^{low}LY6C^{low}$ T-bet^{low} or $CXCR5^{+}PSGL1^{low}LY6C^{int}$T-$bet^{low}$ cells, which give rise to T_{CM} cells. In the second example, the balance between T-bet and BCL-6 would be a principal mechanism regulating the generation of distinct memory cell populations[100,101].

T-bet in B cell memory.

In response to antigen stimulation, activated B cells proliferate and undergo affinity maturation and class-switch recombination (CSR) in order to produce high-affinity antibodies that have different immune effector functions. IgG2a is the most prevalent isotype that is produced in response to intracellular bacterial and viral infections. Cytokines such as IFNγ and IL-27 increase T-bet expression in B cells in a STAT1-dependent and ETS1-dependent manner[102–105] (FIG. 5e). T-bet subsequently induces the expression of Iγ2a transcripts and promotes IFNγ-mediated class-switching to the IgG2a isotype[103]. T-bet-deficient B cells show impaired IgG2a, IgG2b and IgG3 secretion. In addition, ectopic expression of T-bet is sufficient to induce the expression of germline Iγ2a transcripts in *Tbx21*$^{-/-}$ B cells even in the absence of IFNγR signalling[103]. T-bet expression persists in antigen-specific $IgG2a^{+}CD38^{hi}$ memory B cells and it promotes the survival of this cell subset by regulating the transcription of the mature B cell receptor[106]. Furthermore, T-bet drives the migration of memory $IgG2a^{+}$ B cells to sites of inflammation by controlling the expression of the chemokine receptor CXCR3 (REF. 107). These findings identify T-bet as a

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selective inducer of IFNγ-mediated IgG2a class-switching in B cells and a key regulator of B cell migration to inflammatory foci.

Conclusions and perspectives

T-bet has important roles in multiple aspects of a coordinated immune response. It regulates the development of components of the innate immune system, it affects the trafficking of both innate and adaptive immune cells and it controls the polarity of cytokine responses. In this way, T-bet functions as the key molecule in coordinating effective and anatomically appropriate type 1 immunity. Despite the intensive studies that have been ongoing for over a decade, much still remains to be learned about the cell-specific roles of T-bet. Indeed, the recent descriptions of the role of T-bet in the newly discovered ILCs have highlighted further functions of this protean transcription factor. There is still much to be learned about the upstream signals that regulate T-bet expression in specific cell types and how T-bet interacts with other lineage-determining transcription factors *in vivo*. It is tempting to speculate that modulation of T-bet expression may be a very powerful therapeutic target for the treatment of autoimmunity, transplant rejection, infectious diseases and cancer in the not too distant future.

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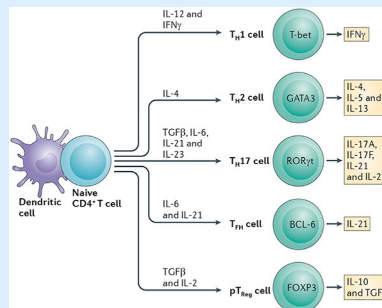
Box 1 |

CD4+ T_H subsets

When a naive CD4+ T cell gets activated by an antigen-presenting cell (APC) in the periphery, it differentiates into one of several T helper (T_H) cell subsets to become a T_H1, T_H2, T_H17, T follicular helper (T_{FH}) or peripherally derived regulatory T (pT_{Reg}) cell. The decision-making process is instructed by the cytokines that are present in the environment during the activation process. Each T_H cell subset is distinguished by a specialized gene expression programme, which is under the control of a lineage-defining transcription factor. The lineage-defining transcription factors are T-bet for the T_H1 cell lineage, GATA-binding protein 3 (GATA3) for the T_H2 cell lineage, retinoic acid receptor-related orphan receptor-γt (RORγt) for the T_H17 cell lineage, B cell lymphoma-6 (BCL-6) for T_{FH} cells and forkhead box P3 (FOXP3) for pT_{Reg} cells (see the figure). CD4+ T_H cell subsets are defined by the signature cytokines that they express, their distinct homing properties and their specialized effector functions, which make them best equipped to target a particular class of pathogen. Thus, T_H1 cells produce interferon-γ (IFNγ) and are particularly effective at activating macrophage microbicidal mechanisms against intracellular bacteria, protozoa and viruses. By contrast, T_H2 cells secrete interleukin-4 (IL-4), IL-5 and IL-13, which are required for the expulsion of extracellular parasites. T_H17 cells have evolved to protect mucosal surfaces against extracellular bacteria and fungi through the production of IL-17A, IL-17F, IL-21 and IL-22, whereas T_{FH} cells support antiviral humoral immunity by promoting antibody class-switching and affinity maturation in germinal centre B cells. In contrast to these protective functions of T_H cells, inappropriate or overwhelming activation of T_H cells can lead to the development of allergies and autoimmune diseases. The most important function of pT_{Reg} cells is to prevent inflammation-mediated tissue injury through the local secretion of suppressive cytokines such as IL-10, IL-35 and transforming growth factor-β (TGFβ), or through the cell–cell contact-mediated inhibition of CD4+ effector cell proliferation.



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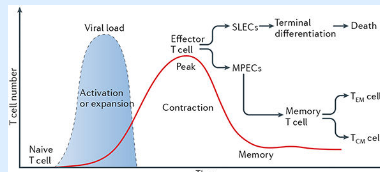
Box 2 |

$CD8^+$ T cell memory

When a naive $CD8^+$ T cell encounters an antigen, it undergoes a differentiation programme that can be divided into three main developmental stages: clonal expansion and differentiation, contraction, and memory formation. The differentiation process that occurs at the peak of the response generates quite a heterogeneous population of $CD8^+$ T cells, which have different fates and memory cell developmental potential. Short-lived effector cells (SLECs) are terminally differentiated effector cells that express low levels of interleukin-7 receptor-α (IL-7Rα) and high levels of killer cell lectin-like receptor subfamily G member 1 (KLRG1) and IL-2Rα. By contrast, memory precursor effector cells (MPECs), which give rise to long-lived, self-renewing memory $CD8^+$ T cells, express high levels of IL-7Rα and low levels of KLRG1 and IL-2Rα. After an infection is cleared, the vast majority of antigen-specific $CD8^+$ T cells that are generated during clonal expansion die. The cells that survive the contraction phase, the MPECs, go on to generate a reservoir of memory $CD8^+$ T cells. Memory T cells can be either effector memory (T_{EM}) cells or central memory (T_{CM}) cells. T_{EM} cells ($KLRG1^{hi}IL\text{-}7R\alpha^{hi}CD62L^{low}CCR7^{low}$) monitor and guard peripheral tissues from reinfection by providing an immediate effector response, whereas T_{CM} cells ($KLRG1^{low}$ $IL\text{-}7R\alpha^{hi}CD62L^{hi}CCR7^{hi}$) reside in lymphoid tissues and give rise to a large number of secondary effector cells during recall responses.



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Experimental autoimmune encephalomyelitis

(EAE). A mouse model of multiple sclerosis. It can be induced by the active immunization of mice with central nervous system (CNS)-derived antigens emulsified in complete Freund's adjuvant (in the case of active EAE) or by adoptive transfer of activated, T cell receptor (TCR)-transgenic $CD4^+$ T cells, in which the TCRs recognize a CNS-derived antigen (in the case of passive EAE).

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Scurfy mice

Mice with a spontaneous mutation in forkhead box P3 (*Foxp3*), which leads to a rapidly fatal lymphoproliferative disease, causing death by ~4 weeks of age.

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Mamammalian target of rapamycin

(mTOR). A serine/threonine protein kinase that regulates cell metabolism, proliferation, survival, protein synthesis and transcription. It is activated by T cell receptor signalling and is sustained by inflammatory cytokines such as interleukin-12.

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AMP-activated protein kinase

(AMPK). A negative regulator of mammalian target of rapamycin (mTOR) kinase activity. It is activated in response to cellular stress and ATP deprivation. It can be also activated by the pharmacological agent metformin.

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Affinity maturation

The process by which B cells produce antibodies that have an increased affinity for antigens during an immune response.

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Class-switch recombination

(CSR). The process by which B cells produce antibodies of different isotypes without changing the antigen specificity of the variable region.

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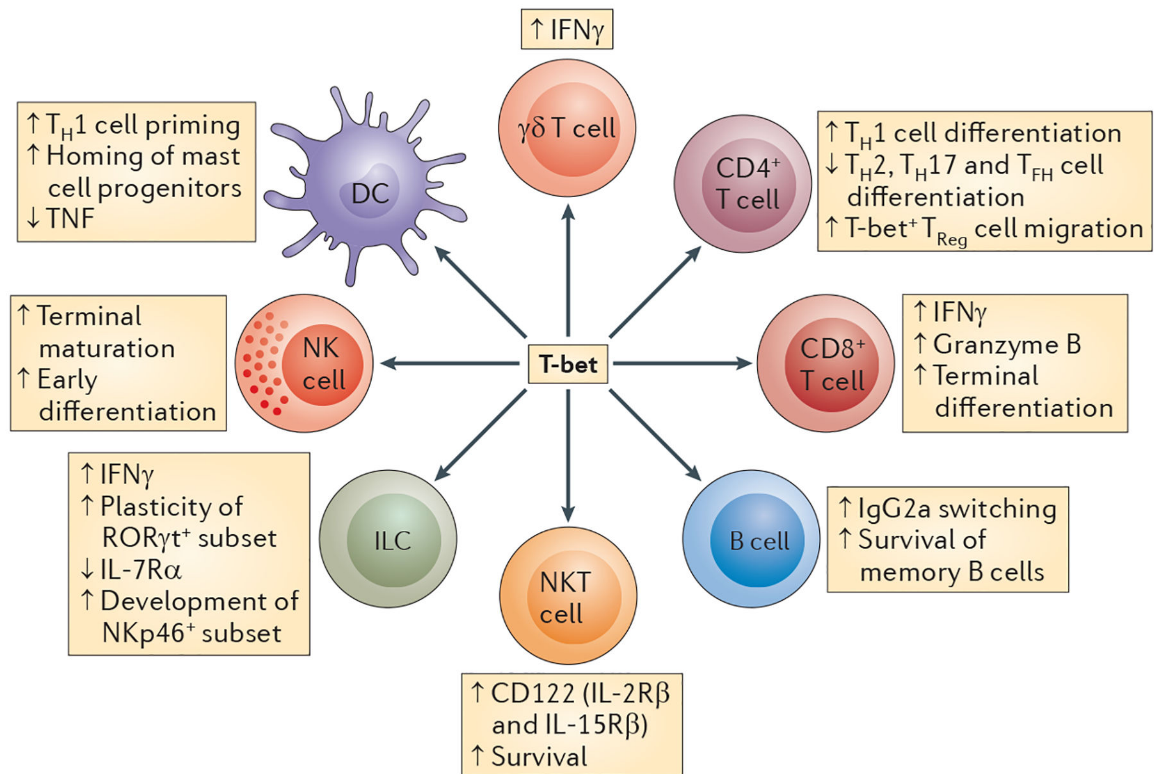

Figure 1 |. Expression and functions of T-bet in immune cells.

T-bet is expressed in multiple cells of the innate and adaptive immune system. Its expression is required for the survival, development and proper functions of immune cells. In the innate immune system, T-bet is expressed in dendritic cells (DCs), natural killer (NK) cells, natural killer T (NKT) cells and innate lymphoid cells (ILCs). In the adaptive immune system, T-bet is expressed in $CD4^+$ and $CD8^+$ T effector cells, B cells, γδ T cells and a subset of regulatory T (T_{Reg}) cells. IFNγ, interferon-γ; IL-7Rα, interleukin-7 receptor-α; NKp46, NK cell p46-related protein; RORγt, retinoic acid receptor-related orphan receptor-γt; T_{FH}, T follicular helper; T_H, T helper; TNF, tumour necrosis factor.

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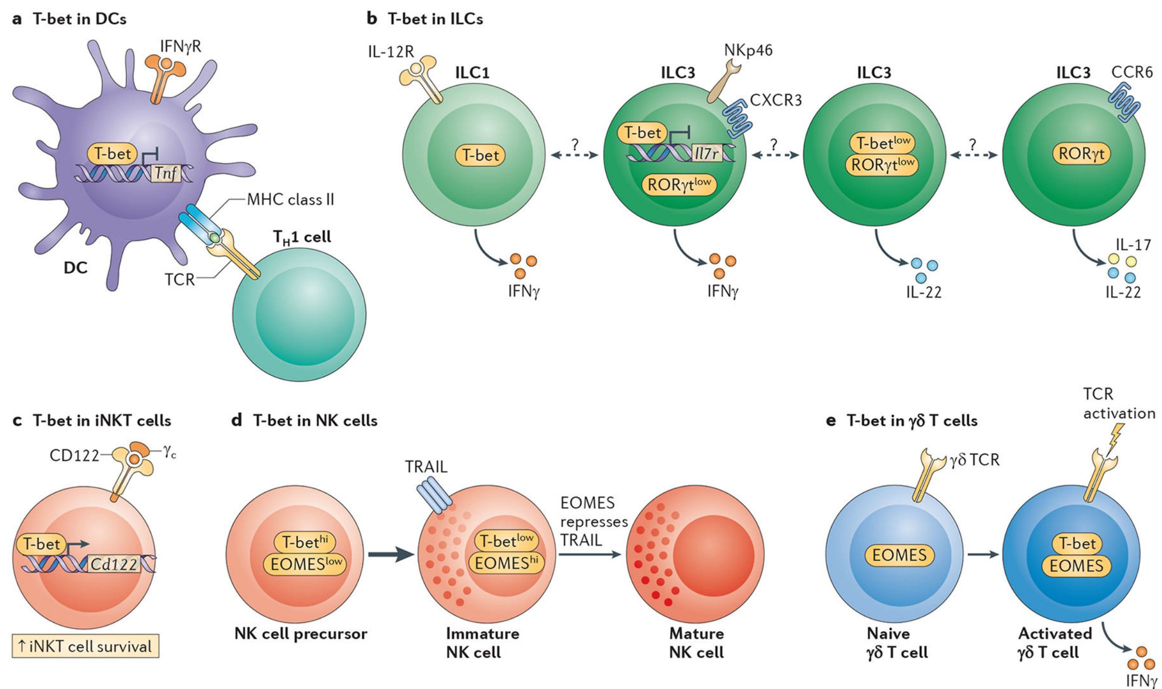

Figure 2 |. T-bet in innate immune cells.

a | T-bet expression in dendritic cells (DCs) is required to properly prime T helper 1 (T_H1) cells. T-bet suppresses tumour necrosis factor (TNF) production in colonic DCs and this is required for the maintenance of mucosal homeostasis. **b** | T-bet is expressed in the innate lymphoid cell 1 (ILC1) subset, which is characterized by the sole production of interferon-γ (IFNγ). ILC3s are dependent on the expression of retinoic acid receptor-related orphan receptor-γt (RORγt) and can be subdivided into CC-chemokine receptor 6 $(CCR6)^+$ and $CCR6^-$ subsets. $CCR6^-$ ILC3s express T-bet. High T-bet expression in these cells is associated with low RORγt and interleukin-7 receptor (IL-7R) expression, and high expression of NK cell p46-related protein (NKp46), CXC-chemokine receptor 3 (CXCR3) and IFNγ. Low T-bet expression in the $CCR6^-$ ILC3 subset is associated with IL-22 expression and low or no expression of NKp46 and IFNγ. $CCR6^+$ ILC3s do not express T-bet but express IL-17 and IL-22. The lineage inter-relationships of these different subpopulations are incompletely defined. **c** | T-bet expression in invariant natural killer T (iNKT) cells promotes their survival through the regulation of CD122 (also known as IL-15Rβ) expression. **d** | T-bet and eomesodermin (EOMES) regulate the maturation process of NK cells in a coordinated fashion. T-bet is expressed at an immature stage of differentiation that is characterized by TNF-related apoptosis-inducing ligand (TRAIL) expression. EOMES expression is required to silence TRAIL expression and to complete the maturation process of NK cells. **e** | T-bet is not expressed in naive γδ T cells. Its expression is rapidly induced following T cell receptor (TCR) engagement. Together with EOMES, T-bet regulates IFNγ production in mature γδ T cells. Dashed line indicates that the developmental relationship between these cells is unclear. γ_c, common cytokine receptor γ-chain; IFNγR, IFNγ receptor.

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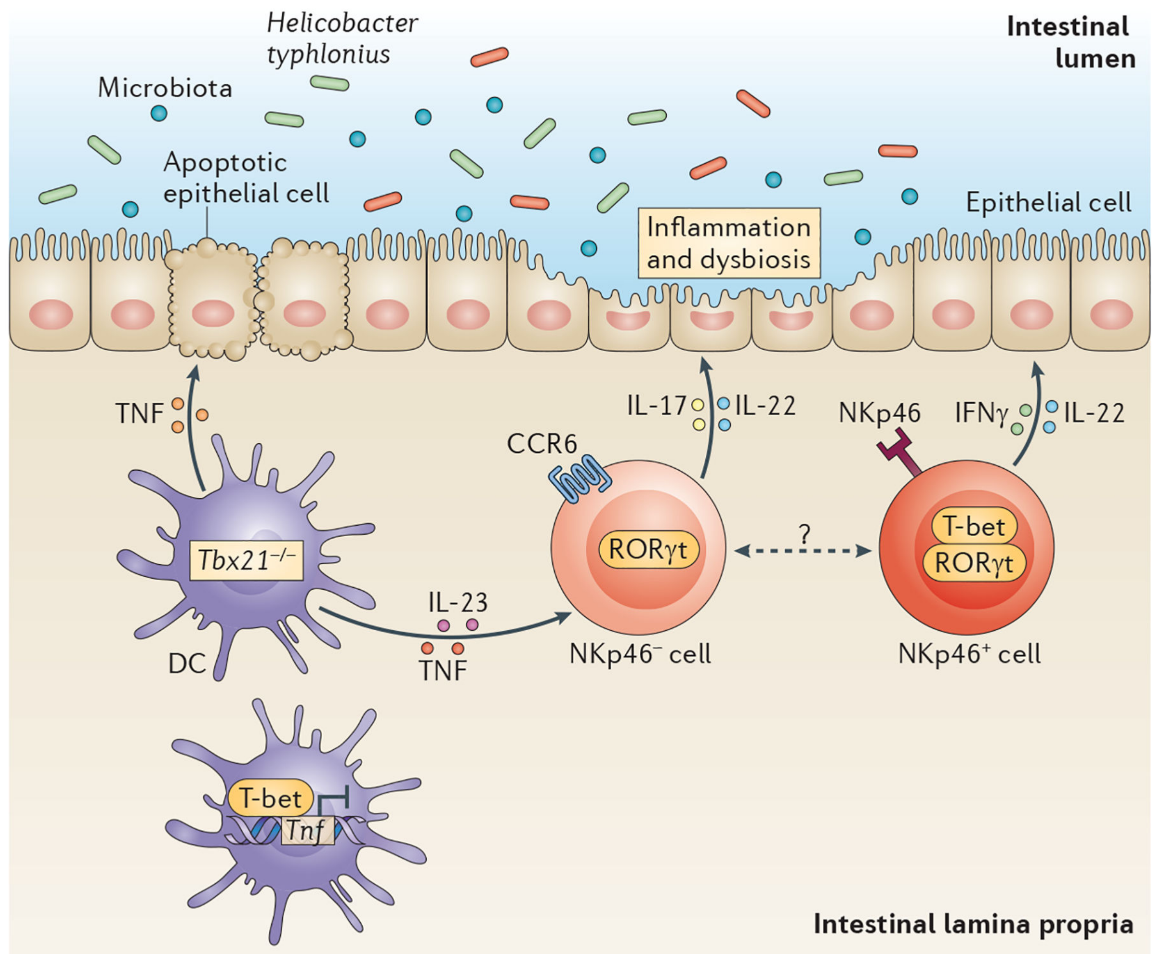

Figure 3 |. T-bet expression in ILCs and DCs in the intestines.

Innate lymphoid cells (ILCs) and T-bet-expressing dendritic cells (DCs) interact in the colonic lamina propria to maintain epithelial integrity. T-bet (encoded by *Tbx21*) represses tumour necrosis factor (TNF) expression in colonic DCs. In the absence of T-bet, TNF from DCs causes colonic epithelial cell apoptosis in the context of the pathobiont, *Helicobacter typhlonius*. TNF from DCs cooperates with interleukin-23 (IL-23) to induce interferon-γ (IFNγ) and IL-17 expression from ILCs. T-bet expression in ILCs regulates the plasticity of cytokine responses by repressing IL-17 and by inducing IFNγ production. This pathology creates an inflammatory milieu that generates a dysbiotic microbiota that is capable of transmitting colonic inflammation to wild-type mice. Dashed line indicates that the developmental relationship between these ILC subsets is unclear. CCR6, CC-chemokine receptor 6; NKp46, NK cell p46-related protein; RORγt, retinoic acid receptor-related orphan receptor-γt.

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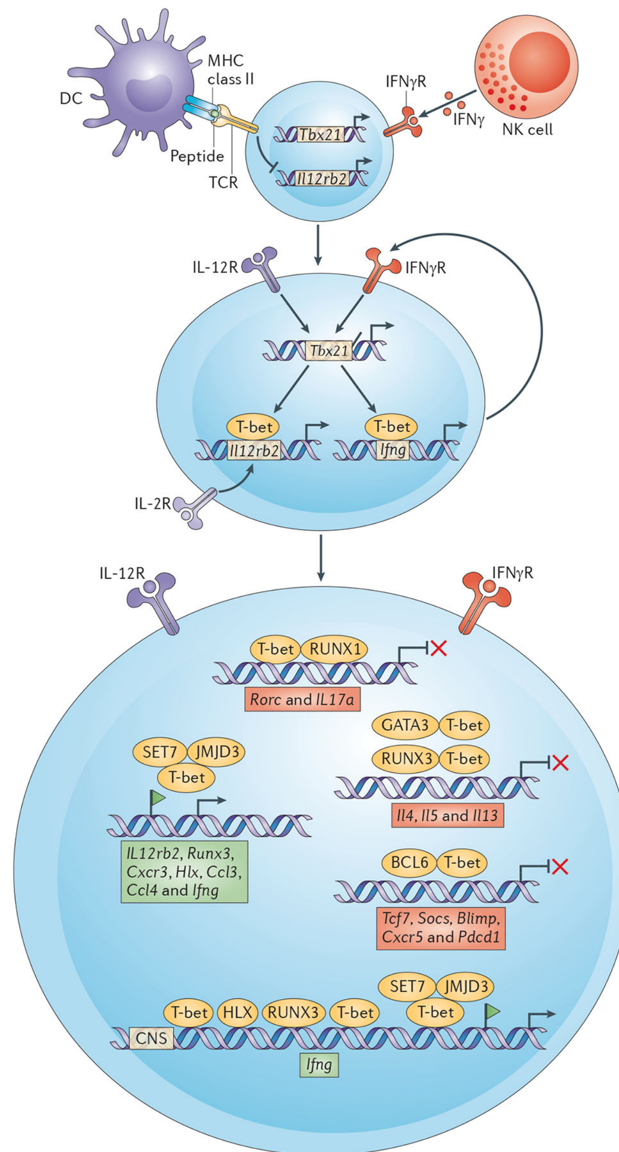

Figure 4 |. Role of T-bet in T_H1 cell differentiation.

T cell receptor (TCR) and interferon-γ (IFNγ)-mediated signalling induces the first wave of T-bet (encoded by *Tbx21*) expression in an interleukin-12 (IL-12)independent manner, as signalling through TCR inhibits the expression of IL-12 receptor-β2 (*Il12rb2)* subunit. Cessation of TCR stimulation and IL-2 signalling induces expression of the IL-12Rβ2 subunit, which thus enables IL-12 signalling. IL-12-mediated activation of signal transducer and activator of transcription 4 (STAT4) prompts the second wave of T-bet expression. IFNγ produced by T helper 1 (T_H1) cells functions in a feedforward loop, which further increases T-bet expression in T_H1 cells. T-bet recruits enzymes (jumonji domain-containing protein 3 (JMJD3) and SET domain-containing protein 7 (SET7; also known as SET9)), which generate chromatin modifications that are associated with gene activation. T-bet-mediated transactivation of the *Ifng* gene is enhanced by H2.0-like homeobox protein 1 (HLX) and runt-related transcription factor 3 (RUNX3) transcription factors, both of which are encoded

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by T-bet target genes. T-bet facilitates CCCTC-binding factor (CTCF) binding and chromatin looping at the *Ifng* locus, which is required for optimal IFNγ expression in T_H1 cells. T-bet induces chemokines, chemokine receptors and other effector molecules that are required for proper migration and function of T_H1 cells. Sequestration of GATA-binding protein 3 (GATA3) by T-bet prevents the GATA3-mediated activation of the *Il4–Il5–Il13* locus, and binding of a T-bet–RUNX3 complex to the *Il4* silencer (DNase I hypersensitivity site 4 (HS4)) prevents the expression of *Il4* in T_H precursor cells. T-bet also binds to the *Gata3* locus, and its binding is associated with the presence of repressive epigenetic marks. RUNX1 enhances the expression of retinoic acid receptor-related orphan receptor-γt (RORγt; encoded by *Rorc*) in T_H17 cells and it functions as a RORγt-specific co-activator of the *Il17a* gene. T-bet binding to RUNX1 prevents *Rorc* and *Il17a* expression in T_H precursor cells. Furthermore, T-bet together with STAT4, silences the *Rorc* locus. T-bet interacts with B cell lymphoma 6 (BCL-6) and recruits it to the promoters of genes that are repressed by T-bet in T_H1 cells (for example, T cell factor (*Tcf7)* and suppressor of cytokine signalling (*Socs*)). By sequestering BCL-6 away from BCL-6 target genes (for example B lymphocyte-induced maturation protein (*Blimp*), CXC-chemokine receptor 5 (*Cxcr5*) and programmed cell death 1 (*Pdcd1*), T-bet effectively blocks T follicular helper (T_{FH}) cell lineage commitment. *Ccl*, CC-chemokine ligand; CNS, conserved non-coding sequence; DC, dendritic cell; IFNγR, IFNγ receptor; NK, natural killer.

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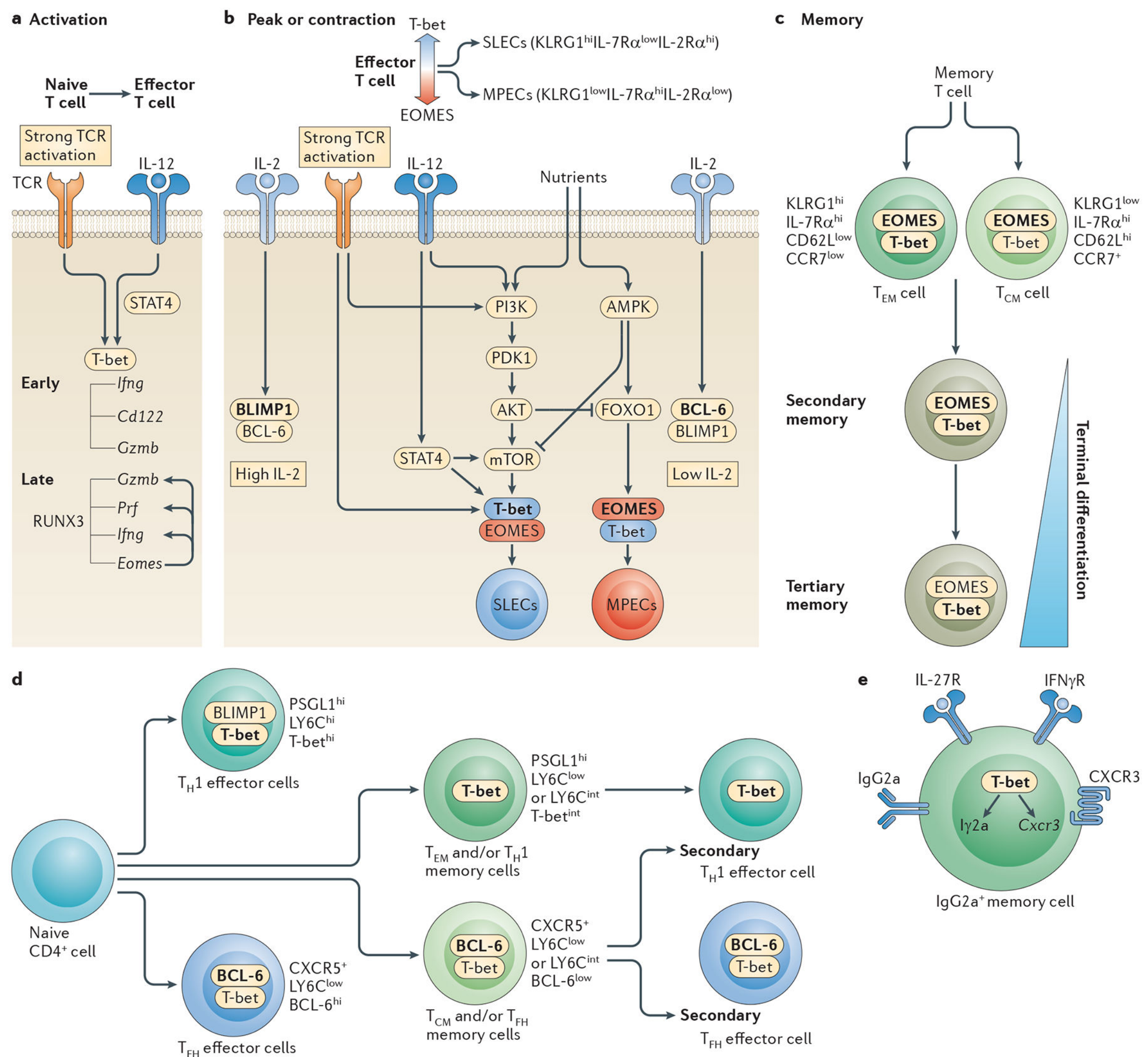

Figure 5 |. T-bet in the generation of B cell and T cell memory.

a | In CD8$^+$ T cells, T-bet (encoded by *Tbx21*) expression is induced by T cell receptor (TCR) and interleukin-12 receptor (IL-12R) signalling, whereas eomesodermin (EOMES) is upregulated later in a runt-related transcription factor 3 (RUNX3)-dependent manner. RUNX3, T-bet and EOMES regulate the expression of interferon-γ (IFNγ), granzyme B (encoded by *Gzmb*), perforin (encoded by *Prf*) and CD122 in CD8$^+$ effector cells. **b** | Strong TCR and IL-12R signalling promotes the terminal differentiation of short-lived effector cells (SLECs) by enhancing and maintaining the activity of the kinase mammalian target of rapamycin (mTOR) downstream of signal transducer and activator of transcription 4 (STAT4). mTOR simultaneously induces T-bet and represses EOMES expression through inhibition of forkhead box O1 (FOXO1). The IL-2R–STAT5 pathway induces the expression of B lymphocyte-induced maturation protein 1 (BLIMP1), inhibiting the expression of B cell lymphoma 6 (BCL-6), which is a transcriptional repressor of the *Tbx21* gene. Inhibition of mTOR activity by rapamycin or by activators of AMP-activated protein kinase (AMPK), such as metformin, supports the development of memory precursor effector cells (MPECs). Similarly, enhanced MPEC formation is observed in low IL-2 and IL-12 conditions. **c** |

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EOMES expression increases and T-bet levels decline in memory $CD8^+$ T cells. Minimal levels of T-bet are maintained for proper expression of CD122, which promotes the homeostatic proliferation of memory cells. T-bet expression is associated with a more differentiated phenotype of effector memory T (T_{EM}) cells and secondary and tertiary memory cells. **d** | P-selectin glycoprotein ligand 1 $(PSGL1)^{hi}$ $LY6C^{hi}$T-bet^{hi} cells are terminally differentiated T helper 1 (T_H1) effector cells. $PSGL1^{hi}LY6C^{low}$T-bet^{int} cells are less differentiated, are longer lived and give rise to T_H1 effector cells after re-challenge. T-bet is expressed at very low levels in T follicular helper (T_{FH}) and T_{FH} memory cells, as a result of BCL-6 expression. **e** | T-bet is a selective inducer of IFNγ-mediated IgG2a class-switching in B cells. T-bet is required for the survival of $IgG2a^+CD38^{hi}$ memory B cells and it promotes the migration of $IgG2a^+$ B cells to inflammatory foci through the transcriptional regulation of CXC-chemokine receptor 3 (CXCR3) expression. CCR7; CC-chemokine receptor 7; IFNγR, IFNγ receptor; KLRG1; killer cell lectin-like receptor subfamily G member 1; PDK1, 3-phosphoinositide-dependent protein kinase 1; PI3K, phosphoinositide 3-kinase; T_{CM}, central memory.