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The evolving role of T-bet in resistance to infection

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Abstract

The identification of T-bet as a key transcription factor associated with the development of IFN γ -producing CD4⁺ T cells predicted a crucial role for T-bet in cell-mediated immunity and in resistance to many intracellular infections. This idea was reinforced by initial reports showing that T-bet-deficient mice were more susceptible to pathogens that survived within the lysosomal system of macrophages. However, subsequent studies revealed IFN γ -dependent, T-bet-independent pathways of resistance to diverse classes of microorganisms that occupy other intracellular niches. Consequently, a more complex picture has emerged of how T-bet and the related transcription factor eomesodermin (EOMES) coordinate many facets of the immune response to bona fide pathogens as well as commensals. This article provides an overview of the discovery and evolutionary relationship between T-bet and EOMES and highlights the studies that have uncovered broader functions of T-bet in innate and adaptive immunity and in the development of the effector and memory T cell populations that mediate long-term resistance to infection.

A major theme in immunology for the past 50 years has been the study of the functional and phenotypic diversity of T cell subsets and their role in protective or pathological responses. T cells as distinct thymus-derived lymphocytes were first described, albeit controversially, in the 1960s^{1,2} and within a few years were accepted as a population distinct from antibody-producing lymphocytes³. These lymphocytes could be further divided into those that helped B cells (CD4⁺ T cells) and those that were cytotoxic (CD8⁺ T cells)⁴. By the 1980s it was appreciated that different subsets of CD4⁺ T helper (T_H) cells predominantly produced either IFN γ (in the case of T_H1 cells) or the combination of IL-4 and IL-5 (in the case of T_H2 cells)⁵. The distinct functions of these subsets were highlighted by studies in which CD4⁺ T cell production of IFN γ was required to activate the antimicrobial activities of

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macrophages that are central to resistance to intracellular infections, whereas CD4⁺ T cells that produce IL-4 promoted resistance to helminth parasites⁶. Since then, as predicted by Mosmann and Coffman, additional subsets of functionally diverse T cells have been described that include regulatory T (T_{reg}) cells⁷, T_H17 cells⁸ and T follicular helper (T_{FH}) cells⁹.

While distinct T cell subsets could be associated with resistance to different classes of pathogens, there was also the realization that aberrant T cell activity contributes to inflammatory and autoimmune conditions^{10–13}. In order to be able to manipulate the immune response to better manage immune-mediated conditions, or promote T cell responses in the context of infection or vaccination, it was necessary to understand the molecular mechanisms that control T cell differentiation. In the 1990s, the ability of the transcription factors MAF and GATA3 to direct the generation of T_H2 cell responses was described^{14,15}, and in 2000, the transcription factor T-bet, encoded by the gene *Tbx21*, was identified as a key regulator of T_H1 cell responses¹⁶. Over the next decade, there were numerous examples of transcription factors that could be linked to individual T cell subsets: forkhead box protein P3 (FOXP3) is essential for the development and function of T_{reg} cells^{17,18}, T_H17 cells require RORγt¹⁹, and BCL-6 directs the generation of T_{FH} cells^{20–22}.

The ability to link different transcription factors with specific T cell subsets provided a framework to understand the mechanisms that underlie the differentiation and plasticity of T cells and reinforced the idea that individual transcription factors (such as T-bet, GATA3, signal transducer and activator of transcription 4 (STAT4) and STAT6) could act as ‘master regulators’ of distinct CD4⁺ T cell fates. This term was originally used to describe transcription factors that regulate developmental lineages²³, but many of these so-called master regulators that are associated with discrete T cell subsets are often co-expressed and are not restricted to CD4⁺ T cells. In addition, variations in the signals propagated downstream of the T cell receptor (TCR) or cytokine receptors influence many aspects of T cell fate, and it is the ability to integrate these diverse inputs that dictates T cell function. It has been proposed that ‘lineage specifying’ is therefore a more precise term that reflects the complex roles of these transcription factors in T cell biology²⁴. In this Review, we highlight the events that led to the discovery of T-bet and eomesodermin (EOMES) and how models of infectious disease have helped to understand the broader functions of T-bet in multiple lymphocyte populations. In addition, synthesis of the current literature highlights the idea that microbial context is a key determinant that helps to explain the threshold for when T-bet becomes essential to control certain infections. Finally, we emphasize the possible contributions of T-bet and variation in its levels of expression to the development of the effector and memory T cell populations that mediate long-term resistance to infection.

The T-box family of transcription factors

The discovery of the T-box family of transcription factors was based on a series of studies in mice to identify the genetic cause of a defect in tail development; a phenotype named brachyury, or T for tail²⁵. Mapping of this locus identified the *T* gene and showed that the brachyury protein contained a conserved DNA-binding motif, the T-box^{26,27}. T-box proteins also interact with other transcription factors, such as homeodomain (encoded by *Hox* genes),

GATA zinc-finger and LIM domain proteins²⁸. The T-box genes are present in all metazoans, make up a large family and, consistent with their initial discovery, are involved in many embryonic developmental processes²⁹. Interestingly, diversification of the TBR1 subfamily of T-box genes in ancient meta zoans coincided with the emergence of adaptive immunity and whole genome duplication events^{29,30}. Thus, amphioxus, a common ancestor placed between vertebrates and invertebrates³¹, lacks an adaptive immune system but does have lymphocyte-like cells³². This marine chordate has a single gene in the Tbr1 subfamily, *AmphiEomes/Tbr1/Tbx21*, which is involved in specification of the endoderm³³. Lampreys share a more recent ancestor with higher vertebrates and have a variable lymphocyte receptor (VLR)-based adaptive immune system³⁴. These jawless vertebrates have two T-box paralogues, *tbr1b* and *tbx21*, but their function in this organism is unclear^{33,35}. The recombination-activating gene (RAG) required for TCR-based and B cell receptor (BCR)-based immunity, as well as the gene encoding IFN γ (*IFNG*), first appeared in the gnathostomes, and this provided the basis for the evolution of novel mechanisms of adaptive immunity to microorganisms^{30,36}. These jawed fish have three related genes, *Tbx21*, *Eomes* and *Tbr1*, that are also present in higher vertebrates²⁹, where *Eomes* functions in mesoderm development and trophoblast formation and is essential for development³⁷. By contrast, the observation that *Tbx21*^{-/-} mice are viable suggests that T-bet has lost developmental functions associated with the other TBR1 subfamily members and may have gained immune functions distinct from those of EOMES and TBR1.

T-bet and EOMES in T cell function

The association of the T_H1 cell and T_H2 cell subsets with resistance to different classes of pathogens implied that there would be a molecular basis that directed differentiation of naive T cells into alternative fates. In the search for a T_H1 cell-specific molecular switch, Glimcher and colleagues^{16,38} identified a T-box transcription factor expressed in CD4⁺ T cells that is required for IFN γ production and resistance to the intracellular parasite *Leishmania major*. Because of its homology with other T-box-containing genes, this novel protein was named T-box expressed in T cells (T-bet). The observation that production of IFN γ was T-bet-independent in natural killer (NK) cells and CD8⁺ T cells indicated that another factor could mediate these events³⁸. When Reiner and colleagues³⁹ used degenerate primers to search for other T-box family members present in activated CD8⁺ T cells, they identified EOMES and showed that, like T-bet, it promoted IFN γ expression. This led the authors to propose that EOMES can “complement the role of T-bet in governing cellular immunity by providing redundancy and, quite likely, cooperativity in the induction of effector genes of T cells and NK cells”. It is now apparent that T-bet and EOMES have different mechanisms of induction, unique functions and possibly even antagonistic activities, perhaps mediated through competition for DNA-binding sites or transcriptional modifiers.

The molecular basis for the activating effects of T-bet and how it interacts with other transcription factors are reviewed in detail elsewhere, and molecular studies on this topic continue to provide insights into the ability of T-bet to recognize remote distal regulatory sequences and coordinate the expression of multiple genes^{40,41}. Indeed, the effects of T-bet extend beyond *trans*-activation of the *IFNG* locus, and T-bet inhibits alternative CD4⁺ T cell

differentiation fates, including T_H2 cell and T_H17 cell development^{16,38,42}. This can be explained by interactions between T-bet and other proteins that result in the induction or inhibition of key factors in T cell differentiation. For instance, T-bet interacts with GATA3 through a tyrosine kinase-mediated interaction, which prevents GATA3 from binding to the *IL5* promoter⁴³. T-bet also cooperates with runt-related transcription factor 3 (RUNX3) to activate the *Ifng* gene and repress the *Il4* gene⁴⁴, while its ability to sequester RUNX1 prevents activation of *Rorc*, and thus limits T_H17 cells⁴⁵. These examples illustrate the interactions between multiple transcription factors that enable T-bet to promote T_H1 cell responses, but it is important to recognize that T-bet was originally identified as a repressor of IL-2 production¹⁶. These inhibitory properties are also apparent during the late stages of T_H1 cell activation, when T-bet recruits BCL-6 to the *IFNG* locus to limit IFN γ production⁴⁶. Furthermore, in differentiated T_H1 cells, T-bet inhibits autocrine type I interferon signalling⁴⁷ and is also associated with repression of programmed cell death 1 (PD1) expression⁴⁸ but with upregulation of T cell immunoglobulin and mucin domain-containing 3 (TIM3; also known as HAVCR2)⁴⁹. Together, these studies highlight that T-bet is not simply a transactivator of the *IFNG* locus but can act as part of a negative-feedback loop to limit T cell responses (discussed below).

Regulation of T-bet expression and function

Induction and activity of T-bet.

In the initial report that identified T-bet, it was recognized that naive CD4⁺ and CD8⁺ T cells are T-bet⁻ and that TCR activation induces T-bet expression¹⁶. Subsequent studies have highlighted that the initial events that lead to a productive T_H1 cell response are characterized by two distinct waves of T-bet expression^{50,51}. Thus, signals through the TCR and IFN γ R (via STAT1) synergize to induce early synthesis of T-bet (FIG. 1). This model is complicated by reports that, for CD4⁺ T cells, the initial IFN γ response may be EOMES-dependent but T-bet-independent⁵². Regardless, after initial T cell activation, local environmental signals associated with infection such as IL-12 (via STAT4 activation) induce a sustained second wave of T-bet expression that stabilizes the T_H1 cell phenotype^{50,53} (FIG. 1). While in vitro systems illustrate the impact of IL-12 on T-bet expression, in vivo exposure to IL-12 is likely to be heterogeneous and has been linked to graded levels of T-bet expression that influence T cell differentiation⁵⁴. The studies described above have focused on conventional $\alpha\beta$ T cells, but the rules that govern T-bet activity are also relevant to other lymphocyte populations that produce IFN γ . Indeed, TCR signals lead to the induction of T-bet in $\gamma\delta$ T cells⁵⁵, and T-bet is also present in innate lymphoid cells (ILCs), NK cells and natural killer T (NKT) cells^{16,38,56–60}.

The ability of IFN γ and IL-12 to reinforce induction of T-bet is primarily linked to a commitment to a T_H1 cell phenotype, but other cytokines such as type I interferons, IL-21 and IL-27 also promote T-bet expression^{61–63} (FIG. 1). It is unclear whether these different cytokines result in any unique effects on T-bet through the levels of T-bet that are induced, its activity or the cofactors that modulate T-bet activity. For example, it seems likely that the quantity and quality of inflammation would influence the expression of additional

transcription factors and/or binding partners that associate with T-bet, ultimately shaping the levels and activities of T-bet in a T cell-intrinsic fashion.

The induction of T-bet represents a key early event in T cell activation, and it is tempting to conflate expression with transcriptional activity. However, there are numerous factors, such as metabolic status and recruitment of chromatin remodelling complexes⁶⁴ or phosphorylation and ubiquitylation⁶⁵, that influence T-bet activity. Indeed, in activated T cells, the subcellular localization of T-bet in the cytoplasm versus the nucleus is variable and has been linked to effector status, cell cycle and protein stability^{66–69}. The cytoplasmic localization of T-bet may reflect a pre-formed pool present in quiescent cells that is mobilized in response to TCR signalling and which would presumably enable enhanced T_H1 cell effector responses. Alternatively, in T cells, the asymmetric apportioning of the proteasome during division does result in differential degradation of T-bet, a process that would require the presence of T-bet in a cytoplasmic compartment⁶⁷. Thus, the presence of cytoplasmic T-bet may provide an indicator of cells that are poised to give rise to progeny with different functions that are governed by the levels of this transcription factor.

T-bet coordinates T cell trafficking.

While IFN γ production is a major characteristic of T_H1 cell activity, there are other proteins, including STAT1, IL-12R β 2, CC-chemokine ligand 3 (CCL3) and CXC-chemokine receptor 3 (CXCR3), that have important roles in the immunobiology of T_H1 cells, and, consonant with its role as a lineage-specifying transcription factor, T-bet can bind to the promoter region of the genes encoding these proteins^{70–73}. There is also evidence that T-bet may influence the T cell–dendritic cell (DC) interactions that are essential for T cell priming. For example, when CD8⁺ T cells are stimulated in vitro with peptide-loaded antigen-presenting cells (APCs), T-bet is required for the acquisition of an effector CD8⁺ T cell lymphoid structures, T cells phenotype (CD62L^{lo}LY6C^{hi}). By contrast, when T cells are activated through the TCR in a system that does not require DC–T cell contact, the generation of effector CD8⁺ T cells is T-bet-independent⁷⁴. One interpretation of these findings is that T-bet is necessary for optimal cellular interactions during T cell priming, and there are several points where this may be relevant. Thus, activated CD8⁺ T cells produce chemokines that create their own optimal priming microenvironment through recruitment of DCs to the site of initial antigen recognition⁷⁵. There is also evidence that a secondary phase CD8⁺ T cell priming is characterized by synaptic interactions between CD8⁺ T cells during a critical differentiation period⁷⁶. Disruption of these events results in an increased ratio of memory (killer cell lectin-like receptor subfamily G member 1 (KLRG1)^{lo}IL-7R^{hi}) to the IFN γ -induced chemokine CXC-chemokine ligand effector cell populations (KLRG1^{hi}IL-7R^{lo}), a phenotype that is also apparent in some infections when T-bet is absent^{54,77,78}. The T-bet target CXCR3 also has a role in CD8⁺ T cell positioning relative to antigen and inflammatory cytokines in secondary lymphoid organs and affects the balance of effector and memory T cell formation⁷⁹. Because the levels of T-bet are in flux during T cell priming, there are many points at which this variation could act in a cell-intrinsic fashion but also in *trans* through promotion of T cell–DC and/or T cell–T cell interactions to influence the magnitude and quality of T cell responses.

After activation and differentiation in secondary lymphoid structures, T cells need to enter the circulation and be able to access sites of inflammation to mediate effector functions or, in the case of some forms of memory, establish tissue residency. The first report that linked T-bet to this process identified platelet selectin glycoprotein ligand 1 (PSGL1), which binds to E-selectin and L-selectin on endothelial cells, and CXCR3 as T-bet targets required on effector T cells for optimal trafficking to the peritoneum⁸⁰ (see FIG. 2a). It is relevant to note that the activation of T-bet drives expression of enzymes, including tyrosyl protein sulfotransferase 2 (TPST2), that alter the PSGL1 post-translational modifications required for binding activity⁸⁰. Indeed, PSGL1 is linked to the ability of TH1 cells to access inflamed skin^{81,82} as well as to their ability to control *Salmonella* infection⁸³. CXCR3, which binds to the IFN γ -induced chemokine CXC-chemokine ligand 10 (CXCL10) that is produced at sites of TH1 cell inflammation, is important for resistance to several intracellular infections that include *Toxoplasma gondii* and respiratory syncytial virus^{84,85}. Furthermore, during infection with lymphocytic choriomeningitis virus (LCMV) or *T. gondii*, the absence of T-bet is associated with reduced effector T cell expression of CXCR3 as well as lower LY6C and KLRG1 expression^{54,77,86,87}. While expression of these surface proteins is frequently used to identify T cell effector status, LY6C and the ligands for KLRG1 (namely, E-cadherin, N-cadherin and R-cadherin) have been implicated in the processes of cellular migration^{88–90}. This link of T-bet to lymphocyte trafficking is not restricted to effector responses: T_{reg} cells that express T-bet and CXCR3 can limit the inflammatory responses in the context of several intracellular infections^{91–93}. Indeed, lineage-tracing experiments indicated that, during infection with *Listeria monocytogenes*, a subset of T_{reg} cells express T-bet stably, and this enables colocalization with effector responses at sites of IFN γ -mediated inflammation⁹⁴ (see FIG. 2b). Thus, T-bet expression coordinates trafficking and behaviour of effector and regulatory T cells associated with TH1 cell-like responses during infection, but FOXP3 selectively opposes the development of a full TH1 cell programme. This interplay between T-bet and FOXP3 is a prominent example of how the effects of T-bet are modified in different T cell populations depending on the cellular environment and co-expression of additional transcription factors.

Perhaps reflecting its evolutionary link to developmental processes, several innate-like lymphocyte populations require T-bet for their maturation and activity^{58,95–98}, as well as for their trafficking. For example, T-bet regulates sphingosine-1-phosphate receptor 5 (S1P5), which promotes lymphocyte egress from lymphoid tissues⁹⁹, and both S1P5-deficient mice and T-bet-deficient mice have increased numbers of NK cells in their bone marrow^{96,100}. S1P5 is also required for homeostatic NK cell positioning within the lymph node medulla, and, after immune challenge, S1P5 promotes a rapid IFN γ response¹⁰¹. Nevertheless, for these innate populations, less is known about the signals that govern T-bet expression, and, while cytokines are likely candidates, the impact of other activating receptors (such as FcRs, LY49, NKp46 and NKG2D) expressed by these cells remains to be explored.

Role of T-bet in infection

T-bet-mediated resistance.

As noted earlier, the IL-12–IFN γ axis is an important pathway required for resistance to many viruses, bacteria and parasites, and the primary example that illustrates the importance of T-bet in CD4⁺ T cells is in mice infected with *L. major* (see TABLE 1). This obligate intracellular protozoan infects macrophages, and control is dependent on an IL-12-driven T_H1 cell response, whereas susceptibility is associated with a T_H2 cell response^{102,103}. While C57Bl/6 mice normally control this infection, the absence of T-bet in this mouse strain leads to reduced IFN γ , increased IL-4 and IL-5 and disease progression³⁸. In other words, the loss of T-bet does not prevent the emergence of an *L. major*-specific CD4⁺ T cell response, but a non-protective T_H2 cell response dominates. This study suggested that T-bet overrides a default pathway that leads to T_H2 cells in order to induce T_H1 cells and foreshadowed the complex relationship between T-bet and GATA3. However, whereas IFN γ is important for resistance to *Mycobacterium tuberculosis* or *Salmonella enterica* subsp. *enterica* serovar Typhimurium, T_H2 cells do not have a prominent role in disease susceptibility^{104,105}. *Tbx21*^{-/-} mice challenged with either of these pathogens have reduced CD4⁺ T cell production of IFN γ and are more susceptible to infection, but this is not accompanied by the emergence of T_H2 cell-type activities^{106,107}. Thus, in these infectious systems, the ability of T-bet to block T_H2 cell development appears irrelevant, and its protective activities track most closely with the induction of IFN γ -producing CD4⁺ T cells.

T-bet-independent resistance.

There has been a tendency to describe antimicrobial responses dominated by the production of IFN γ as T_H1 cell-like regardless of the cellular source of this cytokine (for example, IFN γ can be produced by ILCs, $\gamma\delta$ T cells and CD4⁺ and CD8⁺ T cells during infection). One unintended consequence of this classification is that it implies that the cellular source of IFN γ is not critical for pathogen control, but this becomes an important consideration in systems in which the loss of T-bet is not synonymous with increased susceptibility to infection (see TABLE 1). The role of T-bet-independent IFN γ is most apparent in infections that are not dominated by CD4⁺ T cells and where other lymphocytes are major sources of IFN γ . Thus, murine cytomegalovirus (MCMV) infection induces potent NK cell production of IFN γ that mediates viral control, and while there are reduced numbers of NK cells in *Tbx21*^{-/-} mice, those that are present are sufficient to limit viral replication⁹⁶. Similarly, *Tbx21*^{-/-} mice infected with *L. monocytogenes* have reduced CD4⁺ T cell production of IFN γ , but the NK and CD8⁺ T cell responses appear intact, and these mice control bacterial replication¹⁰⁸. Similarly, the Armstrong strain of LCMV induces an acute viraemia that is cleared via CD8⁺ T cell-dependent and IFN γ R-dependent mechanisms¹⁰⁹. When *Tbx21*^{-/-} mice or those in which T cells lack EOMES are challenged with this strain of LCMV, the CD8⁺ T cells produce normal levels of IFN γ , and there is no obvious alteration in viral control or disease severity¹¹⁰. However, when mice that lacked both T-bet and EOMES in their T cells received the same challenge, there was a major defect in IFN γ production, reduced cytolytic activity and failure to control viral replication¹¹⁰. This phenotype provided evidence for the original prediction of functional redundancy and/or cooperativity between T-bet and EOMES. Unexpectedly at the time, these double knockouts had increased IL-17

production associated with a pathological neutrophil response¹¹⁰, consistent with a role for T-bet in the suppression of *Rorc* and T_H17 cell responses^{42,45}. By contrast, LCMV clone 13 causes a chronic infection with a low viral load, but *Tbx21*^{-/-} mice infected with this strain have reduced numbers of antigen-specific CD8⁺ T cells and are unable to control viral replication⁴⁸. A recent study has shown that the strength of TCR signalling can regulate the relative expression of T-bet and EOMES after infection with LCMV clone 13, and manipulation to increase the ratio of T-bet to EOMES within the CD8⁺ T cells results in better viral control¹¹¹. The variable outcomes associated with different strains of LCMV highlight that the requirement for T-bet (and/or EOMES) to coordinate protective immunity can be profoundly influenced by microbial context.

Microbial context matters.

For some eukaryotic pathogens, such as *T. gondii*, *Plasmodium* spp. and *Trypanosoma cruzi*, their complex life cycles and evasion strategies contribute to chronicity. This in turn requires full integration of cellular and humoral responses for parasite control, and the loss of T-bet can impact on many different facets of protective immunity. Thus, *Tbx21*^{-/-} mice challenged with *T. gondii* have reduced CD4⁺ T cell production of IFN γ , but NK cell production of IFN γ enables parasite control at the initial site of infection^{70,77}. However, in the absence of T-bet, the *T. gondii*-specific CD4⁺ and CD8⁺ T cells express reduced levels of the integrin CD11a as well as CXCR3 (REF.⁷⁷). Both of these molecules regulate different aspects of T cell migration into inflammatory environments^{85,112,113}. Consequently, as *T. gondii* disseminates into secondary sites of infection in the *Tbx21*^{-/-} mice, there is a failure to recruit parasite-specific T cells into the affected tissues to limit parasite replication⁷⁷. A related phenotype is observed in a mouse model of cerebral malaria in which the recruitment of CD8⁺ T cells to the central nervous system causes local damage, but *Tbx21*^{-/-} mice have reduced T cell trafficking to the brain and less neuropathology and are resistant to disease¹¹⁴. These two reports highlight that effector T cell trafficking should be considered a major function of T-bet required for local tissue responses during infection (see FIG. 2). For *T. cruzi*, the role of T-bet appears more complex, as it is required for expansion of parasite-specific CD8⁺ T cell populations but not for the ability of CD4⁺ T cells to produce IFN γ ^{115,116}. Surprisingly, despite apparently normal circulating levels of IFN γ , the *Tbx21*^{-/-} mice still succumb to acute infection associated with the appearance of a polyfunctional population of CD4⁺ T cells that co-produce IFN γ and IL-17 (REFS^{115,116}). Although IL-17 plays a protective role during infection with *T. cruzi*^{117,118}, it remains unclear whether the elevated IL-17 observed in the *Tbx21*^{-/-} mice in this setting contributes to the development of immune pathology. This inverse relationship between T-bet and IL-17 is observed in many systems but can manifest in different ways. In a model of post-influenza bacterial superinfection, the challenge of *Tbx21*^{-/-} mice with influenza results in increased IL-17 production that protects against subsequent challenge with *Streptococcus pneumoniae*¹¹⁹. Furthermore, while IL-17 signalling is protective after infection with the live vaccine strain of *Francisella tularensis*¹²⁰, the susceptibility of *Tbx21*^{-/-} mice to infection with this pathogen is associated with increased IL-17 production¹²¹. Similarly, the observation that RAG mice that lack T-bet can spontaneously develop bacteria-driven colitis provided critical evidence that T-bet functions in innate cells to limit the emergence of colitogenic bacterial species¹²². Further studies on these phenomena revealed that, in the

absence of T-bet, ILCs fail to produce IFN γ but instead produce IL-17 that contributes to the development of mucosal inflammation⁶⁰.

These results using *Tbx21*^{-/-} mice illustrate the range of phenotypes that occur with different viral, bacterial and parasitic organisms and even within strains of the same pathogen. Perhaps one of the main lessons from the opportunity to consider this literature is that the absence of T-bet does not inevitably lead to increased susceptibility to intracellular pathogens. Rather, the type and magnitude of the immune response required for resistance may determine the threshold for when T-bet becomes essential (see FIG. 3). For example, the role of T-bet in host protection is less critical in settings where NK cells and CD8⁺ T cells are major sources of IFN γ , as these are cells in which EOMES can mediate T-bet-independent pathways to IFN γ . When looking at the variable phenotypes shown in TABLE 1, T-bet is essential for the development of T_H1 cells and for resistance to infections that are associated with the lysosomal system. Thus, for *L. major*, *M. tuberculosis*, *S. Typhimurium* and *Francisella* spp., their association with the phagolysosomal system means that their antigens should be readily accessible to the MHC class II antigen-processing machinery, enabling CD4⁺ T cells to recognize infected cells. By contrast, T-bet does not appear so critical to control *T. cruzi* and *Listeria* spp., which reside in the cytosol of infected cells. Consequently, the transporter associated with antigen processing (TAP)-dependent MHC class I antigen-processing pathway would have most ready access to pathogen-derived antigens present in non-lysosomal compartments. Perhaps the most complex phenotype relates to *T. gondii*, which exists within a non-fusogenic parasitophorous vacuole of haematopoietic and non-haematopoietic cells. For this obligate intracellular organism, the pathways that enable infected cells to present antigen are less obvious. Thus, despite the fact that CD4⁺ T cell responses are associated with each of these infections, NK cells or CD8⁺ T cells appear to be sufficient sources of IFN γ and provide a T-bet-independent pathway that enables the development of pathogen-specific T cells. Paradoxically, T-bet-haplosufficient mice (with an intermediate level of T-bet expression) challenged with *M. tuberculosis* have an improved ability to control bacterial replication compared with T-bet-sufficient mice¹²³. Perhaps this observation is related to the finding that T-bet promotes the differentiation of a non-protective CX₃C-chemokine receptor 1 (CX₃CR1)⁺KLRG1⁺ population of intravascular CD4⁺ T cells after *M. tuberculosis* infection¹²⁴. These observations are complimented by experiments showing that ectopic expression of high levels of T-bet compromises the immune response to the neurotropic Theiler's encephalomyelitis virus¹²⁵. The basis for these phenotypes is unclear but may be related to the repressive effects of T-bet (discussed earlier) that are part of a feedback loop that dampens T cell responses.

T-bet and humoral responses

While the sections above have focused on the role of T-bet in cell-mediated immunity, T_H1 cells and their production of IFN γ are also linked to the production of IgG isotypes associated with antibody-dependent cell-mediated cytotoxicity, and early reports indicated that this arm of the immune response was T-bet-dependent³⁸. For example, during infection with LCMV, downstream of STAT4 phosphorylation, T-bet is co-expressed with BCL-6 in T_{FH} cells and coordinates IFN γ production that promotes the germinal centre response¹²⁶. Recent studies in a model of vaccination also established that T-bet is required for the

development of T_{FH} cells that produce IFN γ but that conditional deletion of T-bet after the differentiation of these cells did not affect their ability to make IFN γ ¹²⁷. However, the role of T-bet in humoral immunity is not restricted to T cells, and in lupus-prone mice (in which self nucleic acids may act as an adjuvant), B cell expression of T-bet was required for maximal IgG2a levels, although there was a significant T-bet-independent component¹²⁸. Similarly, other studies found that the ability of T cells to express CD40L and thereby engage CD40 on B cells required for class switching was independent of T-bet but that T cell-independent B cell responses to lipopolysaccharide were T-bet-dependent¹²⁹. These latter observations are consistent with reports that T-bet expression is induced in B cells via BCR signalling that synergizes with IFN γ ¹³⁰ or alternatively after ligation of Toll-like receptor 9 (TLR9) and subsequent MYD88 signalling^{131,132}. Perhaps similar to the ability of T_H1 cells and T_H2 cells to cross-regulate, there are mechanisms to repress T-bet expression in B cells, and treatment with IL-4 antagonizes the induction of T-bet¹³³. These parallels extend to the molecular level, and the expression of T-bet target genes is diminished in germinal centre B cells that express high levels of BCL-6, likely through the direct interaction of these transcription factors¹³⁴. Additionally, the expression of B lymphocyte-induced maturation protein 1 (BLIMP1) in plasma cells appears to antagonize T-bet activity¹³⁴, as does the expression of the transcription factor MYB in germinal centre B cells¹³⁵.

Subsequent studies have highlighted the role of T-bet in IFN γ -mediated class switching. In a model that used alum as the adjuvant, T-bet was dispensable for IFN γ -mediated class-switch recombination to the IgG2b isotype and for the inhibition of the IgG1 isotype, but T-bet did have a role in the generation of IFN γ -mediated IgG2a responses¹³⁶. Similarly, in mice immunized with the haptenated antigen NP-KLH combined with a lipid A adjuvant, T-bet is not required to generate antibody-secreting cells but is required for class-switch recombination to the IgG2a and IgG2c isotypes and for the survival of IgG2a⁺ memory B cells¹³⁴. Among memory B cells, T-bet is expressed in age-associated B cells that appear during humoral autoimmune disease and in the atypical memory B cells that arise following chronic infections^{137,138}. Indeed, in a murine model, B cell-intrinsic T-bet expression is required to control LCMV¹³⁹, and during acute HIV or following vaccination for yellow fever or vaccinia, viral titres correlate with the maintenance of T-bet expression among B cells¹⁴⁰. Thus, a picture has emerged in which T-bet⁺ B cells are not prominent in the commonly used adjuvant–hapten carrier systems but are most apparent in settings where nucleic acids or microbial products act as adjuvants that drive IFN γ production and when these adjuvants can act directly on B cells^{139–141}.

T-bet in chronic infection and memory

The availability of *Tbx21*^{-/-} mice facilitated studies to evaluate how the complete absence of T-bet influences T cell and B cell responses during infection. However, the expression of T-bet and EOMES in lymphocyte populations is not digital, and their maintenance in pathogen-specific T cells during persistent infection implies roles that extend beyond the initial development of effector responses. It has been a challenge to distinguish how the activity of T-bet during T cell priming might affect the quality of long-lived T cell populations versus a role for T-bet in the maintenance of these cells or during a secondary

immune response. Nevertheless, in clinical and experimental infections, variations in the levels of these transcription factors appear biologically relevant. For example, in patients infected with hepatitis B or hepatitis C, the presence of T-bet^{hi} virus-specific CD8⁺ T cells is associated with spontaneous resolution, whereas lower levels of T-bet are characteristic of progressive disease¹⁴². Similarly, HIV-specific CD8⁺ T cells from elite controllers express higher levels of T-bet than those from chronically infected progressors or individuals treated with highly active antiretroviral therapy¹⁴³, and decreased T-bet correlated with loss of effector function¹⁴⁴. The observation that T-bet and EOMES are reciprocally expressed by HIV-specific CD8⁺ T cells and that EOMES is associated with expression of the inhibitory receptors PD1 and CD160 (REF.¹⁴⁵) may help to explain the decreased functionality of CD8⁺ T cells that characterizes HIV progression. The ability to detect variation in T-bet and EOMES expression combined with the application of longitudinal immune profiling to clinical cohorts may help to develop algorithms in which the levels of these transcription factors can be used to predict disease progression and/or responsiveness to immune therapies.

The expression of variable levels of T-bet by pathogen-specific T cells has been noted in many infections, and these have frequently been associated with different stages of T cell activation and effector function. This has perhaps been best studied using LCMV infection, and in this model, short-lived effector T cells (SLECs) express high levels of T-bet, whereas memory precursor effector cells express lower levels of T-bet^{54,78,86,87,146}. These data suggest one model of effector differentiation common to CD4⁺ and CD8⁺ T cells wherein less differentiated T-bet^{int} effector cells can form and persist or further differentiate into terminal T-bet^{hi} effector cells with robust effector function but reduced memory cell potential¹⁴⁶ (see FIG. 4). By contrast, in T-bet-deficient mice infected with LCMV-Armstrong, there is a decrease in effector T cells but enhanced generation of CD27^{hi}KLRG1^{lo} central memory CD8⁺ T cells that confer better protection to a secondary challenge⁷⁸. This unexpected observation suggests that T-bet inhibits memory formation and is consistent with the idea that the magnitude of the primary effector response is inversely correlated with the generation of memory responses^{147,148}. The complex role of T-bet in effector and memory fate decisions is illustrated by the apparent role reversal of T-bet and EOMES in the maintenance of self-renewing CD8⁺ T cells after chronic LCMV challenge; T-bet^{hi} precursors are required to generate EOMES^{hi} terminally differentiated effectors, which display higher expression of inhibitory receptors¹⁴⁹. This contradiction has never been fully resolved, but the report that similar EOMES^{hi}T-bet^{lo} CD8⁺ T cells that express inhibitory receptors at a high level are found in the tumour microenvironment¹⁵⁰ suggests that this transcriptional state is substantially influenced by chronic antigen load. By contrast, as previously mentioned, altering the strength of TCR signalling can increase the proportion of T-bet relative to EOMES, which leads to clearance of this chronic infection, suggesting that the transcriptional state influences the ability to respond to chronic infection¹¹¹.

The infectious examples described above indicate that the impact of changes in the relative levels of these transcription factors is context-dependent and that the specific conclusions from these studies reflect the experimental questions being addressed (that is, mechanisms of exhaustion versus impact on resident tissue memory). For example, in a model of epicutaneous challenge with herpes simplex virus, development of CD8⁺CD103⁺ tissue-

resident memory T (T_{RM}) cells in the skin requires downregulation of EOMES, but low levels of T-bet are necessary to maintain expression of CD122, the β -chain of the IL-15R, which promotes the survival of T_{RM} cells¹⁵¹. However, in the context of a molecular adjuvant designed to directly engage CD40 and TLR2 and/or TLR3, the loss of either T-bet or EOMES (likely downstream of IL-15 and IL-27) compromises clonal expansion¹⁵². This is a finding that runs counter to the majority of the infectious disease literature and highlights that the levels of inflammation associated with infection may override the need for T-bet and EOMES, whereas during subunit vaccination, their critical contributions are more apparent.

Another area that has seen advances relates to reports that link T-bet and EOMES expression with different stages of development of T cell responses: early effectors, proliferative intermediates and various forms of memory (see FIG. 4). This is a topic that is complicated by the variation in the markers used to identify different subsets or developmental stages of T cell activation. As previously noted¹⁵³, “single, or even a handful, of markers is unlikely to reliably predict either the developmental or protective potential of “subsets” that transcends single experimental systems”. In other words, the combination of CD44 and CD62L to distinguish effector and memory populations has been replaced by more complex combinations of surface molecules (adhesion molecules and cytokine and chemokine receptors) and transcription factors that can differ between infectious agents. This is illustrated by the studies with vaccinia or *Listeria* spp. discussed above, the infections of which induce a population of $KLRG1^{hi}T\text{-bet}^{hi}EOMES^{lo}CD8^{+}$ T cells (typically an effector phenotype during LCMV infection) that persists in the memory phase and, despite low proliferative potential, provides optimal protection against re-challenge¹⁵⁴. The integration of an additional persistent infectious disease, such as infection with MCMV, suggests a model in which memory cells that express CD27, CD127 (IL-7RA) and CD122 (IL-15R β) at a high level are derived early during infection, whereas SLECs are continuously generated from this population¹⁵⁵.

A similar theme has emerged during infection with *T. gondii*, in which persistent antigen induces a $CD8^{+}$ T cell memory–effector hybrid intermediate that expresses T-bet, CXCR3 and KLRG1 and gives rise to CXCR3-negative SLECs¹⁵⁶. Certainly, in this setting, T-bet is required for optimal expression of CXCR3 and T cell migration into affected tissues⁷⁷. Additionally, Knolle and colleagues¹⁵⁷ have used expression of the CX₃CL1 (also known as fractalkine) receptor CX₃CR1 to distinguish cytotoxic and proliferative capacity among human and mouse memory $CD8^{+}$ T cells. In murine models with *Listeria* spp. and LCMV infections, after the initial T cell expansion, there was an abrupt transition in which a significant percentage of these cells started to express CX₃CR1. Subsequent studies demonstrated that, in response to LCMV, $CD8^{+}$ central memory T cells that express low levels of CX₃CR1 can give rise to effector memory cell populations that are CX₃CR1^{int} or CX₃CR1^{hi}. Importantly, while T-bet was expressed in each population, the CX₃CR1^{hi} cells were T-bet-dependent¹⁵⁸. These results are consistent with the ability of T-bet to bind to the CX₃CR1 gene locus⁷³, but the impact of CX₃CR1 expression on the function of these cells remains unclear. Interestingly, lineage tracing indicates that $KLRG1^{+}T\text{-bet}^{hi}$ cells that receive intermediate amounts of inflammatory signals can give rise to all memory T cell lineages, including CX₃CR1^{int} peripheral memory cells and T_{RM} cells, and that the relative

levels of T-bet vary between these different subsets¹⁵⁹. Whether these data sets are indicative of a high level of phenotypic plasticity that underlies the ability to generate diverse types of effector and memory populations or that variations in the levels of T-bet influence these events remains to be addressed.

Future directions

This Review has emphasized how mouse models of infection have led to a better appreciation of the broad effects of T-bet and EOMES on lymphocyte biology. A major challenge exists to determine which of these features are shared with humans or whether these transcription factors have distinct functions associated with human diseases. There are numerous examples that illustrate changes in T-bet expression during human infections or lymphoproliferative disorders that correlate with disease progression or resolution^{142–144,160–164}. There are also 40 known polymorphisms of the human *TBX21* gene, one of which has been associated with susceptibility to type 1 diabetes¹⁶⁵, while another is associated with herpes simplex virus 2 incidence¹⁶⁶. How the other 38 known polymorphisms impact human immune responses to infectious disease is unknown.

Since the original paradigm in which T-bet was considered the master regulator of T_H1 cell responses, we have moved through the model of functional redundancy between T-bet and EOMES and to a situation in which the precise role of these factors in T cell fate determination is context-dependent and guided by the inflammatory environment and duration of antigen encounter. Thus, while both T-bet and EOMES can promote IFN γ production, they have different effects on T cell phenotypes and functions, as highlighted by the differential functions of T-bet and EOMES during acute and chronic viral infections, and there is evidence that the relative ratio of T-bet to EOMES matters. It does seem intuitive that T-bet has core functions common to early-stage or late-stage effectors or in different memory populations, but changes in the chromatin landscape or the presence of other transcription factors (such as FOXP3, BLIMP1, BCL-6 and GATA3) that are associated with the diversification of lymphocyte responses would result in the acquisition or loss of regulatory functions. The development of genetic approaches to conditionally delete T-bet¹²⁷ will provide an important tool to understand the functions of T-bet at later stages of T cell (and B cell) development and to test whether T-bet is required for quiescent memory cells to rapidly (re)engage effector activities or give rise to progeny that have the ability to synthesize IFN γ immediately (see FIG. 4).

There have been many studies that have examined the role of T-bet during infection, but there is a paucity of reports that directly assess the contribution of EOMES to the immune response to diverse bacterial and parasitic pathogens. Given the context-dependent function of these factors, as outlined above, a survey of the function of EOMES across different infections may help to understand how and where (or whether) EOMES has a dominant role in the control of a particular type of infection or in immune homeostasis. A good example is the increasingly appreciated role for T-bet in B cells, which lends credence to a possible role for EOMES in these lymphocytes. This resurgence in interest does highlight that, even though a cell-intrinsic role for T-bet in B cell function was described early, the focus on T cells perhaps limited our perspective of T-bet as a lineage-defining factor when in fact it is

broadly expressed in immune cell populations. We now know that there are multiple pathways mediated by diverse stimuli (for example, cytokines, TLR signals and TCR and BCR signalling) that promote NK cell, T cell and B cell expression of T-bet and that this varies enormously among infections. Perhaps the emergence of T-bet contemporaneously with the genes encoding RAG and IFN γ and its ability to coordinate innate and adaptive, cellular and humoral responses are consistent with its function as a central regulator of an evolutionarily conserved response that integrates antigenic and environmental cues to facilitate host protection against a broad spectrum of pathogens.

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Fig. 1 |. The induction and diverse target genes of T-bet.

a | T-bet is induced in T cells through signal transducer and activator of transcription 1 (STAT1) downstream of T cell receptor (TCR) signalling and/or signalling through IFN γ R, IFN α R, IL-27R and IL-21R. T-bet is also induced through STAT4 downstream of signalling through IL-12R. **b** | After T-bet is induced, it binds to an array of genes that exert diverse functions in the cell including activation (via IFN γ , IL12R β 2 and STAT1), cellular trafficking (via CC-chemokine ligand 3 (CCL3), CXC-chemokine receptor 3 (CXCR3), tyrosyl protein sulfotransferase 2 (TPST2) and CD11a) and immune regulation (via IL-2, IL-4, IL-5 and ROR γ t). **c** | T-bet is induced in B cells through STAT1 after ligation of the B cell receptor (BCR) and/or IFN γ R. T-bet can also be induced through MYD88 downstream of Toll-like receptor 9 (TLR9) signalling. pSTAT, phosphorylated STAT. APC, antigen-presenting cell.

Fig. 2 |. Expression of T-bet is linked to T cell trafficking behaviour.

a | T-bet activation is associated with the increased expression of CD11a as well as the expression of enzymes that promote the glycosylation of P-selectin glycoprotein ligand 1 (PSGL1) and its binding to P-selectin, which is required for T cell adhesion to inflamed endothelium and migration into sites of inflammation. Within these tissues, T-bet-mediated expression of CXC-chemokine receptor 3 (CXCR3) enables T cells to respond to IFN γ -induced chemokines, such as CXC-chemokine ligand 9 (CXCL9) and CXCL10, and thus mediate local control of infections in tissues. **b** | The coordinated expression of T-bet by effector T cell and regulatory T (T_{reg}) cell populations would enable these cell types to respond to similar environmental signals and ensure that the regulatory mechanisms mediated by T_{reg} cells occur at sites of T helper 1 (T_H1) cell-like inflammation. Whether T-bet acts in concert with forkhead box P3 (FOXP3) to engage distinct regulatory mechanisms that are required to limit local inflammation is uncertain.

Fig. 3 | T-bet in immunity to pathogens that occupy distinct intracellular niches.

a | Pathogens such as *Leishmania major* are specialized to survive and replicate inside the phagolysosomal system. Microbial antigens at these sites drive the development of MHC class II-restricted T-bet-dependent $CD4^+$ T cells, and the production of $IFN\gamma$ by these cells is the dominant pathway that mediates parasite control. **b** | The ability to escape from the phagolysosomal system into the cytosol of host cells provides a mechanism for *Listeria monocytogenes* to avoid killing, but in this compartment, bacterial antigens are readily presented to MHC class I-restricted $CD8^+$ T cells. In addition, innate responses mediated by natural killer (NK) cells also contribute to the production of $IFN\gamma$. **c** | *Toxoplasma gondii* replicates in a unique parasitophorous vacuole that does not fuse with the lysosomal compartment but fully engages innate and adaptive production of $IFN\gamma$. While the ability to generate parasite-specific $CD4^+$ T cells is partially dependent on T-bet, the magnitude of the NK cell and $CD8^+$ cell response appears intact in the absence of T-bet.

Fig. 4 |. Differential expression and function of T-bet and EOMES during differentiation of effector and memory T cell subsets.

A primary immune challenge results in the activation and expansion of pathogen-specific CD8⁺ T cell populations that give rise to distinct populations of memory precursors as well as effector cells. The relative levels of T-bet and eomesodermin (EOMES) differ in these populations and may be influenced by levels of T cell receptor (TCR) engagement, the process of cell division and the inflammatory environment, factors which in turn influence the expression of surface molecules associated with distinct trafficking and effector functions. After resolution of infection, it is unclear how T-bet and EOMES influence memory responses to secondary challenges or, in the case of persistent infection, whether these transcription factors are required to maintain effective long-lived T cell responses. CX3CR1, CX3C-chemokine receptor 1; CXCR3, CXC-chemokine receptor 3; KLRG1, killer cell lectin-like receptor subfamily G member 1.

Table 1 |

Role of T-bet in resistance to different intracellular infections

Pathogen	IFN γ source	Phenotype seen in <i>Tbx21</i> ^{-/-} mice	Refs
<i>Leishmania major</i>	CD4 ⁺ T cells	More susceptible to infection; reduced T _H 1 cell response and enhanced T _H 2 cell response	38
<i>Mycobacterium tuberculosis</i>	CD4 ⁺ T cells	More susceptible to infection; reduced T _H 1 cell response	106
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium	CD4 ⁺ T cells	More susceptible to infection; reduced T _H 1 cell response	107
Herpes simplex virus 2	CD4 ⁺ T cells	More susceptible to infection	167
<i>Trypanosoma cruzi</i>	CD4 ⁺ T cells, CD8 ⁺ T cells and NK cells	CD8 ⁺ T cell defect; enhanced T _H 17 cell response	116
<i>Francisella tularensis</i>	CD4 ⁺ T cells, CD8 ⁺ T cells, NK cells and neutrophils	More susceptible to infection; intact global IFN γ response but reduced T cell production of IFN γ and increased IL-17 production	121
LCMV clone 13	CD8 ⁺ T cells	More susceptible to infection; reduced CD8 ⁺ T cell response and enhanced PD1 expression	48
LCMV-Armstrong	CD8 ⁺ T cells	Resistant to infection; normal CD8 ⁺ T cell response	110
MCMV	NK cells	Resistant to infection; reduced NK cell response	96
<i>Listeria monocytogenes</i>	CD8 ⁺ T cells and NK cells	Resistant to infection; reduced T _H 1 cell response but normal CD8 ⁺ T cell and NK cell response	108
<i>Toxoplasma gondii</i>	CD4 ⁺ T cells, CD8 ⁺ T cells and NK cells	More susceptible to infection; reduced T _H 1 cell response, normal NK cell and CD8 ⁺ T cell response and altered T cell trafficking	77
Influenza virus	Not defined	Resistant to infection; increased infiltration of neutrophils, eosinophils and B cells into lungs	119

LCMV, lymphocytic choriomeningitis virus; MCMV, mouse cytomegalovirus; NK cell, natural killer cell; PD1, programmed cell death 1; T_H cell, T helper cell.