Review

The T-box Transcription Factor T-bet in Immunity and Autoimmunity

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The T-box transcription factor T-bet (Tbx21) has emerged as a key regulator of type 1-like immunity, playing critical roles in the establishment and/or maintenance of effector cell fates in T and B lymphocytes, as well as dendritic cells and natural killer cells. Several autoimmune diseases, especially those classically considered related to T helper 1 (Th1) immunity, appear to require T-bet, at least as judged in mouse models. This review summarizes a current understanding of T-bet's role in immunity, as well as its importance in autoimmunity, with implications for therapeutic intervention. *Cellular & Molecular Immunology*. 2006;3(2):87-95.

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The role of T-bet in immunity

T-bet in T lymphocytes

T-bet has been most extensively studied and understood in helper T (Th) cells, where it plays a critical role in the development and/or maintenance of Th1 cells, whose signature cytokine is IFN-γ (1). Its initial description correlated its expression with IFN-γ production in CD4, CD8 and natural killer (NK) cells, with ectopic expression inducing IFN-γ, even in Th2 cells (2). T-bet likely plays a critical role in the maintenance of Th1 effector function, since it is induced in a STAT1-dependent fashion in response to IFN-γ itself, remodeling the chromatin of the IFN-γ gene and up-regulating the expression of IL-12Rβ, allowing IL-12-induced growth and stabilization of IFN-γ (3-5) (Tables 1 and 2). This capability of differentiation into Th1 cells *via* T-bet appears to be acquired in the thymus during the $CD4^+$ SP HSA^{hi} to $CD4^+HSA^{lo}$ transition (6).

The modification process at the IFN-γ gene appears to be at least bi-phasic, with initial histone acetylation modifications regulating initial accessibility to the cytokine loci followed by stabilization by T-bet, reducing plasticity of Th1 versus Th2 fates (7-10). Here, T-bet binds monomeric brachyury consensus sites in the IFN-γ promoter (11), and modifies histones at at least 2 distal regulatory elements (12), blocking binding of the corepressor mSin3a (13) – all involving the assistance of probably several co-activators, including the T-bet target gene Hlx (14), Ets1 (15), as well as possibly CREB-binding protein (4) and c-Rel (16). In addition, cell cycle-dependent epigenetic events, including acetylation, likely improve and stabilize the transcriptional efficacy of the T-bet locus itself during Th1 differentiation (17, 18). Thus, T-bet plays a central role in the acquisition of the Th1 cell fate, as established by multiple rounds of epigenetic modifications.

A growing number of cytokines and signaling mediators, in addition to IFN-γ and STAT1, appear relevant to T-bet expression in Th cells *in vivo*. IL-12, for instance, may induce T-bet in Th2 cells to induce a shift to Th0/Th1 (19); and IL-27 can induce T-bet to confer IL-12Rβ expression *via* STAT1 (20-23) – although this pathway may be less important in CD8 cells (24), and T-bet is not required for the generation of Th1 cells *via* IL-27 (25). Other described inducers of T-bet include Notch1 (26); SLAM (CD150), which can promote T-bet expression *via* NF-κB and STAT1 (27); Sem4a, whose deficiency results in defective T-bet and Th1 induction (28); IL-21 (29); GITR (30); and LFA-1 (31). Conversely, T-bet is also negatively regulated by several mediators, especially TGF-β, which can suppress T-bet expression in developing Th1 cells, perhaps by inhibiting Itk, Ca^{2+} signaling, and NFATc1 activation or *via* protein tyrosine phosphatase (PTP) Src homology region 2 domain-containing phosphatase-1 (Shp-1) (32-34). Interestingly, TGF-β-mediated suppression of T-bet may be more important during Th1 commitment and/or development, as opposed to during priming (35, 36). Other potential regulators of T-bet include Vav1 and PPARα, which both

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Table 1. Regulators of T-bet in various immune cell lineages

	$CD4^+$ T	$CD8^+$ T	B	DC	NK
Cytokines					
IFN-γ	+	+	+	+	+
IFN-I	+/-		NE		
IL-12	+		+		+
IL-15	+				+
IL-21	+				+
IL-27	+		+		
TGF-β	-				
Costimulatory receptors					
CD150 (SLAM)	+				
GITR	+				
LFA-1	+				
Notch1	+				
Sema4a	+				
CpG ODNs			+	+	
Other signaling molecules					
Itk	+/-				
PPARa	-				
Shp-1	-				
Vav1	-				

Shown are stimuli reported to affect T-bet expression in the indicated immune cell lineages. (+), induced; (-), repressed; (+/-), conflicting data exist; NE, no apparent effect. GITR, glucocorticoid-induced TNFR-related protein (TNFRSF18); IFN-I, type I interferon; Itk, interleukin-2-inducible T cell kinase; LFA-1, lymphocyte function-associated antigen 1 (integrin α_L, CD11a); ODN, oligodeoxynucleotide; PPAR, peroxisome proliferative activated receptor; Sema4a, sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A; Shp-1, hemopoietic protein tyrosine phosphatase (PTP) Src homology region 2 domain-containing phosphatase-1; SLAM, signaling lymphocytic activation molecule, DC, dendritic cell, NK, natural killer cell.

Table 2. Target genes of T-bet

	$CD4^+$ T	$CD8^+$ T	B	DC	NK
Cytokine-related					
IFN-γ	+		+	+	
IFN-I				+	
IL-2	-				
CD122 (IL-2Rβ)		+			
IL-12Rβ	+				
Osteopontin	+				
Immunoglobulins					
IgG2a			+		
IgG1, IgE			+/-		
Migration/adhesion-related					
CXCR3	+				
FucT-VII	+				
ST3Gal-VI	+				
Cytolysis-related					
Granzyme B					+
Perforin					+
Transcription factors					
Hlx	+				
Runx1					+

Shown are reported T-bet effector genes in the indicated immune cell lineages. (+), induced; (-), repressed; (+/-), conflicting data exist; NE, no apparent effect. FucT-VII, fucosyltransferase VII; IFN-I, type I interferon; ST3Gal-VI, α2,3-sialyltransferase VI; DC, dendritic cell, NK, natural killer cell.

appear to suppress T-bet expression, perhaps *via* the MAP kinase p38 (37, 38). Although the specific means by which these many pathways interact to impact T-bet expression remain incompletely understood, such findings nonetheless indicate that a dynamic synthesis of environmental signals regulates T-bet activation.

T-bet-deficient mice demonstrate impaired Th1 differentiation, including defective IFN-γ production primarily in CD4 and γδ T cells (39, 40). Analogously, they develop a spontaneous Th2-like, asthma-like syndrome consisting of airway hyper-responsiveness, and demonstrate increased susceptibility to experimental asthma models, likely due to enhanced IL-13 expression (41, 42). Interestingly, CD8 T cells require T-bet for full effector function (43), where T-bet appears to cooperate with another T-box transcription factor, eomesodermin, to enforce T cell memory *via* CD122 (IL-2Rβ), which regulates IL-15 responsiveness (44). Thus, T-bet plays essential roles in several facets of type 1-related, inflammatory T cell differentiation.

Although these findings point to a critical role for T-bet in the development of Th1 cells, at least in mice, the role of T-bet in human T cells appears to be less straightforward. On one hand, T-bet expression in human Th cells correlates with Th1 profiles, is heritable and may account for genetic Th1-Th2 biases (45, 46); but on the other hand, human Th cells demonstrate significant plasticity of the Th1/Th2 lineages (47), and T-bet does not always correlate with IFN-γ production (48, 49). As such, continued translational efforts in both human and mouse systems will be required to completely elucidate the specific roles of T-bet in the various T cell effector lineages.

T-bet in B lymphocytes

In addition to T cells, T-bet plays critical roles in B cell effector function, especially type 1-related responses, although its apparent expression in several B cell precursors suggests that it may also play a developmental role (50, 51). In mature B cells, T-bet is induced particularly by IFN-γ, but also by at least IL-12, IL-27 and the toll-like receptor (TLR) 9 ligands CpG oligonucleotides (52-55). Here, T-bet is required to establish and/or maintain a type 1-like cell fate, since T-bet-deficient B cells fail to develop into Th1-inducing B effector 1 (Be1) cells or to undergo class switch recombination (CSR) to IgG2a in response to IFN-γ, a

Table 3. The role of T-bet in autoimmune diseases

	Efficacy of T-bet			Expression of T-bet in human disease
	Deficiency	Over-expression	Knock-down	
Arthritis, inflammatory				low
CAIA	+			
IL-1Ra KO	NE			
KRN	NE			
PIA	-			
S. aureus	-			
Diabetes, type 1				*
RIP-LCMV Tg	+			
B9-23 peptide	NE			
Inflammatory bowel disease				high
AT-scid	+	-		
Oxazolone	-			
Multiple sclerosis				
MOG-EAE	+			
MOG TCR Tg AT	+			
MBP TCR Tg AT			+	
Systemic lupus erythematosus				
MRL/lpr	+			

Shown are the effects of the various indicated T-bet interventions on autoimmune disease models in mice. (+), improvement of disease; (-), worsening of disease; NE, no effect. Indicated also for comparison is the expression levels of T-bet in human disease tissue, when known, as compared to normal controls. AT-scid, $CD4^+CD62L^+$ to scid adoptive transfer; CAIA, collagen antibody-induced arthritis (passive); EAE, experimental autoimmune encephalomyelitis; KRN, K/BxN passive serum transfer arthritis; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; PIA, proteoglycan-induced arthritis; RIP-LCMV Tg, rat insulin promoter-lymphocytic choriomeningitis virus transgenic model for virally-induced diabetes; TCR Tg AT, T cell receptor transgenic adoptive transfer. *, no expression data reported, but T-bet polymphorism(s) have been associated.

STAT1-dependent pathway (56-59) – the latter interestingly in a largely T cell-independent fashion (57). Conversely, T-bet-deficient B cells overproduce the Th2-related Ig isotypes IgG1 and IgE (56). T-bet is also required for the induction of IgG2a CSR in response to CpG oligonucleotides (54) and IL-27 (55), although it is not required for the ability of CpG oligonucleotides to suppress IgE or IgG1 CSR (53, 54, and our unpublished data). Thus, as in T cells, T-bet induces and/or maintains a type 1-like differentiation program in B cells, at least in response to environmental signals such as IFN-γ or TLR9 ligands.

T-bet in other immune cells

T-bet plays similarly critical roles for type 1-like responses in other immune cell lineages, at least in mice. Monocytes and dendritic cells both rapidly induce T-bet expression in response to IFN-γ and CpG oligonucleotides, and T-bet is required in dendritic cells for optimal IFN production and the promotion of Th1 programs in Th cells (60-62). Interestingly, T-bet is furthermore required for proper natural killer (NK) and NKT cell development and effector function (63, 64). Thus, T-bet likely exerts many additional immunomodulatory functions in a diverse array of immune cell lineages.

The role of T-bet in autoimmune diseases

Because Th1 responses have long been associated with autoimmune syndromes, it is not surprising that a growing literature has begun to accumulate evidence for pathogenic roles of T-bet in autoimmunity (Table 3). Indeed, loss of T-bet expression correlates with the development of tolerance, at least in a Th1 TCR transgenic model of autoreactive T cells (65). Thus, enthusiasm continues to be high for a pathogenic role for T-bet in many autoimmune diseases characterized by autoaggressive, type 1-related responses.

Inflammatory bowel disease

To date, T-bet has been most extensively explored in the pathogenesis of inflammatory bowel diseases (IBD). Both Celiac and Crohn's diseases, which have generally been considered Th1-related syndromes, exhibit enhanced T-bet activity and/or expression, perhaps resulting from enhanced antigen-mediated TCR stimulation (66-70). In a Th1-related IBD mouse model – adoptive transfer of $CD4^+CD62L^+$ cells into severe combined immunodeficient (scid) recipients -- T-bet deficiency protects against, while T-bet overexpression promotes, disease, correlating with TGF-β expression and

signaling; while in the Th2-related IBD model oxazolone-induced colitis, T-bet deficiency exacerbates disease (66). Interestingly, T-bet may cooperate with the Th2-associated transcription factor c-Maf, to exert such pathogenic effects, at least in $CD4^+CD62L^+$ adoptive transfer colitis (71). In addition, the ability of IL-27 deficiency to protect mice against dextran sulfate sodium-induced colitis, and the ability of CEACAM1 to effectively treat oxazolone-induced colitis – both Th2-related models -- correlate with reduced T-bet and/or IFN-γ expression (72, 73). Also, the generally Th2-associated cytokine IL-21 is found elevated in Crohn's disease, where its blockade results in an inhibition of T-bet expression (74). Thus, T-bet appears to play a key role in the pathogenesis of inflammatory bowel diseases, at least in some mouse models; however, the mixed observations regarding the role of T-bet in Th1 versus Th2-related pathogenesis suggest that T-bet's role in IBD does not simply reflect regulation of the Th1-Th2 axis.

Multiple sclerosis

A growing body of evidence supports a pathogenic role for T-bet in multiple sclerosis (MS). T-bet-deficient mice are protected from the myelin oligodendrocyte glycoprotein (MOG)-induced and MOG TCR transgenic models of experimental autoimmune encephalomyelitis (EAE) (75), and antisense or siRNAs directed against T-bet are effective in MBP TCR transgenic adoptive transfer models of EAE (76). The worsened EAE that develops in mice deficient in matrix metalloproteinase-12 correlates with enhanced T-bet expression and Th1 activity (77), while the effectiveness of the HMG-CoA-reductase inhibitor lovastatin and γ-secretase inhibitors in EAE correlates with diminished T-bet expression (26, 78). Thus, mouse data has consistently implicated T-bet as a critical mediator of MS pathogenesis; however, given the difficulty of translating findings in murine EAE to human multiple sclerosis (79, 80), caution is reasonable in the interpretation of these findings, which await corroboration with human diseases.

Inflammatory arthritis

Evidence for a pathogenic role for T-bet in inflammatory arthritis remains conflicting. T-bet-deficient mice have recently been reported to be protected from arthritis in the passive collagen antibody-induced arthritis (CAIA) model, interestingly apparently due to a requirement for T-bet in dendritic cells to express Th activation-related inflammatory mediators such as IL-1α, MIP-1α, and thymus- and activation-related chemokine (TARC) (81). However, T-bet-deficient mice are susceptible to the passive K/BxN serum transfer model of arthritis, as well as arthritis that occurs in the setting of IL-1Ra deficiency (81, and our unpublished data); and in fact are more susceptible to arthritis induced by proteoglycan (82) and by *S. aureus* (83). Supporting the latter findings, in humans with rheumatoid arthritis (RA), T-bet expression correlates with lower disease activity (84), and RA peripheral blood contains reduced spontaneous IFN-γ production, a phenotype reversed upon treatment with the TNF-α antagonist infliximab (85). Thus, T-bet may play a largely suppressive role, at least in some RA patients; however, the findings in T-bet-deficient mice with CAIA suggest that T-bet may play a pathogenic role in at least some subsets of inflammatory arthritis.

Diabetes

In type 1 diabetes mellitus (T1DM), findings with T-bet in mice have similarly produced conflicting findings. Although T-bet-deficient mice are susceptible to insulin autoantibody responses and insulitis during B9-23 insulin peptide immunization (86), they are relatively resistant to the rat insulin promoter-lymphocytic choriomeningitis virus transgenic model for virally-induced diabetes due to reduced CD8 cell effector generation and function (87). There, T-bet-deficient mice developed reduced numbers of autoreactive CD8 cells which produced less IFN-γ, but more IL-2 than their wild-type counterparts. Generation, but not apoptosis, expansion or maintenance of these effector cells was defective. Interestingly, T1DM in humans has been associated with two T-bet polymorphisms: His33Gln, which confers increased IFN-γ transactivation, and a CA (14) polymorphism in the 3' UTR (88). Thus, intriguing data suggest that T-bet may play a primary pathogenic role in at least a subset of T1DM patients.

Other autoimmune diseases

T-bet continues to be explored in other autoimmune diseases. For instance, recent studies have demonstrated its upregulated expression or activity in Behcet's and Vogt-Koyanagi-Harada (VKH) disease (89, 90), and T-bet has been implicated in the pathogenesis of psoriasis, a presumed Th1 disease (91). In the MRL/lpr model of systemic lupus erythematosus, T-bet is required for the generation of pathogenic autoantibodies, including the ability of autoreactive B cells to undergo class switching and affinity maturation, and the development of related end-organ disease, such as glomerulonephritis (56). On the other hand, reduced levels of T-bet have been observed in Kawasaki disease (92). Thus, T-bet appears likely to play distinct roles in different autoimmune syndromes, which is consistent with the differential importance of T-bet in the various mouse autoimmunity models, e.g., of inflammatory bowel disease, arthritis or diabetes.

T-bet as a drug target: implications for and of therapeutic intervention

Although traditionally transcription factors have been considered poor therapeutic targets, at least as direct targets of small molecules, their positions as key determinants of immune cell fate and function – such as that of T-bet – have long maintained interest in them (93). Typical means for intervention have included nucleic-acid based strategies, such as decoy oligonucleotides, or antisense or knockdown approaches, and the recent success of antisense and siRNA approaches in mouse EAE indicate that such strategies are indeed applicable T-bet (76). However, they can be fraught

with difficulty, including confounding effects of the nucleic acids themselves upon cellular processes, and/or challenges in developing nucleic acid reagents with desirable absorption, distribution, metabolism, and elimination (ADME) properties *in vivo* (94-96). Other approaches, such as attempts to use small molecules to disrupt binding of transcription factors to DNA or co-factor proteins, have proven difficult due to the lack of well-defined, chemically tractable binding pockets (97).

Recent work, however, suggests that signaling pathways involving specific kinases directly regulate T-bet activity, providing novel targets for therapeutic intervention. Inhibitory synthetic oligodeoxynucleotides, for instance, inhibit pathogenic Th1 responses by inhibiting IFN-γ-induced STAT1 and IL-12-induced STAT3/STAT4 activation, resulting in reduced T-bet expression (98); and the Tec kinase Itk has been suggested to promote Th2 differentiation in Th cells by suppressing T-bet expression (99). T-bet itself, however, is post-translationally regulated by phosphorylation, and Itk has been demonstrated to phosphorylate T-bet at Y525, enabling its ability to bind to and inhibit the transcriptional activity of GATA3 and subsequent expression of Th2 cytokines (100). Analogously, phosphorylation of T-bet at S508 by casein kinase I (CK1) and/or glycogen synthase kinase-3 (GSK3) facilitates the ability of T-bet to bind to and inhibit the transcriptional activity of RELA (NF-κB p65) on IL-2 (101). T-bet is also capable of blocking IL-21 expression by interfering with the transcriptional activity of NFATc2 (102). Such mechanisms may not be universally applicable throughout Th differentiation, since GATA3 can suppress Th1 independent of T-bet (103). Nonetheless, these recent findings in the mouse Th system raise the intriguing possibility that specific kinases might directly regulate the activity of T-bet, such that therapeutic targeting of such relevant kinases might be capable of immunomodulation *via* control of T-bet activity.

Still, it is important to recognize that successful therapeutic inhibition of T-bet could produce untoward immunosuppression. Although T-bet is dispensable for some infections in mice, including listeriosis (104), it appears to contribute to host defense against a growing number of pathogens, at least in experimental models of infection with leishmania, salmonella, vaccinia virus, herpes simplex virus-2, staphylococcus and tuberculosis (39, 83, 104-108). On the other hand, in humans, T-bet has been linked to pathological responses in both human immunodeficiency virus (HIV) infection and human T-lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (109, 110), while filariasis is associated with impaired Th1 and diminished T-bet expression and impaired Th1 responses (111). Similarly, in mice, T-bet has been implicated in the progression of prostate cancer and B cell lymphoma (112, 113), and IL-27 – presumably *via* T-bet – has been shown to exert anti-tumor activity (114); but several human cancers have been linked to T-bet expression, including T cell, B cell and Hodgkin leukemias and/or lymphomas (50, 51, 115-117). Thus, although studies in mice suggest that T-bet may play important roles in host defense, at least during experimental infections and cancer models, many studies in humans simultaneously suggest that T-bet activity may contribute to tumorigenesis and/or pathological responses during infections, suggesting that interfering with T-bet activity may have potentially beneficial or detrimental effects, depending upon the specific clinical setting.

Concluding remarks

In mice, T-bet clearly plays critical roles in T cell effector differentiation as well as pathogenic autoimmune responses in a growing number of autoimmune disease models. However, most of the data are inconsistent, and incompletely understood, such as the incomplete concordance between T-bet, Th1-ness and IFN-γ in human cells; the apparent roles for T-bet in diseases generally considered to be Th2-like, as exhibited by the relationship between IL-21 and T-bet in Crohn's disease or the interaction between T-bet and CEACAM1 in mouse IBD; versus the apparent lack of importance for T-bet in diseases considered Th1-like, such as some mouse arthritis models. In this sense, it is interesting to note that T-bet also influences the capabilities of inflammatory immune cells to migrate to target tissues, perhaps *via* modulation of CXCR3, selectin ligands (92, 118, 119), and osteopontin (Eta-1), which itself may directly influence Th1 polarization (120). In addition, T-bet may be less important in type 1 immunity when induced by cognate antigen or other cytokines like IL-12, IL-18, and/or IL-27 (5, 25, 121); and in some inflammatory disease states, type 1 Th lineages may participate in a regulatory T cell subset (122). Also, the recently-described IL-17-producing Th cell lineage, which has been implicated in the pathogenesis of at least some autoimmune diseases in mice, lack expression of and appear to arise independently of T-bet (123-125). Thus, several outstanding issues remain by which to translate findings with T-bet into human autoimmune diseases. Nonetheless, the accumulating data strongly support a role for T-bet in the pathogenesis of autoimmunity, and hopefully future efforts will continue to consolidate and extend the understanding of the relationships between T-bet, Th1 lineages, IFN-γ production, and autoimmune disease.

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