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T-bet promotes the accumulation of encephalitogenic Th17 cells in the CNS

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Abstract

T-bet enhances the encephalitogenicity of myelin-reactive CD4⁺ T cells, however its mechanism of action is unknown. In this study we show that T-bet confers a competitive advantage for the accumulation of IL-23 conditioned Th17 effector cells in the central nervous system (CNS). Impaired migration of T-bet deficient Th17 cells to the CNS is associated with altered expression of adhesion molecules and chemokine receptors on their cell surface. Our data suggest that therapeutic targeting of T-bet in individuals with Th17-mediated autoimmune demyelinating disease may inhibit inflammatory infiltration of the CNS and, hence, clinical exacerbations.

Graphical abstract

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Keywords

Experimental Autoimmune Encephalomyelitis; Th17 cells; T-bet; adhesion molecules; chemokine receptors

1. Introduction

Experimental autoimmune encephalomyelitis (EAE) is a multifocal demyelinating disease of the central nervous system (CNS) that has clinical and histopathological similarities to multiple sclerosis (MS). It can be induced by the adoptive transfer of myelin antigen-primed and reactivated CD4⁺ T cells into naïve syngeneic hosts. Reactivity of donor T cells against myelin antigens is necessary, but not sufficient, for EAE induction. The properties that myelin-reactive T cells must acquire during differentiation to become pathogenic is the subject of ongoing research.

T-bet (encoded by *Tbx21*), a leukocyte-specific member of the T-box family of transcription factors, has been identified as one of the non-cognate, T cell intrinsic molecules that enhance encephalitogenicity. As a master regulator of Th1 cell differentiation, it induces activation of the IFN γ gene and upregulation of the IL-12 receptor β 2 chain, while suppressing Th2 and Th17 transcriptional programs (Lazarevic et al., 2013). The importance of T-bet in EAE and MS pathogenesis was initially postulated based on the understanding that Th1 cells mediate pathological neuroinflammation (Segal, 2003, Martin and McFarland, 1995). The subsequent discovery that Th17 cells could also drive EAE initially appeared to pose a paradox. However, Th17 cells are plastic and acquire Th1-like characteristics following restimulation with IL-23 by a T-bet dependent pathway (Lee et al., 2009). In fact, fate mapping studies have shown that the majority of CD4⁺ T cells isolated from the CNS at peak EAE are Th17 cells that have upregulated T-bet and IFN γ , and downregulated ROR γ t and IL-17 (referred to as ex-Th17 cells) (Hirota et al., 2011, Kurschus et al., 2010). We have previously demonstrated that, although stable myelin-specific Th17 cell can transfer EAE independently of T-bet, they are significantly less potent than T-bet expressing exTh17 cells (Carbajal et al., 2015, Grifka-Walk et al., 2013).

Since IFN γ is dispensable for the manifestation of clinical EAE (Kroenke et al., 2008, Kroenke et al., 2010, Ferber et al., 1996), the pathway employed by T-bet to promote encephalitogenicity in exTh17 cells remains to be elucidated. Here, we investigated the mechanism of action of T-bet by comparing the fates of T-bet deficient and wild-type MOG-specific CD4⁺ Th17 cells following adoptive transfer, either together or in parallel, into RAG2^{-/-} hosts. Our findings suggest that T-bet facilitates the selective trafficking of CD4⁺ T cells to the CNS, likely by regulating cell surface expression of adhesion molecules and chemokine receptors.

2. Materials and methods

2.1. Mice

8-12-wk-old C57BL/6 CD45.1 congenic (B6.SJL-Ptprca Pepcb/BoyJ), T-bet^{-/-} and RAG2^{-/-} mice were obtained from the Jackson Laboratory and housed in microisolator cages under

specific pathogen-free conditions. T-bet^{-/-} and RAG2^{-/-} mice were subsequently bred in our facility. All animal protocols were approved by the University Committee on Use and Care of Animals.

2.2. EAE Induction

Wild-type (CD45.1+) and T-bet^{-/-} mice were immunized subcutaneously, over the flanks, with 100 µg of an immunodominant myelin oligodendrocyte glycoprotein peptide (MOG₃₅₋₅₅; Biosynthesis, Lewisville, Texas) emulsified in complete Freund's adjuvant (CFA; Difco, Detroit MI). 10-14 days later, draining lymph node cells were harvested, passed through a 70-mm cell strainer (BD Falcon) and cultured with MOG₃₅₋₅₅ (50 µg/ml) in the presence of recombinant IL-23 (8 ng/ml; R&D Systems, Minneapolis, MN), recombinant mouse IL-1α (10 ng/ml; PeproTech, Rocky Hill, NJ), and anti-IFN-γ (clone A11B11). 2×10^6 CD4⁺ T cells from each donor pool were injected, either separately or in combination, per mouse by the intraperitoneal route. The recipients were observed daily for signs of EAE as described previously (Carlson et al., 2008).

2.3. Flow Cytometry

Mice were perfused with PBS and CNS and peripheral tissues were harvested. Spinal cords and lungs were homogenized and digested with DNase I (1mg/mL; Sigma-Aldrich, St. Louis, MO) and collagenase A (2mg/mL) for 30 min at 37 °C. CNS and lung mononuclear cells were isolated over a 30/70% Percoll gradient (GE Healthcare, Little Chalfont, UK). Splenocytes and mesenteric lymph nodes were passed through a 70-µm cell strainer, ACK lysed and washed twice prior to analysis. For assessment of *in vivo* proliferation, mice were injected with BrdU (1mg) by the intraperitoneal route. BrdU incorporation was measured 14-16 hours later with a FITC BrdU Flow Kit (BD Biosciences, San Jose, CA). Apoptotic cells were detected using an Annexin V Staining Kit (ebioscience, San Diego, CA). Data were acquired on a BD FACS Canto II cytometer (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (FlowJo, LLC, Ashland, OR).

2.4 Statistical analysis

Statistical analyses were performed using GraphPad Prism software. Group differences were analyzed by unpaired, two-tailed Student's t test. For comparison of clinical courses, EAE scores from each day were analyzed using an unpaired t test, and p values were corrected for multiple comparisons using the Holm-Sidak method. P-values of 0.05 or less were considered significant.

3. Results

3.1 The clinical course of EAE induced by the co-transfer of T-bet^{-/-} and wild-type effector cells resembles the course induced by WT effectors alone

In a previous study, we found that IL-23 polarized, MOG₃₅₋₅₅-specific T cells generated from T-bet^{-/-} donors induce a delayed and mild form of EAE in comparison to the disease induced by their wild-type counterparts (Grifka-Walk et al., 2013). However, the mechanism by which T-bet enhances encephalitogenicity is unknown. We sought to compare the activity of IL-23 polarized T-bet^{-/-} and wild-type Th17 donor cells *in vivo* in the absence of

endogenous host lymphocytes. To do so, we transferred each donor population, either independently or together, into RAG2^{-/-} mice and monitored recipients for signs of neurological dysfunction on a daily basis. Consistent with our prior results, T-bet^{-/-} donor Th17 cells were slower and less potent than wild-type donor Th17 cells in inducing clinical EAE (Figure 1). The clinical course of mice that received both populations at a 1:1 ratio was indistinguishable from that of mice injected with wild-type Th17 cells alone. This experimental system provided us with the opportunity to assess the proliferation, distribution and properties of wild-type and Tbet^{-/-} autoimmune effector cells within the same host during conventional EAE.

3.2. T-bet expression confers a competitive advantage to wild-type MOG-reactive CD4⁺ T cells for accumulation in the CNS

Representative mice from the group that received equal numbers of wild-type (CD45.1) and T-bet^{-/-} (CD45.2) MOG₃₅₋₅₅-specific Th17 cells were euthanized at peak EAE to measure frequencies of each donor cell type in the CNS and peripheral tissues via flow cytometry. We typically isolated double the number of wild-type over T-bet^{-/-}CD4⁺ donor T cells from either the spinal cord or brain (Fig. 2a and b, and data not shown). Conversely, T-bet^{-/-} CD4⁺ donor T cells were more plentiful than their WT counterparts in the spleen, lungs, blood and mesenteric lymph nodes (Fig. 2a-c). Collectively, these data suggest that the frequency of T-bet^{-/-} donor cells is not globally diminished compared with co-transferred WT donor cells. Rather, their relative paucity in the CNS is specific to that tissue compartment.

3.3. CNS-infiltrating T-bet-deficient and WT donor Th17 cells proliferate, and express apoptotic markers, to a comparable degree

A skewed ratio of wild-type versus T-bet^{-/-} donor Th17 cells in the CNS could result from differences in proliferation or rates of apoptosis. We previously found that MOG-primed T-bet^{-/-} and WT CD4⁺ Th17 expand to a similar extent following rest and reactivation with antigen *in vitro* (Grifka-Walk et al., 2013). Similarly, there was no significant difference in the proliferation of CNS-infiltrating T-bet^{-/-} versus WT donor T cells *in vivo* at peak EAE as measured by BrdU incorporation (Fig. 3a). We also found a similar percentage of Annexin V positive cells among wild-type and T-bet^{-/-} donor T cells isolated from the CNS (Fig. 3b). IL-7 is a survival factor for Th17 cells (Liu et al., 2010). CNS T-bet^{-/-} donor T cells actually expressed higher levels of the IL-7R α chain (CD127) than wild-type donor T cells (Fig. 4a). Together, the latter findings make it unlikely that T-bet^{-/-} cells experience an accelerated rate of cell death post-transfer.

3.4. T-bet-deficient Th17 cells express a distinct array of homing molecules

Since we found no differences in the survival or proliferation of CNS-infiltrating T cells derived from wild-type versus T-bet^{-/-} donors, we speculated that the T-bet^{-/-} Th17 cells are relatively inefficient at trafficking to the CNS. This would be consistent with our observations that disease onset is delayed in recipients of T-bet^{-/-} donor cells alone versus recipients of wild-type donor cells alone (Fig. 1), and that T-bet^{-/-} donor T cells outnumber wild-type donor T cells in the spleens and mesenteric lymph nodes of co-transfer recipients (Fig. 3). In order to investigate the possibility that T-bet expression influences the migration patterns of myelin-reactive Th17 cells, we measured the expression of a panel of chemokine

receptors and adhesion molecules on T-bet^{-/-} and wild-type donor T cells in our co-transfer model by flow cytometric analysis. Wild-type donor T cells expressed higher levels of $\alpha 4\beta 1$ integrin and CXCR3 than Tbet^{-/-} donor T cells in the CNS and spleen, respectively (Fig. 4a and b). In contrast, T-bet^{-/-} donor cells expressed relatively high levels of $\alpha 4\beta 7$ in the spinal cord and spleen, and high levels of CCR6 in the spinal cord (Fig. 4a and b). Wild-type and T-bet^{-/-} donor cells did not differ in expression of sphingosine-1-phosphate receptors, CCR9 or the bioactive form of P-selectin ligand (data not shown).

4. Discussion

In this study we co-transferred T-bet^{-/-} and wild-type MOG-specific Th17 effectors into RAG2^{-/-} hosts and found that the ratios of the donor populations varied across tissue compartments at peak EAE. Hence, wild-type CD4⁺ T cells outnumbered T-bet^{-/-}CD4⁺ T cells in the CNS, while the reverse was the case in spleen, lungs and mesenteric lymph nodes (Fig. 2). Similarly, O'Connor and colleagues reported that T-bet deficiency impairs CNS accumulation and encephalitogenicity of IL-12 conditioned Th1 cells, and results in their retention in the spleen (O'Connor et al., 2013). In our experimental system we found no differences between the donor populations in rates of proliferation or apoptosis that could explain these imbalances, leading us to conclude that divergent migration patterns are responsible.

Consistent with their acquisition of an exTh17 phenotype, a relatively high percent of wild-type T cells in the spleens of host mice expressed CXCR3 (Figure 4b). This was not observed in the CNS (Fig. 4a), which might reflect chemokine-induced downregulation of the receptor at the site of inflammation, as previously described (Meiser et al., 2008). Ligands of CXCR3, including CXCL9 and CXCL10, are upregulated in the CNS during EAE and MS (Groom and Luster, 2011, Balashov et al., 1999, Sorensen et al., 1999), and may play a role in the preferential recruitment and/ or retention of wild-type T cells in the CNS of RAG2^{-/-} co-transfer recipients. However, the importance of CXCR3 expression for T cell accumulation in the CNS during EAE varies across models (Lalor and Segal, 2013, Sporici and Issekutz, 2010, Mony et al., 2014), and its significance in our experimental system has yet to be determined. A high percent of CNS-infiltrating wild-type T cells also expressed $\alpha 4\beta 1$ integrin, which may have facilitated their passage across the blood-brain-barrier (Fig. 4a). That adhesion molecule is critical for efficient CNS trafficking of lymphocytes during EAE and MS; blockade of $\alpha 4$ integrin reduces MS relapses by 68% (Polman et al., 2006). In a recent publication it was suggested that Th1 cells are more dependent on $\alpha 4\beta 1$ / VCAM-1 interactions for CNS infiltration than Th17 cells (Rothhammer et al., 2011). Based on our results, we speculate that the same might be true of exTh17 cells.

Conversely, a relatively high percent of T-bet^{-/-} donor T cells expressed $\alpha 4\beta 7$ integrin, correlating with their accumulation in the mesenteric lymph nodes (Fig. 2c, Fig. 4a and b). This raises the possibility that diversion of T-bet^{-/-} donor cells to the mesenteric lymph nodes, and potentially the gut associated lymphoid tissues, limits their availability for recruitment to the CNS. A minority of T-bet^{-/-} T cells in the CNS expressed CCR6, although at higher frequency than their WT counterparts (Fig. 4a). CCR6-CCL20 interactions have

been reported to drive CNS infiltration by Th17 cells during EAE (Reboldi et al., 2009, Liston et al., 2009, Villares et al., 2009). However, CCR6 is a general biomarker of Th17 cells (Yamazaki et al., 2008, Ivanov et al., 2006) and the fact that it was expressed more frequently on T-bet^{-/-} donor T cells may simply reflect their stability compared with wild-type T cells. In addition to the results presented here, T-bet expression has been shown to alter the levels of chemokine receptors and integrins on other leukocyte subsets. T-bet induces sphingosine-1-phosphate receptor 5 (S1P5) on NK cells and T cells (Jenne et al., 2009). In addition to modulating CXCR3, T-bet enhances the binding of Th1 cells to P-selectin (Lord et al., 2005). T-bet dependent induction of homing molecules is specific for different leukocyte subsets. As mentioned earlier, we found no difference in expression of S1P receptors or bioactive P-selectin ligand when comparing MOG-specific, IL-23 conditioned T-bet^{-/-} versus wild-type CD4⁺ T cells.

In contrast to the data shown in figure 1 of the current study, an independent laboratory reported that IL-23 conditioned, MOG-specific T-bet^{-/-} and wild-type CD4⁺ T cells induce EAE with comparable efficiency and severity (O'Connor et al., 2013). Furthermore, Yeh and colleagues found no defect in the migration of T-bet^{-/-} CD4⁺ T cells to the CNS in MOG-immunized mixed bone marrow chimeric mice that had been reconstituted with a combination of T-bet^{-/-} and wild-type donor cells (Yeh et al., 2014). These discrepancies may reflect the impact of environmental conditions on T cell trafficking patterns and EAE susceptibility. For example, distinctive microbiota of mouse colonies maintained in separate facilities might lead to differences in the cellular composition and cytokine profile of intestine-draining lymph nodes during homeostasis (Diehl et al., 2013). This, in turn, could determine the extent to which donor T cells are driven to, and retained in, the mesenteric lymph nodes. Methodological details may further contribute to the disparate findings. O'Connor and colleagues inject twice the number of donor T cells than we use in adoptive transfer experiments (O'Connor et al., 2013). An increased number of donor T cells may overcome obstacles to CNS accumulation imposed by T-bet deficiency. Yeh and colleagues monitored the migration of T-bet^{-/-} and wild-type CD4⁺ T cells in actively immunized chimeric mice that were exposed to *Mycobacteria tuberculosis* and Bordetella pertussis toxin (Yeh et al., 2014). The pro-inflammatory effects of such stimuli on the T cells themselves and/or on the cerebrovascular endothelium might compensate for the altered pattern of homing molecules on T-bet^{-/-} versus wild-type T cells.

In summary, we have found that expression of T-bet influences the pattern of homing molecules expressed on Th17 polarized encephalitogenic T cells and promotes their accumulation in the CNS. Our results contribute to a growing body of data endorsing T-bet as a general biomarker and therapeutic target in MS, irrespective of the Th lineage of the autoimmune effector cell population driving lesion formation (Yang et al., 2009). In contrast, EAE experiments indicate that therapeutic responsiveness to α 4 integrin- and chemokine receptor- blocking agents might vary depending on the cytokine profile of the dominant encephalitogenic effectors (Rothhammer et al., 2011, Stoolman et al., 2014, Kroenke et al., 2010). Only future experiments will establish the causal relationship between T-bet mediated effects on the expression of specific homing molecules and encephalitogenicity, and determine whether these observations in the EAE model are translatable to the clinic.

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References

- Balashov KE, Rottman JB, Weiner HL, Hancock WW. CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1 α and IP-10 are expressed in demyelinating brain lesions. *Proc Natl Acad Sci U S A*. 1999; 96:6873–8. [PubMed: 10359806]
- Carbajal KS, Mironova Y, Ulrich-Lewis JT, Kulkarni D, Grifka-Walk HM, Huber AK, Shrager P, Giger RJ, Segal BM. Th Cell Diversity in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis. *J Immunol*. 2015; 195:2552–9. [PubMed: 26238492]
- Carlson T, Kroenke M, Rao P, Lane TE, Segal B. The Th17-ELR+ CXC chemokine pathway is essential for the development of central nervous system autoimmune disease. *J Exp Med*. 2008; 205:811–23. [PubMed: 18347102]
- Diehl GE, Longman RS, Zhang JX, Breart B, Galan C, Cuesta A, Schwab SR, Littman DR. Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX(3)CR1(hi) cells. *Nature*. 2013; 494:116–20. [PubMed: 23334413]
- Ferber IA, Brocke S, Taylor-Edwards C, Ridgway W, Dinisco C, Steinman L, Dalton D, Fathman CG. Mice with a disrupted IFN- γ gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). *J Immunol*. 1996; 156:5–7. [PubMed: 8598493]
- Grifka-Walk HM, Lalor SJ, Segal BM. Highly polarized Th17 cells induce EAE via a T-bet independent mechanism. *Eur J Immunol*. 2013; 43:2824–31. [PubMed: 23878008]
- Groom JR, Luster AD. CXCR3 in T cell function. *Exp Cell Res*. 2011; 317:620–31. [PubMed: 21376175]
- Hirota K, Duarte JH, Veldhoen M, Hornsby E, Li Y, Cua DJ, Ahlfors H, Wilhelm C, Tolaini M, Menzel U, Garefalaki A, Potocnik AJ, Stockinger B. Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat Immunol*. 2011; 12:255–63. [PubMed: 21278737]
- Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. The orphan nuclear receptor ROR γ directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell*. 2006; 126:1121–33. [PubMed: 16990136]
- Jenne CN, Enders A, Rivera R, Watson SR, Bankovich AJ, Pereira JP, Xu Y, Roots CM, Beilke JN, Banerjee A, Reiner SL, Miller SA, Weinmann AS, Goodnow CC, Lanier LL, Cyster JG, Chun J. T-bet-dependent SIP5 expression in NK cells promotes egress from lymph nodes and bone marrow. *J Exp Med*. 2009; 206:2469–81. [PubMed: 19808259]
- Kroenke MA, Carlson TJ, Andjelkovic AV, Segal BM. IL-12- and IL-23-modulated T cells induce distinct types of EAE based on histology, CNS chemokine profile, and response to cytokine inhibition. *J Exp Med*. 2008; 205:1535–41. [PubMed: 18573909]
- Kroenke MA, Chensue SW, Segal BM. EAE mediated by a non-IFN- γ /non-IL-17 pathway. *Eur J Immunol*. 2010; 40:2340–8. [PubMed: 20540117]
- Kurschus FC, Croxford AL, Heinen AP, Wörtge S, Ielo D, Waisman A. Genetic proof for the transient nature of the Th17 phenotype. *Eur J Immunol*. 2010; 40:3336–46. [PubMed: 21110317]
- Lalor SJ, Segal BM. Th1-mediated experimental autoimmune encephalomyelitis is CXCR3 independent. *Eur J Immunol*. 2013; 43:2866–74. [PubMed: 23873018]
- Lazarevic V, Glimcher LH, Lord GM. T-bet: a bridge between innate and adaptive immunity. *Nat Rev Immunol*. 2013; 13:777–89. [PubMed: 24113868]
- Lee YK, Turner H, Maynard CL, Oliver JR, Chen D, Elson CO, Weaver CT. Late developmental plasticity in the T helper 17 lineage. *Immunity*. 2009; 30:92–107. [PubMed: 19119024]
- Liu X, Leung S, Wang C, Tan Z, Wang J, Guo TB, Fang L, Zhao Y, Wan B, Qin X, Lu L, Li R, Pan H, Song M, Liu A, Hong J, Lu H, Zhang JZ. Crucial role of interleukin-7 in T helper type 17 survival and expansion in autoimmune disease. *Nat Med*. 2010; 16:191–7. [PubMed: 20062065]

- Lord GM, Rao RM, Choe H, Sullivan BM, Lichtman AH, Luscinskas FW, Glimcher LH. T-bet is required for optimal proinflammatory CD4⁺ T-cell trafficking. *Blood*. 2005; 106:3432–9. [PubMed: 16014561]
- Martin R, Mcfarland HF. Immunological aspects of experimental allergic encephalomyelitis and multiple sclerosis. *Crit Rev Clin Lab Sci*. 1995; 32:121–82. [PubMed: 7598789]
- Meiser A, Mueller A, Wise EL, McDonagh EM, Petit SJ, Saran N, Clark PC, Williams TJ, Pease JE. The chemokine receptor CXCR3 is degraded following internalization and is replenished at the cell surface by de novo synthesis of receptor. *J Immunol*. 2008; 180:6713–24. [PubMed: 18453591]
- Mony JT, Khorrooshi R, Owens T. MOG extracellular domain (p1-125) triggers elevated frequency of CXCR3⁺ CD4⁺ Th1 cells in the CNS of mice and induces greater incidence of severe EAE. *Mult Scler*. 2014; 20:1312–21. [PubMed: 24552747]
- O'connor RA, Cambrook H, Huettner K, Anderton SM. T-bet is essential for Th1-mediated, but not Th17-mediated, CNS autoimmune disease. *Eur J Immunol*. 2013; 43:2818–23. [PubMed: 23878019]
- Polman CH, O'connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, Phillips JT, Lublin FD, Giovannoni G, Wajgt A, Toal M, Lynn F, Panzara MA, Sandroock AW. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med*. 2006; 354:899–910. [PubMed: 16510744]
- Rothhammer V, Heink S, Petermann F, Srivastava R, Claussen MC, Hemmer B, Korn T. Th17 lymphocytes traffic to the central nervous system independently of $\alpha 4$ integrin expression during EAE. *J Exp Med*. 2011; 208:2465–76. [PubMed: 22025301]
- Segal BM. Experimental autoimmune encephalomyelitis: cytokines, effector T cells, and antigen-presenting cells in a prototypical Th1-mediated autoimmune disease. *Curr Allergy Asthma Rep*. 2003; 3:86–93. [PubMed: 12543000]
- Sorensen TL, Tani M, Jensen J, Pierce V, Lucchinetti C, Folcik VA, Qin S, Rottman J, Sellebjerg F, Strieter RM, Frederiksen JL, Ransohoff RM. Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J Clin Invest*. 1999; 103:807–15. [PubMed: 10079101]
- Sporici R, Issekutz TB. CXCR3 blockade inhibits T-cell migration into the CNS during EAE and prevents development of adoptively transferred, but not actively induced, disease. *Eur J Immunol*. 2010; 40:2751–61. [PubMed: 21038468]
- Stoolman JS, Duncker PC, Huber AK, Segal BM. Site-specific chemokine expression regulates central nervous system inflammation and determines clinical phenotype in autoimmune encephalomyelitis. *J Immunol*. 2014; 193:564–70. [PubMed: 24928987]
- Yamazaki T, Yang XO, Chung Y, Fukunaga A, Nurieva R, Pappu B, Martin-Orozco N, Kang HS, Ma L, Panopoulos AD, Craig S, Watowich SS, Jetten AM, Tian Q, Dong C. CCR6 regulates the migration of inflammatory and regulatory T cells. *J Immunol*. 2008; 181:8391–401. [PubMed: 19050256]
- Yang Y, Weiner J, Liu Y, Smith AJ, Huss DJ, Winger R, Peng H, Cravens PD, Racke MK, Lovett-Racke AE. T-bet is essential for encephalitogenicity of both Th1 and Th17 cells. *J Exp Med*. 2009; 206:1549–64. [PubMed: 19546248]
- Yeh WI, McWilliams IL, Harrington LE. IFN γ inhibits Th17 differentiation and function via Tbet-dependent and Tbet-independent mechanisms. *J Neuroimmunol*. 2014; 267:20–7. [PubMed: 24369297]

Abbreviations

CNS	central nervous system
EAE	experimental autoimmune encephalomyelitis
MOG₃₅₋₅₅	myelin oligodendrocyte glycoprotein peptide 35-55

MS multiple sclerosis

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Highlights

- The mechanism by which T-bet enhances Th17 cell encephalitogenicity is investigated
- T-bet expression promotes the accumulation of myelin-reactive T cells in the CNS
- T-bet modulates the pattern of homing molecules on myelin-reactive CD4⁺ T cells
- T-bet might serve as a therapeutic target to block CNS homing of Th17 and Th1 cells

Figure 1. The clinical course of EAE exhibited by RAG2^{-/-} mice injected with a combination of T-bet^{-/-} and wild-type myelin-reactive Th17 cells resembles the clinical course induced by transfer of wild-type Th17 effectors alone

RAG2^{-/-} mice were injected i.p. with IL-23 conditioned, MOG₃₅₋₅₅ specific Th17 cells derived from primed WT or Tbet^{-/-} donors, either separately or in combination (2×10^6 CD4⁺ T cells from each donor pool/ mouse). The recipients were monitored on a daily basis for signs of neurological dysfunction and were rated on a scale of 1-5. The data shown is representative of five experiments with 4-6 mice per group. *p-value < .05

Figure 2. Wild-type donor T cells outnumber T-bet^{-/-} donor T cells in the CNS of co-transfer recipients, while the reverse is true in the spleen, lungs blood and mesenteric lymph nodes RAG-2 KO mice were injected i.p. with a 1:1 combination of wild-type (CD45.1) and T-bet^{-/-} (CD45.2) MOG-specificTh17 cells. Spinal cord mononuclear cells and splenocytes (a and b), as well as mesenteric lymph node cells (MLN), lung and peripheral blood mononuclear cells (a and c), were harvested at peak EAE. The frequencies of CD4⁺ T cells derived from each donor pool were measured via flow cytometric analysis. (a) Data shown is representative of 1 mouse out of 15 with similar findings. (b) Data are representative of 4 experiments with n=3-5 mice/ group/ experiment. *p-value < .05

Figure 3. T-bet^{-/-} and wild-type donor Th17 cells exhibit similar rates of proliferation and apoptosis in the CNS of co-transfer recipients

Spinal cord mononuclear cells were isolated from RAG-2 KO hosts at peak EAE following the adoptive transfer of a 1:1 combination of wild-type (CD45.1) and T-bet^{-/-} (CD45.2) MOG-specific Th17 cells. Mice were injected with BrdU 14 hours prior to euthanasia. (a) Proliferation of CD45.1- and CD45.2- gated CD4⁺ T cells was assessed by incorporation of BrdU. (b) Apoptotic cells were identified and quantified by staining for the surface marker Annexin V. Data are representative of 3 experiments with n=3-6 mice/ group/ experiment.

Figure 4. Adhesion molecule and chemokine receptor profiles differ on T-bet^{-/-} and wild-type donor Th17 cells

Wild-type and T-bet^{-/-} MOG-specific Th17 cells were co-transferred into RAG2^{-/-} hosts, as described in Figure 2. At peak disease, mononuclear cells were isolated from the spinal cord (a) and spleen (b) and CD4⁺ T cells were analyzed for the expression of a panel of homing molecules via flow cytometry. Data is pooled from 2-3 experiments with at least 4 mice/group. *p-value < .05