

Dendritic cell expression of the transcription factor T-bet regulates mast cell progenitor homing to mucosal tissue

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The transcription factor T-bet was identified in CD4⁺ T cells, and it controls interferon γ production and T helper type 1 cell differentiation. T-bet is expressed in certain other leukocytes, and we recently showed (Lord, G.M., R.M. Rao, H. Choe, B.M. Sullivan, A.H. Lichtman, F.W. Luscinskas, and L.H. Glimcher. 2005. *Blood*. 106:3432–3439) that it regulates T cell trafficking. We examined whether T-bet influences homing of mast cell progenitors (MCp) to peripheral tissues. Surprisingly, we found that MCp homing to the lung or small intestine in T-bet^{-/-} mice is reduced. This is reproduced in adhesion studies using bone marrow-derived MCs (BMMCs) from T-bet^{-/-} mice, which showed diminished adhesion to mucosal addressin cellular adhesion molecule-1 (MAdCAM-1) and vascular cell adhesion molecule-1 (VCAM-1), endothelial ligands required for MCp intestinal homing. MCp, their precursors, and BMMCs do not express T-bet, suggesting that T-bet plays an indirect role in homing. However, adoptive transfer experiments revealed that T-bet expression by BM cells is required for MCp homing to the intestine. Furthermore, transfer of WT BM-derived dendritic cells (DCs) to T-bet^{-/-} mice restores normal MCp intestinal homing in vivo and MCp adhesion to MAdCAM-1 and VCAM-1 in vitro. Nonetheless, T-bet^{-/-} mice respond vigorously to intestinal infection with *Trichinella spiralis*, eliminating a role for T-bet in MC recruitment to sites of infection and their activation and function. Therefore, remarkably, T-bet expression by DCs indirectly controls MCp homing to mucosal tissues.

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Abbreviations used: BaP, basophil progenitor; BMDC, BM-derived DC; BMCP, basophil MC progenitor; BMMC, BM-derived MC; CXCL and CXCR, CXC chemokine ligand and receptor, respectively; GMP, granulocyte monocyte progenitor; KC, keratinocyte-derived chemokine; MAdCAM-1, mucosal addressin cellular adhesion molecule-1; MC, mast cell; MCp, MC progenitor(s); MNC, mononuclear cell; PMC, peritoneal MC; SCF, stem cell factor; SDF-1 α , stromal cell-derived factor 1 α ; SIBR, sublethally irradiated BM reconstituted; Tc1, T cytotoxic type 1; VCAM-1, vascular cell adhesion molecule-1.

Mast cell progenitors (MCp) are derived from the hematopoietic stem cells present in the BM and develop along the myelomonocytic pathway. The exact pathway is still somewhat controversial, described in C57BL/6 mice as either via a basophil/MC bipotent progenitor found in the spleen or derived via an alternate path in BM (1, 2). Regardless, MCp transit through the circulation, immigrate into peripheral tissues, and mature into tissue MCs under the influence of the local microenvironment (3–5). Within the peripheral tissues, MCs exhibit different phenotypes according to their tissue location, with distinctive histochemical staining characteristics and differences in the protease

and proteoglycan content of their secretory granules (6–8). They are broadly classified as connective tissue-type MCs, which are found in connective tissues throughout the body, often adjacent to blood microvessels and in the peritoneal cavity of mice and rats, where they have been clearly implicated in the innate immune response to bacteria (9, 10), and mucosal MCs, which appear in mucosal regions within the lung and the intestine. Both connective tissue-type MCs and mucosal MCs act as effector cells during inflammation and play an important role in allergic inflammation of the airways and in the intestinal immune response to helminth infection (11, 12).

Previous studies have proposed a model of basal homing of MCp to the intestine (13, 14). In this tissue, MCp homing is a constitutive,

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The online version of this article contains supplemental material.

dynamic process that appears to be independent of adaptive immunity. The model envisions the regulation of MCp trafficking at the level of integrin CD49d/ β 7 interactions, with its counterreceptors mucosal addressin cellular adhesion molecule-1 (MAdCAM-1) and vascular cell adhesion molecule-1 (VCAM-1) expressed by vascular endothelium (13). Furthermore, the CXC chemokine receptor (CXCR) 2 provides promigratory signals critical to MCp trafficking to the intestine (14, 15).

T-bet is a T-box transcription factor essential to the generation and function of Th1 cells, and its deficiency leads to predominantly Th2-mediated responses (16). T-bet expression has been identified in other leukocytes, including NK cells, DCs, and CD8⁺ cells, where it directs strong transactivation of IFN- γ transcripts (17–19). We previously identified a critical role for T-bet in CD4⁺ T cell trafficking via its ability to regulate selectin-dependent adhesion and surface expression of CXCR3 (20), and more recently T-bet has also been identified as a regulator of NKT cell trafficking (21). We do not know of any studies of the role of T-bet in trafficking of other leukocyte types. In this paper, we investigate the role of T-bet in MCp trafficking and report the unexpected finding that MCp homing to the mucosal tissues of the lung and the intestine is impaired in T-bet^{-/-} mice. This striking observation led us to investigate the hypothesis that T-bet expression by MCp controls recruitment and homing to mucosal tissues. We found that there is no expression of T-bet in MCs at several stages of development. Yet, adoptive transfer of T-bet-deficient BM into sublethally irradiated WT mice still results in impaired homing, demonstrating for the first time that another radiation sensitive BM-derived cell plays an essential role in MCp homing to mucosal tissues. Furthermore, we have shown that this BM-derived cell is most likely a DC, because adoptive transfer of WT BM-derived DCs (BMDCs) into T-bet^{-/-} mice reconstitutes MCp intestinal homing, and co-culture of WT BMDCs with T-bet^{-/-} BM-derived MCs (BMMCs) restored the binding of BMMCs to MAdCAM-1 and VCAM-1 in vitro to normal levels.

RESULTS

MCp homing to the mucosal tissues is impaired in T-bet^{-/-} mice

We have recently shown that CD4⁺ T cell trafficking in T-bet^{-/-} mice is dramatically reduced (20). Our interest in MC trafficking then led us to investigate whether MCp homing to the mucosal tissues is impaired in T-bet^{-/-} mice. We measured the total number of MCp in the intestine and the lung, as well as in the BM and the spleen. T-bet deficiency resulted in a nearly total loss of the MCp reservoir normally present in the small intestine and in a significant decrease in the lung, whereas MCp numbers in the spleen and the BM were comparable to those in WT mice. This finding was consistently observed in both BALB/c (Fig. 1 A) and C57BL/6 (Fig. 1 B) mice strains lacking T-bet. Consistent with previous findings (13), the number of MCp found in the tissues examined in the BALB/c strain was greater than

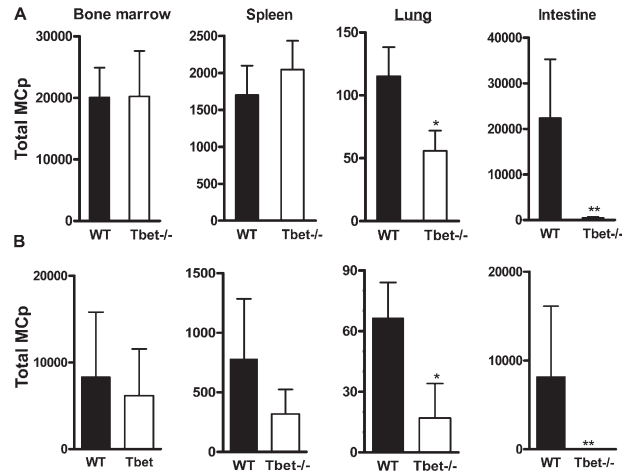


Figure 1. Selective loss of MCp in intestine and lung of T-bet^{-/-} mice. Total numbers of MCp in the intestine, lung, spleen, and BM (one femur) of WT (shaded bars) and T-bet^{-/-} (open bars) mice were determined by limiting dilution MCp assay. Experiments were performed in the BALB/c (A) or C57BL/6 (B) mouse strains. Values are the mean \pm SEM for five separate experiments for BALB/c and two separate experiments for C57BL/6 mice. *, $P < 0.01$; or **, $P < 0.001$ for T-bet relative to WT mice.

that in C57/BL6 mice. Histochemical analysis of tissue sections prepared from intestine and spleen of adult, age-matched T-bet^{-/-} and WT mice also showed that fewer MCs were detected in the intestine of T bet^{-/-} compared with WT mice. In the spleen, which showed no differences in MCp, MCs were also diminished in the T-bet^{-/-} mice (Fig. 2). These data indicate that under basal conditions in the absence of T-bet there is a tissue-specific homing defect in MCp homing to mucosal tissues.

T-bet^{-/-} MCs exhibit impaired binding to VCAM-1 and MAdCAM-1 under flow conditions

Having observed a lack of homing of MCp to the intestine, which is the largest reservoir of committed MCp in the mouse (22), we postulated that T-bet^{-/-} MCp might exhibit diminished α 4 integrin-dependent adhesive interactions with endothelial cell adhesion molecules VCAM-1 and MAdCAM-1, because these endothelial ligands have previously been shown to be essential for MCp homing to the intestine (13). To assess this further, we measured adhesion of WT and T-bet^{-/-} BMMCs to immobilized, recombinant mouse VCAM-1 or MAdCAM-1 under conditions of physiological laminar shear flow. Because the number of MCp in the BM or in circulating blood of adult mice is low (1, 23), it is impossible to isolate quantities sufficient to perform these adhesion assays. Therefore, we and others (24, 25) have used BMMCs generated in vitro as a surrogate model to study the different properties and adhesion pathways of MCs. T-bet^{-/-} BMMCs displayed markedly reduced binding to both VCAM-1 and MAdCAM-1 across a range of shear flow (Fig. 3). Incubation of WT and T-bet^{-/-} BMMCs with blocking

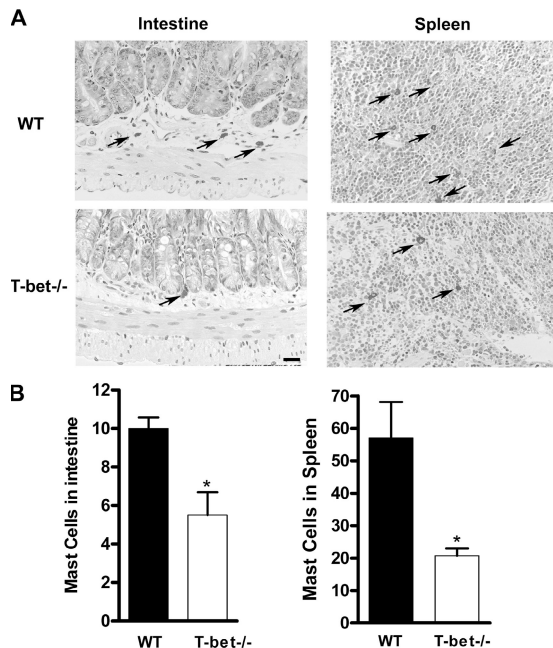


Figure 2. Histochemical analysis of tissue sections prepared from the intestine and spleen of WT and T-bet^{-/-} mice. (A) Sections of small intestine or spleen from WT or T-bet^{-/-} BALB/c mice were incubated with chloroacetate esterase substrate to identify MCs (indicated by the arrows). 1 representative field out of 20 fields analyzed per mouse is shown. Bar, 200 μ m. (B) Quantification of MCs per cross section of tissue. Values are the mean \pm SEM for three BALB/c and four T-bet^{-/-} mice and are the total number of MCs per cross section of jejunum or spleen. *, $P < 0.05$.

antibodies against the $\alpha 4$ integrin chain abolished BMMC binding to VCAM-1 (unpublished data). These data suggest that T-bet affects the adhesive interactions between MCp and intestinal endothelial VCAM-1 and MAdCAM-1. Because MCp express receptors for keratinocyte-derived chemokine (KC/CXC chemokine ligand [CXCL] 1) and chemokines coimmobilized with adhesion molecules are known to trigger leukocyte stable arrest under shear flow conditions (26, 27), we examined whether this or other chemokines (stromal cell-derived factor 1 α [SDF-1 α]) coimmobilized with VCAM-1 or MAdCAM-1 would overcome the adhesion defect. Neither WT nor T-bet^{-/-} MCp showed an increase in adhesion (Fig. S1, A–C, available at <http://www.jem.org/cgi/content/full/jem.20060626/DC1>) nor resistance to detachment after adhesion to MAdCAM-1 (Fig. S1 D), suggesting that MCs and T cells do not exhibit the same recognition response in this assay. We next tested whether surface expression of $\alpha 4$ integrins (CD49d), which bind to VCAM-1 and MAdCAM-1, were altered. FACS analysis revealed very low levels of surface expression of $\alpha 4$ and $\beta 7$ integrins and the chemokine receptor CXCR2 in BMMCs generated from either WT or T-bet^{-/-} mice (Fig. S2), in agreement with previous reports (24, 28, 29). Thus, the defect is not explained by a loss of $\alpha 4\beta 7$ integrin expression or a lack of chemokine-induced arrest under flow conditions.

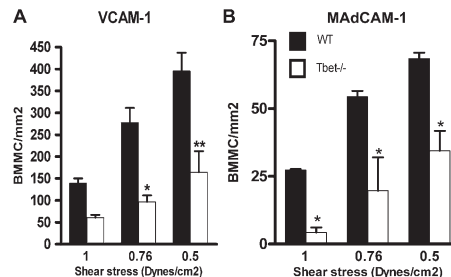


Figure 3. BMMCs have a defect in adhesion to VCAM-1 or MAdCAM-1 under conditions of shear flow.

BMMCs derived from WT BM (shaded bars) or T-bet^{-/-} BM (open bars) were drawn across immobilized VCAM-1 (A) or MAdCAM-1 (B) across a range of estimated shear stress, and adhesive interactions were recorded. BMMC interactions were determined at the end of each shear stress. Values reflect the total number of adherent and rolling cells over time during the range of shear stress examined. Values are the mean \pm SEM from five separate experiments. *, $P < 0.05$; or **, $P < 0.01$ for T-bet relative to WT mice.

T-bet is not expressed in BMMCs or MCp

Because this defect in adhesion suggests a direct role for T-bet in controlling MCp adhesion, the next set of studies determined T-bet expression in cultured BMMCs or other cells along the developmental pathway that we have previously described (1). Western blot analysis revealed that T-bet is not expressed in either T-bet^{-/-} or WT BMMCs under basal conditions or after stimulation with nonphysiologic (PMA + ionomycin) or physiologic (LPS and IgE cross-linking) stimuli (Fig. 4 A). In contrast, T-bet was easily detected in T cytotoxic type 1 cells (Tc1), analyzed in parallel as a positive control for T-bet expression.

We next considered the possibility that T-bet was not expressed in BMMCs because of a loss of expression under the culture conditions required for successful *in vitro* differentiation. Because previous studies have reported that the Lin⁻/c-kit⁺/Sca1⁻ CD34⁺ cell population from adult BM gives rise to MCs (1, 30), we used a high-speed FACS approach on the BM of WT adult mice to isolate these cells and probe the isolated cells for T-bet expression by quantitative real-time PCR, using Th1 cells as a positive control (Fig. 4 B). T-bet was present in the WT BM, but at very low levels. The cell populations sorted from the BM as Lin⁻ or Lin⁺ cells, which correspond to uncommitted or committed BM progenitors (of MCs and other lineages), respectively, also expressed very low levels of T-bet (Fig. 4 B, inset plotted with expanded y axis). The expression of T-bet by whole BM cells is most likely caused by Lin⁺ cells, because T-bet was below detectable levels in the population (Lin⁻) that leads to MCp production. We also tested for the expression of T-bet in other hematopoietic progenitors present in the BM and committed to the myeloid pathway, such as the common myeloid progenitor, the granulocyte monocyte progenitor (GMP), the recently described basophil MC progenitor (BMCP) (1), and the basophil progenitor (BaP), as well as in MCp derived *in vitro* from GMP and mature peritoneal MCs. None of these cells, progenitors or mature MCs,

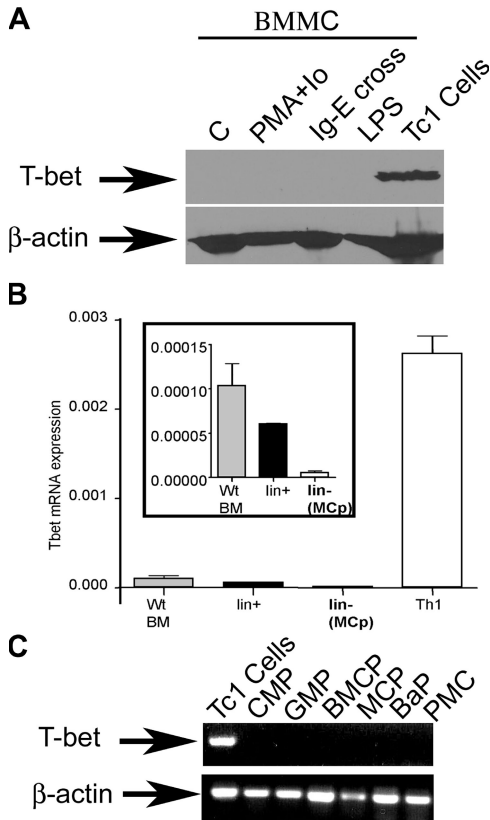


Figure 4. Analysis of T-bet expression in BMMCs and in FACS-sorted MCP and their precursors. (A) BMMCs in vitro derived from WT-BALB/c BM were stimulated as indicated in Materials and methods, and cell lysates were subjected to Western blot analysis for T-bet and β -actin. One representative experiment is shown. (B) Quantitative real-time PCR analysis of mRNA levels of T-bet in FACS-sorted BM MCP, myeloid progenitors, and Th1 cells. Results of real-time PCR are normalized to β -actin and are expressed as the mean \pm SEM for two independent experiments. (C) RT-PCR analysis of T-bet mRNA levels in purified progenitor populations; β -actin is also shown as loading control.

expressed T-bet. In contrast, T bet was easily detected in positive-control Th1 cells (Fig. 4 C). We conclude that T-bet is not expressed in committed MCP, its immediate precursors, or BMMCs derived in vitro, and hence postulate that T-bet plays an indirect role in the homing of MCP to the mucosal tissues.

Reconstitution of MCP in sublethally irradiated BM reconstituted (SIBR) mice with BM cells from T-bet^{-/-} or BALB/c mice

In view of the inability of our assays to detect T-bet expression in MCP or cultured BMMCs, we were forced by the observed striking defect of MCP pools in certain mucosal tissues of T-bet-null mice to pursue other approaches to understanding how T-bet exerts this effect. To define whether the observed homing defect was due exclusively to a defect in the T-bet^{-/-} BM (including MCP or other BM precursors expressing T-bet) or, alternatively, to a defect of the local intestinal microenvironment by itself, we depleted the

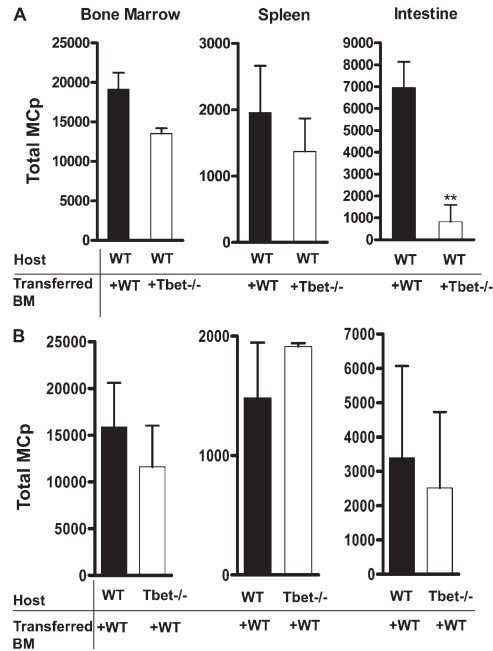


Figure 5. Tissue MCP reconstitution in SIBR BALB/c mice receiving T-bet^{-/-} or WT BM and in SIBR T-bet^{-/-} mice receiving WT BM. (A) Total number of MCP in the intestine, the BM (one femur), and the spleen in SIBR BALB/c mice receiving BALB/c BM (shaded bars) or T-bet^{-/-} BM (open bars) were assessed in parallel by a MCP limiting dilution assay. Values are the mean \pm SEM of five separate experiments. **, P < 0.001 relative to WT BM reconstituted mice. (B) Total number of MCP in the intestine, the BM, and the spleen in SIBR T-bet mice receiving WT BM (open bars). SIBR WT mice receiving WT BM were used as controls (shaded bars) and evaluated in parallel as in A. Values are the mean \pm SEM of three separate experiments.

hematopoietic precursors in WT mice by sublethal irradiation and then reconstituted the animals by adoptive transfer of either WT or T-bet^{-/-} BM cells (SIBR). The number of MCP found in the intestine of WT-SIBR mice reconstituted with T-bet^{-/-} BM was very low compared with that in WT-SIBR mice reconstituted with WT BM. In contrast, the numbers of MCP in the spleen and BM were not significantly different in WT mice reconstituted with T bet^{-/-} BM from those reconstituted with WT BM. (Fig. 5 A). On the other hand, sublethally irradiated T-bet^{-/-} mice reconstituted with BM from WT mice showed total reconstitution of intestinal MCP, as well as of BM and spleen MCP (Fig. 5 B). These results demonstrate that the MCP that arise from the T bet^{-/-} BM show a defect in homing to the intestine despite normal numbers of precursors in the spleen or BM, whereas the MCP arising from the WT BM and transferred into a T-bet^{-/-} host show adequate tissue homing of MCP, including homing to the intestine. These data implicate a defect or loss of T-bet-expressing cells in the BM compartment that is responsible for the lack of MCP adhesion and intestinal-specific homing in the T-bet^{-/-} mice and rule out the possibility of a defect in the intestinal compartment of T-bet^{-/-} mice.

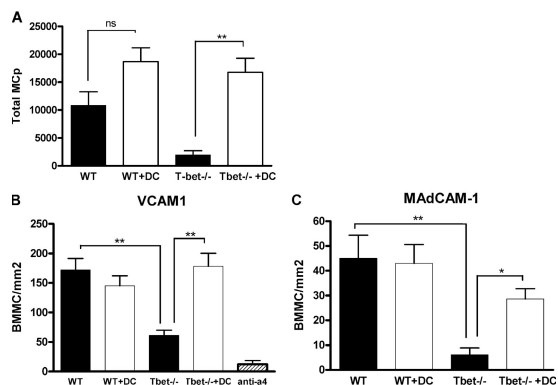


Figure 6. BMDCs induce recovery of T-bet^{-/-} intestinal MCp in vivo and adhesion of T-bet^{-/-} BMMCs to VCAM 1 and MAdCAM 1 under conditions of shear flow in vitro. (A) Total number of MCp in the intestine of WT BALB/c (shaded bars) or T-bet^{-/-} (open bars) mice receiving WT DCs was assessed in parallel by MCp limiting dilution assay. Values are the mean ± SEM of two separate experiments using two mice per group. **, P < 0.01. (B and C) BMMCs derived from WT BM or T-bet^{-/-} BM^{-/-} (shaded bars) mice cultured in the presence or absence of BMDCs (open bars) were drawn across immobilized VCAM-1 (B) or MAdCAM-1 (C) at a shear stress of 0.76 dynes/cm², and adhesive interactions were recorded. Values reflect the total number of adherent and rolling cells. Values are the mean ± SEM of two separate experiments. **, P < 0.01.

T-bet expressed in BMDCs regulates MCp intestinal homing via VCAM-1 and MAdCAM-1 ligands

Because the findings in Fig. 5 demonstrated that a defect in the BM compartment of T-bet^{-/-} mice is responsible for the lack of MCp homing to the intestine, we performed experiments to identify the BM cell type responsible. Based on the literature (18), DCs are good candidates because their development, differentiation, and activation is independent of T-bet, but DCs can up-regulate T-bet and secrete IFN- γ (18). We derived DCs from WT BM by culture in GM-CSF for 7 d and transferred these BMDCs into WT or T-bet^{-/-} mice. Strikingly, T-bet^{-/-} mice given WT BMDCs showed levels of MCp in the intestine comparable to WT mice given BMDCs in parallel when evaluated 14 d later. Transfer of BMDCs into WT mice did not significantly increase the number of MCp (Fig. 6 A). In contrast, T-bet^{-/-} animals that did not receive BMDCs exhibited a dramatic reduction of MCp in the intestine as compared with WT mice.

The role of T-bet⁺ BMDCs in controlling MCp intestinal homing was further investigated by testing whether co-culture of WT BMDCs with T-bet^{-/-} BMMCs restored T-bet BMMC binding to VCAM-1 and MAdCAM-1. Indeed, a 6-h co-culture of T-bet^{-/-} BMMCs with WT BMDCs completely rescued binding to VCAM-1 and MAdCAM-1, as these cells exhibited no binding under conditions of shear flow that was comparable to WT BMMCs (Fig. 6, B and C). BMMC adhesion was abolished by anti- $\alpha 4$ integrin antibody. These data strongly suggest that T-bet in the DCs plays a role in homing of MCp to the intestine by regulating the $\alpha 4\beta 7$ -dependent binding to the endothelial cell ligands

VCAM-1 and MAdCAM-1, which are necessary for the appropriate homing of MCp to the intestine (13).

T-bet^{-/-} mice are able to mount an immune response to *Trichinella spiralis* infection

Infection with *T. spiralis* stimulates a large increase in the number of MCs residing in the small intestine (8). The rapid expulsion of *T. spiralis* is associated with and dependent on the pronounced mastocytosis, which in turn is mediated by a Th2-type response (31–34). Lack of the intestinal MCs and MCp in $\beta 7$ integrin-deficient mice resulted in a delayed MC hyperplasia and delayed rejection of the *T. spiralis* from infected mice (35). Thus, we assessed the intestinal MC response of WT and T-bet^{-/-} mice to infection with *T. spiralis*. Despite the low number of resident MCs and reduced MCp reservoir in the intestine of T-bet^{-/-} mice, a robust mucosal MC response was noted in the T-bet-deficient mice. The response was even greater than that observed in WT mice infected in parallel and resulted in an enhanced elimination of the worms from the intestine (Fig. 7) regardless of the strain background. The enhanced clearance of infection was observed with T-bet^{-/-} mice in a Th2 cell-type background strain (Fig. 7 A, BALB/c mice) and with T-bet^{-/-} mice in a Th1 cell-type background strain (Fig. 7 B, C57BL/6 mice). The local MC hyperplasia in response to the infection was also markedly increased in the T-bet^{-/-} mice, even more so than in WT mice (Fig. 7, C and D). These results are consistent with the view that the T-bet-null mouse is polarized toward the induction of a Th2 cell inflammatory milieu (19), and *T. spiralis*, which provides a potent stimulus, provokes a more vigorous MC proliferation response to this pathogen.

DISCUSSION

We report on the unexpected and novel finding that the transcription factor T-bet expressed in DCs indirectly regulates MCp homing to mucosal tissues. The involvement of T-bet in Th1 T cell polarization, IFN- γ production by DCs, and terminal maturation of NK cells has been documented (16–19), whereas the role for T-bet in leukocyte trafficking is an emerging area. Recent reports showed that T-bet was necessary for trafficking of CD4⁺ T cells (20), CD8⁺ T cells (36), and NKT cells (21) to sites of immune reactions. Our interest in understanding the factors that control MC trafficking (1, 3) led us to study the effect of T-bet deficiency on MCp homing to tissues.

The initial observation of a dramatically lower number of MCp in mucosal tissues in T-bet^{-/-} animals (Fig. 1), specifically in the small intestine as measured by limiting dilution bioassay, was very surprising. That this reduction in MCp was physiologically relevant was corroborated by the observed 50–70% reduction in mature tissue MCs, as detected by histological analysis of the small intestine and spleen (Fig. 2). As some mature MCs in the spleen have been shown to be derived from the intestine, at least after infection with *T. spiralis* (37), the decrease in splenic MC numbers could reflect the decreased numbers of intestinal MCs in the T-bet^{-/-} mice or a maturational defect within the spleen.

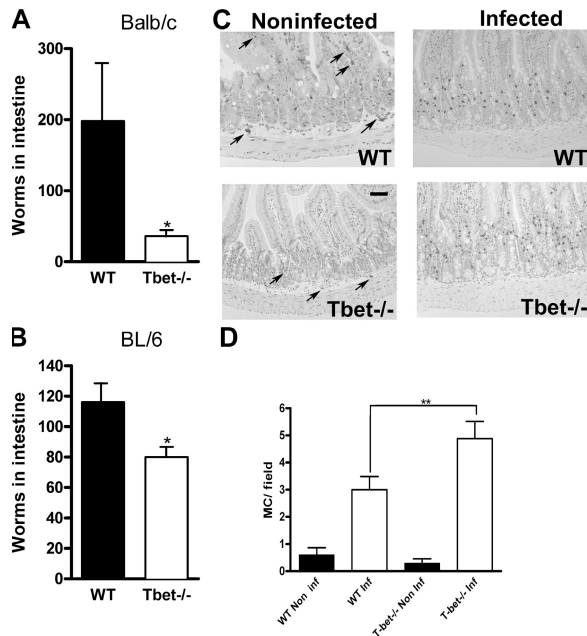


Figure 7. Time course of worm rejection and histological analysis of WT and Tbet^{-/-} mice infected with *T. spiralis*. (A) WT (shaded bars) or Tbet^{-/-} (open bars) mice were infected with 450 worms. Six mice of each strain were analyzed for jejunal worm burden on day 13 after infection. Values are the mean number of worms recovered per mouse ± SEM. The BALB/c (A) and C57/BL6 (B) strains are shown. **, P < 0.01. (C) Histochemical analysis for MCs in the jejunum of noninfected or *T. spiralis*-infected WT and Tbet^{-/-} mice. One representative field is shown for each condition. MCs are indicated by the arrows. Bar, 200 μm. (D) Quantification of MCs in the jejunum of noninfected or *T. spiralis*-infected WT and Tbet^{-/-} mice. Y-axis values represent the number of MCs present in 10–16 fields for each condition. **, P < 0.01.

From earlier studies, we knew that Tbet was necessary for both the biosynthesis of functional selectin ligands and the expression of chemokine receptors in CD4⁺ T cells (20), which guided us to consider that MCP adhesion by itself was defective. Because the hematopoietic pool of committed MCP in mice is estimated to be <30,000 cells (Fig. 1) (1, 23), we used BMMCs cultured in vitro as a surrogate for MCP cells to study their adhesive properties under physiological shear flow conditions. These experiments revealed that Tbet-deficient BMMCs adhered poorly to both VCAM-1 and MAdCAM-1 (Fig. 3). Furthermore, coimmobilized chemokines such as SDF-1α/CXCL12 or KC/CXCL1 were not sufficient to reconstitute or augment adhesion. Despite the decreased adhesion, no loss of α4β7 integrin or CXCR2 expression was detected (Fig. S2), perhaps implicating a defect in α4 integrin-dependent or chemokine receptor signaling.

At this juncture, the picture emerging was that Tbet expression in MCP selectively controlled homing to mucosal tissues (but not development in BM or spleen) via the α4β7 integrin-VCAM-1/MAdCAM-1/CXCR2 adhesion pathway. An unexpected finding was that Tbet transcripts were

below detectable levels in mature MCs isolated from the peritoneal cavity, in BMMCs or in committed intestinal MCP obtained by immunoisolation and high-speed FACS sorting (Fig. 4). We did detect Tbet expression in Lin⁺ BM cells, which are precursors of many other cell types, but not in the uncommitted Lin⁻ population or in other early myeloid progenitors, such as common myeloid progenitor, GMP, and BMCP, or the committed progeny of the BMCP, the MCP or BaP. From these collective findings, we concluded that Tbet cannot directly regulate MCP homing via α4β7 integrin/CXCR2-dependent adhesion. To further understand the role of Tbet, we addressed whether the defect resided in other cellular components of the BM and/or in the intestinal milieu by reconstituting sublethally irradiated WT or Tbet^{-/-}-null animals with BM from WT or Tbet^{-/-} mice. These studies indicated that the defect lies in the BM compartment and not in the intestinal microenvironment (Fig. 5), because Tbet-null mice reconstituted with normal BM cells had similar numbers of intestinal MCP to WT mice reconstituted with WT BM cells. In contrast, WT mice reconstituted with Tbet^{-/-} BM cells showed decreased MCP homing to the intestine. Because the genotype of the BM was critical and not that of the intestine, these results indicate that Tbet expression in BM cells, other than the MCP or its immediate precursors, is critical to proper homing of MCP to tissues. Although surprising, this is similar to the recent observation that TNF-α production by CD11b⁺ BM cells was critical to development and expansion of BMMCs (38) and supports the concept that MC development along the myeloid pathway requires interaction with other leukocytes. Given the restricted expression of Tbet within leukocytes of the BM, the most likely Tbet⁺ cell found in this environment is the DC.

We have tested this hypothesis by adoptive transfer of WT DCs into Tbet^{-/-} mice and found that these animals now exhibited normal homing of MCP to intestine. Furthermore, Tbet^{-/-} BMMCs co-cultured with WT DCs recover the ability to bind to MAdCAM-1 and VCAM-1 at levels that are comparable to WT BMMCs. We conclude that the presence of DCs expressing Tbet in the BM compartment is required for normal α4β7 integrin-dependent binding to MAdCAM-1 and VCAM-1 and MCP intestinal homing.

We further probed whether such a defect in the MCP pool increased the susceptibility of these animals to a serious outcome when challenged with a helminth infection. Based on studies in β7 integrin-deficient mice, which lack MCP and MCs in the intestine and show delayed rejection of an intestinal helminth, we anticipated a similar finding in Tbet-null mice, even though these mice are polarized toward Th2 responses (35, 13). Unexpectedly, the rejection of the worms by Tbet mice was equal to or even better than that by WT mice (Fig. 7), consistent with previous reports of an association of a rapid expulsion of *T. spiralis* with a pronounced mastocytosis mediated by a Th2-type response and the findings that Tbet-null mice are Th2 cell polarized (19, 35).

Our finding suggests that like in the $\beta 7$ integrin-null mice, the adhesion defect in $\alpha 4\beta 7$ integrin/CXCR2-mediated signaling for homing is compensated by other pathways elicited by the potent Th2 cell-mediated immune response that overcomes the initial dependence on the former molecules.

We have yet to identify the specific DC T-bet-dependent mechanisms in BM that are responsible for the defect in the $\alpha 4\beta 7$ integrin-CXCR2 adhesion pathway. We do not suspect that reduced $\alpha 4$ integrin/CXCR2 expression is responsible, because BMMCs derived from WT and T-bet-deficient mice showed equivalent, albeit low, levels of surface-expressed $\alpha 4$ and $\beta 7$ integrins and CXCR2. The loss of the $\alpha 4$ integrin during protracted in vitro culture that we observed (unpublished data) has also been reported previously (28, 29). Another possibility is that T-bet^{-/-} cells have a defect in $\alpha 4$ integrin or CXCR2 signaling that leads to the observed loss of adhesion. We note that one should interpret these results cautiously, because BMMCs are not MCP; they are only a tool, the best in vitro surrogate cells currently available for studying MCP.

T-bet controls a multitude of genes in several cell types, making it difficult to discern how T-bet is indirectly controlling MCP homing to mucosal tissues. Our experiments provide evidence that the transcription factor T-bet expressed in DCs within the BM indirectly imprints a program in developing MCP that ensures appropriate homing to the mucosal tissues. Regardless of the fact that the IFN- γ gene is one of the main direct targets of T-bet (16, 18), we were not able to increase the MCP intestinal homing in T-bet^{-/-} mice by intraperitoneal injection of IFN- γ , and more strikingly, the MCP present in the WT intestine were depleted after IFN- γ treatment, probably due to apoptosis of MCs induced by IFN- γ , as described by other authors (unpublished data) (39). Thus, it seems most likely that IFN- γ is not directly responsible for MCP homing and that some other factor causes the homing defect in T-bet-deficient mice. Nonetheless, these findings are remarkable, as it is the first indication that another innate effector cell directly influences MC development in a way that affects its trafficking in the absence of overt inflammation.

In summary, these observations provide further evidence that the transcription factor T-bet is involved in leukocyte trafficking and show, for the first time, a role for T-bet expression in DCs for the constitutive homing of MCP cells to mucosal tissues.

MATERIALS AND METHODS

Reagents. Dulbecco's PBS and RPMI 1640 were purchased from Invitrogen. The following reagents were obtained from the indicated sources: mouse MAdCAM-1 chimeric protein (T. Yednock, Elan Corporation, San Francisco, CA), recombinant mouse VCAM-1-Fc chimera (R&D Systems), and recombinant mouse IL-3, stem cell factor (SCF), SDF-1 α , GM-CSF, and KC/CXCL1 (PeproTech). The monoclonal antibodies to mouse PSGL-1 (clone 2PH1), CD62L (MEL-14), $\alpha 4$ integrin (clones R1-2 and 9C10), CXCR4 (clone 2B11), MHC class II (anti-H-2K^d; SF1-1.1-FITC), and CD11c (HL3-APC) were purchased from BD Biosciences; anti-mouse CXCR2 was an anti-peptide antibody made in rabbits, as previously described (14). Anti-T-bet antibody (clone 4B10) was obtained from Santa Cruz

Biotechnology, Inc. IgE-DNP, PMA, ionomycin, LPS, fetal calf serum, and polyclonal antibody to β -actin were purchased from Sigma-Aldrich. Gr1 (anti-Ly6G/C; RB6-8C5-PE) was obtained from eBioscience.

Mice. Previously described 6–8-wk-old WT and T-bet^{-/-} mice (19) on C57/BL6 and BALB/c backgrounds were purchased from the Jackson Laboratory. Mice were maintained in a specific pathogen-free barrier unit at the Harvard New Research Building facilities or at the Dana Farber Cancer Institute animal facilities. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee in accordance with policies of the Public Health Service.

MC isolation and cell culture. BM MCs were generated as previously detailed (7). 0.5×10^6 BMMCs/ml were cultured for 4 wk in T-25 culture flasks (Corning) under standard conditions in the presence of 10 ng/ml IL-3 and 10 ng/ml SCF.

BMDC isolation and cell culture. BMDCs were generated as previously detailed (40, 41). BMDCs from WT mice were cultured in the presence of 10 ng/ml GM-CSF, with supplementation on days 0, 3, and 6. On day 7, they were analyzed by FACS. The BMDC purity was 65–75% CD11c⁺, class II⁺, and Gr1⁻, with contaminating cells predominantly Gr-1⁺ cells (Fig. S3, available at <http://www.jem.org/cgi/content/full/jem.20060626/DC1>). This Gr-1 population is likely to contain a mixture of mature granulocytes, monocytes, and macrophages, none of which express T-bet, as previously described (18). 2×10^6 cultured BMDCs/mouse were injected intravenously into T-bet^{-/-} mice. Also on day 7, they were stimulated with 10 U/ml IFN- γ for 1 h and co-cultured with BMMCs (DC/MC = 1:1.5) for 6 h. These co-cultures were tested for adhesion to VCAM-1 and MAdCAM-1 under shear flow conditions.

Adhesion assays under physiological shear flow conditions. Glass coverslips (25-mm diameter; Carolina Biological Supply) were coated with 10 μ g/ml VCAM-1 or 20 μ g/ml MAdCAM-1. BMMC adhesive interactions were examined under conditions of fluid shear stress using a video microscopy system, as previously described (42). Cell accumulation was determined after the initial minute of each flow rate by counting the number of adherent cells in four different fields using a 20 \times phase-contrast objective (Plan Apo; Nikon) and recorded with videomicroscopy connected to Video-Lab software (Ed Marcus Laboratories). Data are the mean \pm SEM ($n = 3$ experiments). $P \leq 0.5$ or less was considered statistically significant using the paired *t* test or one-way analysis of variance for multiple groups.

Preparation of tissue mononuclear cells (MNCs) and MCP limiting dilution assay. The procedure for determining the number of MCP was essentially as previously described (13, 22). Individual tissues from two mice were pooled and processed to isolate MNCs. MNCs were serially diluted in 96-well flat-bottom microtiter plates, and 100 μ l of γ -irradiated (30 Gy) spleen-derived feeder cells in media containing 20 ng/ml IL-3 and 20 ng/ml SCF were added. MNCs were cultured for 12 d before MC colonies were identified and counted with inverted microscopes (CK, Olympus; TMS, Nikon). The MCP concentration is expressed as MCP/tissue and is derived by multiplying the concentration of MCP per 10^6 MNCs by the MNC yield/organ. For the BM, the number represents the total MCP per femur.

T-bet expression by Western blot. BMMCs from WT mice were stimulated for 24 h with 2 μ g/ml LPS, 50 ng/ml PMA plus 2 μ M ionomycin, or IgE cross-linking (1 μ g/ml of mouse α DNP-IgE for 16 h plus 10 ng/ml DNP-OVA). Immunoblot analysis was performed by using the 4B10 mAb, as previously described (16).

Sorting of MCP, real-time PCR, and semiquantitative RT-PCR. Sorting of myeloid progenitors was as previously described (30). Myeloid progenitors were sorted as Lin⁻ Sca-1⁻ c-kit⁺ CD34⁺, as previously described (43). BaP or MCP sorting was as previously described (1). Total RNA

was extracted from the sorted cells or from in vitro-generated mouse Th1 cells or Tc1 cells (provided by N. Grabie, Brigham and Women's Hospital, Boston, MA) as positive controls using TRIzol solution (Invitrogen) and reverse transcribed using a cDNA synthesis kit (iScript; Bio-Rad Laboratories). The amount of amplicon generated was monitored with an ABI Prism 7700 (Applied Biosystems). A specific probe labeled with both reporter and a quencher dye was added into the Taqman PCR mix at the beginning of the reaction. The cycle number was normalized to β -actin.

For semiquantitative PCR, the following primers were used to amplify T-bet and β -actin: T-bet (forward), 5'-GCCAGGGAACCGCTTATATGTC-3'; T-bet (reverse), 5'-CTGTGAGATCATATCCTTGGGCTG-3'; β -actin (forward), 5'-GTGGGCCGCTCTAGGCACCA-3'; and β -actin (reverse), 5'-CGGTTGGCCTTAGGGTTCAGGGGGG-3'.

Immunohistochemistry. Thin sections (1.5- μ m thickness) of tissue were fixed with 4% paraformaldehyde and subjected to the chloroacetate esterase reactivity cytochemistry procedure for MC histology analysis, as previously described (8). The results are expressed as the number of MCs per cross-sectional area of the intestine or spleen.

T. spiralis infection and worm burden determination. Trichinella larvae were obtained as detailed previously (8). Mice were infected with ~450 larvae suspended in 200 μ l PBS by direct gastric installation. Worm burden was determined after 13 d, as previously described (44). Each experimental group comprised six animals.

Sublethal irradiation with BM reconstitution. Animals were irradiated (GammaCell-40; Atomic Energy of Canada) for 4.5 min, 5 gray (500 rad). 2 h after irradiation, mice were given intravenous injections of 10^7 BM cells that had been isolated from femurs and tibias of syngenic or congenic animals. 14 d after irradiation, mice were killed, and MCP limiting dilution assays were performed.

Statistical analysis. Data are the mean \pm SEM ($n = 5$ separate experiments with two mice per group in each experiment), unless indicated otherwise. $P \leq 0.05$ was considered statistically significant by the paired t test or one-way analysis of variance for multiple groups using Prism software (version 4; GraphPad).

Online supplemental material. The online supplemental material contains additional information about adhesion studies under flow conditions, as well as characterization of DCs and BMMCs. Fig. S1 evaluates the adhesion of BMMCs under shear flow conditions to VCAM-1 and MAdCAM-1, co-immobilized with the chemokines KC and SDF-1 α . Fig. S2 shows expression of CXCR2 and $\alpha 4$ and $\beta 7$ integrins in BMMCs by FACS analysis. Fig. S3 shows the phenotypes of the DCs used in our assays. Online supplemental material is available at <http://www.jem.org/cgi/content/full/jem.20060626/DC1>.

We wish to thank Dr. Ravi Rao for his helpful discussions regarding T-bet function in MC biology, Dr. Nir Grabie for his gift of mouse Tc1 lymphocytes, Dr. Gail Newton for help with designing primers for T-bet RT-PCR, and Drs. Daniel Friend and Yuhui Xu for MC histological analysis.

These studies were supported by grants from the Fulbright-Spanish Ministry of Education and Science (to P. Alcaide), the P.E. Lindahl Fund from the Royal Swedish Academy of Sciences (to J. Hallgren), the National Institutes of Health (HL36028, HL53993, and HL56985 to F.W. Luscinskas; CA112663 and AI56296 to L.H. Glimcher; and HL 036110 and AI 031599 to M.F. Gurish), and the Ellison Medical Foundation (L.H. Glimcher).

The authors have no conflicting financial interests.

Submitted: 21 March 2006

Accepted: 18 January 2007

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