Receptor Tim-3 Expressed on Innate Immune Cells

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CD4+ T helper 1 (T(H)1) cells are important mediators of inflammation and are regulated by numerous pathways, including the negative immune receptor Tim-3. We found that Tim-3 is constitutively expressed on cells of the innate immune system in both mice and humans, and that it can synergize with Toll-like receptors. Moreover, an antibody agonist of Tim-3 acted as an adjuvant during induced immune responses, and Tim-3 ligation induced distinct signaling events in T cells and dendritic cells; the latter finding could explain the apparent divergent functions of Tim-3 in these cell types. Thus, by virtue of differential expression on innate versus adaptive immune cells, Tim-3 can either promote or terminate T(H)1 immunity and may be able to influence a range of inflammatory conditions.

Inflammatory responses are regulated through multiple pathways that often involve subsets of CD4+ T helper cells, and much effort has been devoted to understanding the key pathways that regulate these cells. Tim-3 was identified as a member of the TIM (T cell immunoglobulin and mucin domain) family, specifically expressed on CD4+ T(H)1 and not T(H)2 lymphocytes (1). We have shown that interactions of Tim-3 and its ligand play a role in regulating T(H)1 responses as well as contributing to T cell tolerance (2, 3). More recently, galectin-9 was identified as a Tim-3 ligand that could dampen T(H)1 immunity by inducing cell death in effector T(H)1 cells (4). Moreover, Tim-3 expression is low in T cell clones isolated from the cerebrospinal fluid of patients with multiple sclerosis (MS) (5). Collectively, these data support a key role for Tim-3 in regulating T(H)1 responses and human autoimmune diseases.

In addition to its expression in T cells, Tim-3 mRNA is present in CD11b+ cells (1) and other non-T cells, although the functional relevance of this is not known (6–8). Further analysis here revealed that Tim-3 was specifically restricted to CD11b+ dendritic cells (DCs) and not CD11b+ macrophages (Fig. 1A). Analysis of monocytes and DCs from peripheral blood of healthy human subjects also revealed that human DCs expressed high levels of TIM-3, although it was also detected at low levels in human monocytes (Fig. 1B).

We next tested the production of cytokines from wild-type and Tim3−/− DCs in response to lipopolysaccharide (LPS) as a positive control, or galectin-9 plus LPS. Although a small level of tumor necrosis factor–α (TNF-α) was secreted in response to LPS stimulation alone, this was much greater in combination with galectin-9 and considerably diminished in Tim3−/− DCs (Fig. 1C).

To more specifically study the effects of Tim-3 triggering in DCs, we generated an antibody agonist of Tim-3 (anti-Tim-3) (9). Because we observed that DCs produce TNF-α ex vivo in response to Tim-3 ligation, we stimulated the dendritic cell line D2SC1 with anti–Tim-3. Consistent with the previous cytokine expression data, engagement of Tim-3 led to a specific induction of nuclear factor κB (NF-κB) (Fig. 1D), confirming the induction of an inflammatory transcription factor cascade. Using a blocking anti-Tim-3, we observed 75% inhibition of galectin-9–mediated TNF-α secretion from human monocytes (Fig. 1E). Collectively, these data show that Tim-3 is highly expressed by cells of the innate immune system in both mice and humans, and that its expression on antigen-presenting cells promotes the secretion of proinflammatory cytokines from monocytes and dendritic cells.

Peripheral bone marrow–derived monocytes give rise to microglia in the central nervous system (CNS) (10, 11), and both resident microglia and infiltrating monocytes have been shown to contribute to CNS inflammation (12). To explore possible TIM-3 expression on monocyte cells in the CNS, we first examined its expression in autopsy tissue from subjects with no evident inflammatory disease (9). TIM-3 staining was apparent in white but not gray matter parenchyma on cells with a histological appearance consistent with microglia (Fig. 2A). Staining with CD11b confirmed the expression of TIM-3 on microglia in CNS white matter (Fig. 2B). We
Fig. 2. TIM-3 expression in human microglia. (A) Tissue sections from white and gray matter regions of human noninflamed CNS tissue were stained with TIM-3-specific mAb (magnification, 20×). (B) Dual immunofluorescence of noninflamed CNS white matter tissue using CD11b and TIM-3 mAbs (magnification, 20×; insets, 40×). (C) Quantitative RT-PCR analysis of TIM-3 mRNA levels in microglia isolated using LCM from white and gray matter regions of CNS tissue. Error bars represent SD in TIM-3 mRNA levels among five experiments. Gray matter microglia express significantly lower levels of TIM-3 (P = 0.02, two-tailed t test). (D) Microglia were isolated from noninflamed (control) human CNS tissue (n = 2), normal-appearing white matter (NAWM) regions of MS tissue (n = 2), the center (n = 4) or border (n = 3) regions of active MS plaques, or glioblastoma multiforme (GBM) tumor specimens (n = 3), and levels of TIM-3 were determined by quantitative RT-PCR. Error bars represent SEM. (E) Astrocytes were isolated by LCM from noninflamed white matter (n = 2) and from MS plaques (n = 5) from two different brain specimens. Galectin-9 expression was determined by RT-PCR. Error bars represent SEM.

Fig. 3. Analysis of Tim-3 on murine CNS monocytes and microglia. (A) CNS mononuclear cells from a mouse with EAE were stained with CD11b, CD45, and either Tim-3 mAb or rat IgG1 isotype control. Tim-3 (open histogram) and isotype control (shaded histogram) staining on CNS microglia (CD45lo) and infiltrating monocytes (CD45hi) is shown. Numbers indicate percentage of cells in each gate. (B) Mice were immunized for EAE and killed at the indicated stages of disease. Each bar represents the mean of two or three individual mice. Error bars represent SEM. (C) Mice were immunized with 100 μg of myelin PLP 139-151 emulsified in IFA, IFA containing 100 μg of mouse IgG1, IFA containing 100 μg of anti-Tim-3, or complete Freund’s adjuvant (CFA) supplemented with Mycobacterium tuberculosis (4 mg/ml). Immunized mice were monitored for the development of EAE. The mean clinical disease score in each group is shown. Results for two independent experiments (n = 4 or n = 5, respectively) are represented. (D) Linear regression curves of the acute phase of disease for anti-Tim-3 and mouse IgG1 groups are shown for the experiments represented in (C). The slopes are significantly different between these groups for experiments 1 and 2 (P = 0.02113 and P < 0.0001, respectively). The 95% confidence intervals for each curve are represented with dashed lines.
then used laser capture microdissection (LCM) to isolate CD11b+ cells from normal white and gray matter tissues for quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis (9). In these analyses, little or no TIM-3 mRNA could be detected in microglia obtained from gray matter tissue, whereas it was clearly present on microglia from white matter tissue (Fig. 2C).

To test the possible consequences of TIM-3 in the CNS, we examined TIM-3 expression on infiltrating monocytes and microglia isolated from the white matter CNS tissue of patients with MS and from CNS glioblastoma multiforme (GBM) tumors. Although lymphocytes as well as monocytes and microglia are associated with these pathologies, the cytokine profiles differ considerably (13, 14). Thus, the Th1 cytokines interferon-γ (IFN-γ) and TNF-α associate with MS but not with GBM tissues. Monocytes and microglia captured from the active border regions of MS lesions expressed higher levels of TIM-3 than did those captured from the quiescent center of MS lesions, from adjacent normal-appearing white matter, or from noninflamed white matter tissue (Fig. 2D). In contrast, TIM-3 expression was significantly lower in monocytes and microglia obtained from GBM tissues, relative to those obtained from control tissue or MS tissue.

Although galectin-9 is undetectable on resting human astrocytes, it can be up-regulated by the cytokines interleukin-1 and IFN-γ (15–17), leading us to examine whether it might be induced on astrocytes present in MS lesions. Indeed, galectin-9 levels were significantly elevated on astrocytes present in MS lesions relative to normal human CNS tissue (Fig. 2F). Collectively, these data reveal that both TIM-3 and its ligand are up-regulated on microglia and astrocytes, respectively, in inflamed white matter tissue.

To further explore the mechanism underlying selective TIM-3 expression on white matter microglia, we examined TIM-3 expression on peripheral CD11b+ macrophages in mice immunized for the induction of experimental autoimmune encephalomyelitis (EAE), a murine model of MS (18). In these animals, activated peripheral CD11b+ cells failed to express TIM-3, although CD11b+ monocytes infiltrating the CNS from the periphery, distinguished from resident microglia by higher expression of CD45 (19, 20), did express TIM-3 (Fig. 3A and fig. S1). Moreover, levels of TIM-3 on both microglia and infiltrating monocytes peaked just before the peak of clinical disease (Fig. 3B).

The earlier experiments showing high TIM-3 on DCs and TNF-α secretion are consistent with the notion that triggering TIM-3 on DCs promotes inflammatory Th1 responses. To examine this further, we induced EAE by immunizing mice with myelin proteolipid protein (PLP) 139–151, emulsified in incomplete Freund’s adjuvant (IFA) containing either anti-Tim-3 or an isotype control. We observed that immunization in the presence of anti–Tim-3 led to disease with greater severity (Fig. 3C), which also showed a statistically significant difference in disease course.

To address whether Tim-3 engagement induces distinct signaling in innate and adaptive immune cells, we stimulated a Tim-3–expressing CD4+ T cell clone and a DC cell line with anti–Tim-3 and examined them for tyrosine phosphorylation. Differences were observed in the proximal signaling pathways triggered by Tim-3 in T cells and DCs (Fig. 4A). Specifically, tyrosine phosphorylation was induced in two molecules after Tim-3 engagement in T cells but not in DCs, and phosphorylation of a third molecule was induced in DCs but not in T cells. In contrast, engagement of Tim-3 led to similar degrees of extracellular signal–regulated kinase activation and degradation of the NF-κB inhibitor IκBα in the two cell types (Fig. 4, B and C). Although the magnitude of ERK phosphorylation induced by Tim-3 appeared to be lower in the DCs, phosphorylation induced with a positive control stimulus [phorbol 12-myristate 13-acetate (PMA)] was also lower in these cells.

Our results show that Tim-3 serves opposing roles in the innate and adaptive immune systems. In the naïve resting immune system, Tim-3 promotes inflammation. This is consistent with our data indicating that Tim-3 is primarily expressed on DCs in the naïve state and that Tim-3 synergizes with Toll-like receptors to promote TNF-α secretion. Once Th1 responses are generated, Tim-3 is expressed on terminally differentiated Th1 cells, which will outnumber DCs and induce the up-regulation of galectin-9 via their production of IFN-γ (15, 16). Finally, galectin-9 triggers Tim-3 on Th1 cells to terminate Th1 immunity. In sum, our data show how a single molecule, Tim-3, by virtue of differential expression on cells of the innate and adaptive immune systems, can both promote inflammation and terminate Th1 immunity. Thus, Tim-3 may represent a valid therapeutic target in a wide range of peripheral and organ-specific human inflammatory diseases.

References and Notes
9. See supporting material on Science Online.
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Materials and Methods
Fig. S1
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