TIM-3 and Its Regulatory Role in Immune Responses

Chen Zhu, Ana C. Anderson, and Vijay K. Kuchroo

Contents

Abstract T cell immunoglobulin mucin-(TIM)-3 was first identified as a molecule specifically expressed on IFN-γ-secreting $CD4^+$ T helper 1 (Th1) and $CD8^+$ T cytotoxic (Tc1) cells in both mice and humans. TIM-3 acts as a negative regulator of Th1/Tc1 cell function by triggering cell death upon interaction with its ligand, galectin-9. This negative regulatory function of TIM-3 has now been expanded to include its involvement in establishing and/or maintaining a state of T cell dysfunction or "exhaustion" observed in chronic viral diseases. In addition, it is now appreciated that TIM-3 has other ligands and is expressed on other cell types, where it may function differently. Given that an increasing body of data support an important role for TIM-3 in both autoimmune and chronic inflammatory diseases

V.K. Kuchroo (✉), C. Zhu, and A.C. Anderson
Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA 02115, USA
e-mail: vkuchroo@rics.bwh.harvard.edu

R. Ahmed and T. Honjo (eds.), *Negative Co-Receptors and Ligands*,
Current Topics in Microbiology and Immunology 350, DOI 10.1007/82_2010_84

in humans, deciphering the function of TIM-3 on different cell types during different immune conditions and how these can be regulated will be critical for harnessing the therapeutic potential of TIM-3 for the treatment of disease.

1 Introduction

T-cell immunoglobulin and mucin-(TIM)-3 domain was first discovered in 2002 as a molecule specifically expressed on IFN-γ-producing $CD4^+$ T helper 1 (Th1) and $CD8^+$ T cytotoxic 1 (Tc1) cells in the mouse (Monney et al. 2002). Later, it was found that TIM-3 is also specifically expressed on IFN-γ-producing T cells in humans (Khademi et al. 2004). The specific expression of TIM-3 on Th1 cells catalyzed investigation into its potential role as a regulator of Th1 cells. Indeed, it is now known that ligation of TIM-3 triggers cell death in Th1 cells in mice (Zhu et al. 2005). Other studies support that TIM-3 also acts as a negative regulator of human Th1 T cells (Hastings et al. 2009; Koguchi et al. 2006; Yang et al. 2008).

The importance of TIM-3 in regulating T cell responses is underscored by the fact that both TIM-3 expression and its negative regulatory function is dysregulated in patients with multiple sclerosis (MS) and that both these defects are reversed following treatment (Koguchi et al. 2006; Yang et al. 2008). Moreover, the negative regulatory role for TIM-3 in T cells has recently been extended to dysfunctional or "exhausted" T cells in chronic viral infections such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV) (Golden-Mason et al. 2009; Jones et al. 2008). Here, blockade of TIM-3 signaling has been shown to partially restore T cell function of otherwise "exhausted" T cells. Collectively, these data strongly support an important role for TIM-3 as a negative regulator of T cell responses and highlight the importance of this pathway as a therapeutic target in human diseases.

However, it is now appreciated that TIM-3 is not only expressed on T cells but also on other cell types such as dendritic cells (DCs) in both mice and humans and on monocytes in humans (Anderson et al. 2007). Further, TIM-3 is expressed on mast cells (Nakae et al. 2007), melanoma (Wiener et al. 2007), and on lymphoma-derived endothelium (Huang et al. 2010), where it may be involved in promoting tumor progression by inhibiting anti-tumor $CD4^+$ T cell responses (Huang et al. 2010). How these diverse functions of TIM-3 in different cell types are regulated and which one predominates in different disease states is not clear at this stage.

2 Protein Structure

Mouse TIM-3 is a 281 amino acid (aa) type I transmembrane glycoprotein that contains a membrane distal immunoglobulin variable (IgV) domain and a membrane proximal mucin domain. Human TIM-3 is 302 aa in length and shares 63% aa identity with mouse TIM-3 (Monney et al. 2002). Further, a putative soluble mouse TIM-3 splice variant has been identified in cDNA generated from concanavalin

A–activated splenocytes. The predicted protein sequence of this TIM-3 isoform contains only the signal peptide, immunoglobulin V (IgV), and cytoplasmic domain, lacking the mucin domain and transmembrane region (Sabatos et al. 2003).

TIM-3 belongs to the immunoglobulin super family (IgSF) (Bork et al. 1994) and recent studies have revealed the 3D structure of the IgV domain of TIM-3 as well as other TIM proteins (Cao et al. 2007; Santiago et al. 2007a, b). TIM-3 IgV domains consist of two anti-parallel β sheets that are tethered by a disulfide bond. Additional two disulfide bonds are formed by four noncanonical cysteines that are invariant within TIM proteins and unique among IgSF members. They stabilize the IgV domain of TIM-3 and reorient the CC′ loop so that it is in close proximity to the FG loop resulting in formation of a "cleft" or "pocket" structure in TIM-3 as well as other TIM proteins (Fig. 1) (Cao et al. 2007; Santiago et al. 2007b). This unique cleft structure is not found in other IgSF proteins and has been predicted to be involved in ligand binding (see below).

In the cytoplasmic region of both human and mouse TIM-3, there is a highly conserved region containing five tyrosine residues. Galectin-9 triggering of TIM-3 results in tyrosine phosphorylation of these residues, indicating that some, if not all, of these tyrosines are involved in TIM-3 signaling (van de Weyer et al. 2006). Otherwise, protein sequence analysis does not reveal any other homology to known inhibitory domains such as an immunoreceptor tyrosine-based inhibitory motif or immunoreceptor tyrosine-based switch motif. Thus, much remains to be elucidated regarding the signaling pathways recruited by TIM-3.

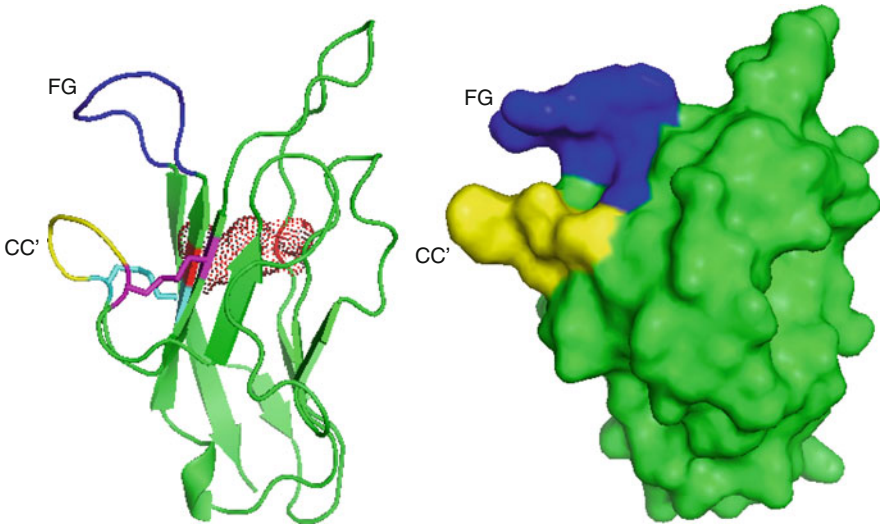

Fig. 1 Mouse TIM-3 IgV domain. (**a**) Ribbon diagram. *Sticks* represent two noncanonical disulfide bonds that reorient the CC′ loop thereby forming a "cleft" or "pocket" structure together with the FG loop in TIM-3 as well as other TIM proteins. Dots represent the disulfide bond that exist in all IgSF proteins. (**b**) Surface representation. Structure simulation was done by PyMOL

3 TIM-3 Ligands

3.1 Identification of Galectin-9 as a TIM-3 Ligand

In an attempt to identify the TIM-3 ligand, we screened a number of T cell lines and lymphomas for their ability to bind TIM-3-Ig fusion protein. TK-1 $CD8^+$ T cell lymphoma was found to have the strongest binding to TIM-3-Ig fusion protein, suggesting a high level of TIM-3 ligand expression on this cell type. Subsequent pull-down of proteins bound to TIM-3-Ig identified a 35-kDa cell surface molecule that only bound TIM-3-Ig but not control hIgG1. This molecule was later identified as galectin-9 by mass spectrometry (Zhu et al. 2005).

Galectins, a group of S-type lectins, are a family of carbohydrate-binding proteins that exhibit important functions in regulating immune cell homeostasis and inflammation (Rabinovich and Toscano 2009). Galectin-9 binding to TIM-3 is dependent on its carbohydrate recognition domain that recognizes the oligosaccharide chains on the TIM-3 IgV domain. Galectin-9 is expressed on a variety of cell types and it is up-regulated by IFN-γ (Imaizumi et al. 2002). In vitro analyses revealed that galectin-9 predominantly induces intracellular calcium flux, cell aggregation, and death of Th1 but not Th2 cells, and this process is dependent on the presence of TIM-3 on Th1 cells, as TIM-3 deficient Th1 cells are relatively resistant to galectin-9-induced cell death. Furthermore, administration of galectin-9 in vivo during an ongoing immune response dampens inflammation by specifically eliminating antigen specific IFN-γ-producing T cells, thereby attenuating disease progress in experimental autoimmune encephalomyelitis (EAE) (Zhu et al. 2005). Thus, the galectin-9–TIM-3 pathway provides a negative feedback loop by which Th1 cells are regulated to prevent uncontrolled Th1 responses which otherwise could be detrimental to the host.

Subsequent studies in other disease models have demonstrated that galectin-9 triggering of TIM-3 attenuates Th1 and Th17 responses, thereby exhibiting therapeutic potential in skin inflammation (Niwa et al. 2009), experimental autoimmune arthritis (Seki et al. 2008), and herpes simplex virus (HSV)-induced ocular inflammation (Sehrawat et al. 2009). Thus, although TIM-3 is expressed at low levels on Th17 cells (Chen et al. 2006), it may have a role in attenuating Th17 responses. Further, galectin-9 has been shown to induce cell death in $TIM\text{-}3^+CD8^+$ alloreactive T cells, thereby reducing cytotoxicity and prolonging survival of skin grafts (Wang et al. 2007).

Interestingly, the galectin-9–TIM-3 interaction does not always lead to suppression of immune responses. Triggering of TIM-3 on innate immune cells in both mice and humans exhibits an opposite role (Anderson et al. 2007) (see below). The galectin-9–TIM-3 pathway in DCs actually synergizes with Toll-like receptor (TLR) signaling and promotes Th1 immunity. In addition, administration of galectin-9 prolongs the survival of Meth-A tumor-bearing mice by increasing the number of IFN-γ-producing $TIM\text{-}3^+CD8^+$ T cells with enhanced cytolytic function as a result of an increase in the number of $TIM\text{-}3^+CD86^+$ mature DCs (Nagahara et al. 2008).

These observations support another interesting biological role for the galectin-9–TIM-3 pathway, enhancement of adaptive immunity via galectin-9-induced maturation of TIM-3^+ antigen presenting cells.

Although the function of the galectin-9–TIM-3 pathway in immune responses has been extensively studied, multiple lines of evidence suggested the existence of other TIM-3 ligands. (1) At least one additional membrane protein specifically associates with TIM-3-Ig (our unpublished data); (2) Bacterially expressed TIM-3 tetramer that lacks carbohydrate modification binds to a broad panel of cell types. That these interactions do not require TIM-3 carbohydrate moieties excludes the possibility of them being galectin-9-dependent. (3) The crystal structure of the TIM-3 IgV domain revealed a potential ligand binding site at the CC′-FG cleft; however, the potential N and O-linked glycosylation sites in the TIM-3 IgV domain are not proximal to this region. Overall, the topological features of TIM-3 indicate the existence of other independent TIM-3 ligands that bind to discrete regions on the TIM-3 IgV domain.

3.2 Phosphatidylserine as a TIM-3 Ligand

As mentioned above, it has been predicted that the unique cleft present in TIM family proteins participates in ligand binding. Indeed, it is now known that phosphatidylserine (PtdSer) binds the cleft region of both TIM-1 and TIM-4 and is functionally involved in recognition and uptake of apoptotic cells (Miyanishi et al. 2007; Santiago et al. 2007a). TIM-3, on the other hand, exhibits a different FG loop structure and thus the cleft present in TIM-3 is a bit different from that of the other TIMs (Santiago et al. 2007a). Nevertheless, Nakayama and colleagues recently reported that phosphatidylserine (PtdSer) may be another ligand for TIM-3 (Nakayama et al. 2009). Expression of TIM-3 in NKR cells, a rat kidney cell line that does not express TIM-3, resulted in gain of PtdSer binding and internalization of apoptotic cells. They further found that expression of TIM-3 on peritoneal exudate $Mac1^+$ cells (PEMs), monocytes, and splenic $CD8^+$ DCs was found to be involved in apoptotic cell uptake. Accordingly, blockade of the TIM-3 pathway by an anti-TIM-3 antibody resulted in increased anti-dsDNA autoantibody in the serum and reduced cross-presentation of apoptotic cell-associated antigens (Nakayama et al. 2009). Although PtdSer can bind to the TIM-3 cleft region, its binding affinity is much weaker than that of TIM-1 or TIM-4. Furthermore, allelic variants of TIM-3 display a differential capacity to bind to PtdSer and to phagocytose apoptotic cells, with the BALB/c allele demonstrating stronger affinity and phagocytic properties than the C.D2Es-Hba (HBA) allele (DeKruyff et al. 2010). Lastly, TIM-3 mediated phagocytic function is cell type dependent, as TIM-3 transfected T cell or B cell lines were able to form conjugates with but failed to engulf apoptotic cells (DeKruyff et al. 2010). Given that the bulk of the data showing TIM-3 binding to PtdSer come from experiments with transfected cell lines and that there is no obvious defect in apoptotic cell uptake in TIM-3 deficient mice (unpublished observations), raises the

question as to how physiologically relevant is the binding of TIM-3 to PtdSer. Further investigation will help clarify this issue.

3.3 Carbohydrate Ligands for TIM-3

Other lines of evidence suggest that TIM-3 may also bind carbohydrate moieties. Wilker and colleagues performed a glycan array screen and identified a set of glycan moieties exhibiting high affinity for the TIM-3 IgV domain (Wilker et al. 2007). Direct evidence was found using IdlD CHO cells, a UDP-galactose/UDP-*N*-acetylglucosamine 4-epimerase defective cell line. While IdlD CHO cells lack the ability to synthesize complete N-linked, O-linked, and lipid-linked glycoconjugates de novo, the cells can uptake galactose (Gal) and *N*-acetylgalactosamine (GalNAc) from culture medium and generate these glycoconjugates through salvage pathways. When these cells were stained with TIM-3 tetramer, it was found that TIM-3 retained binding to the cells grown in media with either 10% or 3% serum. However, the binding of TIM-3 tetramer to the cells grown in 1% serum was significantly reduced. Importantly, TIM-3 tetramer binding to IdlD CHO cells was restored when 1% serum was supplemented with Gal and GalNAc (Wilker et al. 2007). These results support that certain glycan moieties can act as TIM-3 ligands. The functional role of such interactions remains unknown.

4 Expression of TIM-3

Since its discovery on T cells, it is now appreciated that TIM-3 is expressed constitutively on other cell types and can be induced on some cells in pathological conditions. In the naïve or unimmunized state in mice, TIM-3 is expressed primarily on DCs at high levels (Anderson et al. 2007) and on a small percentage of effector/memory ($CD44^{hi}CD62L^{low}$) CD4 and CD8 T cells (Zhu et al. 2005).

During in vitro Th1 polarization, TIM-3 expression gradually increases until it reaches a stable, high expression level on terminally differentiated Th1 cells (Monney et al. 2002; Sanchez-Fueyo et al. 2003). When EAE is induced in mice, TIM-3 expression is found on $CD4^+$ and $CD8^+$ T cells that infiltrate the central nervous system (CNS) during the disease onset. These TIM-3^+ T cells decrease in the CNS as disease progresses, indicating an active role for TIM-3 in the initiation of EAE (Monney et al. 2002). Interestingly, recent studies of viral antigen specific $CD4^+$ and $CD8^+$ T cells from patients with chronic viral infection, showed that TIM-3 is expressed by a distinct population of "exhausted" T cells that fail to respond to viral antigens (Jones et al. 2008; Golden-Mason et al. 2009) (discussed below). The expression of TIM-3 on both functional and non-functional or "exhausted" T cells in two different disease states may indicate that TIM-3 integrates different extracellular signals present in these different immune milieus thereby delivering distinct signaling events to regulate T cell function.

In the naïve state, TIM-3 is not expressed on peripheral $CD11b^+$ cells but is expressed in $CD11b^+$ microglia that are resident in the CNS (Anderson et al. 2007) and can be induced in $CD11b^+$ peritoneal macrophages after treatment with thioglycollate (Nakayama et al. 2009). In the peritoneum, TIM-3 is additionally expressed on peritoneal mast cells (Nakae et al. 2007).

In humans, TIM-3 is also expressed on IFN-γ-secreting cells (Khademi et al. 2004) and is expressed constitutively at high levels on DCs and at lower levels on monocytes (Anderson et al. 2007). In cancer, TIM-3 expression has been noted on melanoma cells (Wiener et al. 2007) and lymphoma associated endothelium (Huang et al. 2010). While the differential roles and contributions of TIM-3 expression on T cells versus other innate and non-immune cells types remains to be ironed out, the wealth of data supporting an important role for TIM-3 in regulating the immune responses in both animal models and in human diseases, prompted us to begin examining the transcriptional regulation of TIM-3 expression in the two major cell types that express TIM-3, T cells and DCs.

4.1 Transcriptional Control of TIM-3 Expression

We have examined the role of Th1-associated transcription factors in regulating TIM-3 expression and found that TIM-3 expression is in part regulated by the Th1-specific transcription factor T-bet in both T cells and DCs (Anderson et al. 2010). We have found that T-bet directly binds to the TIM-3 promoter. In addition, we have found that the role of T-bet is not secondary to its induction of IFN-γ as T-bet can drive TIM-3 expression in the absence of IFN-γ and IFN-$\gamma R^{-/-}$ cells do not exhibit defects in TIM-3 expression. We have also examined a role for STAT-4 in regulating TIM-3 expression but found that STAT-$4^{-/-}$ cells exhibit only a modest, if any, defect in TIM-3 expression. Given that TIM-3 is stably expressed in Th1 cells only after several rounds of in vitro polarization but T-bet is upregulated early during Th1 differentiation (Szabo et al. 2000) suggests that other transcription factors may be involved in TIM-3 expression. Indeed, that T-$bet^{-/-}$ cells are not completely deficient in TIM-3 expression points to the involvement of other transcription factors in transactivating TIM-3 expression; however, these remain to be identified. In addition, it remains to be seen how TIM-3 expression is regulated in non-immune cell types.

5 TIM-3 in Disease

5.1 Genetic Basis for Role of TIM-3 in Disease

Genetic data suggest a role for TIM-3 expression and/or function in immune-mediated diseases in animal models and humans. The locus that encodes the TIM

gene family has shown linkage to disease susceptibility in several different autoimmune disease models such as EAE (locus EAE 6a), diabetes (Idd4), and SLE (lbw8) (Butterfield et al. 1998; Grattan et al. 2002; Kono et al. 1994). Similarly, a major locus for airway hyper-reactivity in mice and a syntenic locus on 5q33 in humans associated with asthma overlaps with the TIM gene locus (McIntire et al. 2001). Furthermore, comparisons of the TIM family genes in different strains of mice have revealed polymorphisms in TIM-1 and TIM-3, with Th1 prone strains (i.e., C57BL/6) and Th2 prone strains (i.e., Balb/c) expressing different TIM-1 and TIM-3 alleles, further supporting that genetic differences in the genes encoding TIM family proteins impact on disease.

In humans, several single nucleotide polymorphisms (SNPs) have been identified in the TIM-3 gene; one is found in the coding region (exon 3) and results in an amino acid change. An analysis of TIM-3 genotype and allelic frequencies among several hundred patients with rheumatoid arthritis and control subjects suggested that a SNP in the coding region of TIM-3 may be associated with susceptibility to rheumatoid arthritis (Chae et al. 2004b). This group has similarly identified SNPs in the promoter and coding regions of TIM-3 that may be associated with atopic disease (Chae et al. 2004a). Another group has also observed that a SNP in the coding region of TIM-3 is highly associated with atopic disease (Graves et al. 2005). While the functional and biological consequences of TIM-3 SNPs are presently unknown, current data in both humans and mice point to the TIM family genes, specifically TIM-1 and TIM-3, as important regulators of Th1/Th2 immunity, and possibly important determinants of susceptibility to both autoimmune and allergic diseases.

6 TIM-3 in Autoimmune Diseases

The importance of TIM-3 in regulating the immune response was first suggested by experiments that involved manipulation of the TIM-3 pathway in experimental disease models (Monney et al. 2002; Sabatos et al. 2003; Sanchez-Fueyo et al. 2003). Since then, several observations regarding TIM-3 expression and function in different disease states in humans further support the importance of TIM-3 in immune regulation. First, it has been noted that TIM-3 expression is dysregulated in patients with MS in that T cell clones isolated from the cerebrospinal fluid (CSF) clones from MS patients secrete significantly higher levels of IFN-γ than clones from the CSF of control subjects, yet the CSF clones from MS patients express lower levels of TIM-3 (Koguchi et al. 2006). Moreover, further Th1 polarization in vitro significantly augmented IFN-γ secretion but not TIM-3 expression among CSF clones from MS patients relative to those from control subjects. Tolerance induced by costimulatory blockade in vitro was less effective among CSF clones from MS patients that expressed lower amounts of TIM-3, consistent with previous reports that TIM-3 influences tolerance induction in a variety of murine models (Sabatos et al. 2003; Sanchez-Fueyo et al. 2003). Interestingly, T cells from MS

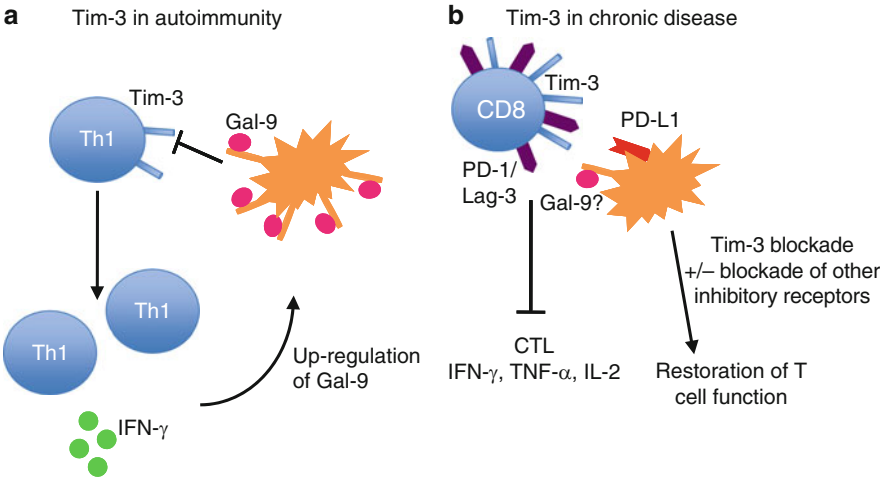

Fig. 2 Dysregulation of TIM-3 in different disease states. (**a**) In autoimmunity, IFN-γ secretion by $CD4^+$ Th1 cells up-regulates expression of the TIM-3 ligand, galectin-9. However, low T cell expression of TIM-3 allows cells to escape galectin-9-induced cell death. Consequently, autoreactive proinflammatory cells expand. Treatments that increase TIM-3 expression restore galectin-9-mediated negative regulation of IFN-γ-secreting $CD4^+$ Th1 T cells. The factor(s) responsible for the dysregulation of TIM-3 expression in $CD4^+$ Th1 cells in patients with autoimmune disease are not known. (**b**) In chronic conditions, TIM-3 expression on $CD8^+$ T cells either with or without co-expression of other inhibitory ligands, such as PD-1 or Lag-3, is associated with T cell exhaustion. Blockade of TIM-3/TIM-3–ligand interactions either alone or in combination with blockade of other inhibitory receptors restores effector function to T cells. Whether galectin-9 is involved in this function of TIM-3 and, if so, how these cells escape galectin-9-mediated cell death is not known

patients who have undergone treatment with glatiramer acetate or IFN-β for MS exhibit a restoration of TIM-3 expression (Yang et al. 2008). Furthermore, the ability of TIM-3 blockade to augment T cell proliferation and IFN-γ production is also restored in T cells from MS patients after treatment. Collectively, these data support that TIM-3 is an important negative regulator of T cell function and suggest that low-level expression of TIM-3 in T cells from MS patients allows pathogenic, autoreactive T cells to escape negative regulation by TIM-3 (Fig. 2).

6.1 TIM-3 in Chronic Viral Infection

A second disease state where TIM-3 appears to play a critical negative regulatory role in T cells is chronic viral infection. Here, it has been observed that virus-specific T cells develop an impaired or dysfunctional phenotype characterized by failure to proliferate and exert effector functions such as cytotoxicity and cytokine secretion in response to antigen stimulation. This phenomenon has been termed

T cell "exhaustion" and was first described in T cells in mice chronically infected with lymphocytic choriomeningitis virus (LCMV) (Zajac et al. 1998). Further studies identified that "exhausted" T cells exhibit sustained expression of the inhibitory molecule programmed cell death 1 (PD-1) and that blockade of PD-1 and PD-1 ligand (PD-L1) interactions can partially reverse T cell "exhaustion" and restore antigen specific T cell responses in LCMV infected mice (Barber et al. 2006). Importantly, T cell "exhaustion" also occurs during chronic viral infections in humans (Klenerman and Hill 2005) and $CD8^+$ T cells in humans chronically infected with HIV (Day et al. 2006; Petrovas et al. 2006; Trautmann et al. 2006), hepatitis B virus (HBV) (Boettler et al. 2006), and HCV (Urbani et al. 2006) express high levels of PD-1 and blockade of PD-1/PD-L interactions can partially restore T cell function in vitro.

Interestingly, a recent study in patients with HIV has shown that TIM-3 is also upregulated on "exhausted" $CD8^+$ T cells (Fig. 2) and that TIM-3 and PD-1 mark distinct populations of "exhausted" cells (Jones et al. 2008). T cells positive for both PD-1 and TIM-3 were rare. Similarly, another group has shown that TIM-3 is upregulated on "exhausted" T cells in patients with HCV (Golden-Mason et al. 2009). In this case, cells that co-express TIM-3 and PD-1 are the most abundant fraction among HCV-specific $CD8^+$ T cells. In both studies, blocking TIM-3 partially restored T cell proliferation and enhanced cytokine production. Given that blockade of the TIM-3 and PD-1 pathways has each been shown individually to partially restore function to "exhausted" T cells and the fact that these molecules are expressed on distinct and overlapping T cell populations in chronically infected patients raises the possibility that blockade of both pathways may prove most effective in restoring function to "exhausted" T cells. Indeed, combined blockade of PD-1 and TIM-3 during the priming/differentiation phase of Friend virus (FV) infection has been shown to restore $CD8^+$ T cell functionality and virus control to otherwise nonresponsive or "exhausted" T cells (Takamura et al. 2010).

While it is clear that TIM-3 plays an important role in T cell exhaustion, many questions remain. Whether galectin-9 is involved in this function of TIM-3 has not been addressed experimentally. If galectin-9 is involved, then why do these $TIM\text{-}3^+$ cells persist and escape galectin-9-induced cell death? Some answers may lie in the elucidation of the TIM-3 signaling cascade in exhausted T cells versus bona fide $TIM\text{-}3^+$ IFN-γ-secreting Tc1 cells. Another possibility is that integration of signals through other inhibitory receptors changes the response to TIM-3 ligation in exhausted T cells. Further breakdown of the distribution of inhibitory receptors (PD-1, TIM-3, Lag-3, and CTLA-4) on exhausted T cells and how these define different subpopulations of exhausted cells will advance our understanding of how exhaustion is induced, maintained, and most effectively reversed.

Several lines of evidence also suggest that during chronic viral infection and virus-associated malignancy, an elevated expression of galectin-9, may be related to suppression of adaptive immune responses. In chronic HCV infection, it was reported that an increased expression of galectin-9 in serum and in Kupffer cells during chronic infection is associated with expansion of $CD4^+CD25^+FoxP3^+C\text{-}D127^{lo}$ regulatory T cells, contraction of $CD4^+$ effector T cells, and apoptosis

of HCV-specific CTLs (Mengshol et al. 2010). In Epstein–Barr virus (EBV)-associated nasopharyngeal carcinoma (NPC), one of the most common virus-associated human malignancies, it has been reported that NPC cells release galectin-9-containing exosomes that induce massive apoptosis in EBV-specific $CD4^+$ T cells, which can be inhibited by both anti-TIM-3 and anti-galectin-9 blocking antibodies (Klibi et al. 2009). These observations indicate that the galectin-9–TIM-3 pathway can be adopted to escape immune surveillance during both chronic viral infection and tumor progression. Thus, blockade of the galectin-9–TIM-3 pathway might help to reinvigorate anti-viral and anti-tumor immunity and thereby improving the clinical efficacy of current immunotherapies.

6.2 TIM-3 in Other Diseases

It has been shown in a mouse model of acute graft-versus-host disease (aGVHD) that TIM-3 expression is dramatically upregulated in both donor and host-derived hepatic $CD8^+$ T cells. Blockade of the TIM-3 signaling pathway with anti-TIM-3 antibodies results in significantly increased IFN-γ expression by splenic and hepatic $CD4^+$ and $CD8^+$ T cells and exacerbates aGVHD (Oikawa et al. 2006). This result demonstrates that TIM-3 is crucial in the regulation of hepatic $CD8^+$ T cell homeostasis and tolerance.

In a mouse model of coxsackievirus B3 (CVB3)-induced autoimmune heart disease, the TIM-3 signaling pathway has been shown to affect the adaptive immune system through effects on the innate immune system. Specifically, blockade of TIM-3 with anti-TIM-3 antibody in vivo exacerbates acute myocarditis due to reduced TIM-3 and CD80 expression on mast cells and macrophages and the amount of intracellular CTLA-4 in $CD4^+$ T cells (Frisancho-Kiss et al. 2006), resulting in increased macrophages/neutrophils and reduced Treg populations in the heart (Frisancho-Kiss et al. 2006).

7 Conclusions

Since the initial discovery of TIM-3, much progress has been made in characterizing TIM-3 ligands and TIM-3 function in immune responses in different disease states. It is now well appreciated that besides Th1 and Tc1 cells, TIM-3 is also expressed on DCs and macrophages, and even on non-immune cells during tumor development, suggesting a complex biological role for TIM-3. While the role of TIM-3 on non-lymphoid cells is still being investigated, accumulating evidence suggests that TIM-3 negatively regulates the functions of Th1 and Tc1 cells. Reduced TIM-3 expression correlates with increased IFN-γ production of CSF T cell clones in MS patients and escape from TIM-3-mediated regulation. In contrast, sustained expression of TIM-3 contributes to the exhausted phenotype of viral

antigen specific $CD8^+$ T cells in chronic HIV and HCV infection. Elucidating the mechanism(s) by which TIM-3 impacts on T cell function in different human autoimmune diseases and chronic viral infections will provide new therapeutic targets for treating these diseases.

References

Anderson AC, Anderson DE, Bregoli L, Hastings WD, Kassam N, Lei C, Chandwaskar R, Karman J, Su EW, Hirashima M et al (2007) Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells. Science 318:1141–1143

Anderson AC, Lord GM, Dardalhon V, Lee DH, Sabatos-Peyton CA, Glimcher LH, Kuchroo VK (2010) T-bet, a Th1 transcription factor regulates the expression of Tim-3. Eur J Immunol 40:859–866

Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R (2006) Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 439:682–687

Boettler T, Panther E, Bengsch B, Nazarova N, Spangenberg HC, Blum HE, Thimme R (2006) Expression of the interleukin-7 receptor alpha chain (CD127) on virus-specific $CD8^+$ T cells identifies functionally and phenotypically defined memory T cells during acute resolving hepatitis B virus infection. J Virol 80:3532–3540

Bork P, Holm L, Sander C (1994) The immunoglobulin fold. Structural classification, sequence patterns and common core. J Mol Biol 242:309–320

Butterfield RJ, Sudweeks JD, Blankenhorn EP, Korngold R, Marini JC, Todd JA, Roper RJ, Teuscher C (1998) New genetic loci that control susceptibility and symptoms of experimental allergic encephalomyelitis in inbred mice. J Immunol 161:1860–1867

Cao E, Zang X, Ramagopal UA, Mukhopadhaya A, Fedorov A, Fedorov E, Zencheck WD, Lary JW, Cole JL, Deng H et al (2007) T cell immunoglobulin mucin-3 crystal structure reveals a galectin-9-independent ligand-binding surface. Immunity 26:311–321

Chae SC, Park YR, Lee YC, Lee JH, Chung HT (2004a) The association of TIM-3 gene polymorphism with atopic disease in Korean population. Hum Immunol 65:1427–1431

Chae SC, Park YR, Shim SC, Yoon KS, Chung HT (2004b) The polymorphisms of Th1 cell surface gene Tim-3 are associated in a Korean population with rheumatoid arthritis. Immunol Lett 95:91–95

Chen Y, Langrish CL, McKenzie B, Joyce-Shaikh B, Stumhofer JS, McClanahan T, Blumenschein W, Churakovsa T, Low J, Presta L et al (2006) Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. J Clin Invest 116:1317–1326

Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, Mackey EW, Miller JD, Leslie AJ, DePierres C et al (2006) PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature 443:350–354

DeKruyff RH, Bu X, Ballesteros A, Santiago C, Chim YL, Lee HH, Karisola P, Pichavant M, Kaplan GG, Umetsu DT et al (2010) T cell/transmembrane, Ig, and mucin-3 allelic variants differentially recognize phosphatidylserine and mediate phagocytosis of apoptotic cells. J Immunol 184:1918–1930

Frisancho-Kiss S, Nyland JF, Davis SE, Barrett MA, Gatewood SJ, Njoku DB, Cihakova D, Silbergeld EK, Rose NR, Fairweather D (2006) Cutting edge: T cell Ig mucin-3 reduces inflammatory heart disease by increasing CTLA-4 during innate immunity. J Immunol 176:6411–6415

Golden-Mason L, Palmer BE, Kassam N, Townshend-Bulson L, Livingston S, McMahon BJ, Castelblanco N, Kuchroo V, Gretch DR, Rosen HR (2009) Negative immune regulator Tim-3

is overexpressed on T cells in hepatitis C virus infection and its blockade rescues dysfunctional $CD4^+$ and $CD8^+$ T cells. J Virol 83:9122–9130

Grattan M, Mi QS, Meagher C, Delovitch TL (2002) Congenic mapping of the diabetogenic locus Idd4 to a 5.2-cM region of chromosome 11 in NOD mice: identification of two potential candidate subloci. Diabetes 51:215–223

Graves PE, Siroux V, Guerra S, Klimecki WT, Martinez FD (2005) Association of atopy and eczema with polymorphisms in T-cell immunoglobulin domain and mucin domain-IL-2-inducible T-cell kinase gene cluster in chromosome 5 q 33. J Allergy Clin Immunol 116:650–656

Hastings WD, Anderson DE, Kassam N, Koguchi K, Greenfield EA, Kent SC, Zheng XX, Strom TB, Hafler DA, Kuchroo VK (2009) TIM-3 is expressed on activated human $CD4^+$ T cells and regulates Th1 and Th17 cytokines. Eur J Immunol 39:2492–2501

Huang X, Bai X, Cao Y, Wu J, Huang M, Tang D, Tao S, Zhu T, Liu Y, Yang Y et al (2010) Lymphoma endothelium preferentially expresses Tim-3 and facilitates the progression of lymphoma by mediating immune evasion. J Exp Med 207:505–520

Imaizumi T, Kumagai M, Sasaki N, Kurotaki H, Mori F, Seki M, Nishi N, Fujimoto K, Tanji K, Shibata T et al (2002) Interferon-gamma stimulates the expression of galectin-9 in cultured human endothelial cells. J Leukoc Biol 72:486–491

Jones RB, Ndhlovu LC, Barbour JD, Sheth PM, Jha AR, Long BR, Wong JC, Satkunarajah M, Schweneker M, Chapman JM et al (2008) Tim-3 expression defines a novel population of dysfunctional T cells with highly elevated frequencies in progressive HIV-1 infection. J Exp Med 205:2763–2779

Khademi M, Illes Z, Gielen AW, Marta M, Takazawa N, Baecher-Allan C, Brundin L, Hannerz J, Martin C, Harris RA et al (2004) T Cell Ig- and mucin-domain-containing molecule-3 (TIM-3) and TIM-1 molecules are differentially expressed on human Th1 and Th2 cells and in cerebrospinal fluid-derived mononuclear cells in multiple sclerosis. J Immunol 172:7169–7176

Klenerman P, Hill A (2005) T cells and viral persistence: lessons from diverse infections. Nat Immunol 6:873–879

Klibi J, Niki T, Riedel A, Pioche-Durieu C, Souquere S, Rubinstein E, Le Moulec S, Guigay J, Hirashima M, Guemira F et al (2009) Blood diffusion and Th1-suppressive effects of galectin-9-containing exosomes released by Epstein-Barr virus-infected nasopharyngeal carcinoma cells. Blood 113:1957–1966

Koguchi K, Anderson DE, Yang L, O'Connor KC, Kuchroo VK, Hafler DA (2006) Dysregulated T cell expression of TIM3 in multiple sclerosis. J Exp Med 203:1413–1418

Kono DH, Burlingame RW, Owens DG, Kuramochi A, Balderas RS, Balomenos D, Theofilopoulos AN (1994) Lupus susceptibility loci in New Zealand mice. Proc Natl Acad Sci USA 91:10168–10172

McIntire JJ, Umetsu SE, Akbari O, Potter M, Kuchroo VK, Barsh GS, Freeman GJ, Umetsu DT, DeKruyff RH (2001) Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family. Nat Immunol 2:1109–1116

Mengshol JA, Golden-Mason L, Arikawa T, Smith M, Niki T, McWilliams R, Randall JA, McMahan R, Zimmerman MA, Rangachari M, Dobrinskikh E, Busson P, Polyak SJ, Hirashima M, Rosen HR (2010) A crucial role for Kupffer cell-derived galectin-9 in regulation of T cell immunity in hepatitis C infection. PLoS One 5(3):e9504

Miyanishi M, Tada K, Koike M, Uchiyama Y, Kitamura, Nagata S (2007) Identification of Tim4 as a phosphatidylserine receptor. Nature 450:435–439

Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, Manning S, Greenfield EA, Coyle AJ, Sobel RA et al (2002) Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. Nature 415:536–541

Nagahara K, Arikawa T, Oomizu S, Kontani K, Nobumoto A, Tateno H, Watanabe K, Niki T, Katoh S, Miyake M et al (2008) Galectin-9 increases Tim-3+ dendritic cells and $CD8^+$ T cells and enhances antitumor immunity via galectin-9-Tim-3 interactions. J Immunol 181:7660–7669

Nakae S, Iikura M, Suto H, Akiba H, Umetsu DT, Dekruyff RH, Saito H, Galli SJ (2007) TIM-1 and TIM-3 enhancement of Th2 cytokine production by mast cells. Blood 110:2565–2568

Nakayama M, Akiba H, Takeda K, Kojima Y, Hashiguchi M, Azuma M, Yagita H, Okumura K (2009) Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation. Blood 113:3821–3830

Niwa H, Satoh T, Matsushima Y, Hosoya K, Saeki K, Niki T, Hirashima M, Yokozeki H (2009) Stable form of galectin-9, a Tim-3 ligand, inhibits contact hypersensitivity and psoriatic reactions: a potent therapeutic tool for Th1- and/or Th17-mediated skin inflammation. Clin Immunol 132:184–194

Oikawa T, Kamimura Y, Akiba H, Yagita H, Okumura K, Takahashi H, Zeniya M, Tajiri H, Azuma M (2006) Preferential involvement of Tim-3 in the regulation of hepatic $CD8^+$ T cells in murine acute graft-versus-host disease. J Immunol 177:4281–4287

Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, Adams WC, Precopio ML, Schacker T, Roederer M, Douek DC et al (2006) PD-1 is a regulator of virus-specific $CD8^+$ T cell survival in HIV infection. J Exp Med 203:2281–2292

Rabinovich GA, Toscano MA (2009) Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. Nat Rev Immunol 9:338–352

Sabatos CA, Chakravarti S, Cha E, Schubart A, Sanchez-Fueyo A, Zheng XX, Coyle AJ, Strom TB, Freeman GJ, Kuchroo VK (2003) Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance. Nat Immunol 4:1102–1110

Sanchez-Fueyo A, Tian J, Picarella D, Domenig C, Zheng XX, Sabatos CA, Manlongat N, Bender O, Kamradt T, Kuchroo VK et al (2003) Tim-3 inhibits T helper type 1-mediated auto- and alloimmune responses and promotes immunological tolerance. Nat Immunol 4:1093–1101

Santiago C, Ballesteros A, Martinez-Munoz L, Mellado M, Kaplan GG, Freeman GJ, Casasnovas JM (2007a) Structures of T cell immunoglobulin mucin protein 4 show a metal-Ion-dependent ligand binding site where phosphatidylserine binds. Immunity 27:941–951

Santiago C, Ballesteros A, Tami C, Martinez-Munoz L, Kaplan GG, Casasnovas JM (2007b) Structures of T cell immunoglobulin mucin receptors 1 and 2 reveal mechanisms for regulation of immune responses by the TIM receptor family. Immunity 26:299–310

Sehrawat S, Suryawanshi A, Hirashima M, Rouse BT (2009) Role of Tim-3/galectin-9 inhibitory interaction in viral-induced immunopathology: shifting the balance toward regulators. J Immunol 182:3191–3201

Seki M, Oomizu S, Sakata KM, Sakata A, Arikawa T, Watanabe K, Ito K, Takeshita K, Niki T, Saita N et al (2008) Galectin-9 suppresses the generation of Th17, promotes the induction of regulatory T cells, and regulates experimental autoimmune arthritis. Clin Immunol 127:78–88

Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH (2000) A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 100:655–669

Takamura S, Tsuji-Kawahara S, Yagita H, Akiba H, Sakamoto M, Chikaishi T, Kato M, Miyazawa M (2010) Premature terminal exhaustion of Friend virus-specific effector $CD8^+$ T cells by rapid induction of multiple inhibitory receptors. J Immunol 184:4696–4707

Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, Boulassel MR, Delwart E, Sepulveda H, Balderas RS et al (2006) Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. Nat Med 12:1198–1202

Urbani S, Amadei B, Tola D, Massari M, Schivazappa S, Missale G, Ferrari C (2006) PD-1 expression in acute hepatitis C virus (HCV) infection is associated with HCV-specific CD8 exhaustion. J Virol 80:11398–11403

van de Weyer PS, Muehlfeit M, Klose C, Bonventre JV, Walz G, Kuehn EW (2006) A highly conserved tyrosine of Tim-3 is phosphorylated upon stimulation by its ligand galectin-9. Biochem Biophys Res Commun 351:571–576

Wang F, He W, Zhou H, Yuan J, Wu K, Xu L, Chen ZK (2007) The Tim-3 ligand galectin-9 negatively regulates CD8+ alloreactive T cell and prolongs survival of skin graft. Cell Immunol 250:68–74

Wiener Z, Kohalmi B, Pocza P, Jeager J, Tolgyesi G, Toth S, Gorbe E, Papp Z, Falus A (2007) TIM-3 is expressed in melanoma cells and is upregulated in TGF-beta stimulated mast cells. J Invest Dermatol 127:906–914

Wilker PR, Sedy JR, Grigura V, Murphy TL, Murphy KM (2007) Evidence for carbohydrate recognition and homotypic and heterotypic binding by the TIM family. Int Immunol 19:763–773

Yang L, Anderson DE, Kuchroo J, Hafler DA (2008) Lack of TIM-3 immunoregulation in multiple sclerosis. J Immunol 180:4409–4414

Zajac AJ, Blattman JN, Murali-Krishna K, Sourdive DJ, Suresh M, Altman JD, Ahmed R (1998) Viral immune evasion due to persistence of activated T cells without effector function. J Exp Med 188:2205–2213

Zhu C, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ, Zheng XX, Strom TB, Kuchroo VK (2005) The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol 6:1245–1252