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Emerging Tim-3 functions in anti-microbial and tumor immunity

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Abstract

T cell immunoglobulin-3 (Tim-3) was identified as a marker of differentiated IFN- γ -producing CD4⁺ T helper type 1 and CD8⁺ T cytotoxic type 1 cells. The interaction of Tim-3 with its ligand, galectin-9 (Gal-9), induces cell death and in vivo blockade of this interaction results in exacerbated autoimmunity and abrogation of tolerance in experimental models, establishing Tim-3 as a negative regulatory molecule. Recent studies have uncovered additional mechanisms by which Tim-3 negatively regulates T cell responses, such as by promoting the development of CD8⁺ T cell exhaustion and inducing expansion of myeloid-derived suppressor cells (MDSC). In contrast to this inhibitory effect on T cells, Tim-3-Gal-9 interaction promotes macrophage clearance of intracellular pathogens. Here, we will focus on the emerging role for Tim-3 in tumor and anti-microbial immunity.

The known roles of Tim-3 in T cells

Tim-3 was first discovered in 2002 as a molecule expressed on IFN- γ -producing CD4⁺ T helper type 1 (Th1) and on CD8⁺ T cytotoxic type 1 (Tc1) cells ¹. The S-type lectin galectin-9 (Gal-9) was then identified as a Tim-3 ligand. Gal-9 is a soluble molecule that is widely expressed, is upregulated by IFN- γ ² and binds to oligosaccharides on the Tim-3 IgV domain. Gal-9 triggering of Tim-3 on Th1 cells has been shown to induce cell death (³ and Figure 1A). Thus, Tim-3 came to be known as a negative regulatory molecule important for abrogating Th1 and Tc1 driven immune responses.

Tim-3 belongs to the Tim family of molecules that in mice contains 8 members (Tim-1–8). Of these, only Tim-1, Tim-3 and Tim-4 are expressed in humans. All of the Tim molecules share a common structural organization consisting of an N-terminal IgV domain followed by a mucin domain, a transmembrane domain and a cytoplasmic tail. Of note are the four non-canonical cysteines in the IgV domain that are conserved in all the Tims in both mouse and man, which result in the formation of a unique binding cleft not seen in the Ig domain of any other Ig superfamily members ^{4,5}. This cleft has been shown to be important for the binding of Tim-1, 3 and 4 to phosphatidyl serine (PS) and for the clearance of apoptotic bodies by cells expressing these Tim molecules such as macrophages and dendritic cells (DCs) ^{6–10}.

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Consistent with its role as an inhibitory molecule, blockade of the Tim-3–Tim-3L pathway *in vivo* by either blocking antibody or soluble Tim-3 Ig fusion protein (Tim-3Ig), which serves to block Tim-3–Gal-9 interactions, exacerbates experimental allergic encephalomyelitis (EAE) and Type-1 diabetes (T1D)^{1,11}. Similarly, abrogation of Tim-3 signaling either with blocking antibody or by RNA interference increases the secretion of IFN- γ by activated human T cells^{12,13}. The physiological relevance of this negative regulatory function of Tim-3 is highlighted by the fact that Tim3 expression is down-regulated and IFN- γ is increased in T cell clones isolated from the cerebrospinal fluid of patients with MS relative to those from healthy control subjects, suggesting that low expression of Tim-3 allows for auto-pathogenic cells to escape regulation¹³. Interestingly, blocking Tim-3 during CD4⁺ T cell stimulation does not enhance IFN- γ secretion in cells from MS patients who have not received treatment, suggesting that impaired immunoregulation in these individuals might be due not only to reduced expression of Tim-3 but also a functional defect in Tim-3-mediated signaling. CD4⁺ T cells from MS patients who have undergone treatment with two different therapeutic regimens exhibited a restoration of both Tim-3 expression and functional responsiveness to Tim-3 blockade¹⁴, suggesting that restoration of Tim-3-mediated regulation of Th1 cells may underlie the clinical effect and that Tim-3 could be an important target for treatment of MS.

Tim-3 is also involved in the induction of peripheral tolerance. Administration of Tim-3Ig abrogates the development of tolerance in Th1 cells and Tim-3-deficient mice are refractory to induction of tolerance by administration of high dose aqueous antigen¹⁵. Of note, it has been shown that Tim-3 can regulate auto- and alloimmunity by modulating the capacity of regulatory T cells to dampen inflammatory responses¹¹. In addition, studies in a murine graft-versus-host disease model and a hepatitis B infection model support a role for Tim-3 in negatively regulating IFN- γ -producing CD8⁺ Tc1 cells^{16,17}.

New findings regarding the inhibitory role of Tim-3 have emerged over the past few years and it is now well established that Tim-3 is highly expressed on “exhausted” or impaired CD8 T cells in various chronic viral infections^{18–21} and in tumor-bearing hosts^{22,23,24,25} (Figure 1B). More importantly, the function of these impaired CD8 cells can be restored by blocking the Tim-3–Tim-3L pathway, thereby supporting the potential of targeting this pathway to improve immunity in chronic viral infections and cancer. Furthermore, it has now been shown that Tim-3 on CD4⁺ Th1 cells promotes clearance of intracellular pathogens via recognition of Gal-9 on macrophages²⁶ (Figure 1D). In this review, these recently revealed functions of Tim-3 in anti-microbial and tumor immunity are discussed in detail.

Tim-3 in exhausted CD8⁺ T cells

A recent breakthrough regarding the role of Tim-3 in CD8⁺ T cells came from the examination of “exhausted” T cells. T cell “exhaustion” describes a state of T cell dysfunction that was initially observed during chronic lymphocytic choriomeningitis virus (LCMV) infection in mice^{27,28}. “Exhausted” T cells fail both to proliferate and exert effector functions such as cytotoxicity and cytokine secretion in response to antigen stimulation. Initial studies identified that “exhausted” T cells are characterized by 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^{29,30}. T cell “exhaustion” also occurs during chronic infections in humans (reviewed in³¹). CD8⁺ T cells in humans chronically infected with human immunodeficiency virus (HIV)^{32–34}, hepatitis B virus (HBV)^{35,36} and hepatitis C virus (HCV)^{37,38} express high levels of PD-1 and blocking of PD-1–PD-L1 interactions can partially restore T cell function *in vitro*.

The PD-1–PD-L1 pathway is also involved in T cell “exhaustion” in cancer. PD-1 expression is found on tumor infiltrating CD8⁺ T cells in multiple solid tumors^{39–41} and on antigen specific CD8⁺ T cells in hosts with non-solid tumors^{42,43}. These PD-1⁺ T cells are dysfunctional and high expression of PD-L1 on tumors has been associated with poor prognosis^{44–46}. Importantly, interference with PD-1–PD-L1 signaling either through antibody blockade or PD-1 deficiency has been shown to improve clinical outcome and partially restore functional T cell responses in both human cancers and experimental models^{39,42,43,47}. While, these data support that targeting the PD-1–PD-L1 pathway can reverse T cell exhaustion and improve anti-tumor immunity, it has been shown that targeting the PD-1–PD-L1 pathway does not always result in reversal of T cell “exhaustion”^{33,40} and that PD-1 expression is not always associated with “exhausted” phenotype in tumor-bearing hosts⁴⁸. This indicates that other pathways are likely involved. Therefore, to fully restore function in exhausted T cells, it is important to identify the additional pathways and mechanisms that may be involved.

A recent study in patients with HIV has shown that Tim-3 is upregulated on “exhausted” CD8⁺ T cells²¹. In HIV patients, Tim-3 marks a distinct population of exhausted cells from those expressing PD-1. Similarly, Tim-3 has also been found on exhausted T cells in patients with HCV^{20,49} and HBV⁵⁰. In HCV, cells that co-express Tim-3 and PD-1 are the most abundant fraction among HCV-specific CD8⁺ T cells.

Recent studies have examined Tim-3 expression in both acute and chronic models of viral infection. In acute LCMV and HSV infection, Tim-3 is expressed on CD8⁺ T cells; however, this expression is transient and is found on only a small fraction of cells^{[18] 51}. Blockade of Tim-3–Gal-9 signals during the acute phase of HSV results in increased effector and memory CD8⁺ T cell responses and more efficient viral control⁵¹. In contrast, virus-specific CD8⁺ T cells in both chronic LCMV and Friend virus infection exhibit sustained high-level expression of Tim-3 on a large fraction of cells. These cells co-express PD-1 and exhibit impaired effector cytokine production^{19,52}. Interestingly, in these experimental models of viral infection, as well as in T cells from patients chronically infected with HIV and HCV, blocking both the Tim-3 and PD-1 pathways restores T cell proliferation and enhances cytokine production. Thus, in chronic viral infections both the Tim-3 and PD-1 pathways seem to impact on T cell “exhaustion” (Figure 1B).

Tim-3 in cancer

As is the case in chronic viral infections, both CD4⁺ and CD8⁺ tumor infiltrating lymphocytes (TILs) in mice bearing the solid tumors, CT-26, 4T1 and B16, (murine models for colon adenocarcinoma, mammary adenocarcinoma, and melanoma, respectively) co-express Tim-3 and PD-1. Tim-3⁺PD-1⁺ T cells represent a major population within the CD8⁺ TILs in all three models and virtually all Tim-3⁺ TILs co-express PD-1²³. Interestingly, upregulation of Tim-3 has also been observed in PD-1⁺ tumor antigen specific CD8⁺ T cells in the blood of patients with advanced melanoma²². In both cases, Tim-3⁺PD-1⁺ CD8⁺ cells represent the most impaired population of CD8⁺ T cells in tumor-bearing hosts in that they exhibit defects in proliferation and produce the least amount of IL-2, TNF, and IFN- γ . Of note, examination of CD8⁺ TILs in the murine CT-26 tumor model revealed that the PD-1⁺ single positive TILs that do not express Tim-3 on the cell surface, produced the most IFN- γ among all the populations of TILs. Furthermore, this population was less impaired in progression through cell cycle, as well as in the production of IL-2 and TNF, than the Tim-3⁺PD-1⁺ TILs. Collectively these data support the idea that the Tim-3⁺PD-1⁺ TILs represent the most exhausted TILs and that Tim-3⁺PD-1⁺ TILs may contain a mixture of “exhausted” T cells and bona fide effector T cells.

As the blockade of either the PD-1 or Tim-3 signaling pathways can improve T cell function in the context of chronic infections^{20,21,29,53} this raised the possibility that combined targeting of these two pathways may prove to be the most efficacious means to restore anti-tumor immunity *in vivo*. Indeed, treatment with anti-Tim-3 alone had little or no effect and treatment with anti-PD-L1 alone showed a trend towards delayed tumor growth that did not reach statistical significance. However, combined treatment with anti-Tim-3 and anti-PD-L1 resulted in a dramatic reduction in tumor growth with 50% of the mice exhibiting complete tumor regression²³. These data support the notion that the combined targeting of the Tim-3 and PD-1 signaling pathways is effective in restoring anti-tumor immunity *in vivo*. Indeed, treatment with anti-Tim-3 plus anti-PD-L1 antibody restores effector function in TILs cultured *ex vivo*.

In line with these observations, the synergistic effect of blocking both pathways was also observed in samples from melanoma patients²². When tumor-antigen specific CD8⁺ T cells from PBMC of melanoma patients were activated *in vitro* with specific antigen, the strongest increase in IFN- γ , TNF, and IL-2 production was observed in the cultures when both the Tim-3 and PD-1 signaling pathways were blocked with anti-Tim-3 and anti-PD-L1 antibodies. Interestingly, combination of anti-Tim3 and anti-PD-L1 also increased the frequency of proliferating antigen specific CD8⁺ cells, thereby increasing the total number of tumor-antigen specific CD8⁺ T cells in the culture.

Exhausted CD8⁺ T cells that co-express Tim-3 and PD-1 have also been found in mice with advanced acute myelogenous leukemia (AML)²⁴. Interestingly in AML, the frequency of Tim-3⁺PD-1⁺ CD8⁺ T cells also increases as disease progresses. Mirroring the result obtained in solid tumors, the treatment of mice with AML with anti-PD-L1 and Tim-3Ig results in significant reduction in tumor burden and superior survival advantage over mice receiving either treatment alone. Collectively, current data support that the combined targeting of the Tim-3 and PD-1 pathways may be most effective in restoring function of antigen specific CD8⁺ T cells in both solid and non-solid cancers in humans and as well as in experimental models.

While current data strongly implicate Tim-3 in T cell exhaustion, whether gal-9 or another Tim-3 ligand is involved in T cell exhaustion remains to be addressed. Gal-9 is upregulated by IFN γ ²; however, T cell exhaustion is associated with low IFN γ , thereby raising the possibility that when gal-9 is low Tim-3⁺ T cells may escape gal-9 triggered cell death. Counter to this is the observation that in chronic HCV infection, serum levels of gal-9 are high relative to healthy controls⁵⁴. Clearly, more investigation into the expression of gal-9 in different chronic conditions is required in order to determine its role in T cell exhaustion. (see Box 1.)

Role of Tim-3 in promoting myeloid-derived suppressor cells

Although Tim-3 inhibits T cell responses directly by inducing cell death and exhaustion, recent data show that Tim-3 expressed on T cells also suppresses immune responses indirectly by inducing expansion of myeloid derived suppressor cells (MDSCs).

MDSC are heterogeneous CD11b⁺Gr-1⁺ myeloid cells that expand in large numbers in tumor-bearing individuals and in infection, trauma, and autoimmunity. MDSC are potent suppressors of T cell responses and their presence is correlated with poor clinical outcome in cancer⁵⁵. MDSC can be subdivided into two classes, monocytic (Ly6C^{high}Ly6G⁻) and granulocytic (Ly6C^{low}Ly6G⁺) cells. Transgenic overexpression of Tim-3 on T cells results in inhibition of T cell responses and an increase in CD11b⁺Ly6G⁺ cells that share morphology consistent with granulocytic MDSC⁵⁶. Moreover, they exhibit a molecular signature consistent with CD11b⁺ MDSC cells from tumor-bearing hosts. Similarly,

overexpression of Gal-9 under the actin promoter results in an increase in CD11b⁺Ly6G⁺ cells and inhibition of immune responses. Loss of Tim-3 reduces the levels of CD11b⁺Ly6G⁺ cells and generates normal immune responses in Gal-9 transgenic mice. The physiological relevance of the role of Tim-3 in modulating the CD11b⁺ population was demonstrated by implanting tumors into Tim-3 Tg mice. Accelerated growth of EL4 lymphoma was observed in Tim-3 Tg mice and the frequency of MDSCs in the spleen was increased compared to wild-type (WT) mice. Furthermore, treatment with the anti-Tim-3 antibody suppressed EL4 growth, which was coincident with less expansion of MDSC. Lastly, there was less tumor growth and MDSC expansion in Tim-3-deficient mice implanted with 4T1 breast cancer. These data together demonstrate that Tim-3-Gal-9 recognition can also impact on tumor growth by regulating the expansion of MDSC that are known to inhibit immune responses (Figure 1C).

The role of Tim-3 in anti-microbial immunity

Chronic bacterial pathogens such as *Mycobacterium tuberculosis* (*Mtb*) evade humoral immunity by adapting to the normally hostile intracellular environment of macrophages, where they replicate and persist. Host resistance against *Mtb* relies on Th1 mediated immunity⁵⁷. Although IFN- γ produced by Th1 cells plays an important role in controlling intracellular pathogens, there is also evidence for a cognate interaction between T cells and infected macrophages to suppress bacterial growth. The expression of Tim-3 on Th1 cells raises the question of whether Tim-3 provides that cognate signal and plays a role in immunity against intracellular pathogens like *Mtb*. Indeed, the accumulation of Tim-3⁺ T cells in the lungs following *Mtb* infection and the pulmonary expression of Gal9 support a function for Tim3 and Gal9 during host immunity to *Mtb*²⁶. Administration of Tim-3-Ig reduces the pulmonary bacterial load in both WT and *Rag*^{-/-} mice, indicating that Tim3 binding to innate cells in the lung can activate antibacterial activity. Indeed, *in vitro* experiments confirmed that binding of Tim-3-Ig to cell surface Gal9 expressed by infected macrophages activates an antimicrobial program that restricts intracellular replication of *Mtb*. The antimycobacterial activity stimulated by Tim-3-Gal-9 is independent of the “canonical” antibacterial pathways as it occurs in the absence of IFN- γ signaling and does not require nitric oxide synthase. Instead, the pathways activated by Tim-3-Gal9 binding lead to caspase-1-dependent secretion of IL-1 β and autocrine signaling via the IL-1 receptor resulting in suppression of the bacterial growth²⁶ (Figure 1D). Thus, Tim-3⁺ antigen-specific T cells activate *Mtb*-infected cells to produce cytokines including IL-1, which promote host resistance to *Mtb*.

Concluding remarks

Over the past decade, the great emphasis on learning how activated immune cells are regulated to avoid hyperactivation has led to the discovery of several negative regulatory molecules. These negative regulatory molecules ensure that an effector T cell population contracts in a timely manner, such that they do not induce immunopathology, particularly during a chronic immune response. While well-known inhibitory receptors such as CTLA-4 and PD-1 are rapidly up-regulated on all activated T cells, Tim-3 is specifically expressed on IFN- γ producing effector Th1 and Tc1 cells and thus Tim-3 plays a more specialized negative regulatory role. Expression of Tim-3 on T cells has two major functions: 1) to dampen the response of both CD4⁺ and CD8⁺ effector T cells through induction of cell death/exhaustion and/or by promoting MDSC expansion; 2) to enhance the ability of macrophages to eliminate intracellular pathogens through binding to Gal-9.

These studies point to an very important role for Tim-3 in inducing T cell exhaustion and death, but it is not clear how the expression of Tim-3 is induced on the surface of Th1 cells

except that T-bet (a master transcription factor of Th1 cells) partly regulates its cell surface expression. In addition, downstream intracellular signaling pathways emanating from Tim-3 that lead to T cell exhaustion/death have not been identified. Elucidation of the Tim-3 signaling pathway that leads to induction of T cell exhaustion versus death will provide new targets for modulating anti-tumor immunity in cancer and anti-viral immunity in chronic viral infections.

Box1 Tim-3: unanswered questions

T cell exhaustion and cancer

- What factors regulate Tim-3 expression?
- How is Tim-3 taking part in inducing or maintaining exhaustion?
- Is Gal-9 triggering of Tim-3 important in exhaustion?
- Tim-3 is also highly expressed on CD4⁺ TILs. What is the role of Tim-3 on these cells?
- Why do Tim-3⁺ cells persist in chronic conditions? Do they escape death or are they constantly being generated?

Anti-microbial immunity

- How does the Tim-3Gal9 interaction lead to macrophage activation?
- How does Gal-9, a soluble molecule, transduce a signal in innate immune cells to promote IL-1 β secretion?
- What are the cell surface interacting partners of Gal9?
- Does Tim-3-mediated T cell exhaustion take place in chronic bacterial infection?
- Do genetic polymorphisms in loci encoding Tim-3 and Gal-9 modulate susceptibility to infectious diseases in humans?

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Figure 1.

Model of Tim-3 function in the immune response

(a) In acute inflammation, Tim-3 is expressed on terminally differentiated IFN- γ producing CD4⁺ and CD8⁺ T cells. Upon recognition of its ligand, galectin-9, Tim-3 expressing T cells undergo apoptosis. (b), In chronic inflammation, Tim-3 is co-expressed with PD-1 on dysfunctional or “exhausted” CD8⁺ T cells. Combined targeting of both the PD-1–PD-L1 and Tim-3–Tim-3L pathways successfully restores CD8⁺ T cell effector function and ameliorates chronic disease. (c), Tim-3 on T cells interacts with Gal-9 on MDSC precursors to promotes MDSC expansion, which in turn suppresses T cell responses. (d), Tim-3 on CD4⁺ T cells can also act to facilitate killing of intracellular pathogens in macrophages through interaction with gal-9 and a mechanism involving IL-1 β and caspase-1. Overall, Tim-3 on CD4⁺ and CD8⁺ T cells harnesses multiple mechanisms to negatively regulate effector T cell responses and to terminate IFN- γ –mediated inflammation while preserving or enhancing the ability of innate cells to kill intracellular pathogens.