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TIM-3 and Its Immunoregulatory Role in HIV Infection

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Abstract

The chronic nature of Human Immunodeficiency Virus Type 1 (HIV) infection leads to the functional exhaustion of HIV specific CD8$^+$ T cells. Although multiple markers of exhaustion have been identified so far, the recently discovered T cell immunoglobulin and mucin domain 3 (TIM-3) molecules distinguishes bona fide exhausted T cells with replicative senescence and functional impairment. TIM-3 is expressed on T helper type 1 (TH1) and T cytotoxic type 1 (TC1) T cells during chronic HIV infection and once ligated to its ligand transmits negative signaling to arrest T cell function. This negative effect of TIM-3 ligation could be exploited to our advantage as a therapeutic approach to treat HIV-infected individuals. In addition to exhaustion, altered TIM-3 expression has been implicated in autoimmunity and tolerance abrogation.

Keywords: Human Immunodeficiency Virus (HIV); TIM-3; T cells; Immunoregulation

Introduction

The hallmarks of Human Immunodeficiency Virus Type 1 (HIV) infection are immune dysfunction and immune dysregulation [1]. Early in infection, HIV preferentially infects CD4$^+$ T cells and through mechanisms that are not completely understood, leads to the elimination of this target population [2-5]. While the CD4$^+$ T cells are being depleted, HIV specific CD8$^+$ T cells proliferate and mount an effective immune response to contain the virus [6-9]. In the absence of CD4$^+$ T cells, the central organizers of cellular and humoral immunity, effective immune responses against HIV are compromised [10-12]. Without proper CD4$^+$ T cell help, B lymphocytes fail to produce and secrete antibodies, and CD8$^+$ T cells fail to sustain an effective anti-viral response to clear the infection [13,14]. Lack of viral clearance during the acute phase leads to a chronic virus infection with continual stimulation of HIV specific CD8$^+$ T cells, rendering them exhausted [15-17]. The exhausted state of the CD8$^+$ T cells is exacerbated by the absence of CD4$^+$ T cell help [18-20].

Exhausted T cells exist in an altered state of differentiation that is characterized by a stepwise hierarchical loss of effector functions. Exhausted T cells lose proliferative capacity and IL-2 production initially, which is then followed by a loss of TNF-α production and, in extreme cases of exhaustion, T cells even fail to produce IFN-γ [21,22] (Figure 1). In addition, these exhausted cells are poised for apoptosis [18-20]. To date, multiple markers of exhaustion, which are upregulated during chronic stages of viral infection, have been identified. Program death 1 (PD-1), a member of the extended CD28 family of T cell regulator [23,24], was first identified as the primary marker of exhaustion in 2006 [25-27]. However, in HIV infection, PD-1 expressing cells retain their ability to produce cytokines indicating that other inhibitory receptors may be controlling T-cell exhaustion [28-30]. Recently, our lab identified T cell immunoglobulin and mucin domain 3 (TIM-3) as an exhaustion marker on T cells during chronic HIV infection. Further studies have identified TIM-3 as a functional late-stage T-cell exhaustion marker [31,32]. This review will focus on the recent advances in TIM-3 immunoregulatory biology in the context of HIV following a brief overview of TIM-3 biology.

TIM-3 Biology

TIM-3 is a type 1 transmembrane protein consisting of an N-terminal immunoglobulin variable (IgV)-like domain, a central mucin domain, followed by a transmembrane domain and a short intercellular tail that has potential tyrosine phosphorylation motifs putatively involved in signaling events when TIM-3 interacts with its ligand (Figure 2). TIM-3 was initially identified as a molecule expressed exclusively on the surface of T helper type 1 (T_H1) cells and conferred sensitivity to cell death through a mixture of apoptotic and necrotic pathways upon ligation with its ligand [33-35]. To date, TIM-3 expression has been detected on the surface of T_H17 cells, natural killer (NK) T cells, dendritic cells and macrophages [36-38]. The putative

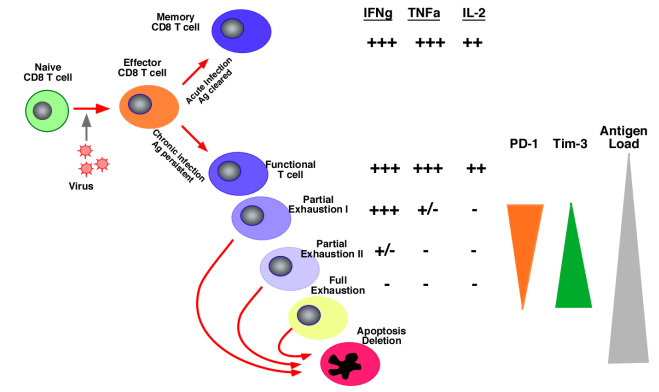

Figure 1: A current proposed model of T cell exhaustion in chronic virus infections. Chronic virus infections are associated with T cell exhaustion, that progresses with duration of infection and antigen presence, exemplified by incremental deficiencies in the ability of virus-specific T cells to produce cytokines, proliferate and to survive. PD-1 is involved early on in T cell exhaustion, and is associated with milder deficiencies in human T cell function, whereas Tim-3 is associated with severe T cell exhaustion.

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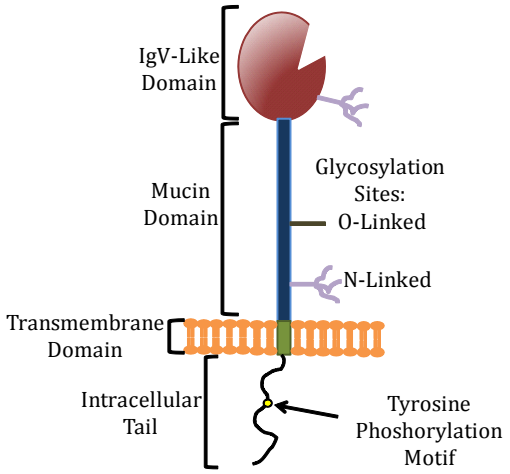

Figure 2: Tim-3 is a type I transmembrane glycoprotein with an extracellular Ig-V-like and mucin domains. The protein contains 2 N-linked and 1 O-linked glycosylation sites.

ligands for human TIM-3 are Galectin-9 and phosphatidylserine [39-41].

In addition to being implicated in the aforementioned exhaustion of T cells in chronic HIV infection, lower levels of TIM-3 expression with ensuing hyper-immune responses has been implicated in autoimmunity and peripheral tolerance abrogation. In experimental autoimmune encephalomyelitis (EAE), a mouse model of human multiple sclerosis (MS), blocking TIM-3 interaction by administering anti-TIM-3 antibody during the induction of EAE led to the exacerbation of the disease and elevated levels of circulating activated macrophages [33]. In a human study, T cell clones from cerebrospinal fluid from MS patients had lower levels of TIM-3 expression and higher levels of secreted IFN-γ than those from healthy control subjects [42]. Furthermore, knocking down TIM-3 expression on T cells from healthy donors by siRNA induced significantly elevated levels of secreted IFN-γ from T_H1 cells [42]. Moreover, in non-obese diabetic mice, blocking TIM-3 accelerated the development of diabetes [34]. Peripheral tolerance can be abrogated by blocking the TIM-3 pathway during ongoing immune response by inducing T_H1 hyper proliferation and TIM-3 deficient mice are refractory to high-dose tolerance induction [35]. Thus, altered or delayed expression of TIM-3 can lead to autoimmunity and tolerance abrogation.

Conversely, higher levels of TIM-3 expression have been implicated in exhaustion during chronic HIV infection. However, HIV by itself can not directly induce Tim-3 expression on T cells [43]. We demonstrated TIM-3 expressing T_H1 and T cytotoxic type 1 (T_C1) T cells during chronic HIV infection as a distinct population of exhausted T cells [44]. TIM-3 expression on exhausted T cells correlated with clinical parameters of HIV progression.

Generalized chronic immune activation, measured by CD38 expression, and viral load positively correlated with TIM-3 expression, while absolute $CD4^+$ T count negatively correlated with TIM-3 expression. Blocking TIM-3-TIM-3L pathway *ex vivo* using a soluble Tim-3 decoy to mop up the ligand enhanced HIV specific responses by HIV specific T cells. Thus, accumulating evidence suggests that the immune response needs to balance the expression of TIM-3 to tone down the initial immune activation for appropriate immune outcome. Dysregulated TIM-3 expression can lead to dysfunctional immune effects in the form of autoimmunity, peripheral tolerance abrogation and T cell exhaustion.

TIM-3 on $CD8^+$ T cells Reduces HIV Replication and Persistence in $CD4^+$ T cells

The inhibitory nature of TIM-3-TIM-3L interaction could be exploited to limit HIV infection of activated $CD4^+$ T cells during the initial phases of HIV infection. Activated $CD4^+$ T cells that express TIM-3 will downregulate the expression of HIV co-receptors CCR5, CXCR4 and α4β7 and concomitantly upregulate p21, a cycline-dependent kinase that has been associated with replication control of HIV virus [45], when incubated with Galectin-9 [46]. Furthermore, TIM-3-Galectin-9 interaction can prevent newer HIV infection of activated $CD4^+$ T cells and limit HIV replication in $CD4^+$ T cells already infected with HIV. This HIV infection inhibitory phenomenon of TIM-3-Galectin-9 interaction is desirable during acute phase of HIV infection to limit HIV infection and replication in activated $CD4^+$ T cells. However, due to the chronic nature of HIV infection, increased TIM-3-Galectin-9 interaction will render T_H1 and T_C1 cells exhausted. Nevertheless, a recent study has demonstrated that in certain HIV infected individuals, TIM-3-Galectin-9 interaction could protect against rampant HIV replication.

Elahi et al. [46] have shown that during the chronic phase of HIV infection in elite controllers (ECs) the interaction between TIM-3 and Galectin-9 from HIV-specific $CD8^+$ T cells and regulatory T cell (T_{reg}), respectively, can confer protection against HIV propagation. ECs are a rare population of chronically HIV-infected individuals endowed with the capacity to control HIV replication with an intact robust and functionally healthy $CD4^+$ T cell population without the intervention of any antiretroviral therapy. ECs are spared from HIV disease progression and lead a longer AIDS-free status in most part due to the presence of HIV specific $CD8^+$ T cells that express specific human leukocyte antigen (HLA) class I alleles, particularly HLA-B*27 and HLA-B*57 [48,49]. Even then, due to the chronic nature of HIV-infection, the HIV-specific $CD8^+$ T cells from ECs express TIM-3 and other inhibitory markers after antigenic stimulation.

Elahi et al. [47] has shown that Treg cells, which are known to constitutively express Galectin-9 [50], could differentially suppress proliferation of HIV specific $CD8^+$ T cells based on the expression of different HLA class I alleles and Tim-3 levels. In co-culture assays with Treg cells, proliferation of HIV specific $CD8^+$ T cells expressing nonprotective HLA class I molecules was restricted but that of HLA-B*27 and HLA-B*57 restricted HIV specific $CD8^+$ T cells was not, despite the protective and nonprotective $CD8^+$ T cells being from the same ECs. Later it was shown that nonprotective HLA class I restricted HIV specific $CD8^+$ T cells expressed significantly more TIM-3 on their surface than the protective HLA class I restricted HIV specific $CD8^+$ T cells. The proliferative suppression was dependent on the TIM-3 and Galectin-9 interaction between the HIV specific $CD8^+$ T cells and T_{reg} cells, respectively.

Since it is already known that HLA-B*27 and HLA-B*57 restricted HIV specific $CD8^+$ T cells express significantly more granzyme B on a per cell basis than HIV specific $CD8^+$ T cell restricted by other HLA class I alleles [51], the authors showed that the ligation of TIM-3, expressed on the surface of HLA-B*27 and HLA-B*57 restricted HIV specific $CD8^+$ T cells, with Galectin-9, expressed on the surface of T_{reg}

cells or T_H1 cells, led to the deletion of T_{reg} and other T_H1 cells through granzyme B mediated pathways. In the case of HIV specific $CD8^+$ T cells restricted by non-protective HLA class I alleles, expression of significantly higher levels of TIM-3 ensures their demise when ligated with Galectin-9 expressed on T_{reg} cells, leaving the host with unchecked virus levels. Although still unclear why HLA-B*27 and HLA-B*57 restricted HIV specific $CD8^+$ T cells expressed significantly less TIM-3 on their surface after antigenic stimulation than the HIV specific $CD8^+$ T cells restricted by non- protective HLA class I alleles, the former was less susceptible to apoptosis, but capable of eliminating T_{reg} and other T_H1 cells potentially infected with HIV or harboring HIV DNA through undefined mechanisms. Thus, TIM-3 levels expressed on the surface of HIV specific $CD8^+$ T cells restricted by particular HLA class I alleles could confer either protection against or susceptibility to rampant HIV replication.

Bat3 as an Immunoregulator of TIM-3 Mediated Exhaustion

It is crucial to understand TIM-3-TIM-3L signaling events in order to exploit the inhibitory nature of TIM-3-TIM-3L interaction as a therapeutic intervention for autoimmunity, tolerance abrogation or exhaustion. In EAE, MS, diabetes and other autoimmune diseases it would be desirable to have enhanced TIM-3-TIM-3L signaling in T_H1 and T_C1 cells to tone down autoimmunity so as to limit disease severity. Conversely in chronic infections and cancer the opposite would be desirable to rescue the functions of exhausted T_H1 and T_C1 T cells to eliminate pathogens and cancer.

Recently Rangachari et al. [52] identified Bat3 as the intracellular binding partner of TIM-3 by using a yeast two-hydrid system. Bat3 is a proline rich cytoplasmic protein that regulates mammalian cell proliferation and death. The Bat3 binding region on mouse TIM-3 was mapped to the tyrosine residues near the C-terminus of the cytoplasmic tail. Interestingly, treatment with Galectin-9 resulted in abrogation of the Bat3-Tim-3 interaction. In addition, overexpressing Bat3 overcame the negative regulatory effects of Tim-3, resulting in enhanced secretion of IFN-γ and IL-2, and earlier onset of EAE with greater clinical severity. Thus, Bat3 overexpression promotes T_H1 cell response. Conversely, knocking down Bat3 reduced EAE severity by leaving the inhibitory effects of TIM3 intact with T_H1 cells produced less IFN-γ but substantially more IL-10 than control T_H1 cells. Furthermore, these T_H1 cells had higher frequency of TIM-3 expression.

To further explore the relationship between Bat3 expression and T cell exhaustion, the authors observed that Bat3 deleted T_H1 cells had an overall exhausted-like phenotype. These cells produced less IFN-γ and IL-2, expressed higher levels of TIM-3 and a number of other exhaustion markers, among them the transcription factors Prdm1 and Pbx3, as well as the inhibitory surface marker Lag3. Following establishment of Bat3's role in T-cell exhaustion, the authors postulated that exhausted T cells express altered endogenous levels of Bat3. In a tumor mouse model, $TIM\text{-}3^+PD\text{-}1^+$ tumor infiltrating lymphocytes (TILs) expressed 50% lower amount of Bat3 mRNA relative to TIM-3-$PD\text{-}1^+$ TILs. Similarly, $TIM\text{-}3^+PD\text{-}1^+$ $CD4^+$ T cells from chronic treatment-naïve HIV patients expressed significantly less *Bat3* mRNA in comparison to those from TIM-3-$PD\text{-}1^+$ $CD4^+$ T cells.

Even though Bat3 has been identified as a negative regulator of TIM-3, further studies are needed to understand why knocking down Bat3 in T cells would confer an exhaustion-like phenotype. This could possibly be due to higher Tim3 levels. Being a chaperone, Bat3 may act by downregulating surface levels of Tim-3, and thus restricting access to Tim-3 ligands and resulting inhibitory signaling. We still do not understand the complete relationship between Bat3 and TIM-3. Further explorations are needed to reveal the downstream signaling events of TIM-3-TIM-3L interactions and how this interactions influence an effective immune response in healthy immune system.

$TIM\text{-}3^+$ T cells as a Possible Therapeutic Agent

One of the correlates of immune protection in ECs is that they possess higher frequencies of polyfunctional $CD8^+$ T cells than chronic progressors (CPs) [53-56]. Polyfunctionality of T cells is defined as the ability of the responding T cells to produce multiple functions on per cell basis, including but not limiting to their ability to proliferate, secrete cytokines, influence the function of other cells and mediate cytolysis of infected cells by secreting perforin and granzymes. The TH1 transcription factor T-bet has been associated with the immune control of HIV by the polyfunctional T cells from ECs [57]. It is the quality of the functions of the responding T cells rather than the quantity of functionally responding T cells that defines HIV viral control in ECs [53-56].

We have recently shown that although $TIM\text{-}3^+$ $CD8^+$ T cells have lower cytokine production and proliferative abilities compared to TIM-3-$CD8^+$ T cells, they have significantly higher contents of perforin and the transcription factor T-bet [58]. However, $TIM\text{-}3^+$ $CD8^+$ T cells were impaired in their ability to degranulate compared with TIM-3-$CD8^+$ T cells. Stimulating sorted $CD8^+$ T cells from treatment naïve chronically HIV-infected individuals with an HIV gag peptide pool in the presence of an antagonistic anti-TIM-3 antibody enhanced the release of perforin via degranulation in $TIM\text{-}3^+$ $CD8^+$ T cells. In addition, Tim-3 was found to correlate with the loss of $CD8^+$ T cell cytotoxic activity. $Tim\text{-}3^+$ $CD8^+$ T-cells pretreated with a Tim-3 antagonistic antibody were more efficient at killing autologous HIV-infected $CD4^+$ T cells in a co-culture assay. Furthermore, as a supporting assay, $TIM\text{-}3^+$ $CD8^+$ T cells treated with the same antagonistic antibody were able to transfer significantly higher levels of granzyme B inside the autologous infected $CD4^+$ T cells.

This proof-of-concept *in vitro* study provides us with a conceptual model to rescue exhausted $CD8^+$ T cell effector functions to eliminate HIV infected cells *in vivo*. Blocking TIM-3 might enhance polyfunctionality of exhausted $TIM\text{-}3^+$ $CD8^+$ T cells since $TIM\text{-}3^+CD8^+$ T cells express higher levels of T-bet, which is associated with immune control of HIV by polyfunctional T cells in ECs. However, our *in vitro* blocking experiments only conferred partial rescue of effector function. Other inhibitory receptors could be synergistically inhibiting T cell functions [3-5] suggesting the need for therapies targeting multiple inhibitory receptors. A study by Sakuishi et al. [59] showed that blocking Tim-3 and PD-1 in a mouse tumor model resulted in better tumor infiltrating lymphocyte function when the treatments were given in combination. This proof-of-concept *in vivo* study provides evidence that blocking TIM-3 in combination with other inhibitory receptors could be used to improve the disease course during chronic infections like HIV and treatment of cancer.

Conclusion

TIM-3 is a negative immunoregulator of T cell activation. The nuances of dysregulated TIM-3 mediated effects downstream of TIM-3-TIM-3L interaction still require better understanding in the context of autoimmunity, tolerance abrogation and exhaustion vis-à-

vis healthy immune system. Although research has elucidated much of TIM-3's function in the last decade, less is understood about the TIM-3's mechanism of action. Further studies are required to better understand TIM-3 regulation in the aim to develop therapies for TIM-3 manipulation for better disease treatment outcomes.

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