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Regulation of T-Cell Immunity by T-Cell Immunoglobulin and Mucin Domain Proteins

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Abstract

The ability of T helper (T_H) precursor cells to differentiate into T effector populations confers the adaptive immune system with a means to protect the host from microbes and react to "foreign" antigenic tissues. T-cell immunoglobulin and mucin domain (TIM) proteins have recently been shown to be novel and critical regulators of T cell subset-driven dependent immune responsiveness. A dichotomy is emerging as to how Tim-3– and Tim-2– related signals respectively impact T_H1 and T_H2 responses. By comparison, the influence of the Tim-1 pathway seems to be broader and is probably not restricted to a specific type of T helper response. Beyond the mere control of the T_H1/T_H2 balance, Tim proteins are likely to target other regulatory components of the T cell response. Likewise, it is tempting to speculate that Tim proteins might also modulate the function of other T helper cell subsets such as T_H3, T_R1 and T_H17 cells, among others.

Keywords

Immunity; T cell; T-cell immunoglobulin mucin proteins

The ability of T helper (T_H) precursor cells to differentiate into T effector populations confers on the adaptive immune system its capacity to protect against foreign antigenic tissues. A defect in the control of T_H cell activation has been shown to lead to a number of inflammatory disorders. In this regard, the presence of alloreactive T_H1 cells, which produce interferon (IFN)-γ and interleukin (IL)-2, is commonly associated with allograft rejection (1, 2) and induction of organ-specific autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE) and type 1 diabetes (3, 4). In contrast, expression of T_H2 cytokines (classically IL-4 and IL-13) has been observed in atopic and allergic diseases (4, 5) and has been linked, albeit not always causally, to the acquisition of transplant tolerance (1, 2). Recently, T cell immunoglobulin mucin (TIM) proteins have been shown to be novel and critical regulators of the various stages of the immune response, including initiation of the effector functions of T_H1 and T_H2 cells.

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The TIM Family

TIM proteins are an evolutionarily conserved family and have been described in rodents (mouse and rat), monkeys, and humans. Eight *Tim* genes (*Tim-1* to *Tim-8*) have been identified in the mouse genome (6), but only four proteins (Tim-1 to Tim-4). In humans, three *TIM* genes (*TIM-1, TIM-3* and *TIM-4*) have been identified (6). With the exception of TIM-4, which exhibits a short intracytoplasmic domain with no reported phosphorylation sites, TIM proteins share a common structure consisting of cell surface glycoproteins with common structural motifs, including a signal peptide, immunoglobulin (Ig) domain, mucin domain, transmembrane domain, and intracellular tail with phosphorylation sites. Soluble forms of TIM-1 and TIM-3 have been reported, indicating that TIM proteins could act as functional receptors through the use of their cytoplasmic tail or as decoy molecules (7–9).

TIM-3 Regulates T_H1 Response

Tim-3 was originally discovered as a result of extensive screening of antibodies to identify specific cell surface markers of T_H1 or T_H2 clones (10). The expression of Tim-3 is detectable only after several rounds of stimulation on CD4 and CD8 cells under T_H1 conditions, and appears to be a specific marker for terminally differentiated T_H1 cells (Fig. 1) (8, 11). The distribution of Tim-3 is not restricted to the T cell compartment, and Tim-3 expression has been reported on mouse dendritic cells (DCs; our unpublished data), mouse monocytes and macrophages (12), as well as human natural killer (NK) and NK-T cells (13).

Tim-3 functions, in vitro and in vivo, as a negative regulator of T_H1-type response. Thus, abrogation of the Tim-3–Tim-3 ligand interaction through the use of Tim-3 Ig or Tim-3 knockout mice results in hyperproliferation of T_H1 clones associated with a vigorous enhancement of T_H1-type cytokine secretion (8, 11). Moreover, when Tim-3 pathway was blocked in a variety of T_H1-driven murine models, conventional strategies to induce tolerance such as oral antigen tolerance or costimulatory blockade were abrogated (8, 11).

In keeping with the kinetics of the expression of Tim-3 on terminally differentiated T_H1 clones, Tim-3 pathway was speculated to be associated with the regulation of the T_H1 cell clone size. Recent reports emphasize this role and unravel some of the mechanisms of action of Tim-3. The expression of Tim-3 on human T cells directly regulates proliferation and secretion of IFN-γ (Fig. 1) (14). Koguchi et al. demonstrated that Tim-3 expression is dysregulated in clones derived from the cerebrospinal fluid of patients with multiple sclerosis. Indeed, these clones secret higher amounts of IFN-γ than the clones derived from healthy individuals, but, paradoxically express lower amounts of Tim-3 transcripts. Tim-3 ligand was later identified as galectin-9 (15). Galectins represent a highly conserved family of molecules that have been proposed to modulate cell adhesion, cell proliferation and cell death (16, 17). In vivo administration of galectin-9 results in selective loss of IFN-γ producing cells and suppression of T_H1 autoimmunity. Thus, it appears that galectin-9 triggered activation of the Tim-3 pathway ensures an effective termination of T_H1 driven immunity (Fig. 1).

Modulation of the regulatory T cell compartment ($CD4^+CD25^+$ T cells) by the Tim-3 pathway extends the spectrum of Tim-3 biological effects. In a peripheral tolerance transplantation model, Tim-3-Ig has been shown to neutralize the capacity of donor specific transfusion plus anti-CD154 monoclonal antibody (mAb) to enhance the suppressive functions of $CD4^+CD25^+$ T cells (Fig. 1) (11). However, Tim-3-Ig does not interfere with the suppressive effects of regulatory T cells. Moreover, blocking anti-Tim-3 mAb inhibits the migration of regulatory $CD25^+Foxp3^+$ T cells into the heart during the adaptive immune response (12).

TIM-2 Regulates T_H2 Response

Whereas human TIM-3 and TIM-4 have apparent ortholog in mice, based on sequence homology, human TIM-1 is almost equally homologous to mouse TIM-1 (41%) and mouse Tim-2 (36%), which are 66% homologous to each other. One has to keep in mind this ambiguity, which partially impaired our ability to extrapolate the data generated in mice models. Tim-2 was originally identified from a cDNA library of T cell stimulated by ConA (18). Expression of Tim-2 by activated but not by naïve T cells was rediscovered through the identification of Tim-2 ligand, Sema4A (19), and Chakravarti et al. demonstrated that Tim-2 is preferentially expressed by T_H2 differentiated clones (Fig. 2) (20).

Blockade of Tim-2–Tim-2 ligand interaction through the administration of Tim-2 Ig results in T cell hyperproliferation and the production of T_H2-type cytokines (20). This observation was confirmed when the Tim-2 deficient mouse was developed by Rennert et al. (21). The absence of Tim-2 does not affect the normal resting immune system or the primary activation of naïve T cells. However, when the Tim-2 deficient mouse was immunized subcutaneously with keyhole limpet hemocyanin (KLH) in complete Freund adjuvant, and $CD4^+$ T cells from the draining lymph nodes restimulated in vitro with KLH, the in vitro recall response was markedly higher, with higher levels of T_H2-type cytokines. This phenotype was further confirmed in a model of airway inflammation known to be dependant on T_H2 cytokines. The implication of Tim-2 in the regulation of T_H2 response is suggested to be important in the expansion or survival of the T_H2 subset rather than in the initiation of the differentiation process (Fig. 2). Indeed, lung inflammation and T_H2-type cytokines were markedly increased when Tim-2 Ig was administered to immunized mice just before the challenge phase (21).

A new role of Tim-2 has been recently revealed with the identification of Tim-2 as a receptor for H-ferritin endocytosis (22). Among the various activities of H-ferritin, this molecule has been shown to be essential for the antiapoptotic effect of NF-κB, to impair maturation of the B cells in vitro and to be a strong immunosuppressor in vivo. These phenomena may partially explain the hyperproliferation of T cells observed when the Tim-2 pathway is blocked.

TIM-1 Regulates T-Cell Response

Whereas Tim-2 and Tim-3 regulate T_H2 and T_H1-type response, respectively, presumably in the late phase of the immune response, the role of Tim-1 in the regulation of the immune response is not clearly defined. Tim-1, the first member of the Tim protein family to be described, was originally identified in African green monkeys as a cellular receptor for hepatitis A virus (HAV) (23). An ortholog was then described in humans (24). Ichimura et al. identified kidney injury molecule (KIM)-1, another ortholog of Tim-1 in the rat (25). Kim-1 was hypothesized to play an important role in the restoration of the morphological integrity and function of postischemic kidney. Murine Tim-1 was later identified as an orthologue of both human Tim-1 and rat Kim-1 (6).

Tim-1 was originally considered to be a membrane protein with certain alleles associated with the susceptibility to allergic and autoimmune diseases (18, 26). In the initial report in which McIntire et al. examined congenic mice that differed at the homologous chromosomal segment to human chromosome 5q23-35 (where the asthma susceptibility genes are located), the authors identified a T cell and airway phenotype regulator (*Tapr*) and linked *Tapr* with Tim-1. The authors proposed that HAV, via its interaction to Tim-1, may reduce T_H2 differentiation and thereby reduce the likelihood of developing asthma. Asthma susceptible alleles of *TIM*-1 may have been preserved through human evolution because they provide a protective effect, such as resistance to HAV-induced hepatitis or resistance to

autoimmune disease. The association of asthma atopy and variants of TIM-1 have been further reported in some populations such as the Korean population (27, 28) or African American population (29). Similarly, correlation between TIM-1 variants and HAV infection status varied from one study to another (18, 29).

Umetsu et al. provided the first mechanistic analysis of the immunological function of Tim-1 with the 3B3 anti-Tim-1 mAb (30). Stimulating the Tim-1 pathway in vitro or in vivo results in a strong enhancement of the proliferation of T lymphocytes, as well as an enhancement of T_H1 and T_H2 cytokine production. Furthermore, the ability to induce respiratory tolerance was impaired when anti-Tim-1 mAb was administered. Using the same mAb, the development of allotransplantation tolerance was inhibited when mice received anti-Tim-1 mAb in association with anti-CD154 mAb as tolerizing regimen (submitted manuscript). Given the enhancement of T cell proliferation, the increase of both T_H1– and T_H2–type cytokine production and the strong effect on development of tolerance in T_H1– and T_H2–type murine models, Tim-1 seems to regulate the initiation phase of T cell immune response rather than the differentiation phase (Fig. 3). This hypothesis is further supported by the analysis of Tim-4, which was identified as the ligand for Tim-1 (31). In vivo administration of Tim-4 Ig to SJL/J mice in conjunction with immunization with PLP results in an enhancement of T cell proliferation and T_H1 cytokine production (Fig. 3). Elements of NFAT/AP-1 (32) or calcineurin (submitted manuscript) have been related to the Tim-1 pathway. Detailed cellular and molecular studies are still needed to elucidate the role of Tim-1 in immunity, and the generation of new experimental tools such as KO animals will allow the study of the physiological relevance of this pathway. In both humans and mice, Tim-4 is the ligand for Tim-1, thereby indicating that mouse Tim-1, not Tim-2, is the ortholog for human TIM-1.

As initially suspected by genetic and epidemiological studies, the role of TIM proteins in regulating T helper cell responses has recently been confirmed in various experimental systems. A dichotomy is emerging as to how Tim-3– and Tim-2–related signals, respectively, impact T_H1 and T_H2 responses. By comparison, the influence of the Tim-1 pathway seems to be broader and is probably not restricted to a specific type of T helper response.

Beyond the mere control of the T_H1/T_H2 balance, the Tim proteins are likely to target other regulatory components of the T cell response. In this regard, we certainly need to address whether and how Tim proteins affect the function of the two major regulators of T cell immunity, dendritic cells and $Foxp3^+$ T cells.

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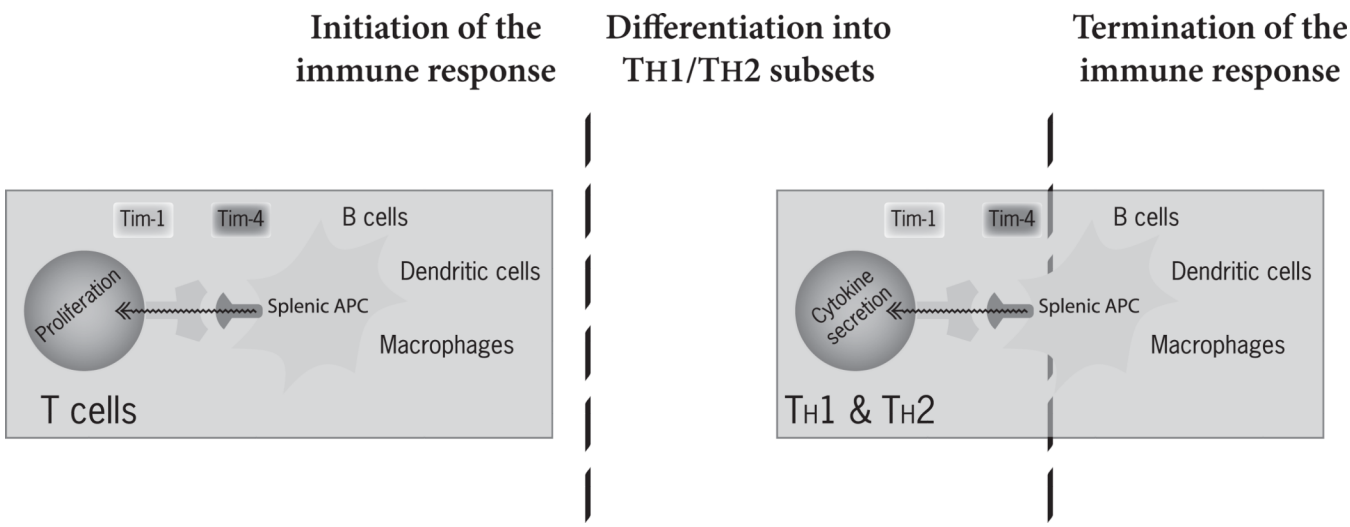

Figure 1.

Tim-3 ensures an effective termination of T_H1 driven immunity and inhibits the function of $CD8^+$ T cells. Tim-3 is expressed preferentially by terminally differentiated T_H1 clones, $CD4^+CD25^+$ regulatory T cells and activated $CD8^+$ T cells. Interaction between galectin-9 and Tim-3 induces the deletion of antigen-specific T_H1 clones. Galectin-9 is expressed by a variety of cells including regulatory T cells. Tim-3–Tim-3 ligand interaction delivers a negative signal to $CD8^+$ T cells that inhibits their proliferation and their cytotoxic functions.

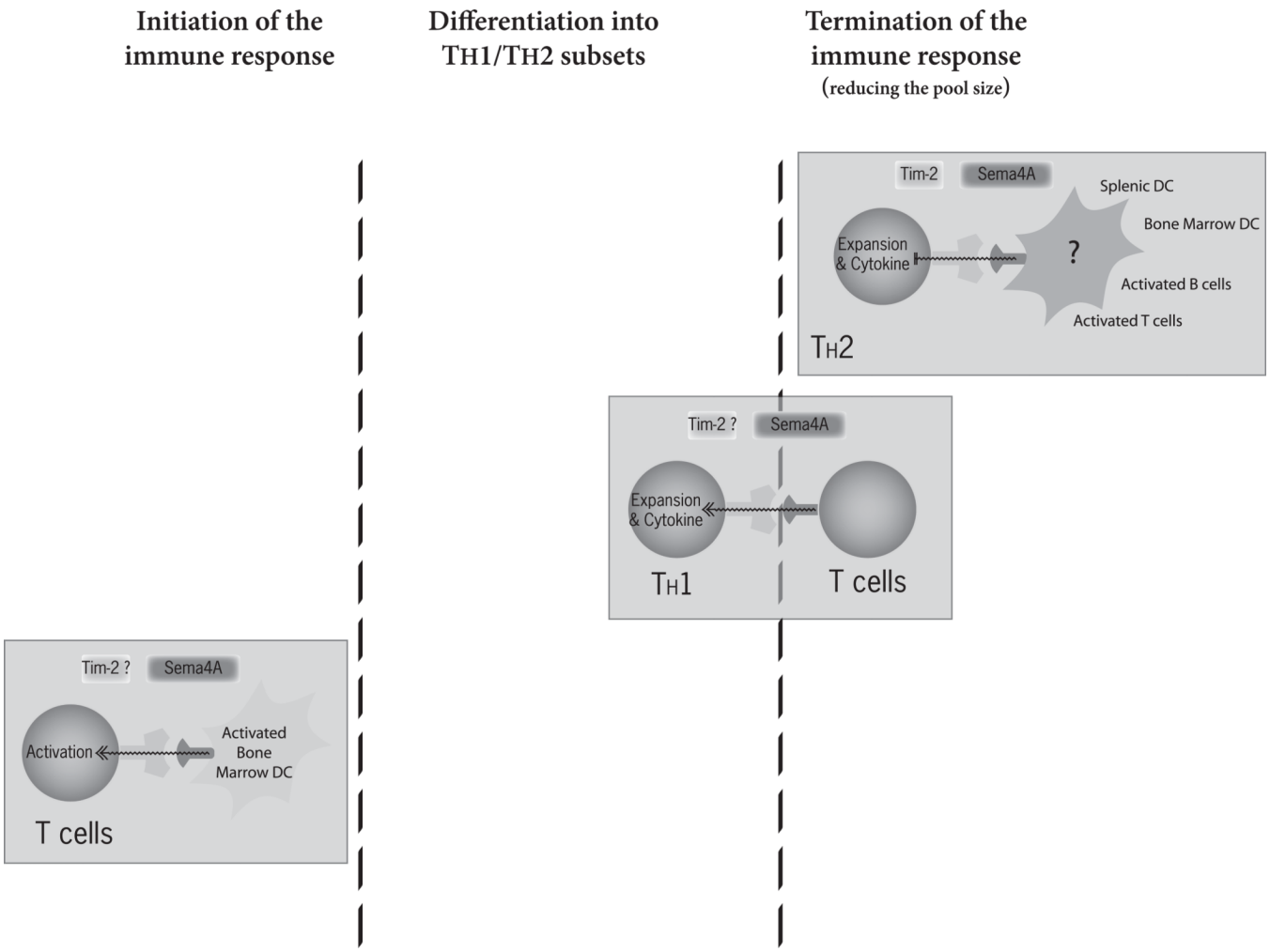

Figure 2.

Tim-2 negatively regulates the T_H2 pool size. Tim-2 is preferentially expressed by terminally differentiated T_H2 T cells, whereas Tim-2 ligand is detected on activated antigen presenting cells (such as activated B cells, macrophages, and mature dendritic cells). Sema4A, which has a similar cellular pattern of expression as Tim-2 ligand, interacts with Tim-2 to negatively regulate the T_H2 pool size by inhibiting the proliferation and the secretion of cytokines by T_H2 clones. Sema4A expressed by bone marrow DCs enhances the initiation of the immune response by promoting the proliferation of naïve T cells, whereas Sema4A expressed by T cells enhances their proliferation and the secretion of cytokines by T_H1 clones in an autocrine manner or via T cell–T cell interaction.

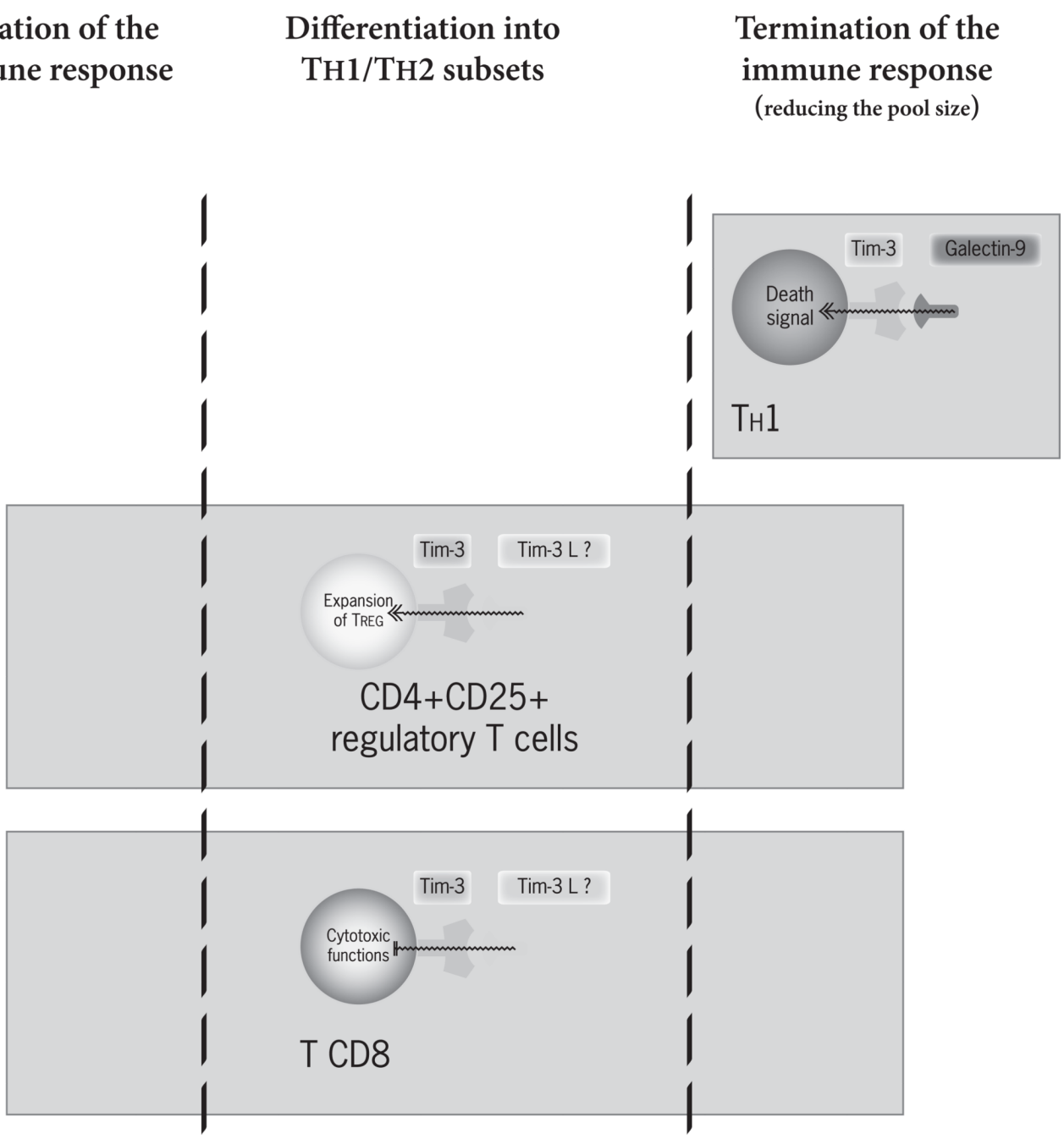

Figure 3.

Tim-1 regulates the initiation of the T cell immune response. Tim-1 is expressed at low levels on undifferentiated T cells and is upregulated after stimulation. Tim-1 pathway, stimulated at least in part by Tim-4 expressed by splenic APCs, enhances the proliferation of T lymphocytes (CD4 and CD8) and the cytokine production regardless of the nature of differentiated T lymphocytes (T_H1 or T_H2).