

The role of TIM-containing molecules in airway disease and their potential as therapeutic targets

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Abstract: T cell immunoglobulin and mucin-domain (TIM)-containing molecules have emerged as promising therapeutic targets to correct abnormal immune function in several autoimmune and chronic inflammatory conditions. Despite the initial discovery linking TIM-containing molecules and the airway hyperreactivity regulatory locus in mice, there is a paucity of studies on the function of TIM-containing molecules in lung inflammatory disease. Initially, studies were limited to mice models of asthma. More recently however, TIM-containing molecules have been implicated in an ever-expanding list of airway conditions that includes pneumonia, tuberculosis, influenza, sarcoidosis, lung cancer, and cystic fibrosis. This present review discusses the role of TIM-containing molecules and their ligands in the lung, as well as their potential as therapeutic targets in airway disease.

Keywords: T cell immunoglobulin and mucin-domain, inflammation, galectin-9, airway disease

Introduction

T cell immunoglobulin and mucin-domain (TIM) molecules are key regulators of immune responses.¹⁻⁴ TIM proteins have also been associated with several human inflammatory conditions,^{3,5} including rheumatoid arthritis,⁶ asthma,⁷ systemic lupus erythematosus,¹ multiple sclerosis,⁸ diabetes,⁹ and, more recently, in tumor^{10,11} and antimicrobial immunity.¹² However, the role of TIM-containing molecules in airway disease is only beginning to be unraveled. In this review, we consolidate the literature to discuss the prospective role of TIM-containing molecules as key inflammatory mediators with the potential to be utilized as therapeutic targets in the treatment of a variety of human diseases. Our literature review was carried out using the MEDLINE[®] database (from 1987 to 2012),¹³ Google Scholar,¹⁴ and The Cochrane Library¹⁵ database using several appropriate generic terms.

TIM structure and signaling

The TIM gene family is encoded on chromosome 11B1.1 in mice (TIM1-8) and chromosome 5q33.2 in humans. There are four TIM proteins characterized in mice – TIM-1, TIM-2, TIM-3, and TIM-4 – but only three members of the family have been identified in humans: TIM-1, TIM-3, and TIM-4. Human TIM-3 shares 63% homology with mouse TIM-3 and TIM-4 shares 49% homology with the mouse ortholog TIM-4. In contrast, human TIM-1 does not seem to have a clear ortholog in mouse as it shares 42% and 32% amino-acid sequence with murine TIM-1 and TIM-2, respectively.¹⁶ All TIM-containing

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molecules share a similar structure as type I membrane proteins, consisting of an N-terminal immunoglobulin variable (IgV)-like domain, a mucin-like domain, a transmembrane region, and an intracellular tail (Figure 1).

Human TIM IgV-like domains share 40% homology and are cysteine rich, suggestive of a highly cross-linked structure.¹⁷ In contrast, the mucin-domain presents in an extended conformation. The size of the mucin-domain varies depending on the receptor, with TIM-3 being the shortest. TIM-1 and TIM-3 are involved in intracellular signaling through tyrosine-phosphorylation motifs in the cytoplasmic domain, whereas TIM-4 does not contain a conserved motif, indicating that it exerts its function by association with other receptors. An important feature of TIM-containing molecule structure is the high level of glycosylation that enables ligand binding¹⁷ and increases resistance to proteolytic cleavage. All TIM receptors are predicted to be N-glycosylated in the immunoglobulin (Ig)-domain and at different positions close to the membrane, and O-glycosylated in the mucin-domain. However, the level of glycosylation varies from one predicted site in TIM-3 to up to 57 in TIM-1 (Figure 1).

TIM-1 was initially identified as hepatitis A virus receptor in monkeys¹⁸ and in humans.¹⁹ It was also cloned as kidney injury molecule-1.²⁰ TIM-1 is overexpressed in injured renal epithelium and can be cleaved off the cell membrane by metalloproteases.²¹ Shedded TIM-1 can be detected in urine after kidney injury and it has been proposed as a urinary marker of renal injury.^{20,22} Interest in TIM-containing molecules has

grown dramatically since the discovery of differential TIM expression in T helper (Th) cells and their immunomodulatory properties. TIM-1 is expressed in Th-2 cells, whereas TIM-3 is found in Th-1 cells.⁸ TIM signaling has mostly been studied in Th cells in mice. However, the TIM-1 signaling mechanisms in Th-2 cells are poorly understood. Studies in mice suggest that, in contrast to TIM-3, TIM-1 acts as a positive regulator of Th-2 cell function,²³ whereas TIM-2 has been shown to act as a negative regulator of Th-2 responses.²⁴ A TIM-2 human ortholog has not been identified, despite its close sequence homology with murine TIM-1.

Unlike other members of the TIM family, TIM-4 is not expressed in T cells but is found in antigen-presenting cells, including dendritic cells and macrophages.¹⁶ The exact role of TIM-4 is not fully understood. TIM-4 in macrophages has been found to bind phosphatidylserine on apoptotic cells, mediating apoptotic body clearance^{25,26} and raising the possibility of a role in the development of tolerance.²⁷ In addition, TIM-4 is believed to bind to TIM-1 or self-ligate and act as a co-stimulatory molecule of T cell function.⁴ TIM-4 function in T cells is complex and appears to work in a bimodal fashion. Both inhibitory and stimulatory responses have been reported depending on the activation status of the T cell, the level of TIM-4 expression, and whether co-stimulatory cells express TIM-4.^{4,16} Thus, TIM-4 is capable of modulating the immune response despite the absence of downstream cytosolic signaling motifs.

TIM-3 signaling in Th-1 cells is better characterized. Galectin-9 binds to TIM-3 in a glycosylation-dependent

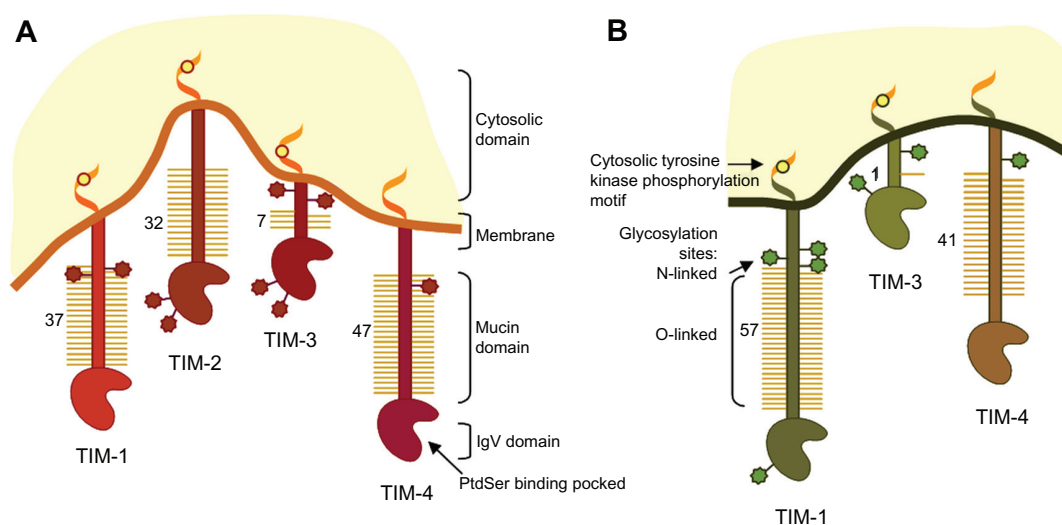


Figure 1 Schematic representation of mouse and human T cell immunoglobulin and mucin-domain (TIM)-containing molecules.

Notes: Protein structure and positions of the glycosylated sites of mouse TIM-1, -2, -3 and -4 and human TIM-1, -3 and -4 are shown. N-glycosylation in the immunoglobulin-domain and at different positions close to the membrane and O-glycosylation in the mucin-domain are positioned approximately.

Abbreviations: IgV, immunoglobulin variable; PtdSer, phosphatidylserine.

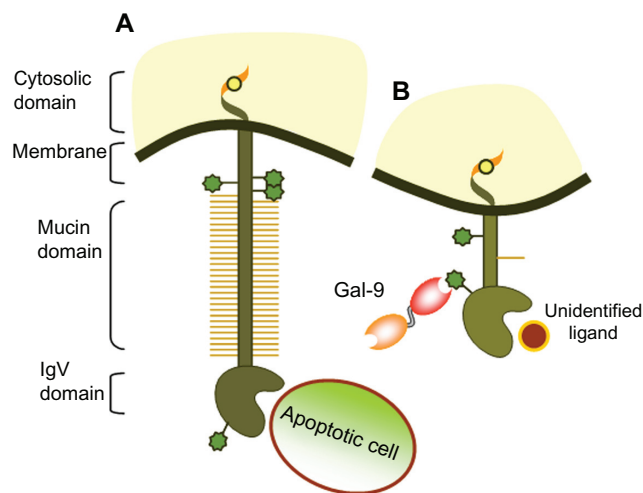


Figure 2 Schematic representation of T cell immunoglobulin and mucin-domain (TIM)-containing proteins and their ligands. **(A)** TIM-1 can bind to phosphatidylserine on apoptotic bodies through the FG-CC' binding region in the N-terminal immunoglobulin variable (IgV) domain conferring phagocytic characteristics to epithelial cells. TIM-1 can also bind to soluble TIM-4 or ligate to itself, leading to T cell activation and T-helper (Th)-2 expansion. **(B)** TIM-3 binds to galectin-9 (Gal-9) through N-linked carbohydrates in the IgV domain, driving a Th-1-mediated inflammatory response. **Note:** The ligand binding to the FG-CC' cleft on the opposite side of the IgV domain has not yet been identified.

manner (Figure 2). Although the downstream signaling events remain poorly characterized, TIM-3 activation causes phosphorylation of the tyrosine motif Y265 in the cytosolic domain.²⁸ Other tyrosine residues of TIM-3 appear to be responsible for signal transduction in T cell receptor-dependent mechanisms.²⁹ TIM-3 engagement by galectin-9 has been shown to induce apoptosis of Th-1 cells.³⁰ Blockade of galectin-TIM-3 interaction induced an exacerbation of Th-1-driven immune response and increased the level of macrophage activation in a mouse model of autoimmune disease.³¹ In humans, TIM-3 blockade with monoclonal antibodies revealed that human TIM-3 signaling regulates cytokine expression at the transcriptional level rather than controlling Th-1 cell expansion as it does in mice.³² Collectively, these data suggest an inhibitory role for TIM-3 in Th-1-driven immunity.

TIM-3 has also been reported to play a role in the induction of peripheral tolerance. Blockade of TIM-3 function by administration of soluble TIM-3 prevented the development of tolerance in Th-1 cells.³³ Furthermore, TIM-3-deficient mice are resistant to tolerance induction by administration of high-dose antigen.³³ TIM-3 has been shown to regulate both auto- and allo-immune tolerance by modulating T-regulatory cell-mediated inflammatory responses.⁹ This TIM-3 function has been confirmed in a murine model of graft-versus-host disease.³⁴ Perhaps

one of the most exciting functions of TIM-3 is its role in T cell exhaustion during chronic viral infections and in tumor immunity (Figure 3). Indeed, blockade of TIM-3 in these settings restored normal T cell function.^{10,11} More recently, it was discovered that TIM-3 is also expressed in other subsets of T cells, and cells from the innate immune system including monocytes, dendritic cells, mast cells, and microglia.^{3,4} Thus, the role of TIM-3 is not limited to the adaptive immune response. For instance, TIM-3 is involved in macrophage phagocytic³⁵ and bactericidal activity.^{12,36}

In addition to the immunomodulatory properties, TIM-containing molecules also play an important role in apoptotic body clearance via phosphatidylserine recognition.²⁷ TIM-4²⁶ and TIM-1²⁵ have been shown to bind to phosphatidylserine and mediate phagocytosis of apoptotic cells. More recently, it was demonstrated that TIM-3 presence in the phagocytic cup is required for apoptotic clearance by macrophages.³⁵ The expression of TIM receptors confers phagocytic properties even in “nonprofessional” phagocytic cells. Indeed, TIM-1 expression in endothelial cells has been reported to confer phagocytic capacity to this cell type.^{3,37}

Galectin-9 signaling through TIM-3

Galectin-9 was identified as the first ligand of TIM-3.³⁰ In line with its lectin nature, galectin-9 binds to TIM-3 in a carbohydrate-dependent manner, interacting with the N-glycosylated site in the IgV domain. Galectins have been shown to regulate immune homeostasis and inflammation.³⁸ Mammalian galectins comprise a large family of S-lectin proteins characterized by their affinity for β -galactosidase sugars and the conserved specific sequence motif in the carbohydrate recognition domain (CRD).³⁹

Human galectins have been grouped into three classes according to their structure (Figure 4):⁴⁰ (1) prototypical galectins, which contain a single CRD and may appear associated in the form of homodimers; (2) chimeric galectins, which contain a single CRD and a long amino-terminal domain; and (3) tandem-repeat galectins, which contain two CRD domains linked by a small peptide chain. Galectin-9 belongs to the tandem-repeat class. To date, three galectin-9 isoforms have been identified that only differ in the length of the polypeptide linker region (Figure 5). The short-size galectin-9 has a peptide linker of 14 amino acids and the medium- and the long-size forms have a linker of 26 and 58 amino acids, respectively.⁴¹ This relatively long polypeptide chain makes galectin-9 very susceptible to proteolytic cleav-

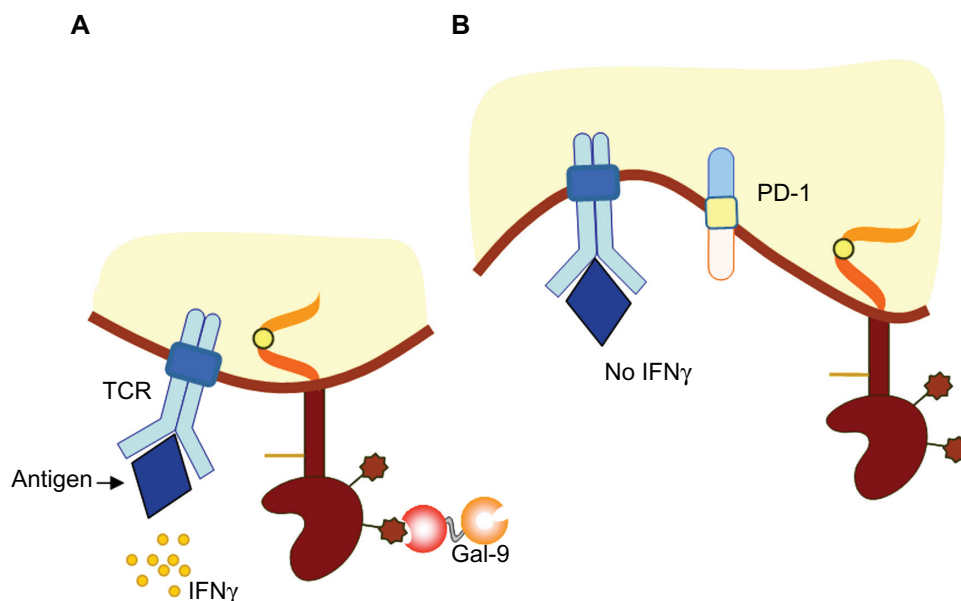


Figure 3 Model of T cell immunoglobulin and mucin-domain (TIM)-containing molecule-3 function in the immune response. **(A)** During acute inflammation, TIM-3 is expressed on terminally differentiated interferon-gamma (IFN- γ)-producing CD4+ and CD8+ T cells. TIM-3-expressing T cells undergo apoptosis following recognition of the TIM-3 ligand galectin-9 (Gal-9). **(B)** During chronic inflammation, dysfunctional or exhausted CD8+ T cells express both TIM-3 and programmed death-1 receptor (PD-1).

Note: Combined targeting of the PD-1/PD-ligand 1 and TIM-3/TIM-3 ligand pathways restores CD8+ T cell effector function and ameliorates chronic disease.

Abbreviation: TCR, T cell receptor.

age,⁴² resulting in inactivation of the molecule.⁴³ Recently, a recombinant form of galectin-9 lacking the linker chain has been shown to be functional and resistant to proteases (Figure 5).⁴²

Galectin-9 was first identified in embryonic mouse kidney and found to be ubiquitously expressed in mouse and rat tissue.⁴⁴ Galectin-9 is also widely distributed in human tissues and expressed in several cell types.⁴⁵ Galectin-9 expression has been reported in epithelial tissues such as endometrium,⁴⁶ cervix,⁴⁷ and intestine.⁴⁸ Galectin-9 expression in the oral-nasopharyngeal tract has been located to fibroblasts in nasal polyps,⁴⁹ periodontal ligaments in the oral cavity,⁵⁰ and Epstein–Barr virus-related nasopharyngeal carcinoma cells.⁵¹ Galectin-9⁵² is also abundantly expressed in human endothelial cells^{52,53} and melanocytes⁵⁴ and is broadly expressed in the immune system, including in bone marrow, the spleen, the thymus, and lymph nodes. Within immune cells, galectin-9 expression has been demonstrated in myeloid lineage cells⁵⁵ including Kupffer cells,⁵⁶ microglia,⁵⁷ astrocytes,⁵⁸ dendritic cells,⁵⁹ and macrophages.¹² Galectin-9 has also been shown to be constitutively expressed in mast cells⁵⁴ and, to a lesser extent, in the Jurkat T lymphocyte cell line.⁴¹ Regulatory T cells also express galectin-9.⁶⁰ Although galectin-9 was not detected in human promyelocytic leukemia cells, it was found to be expressed in neutrophils in the lung of ovalbumin-challenged mice.⁶¹ The most interesting feature of galectin-9 expression is that,

although most immune cells exhibit constitutive expression, the extent of expression depends on the stage of cell activation and differentiation.⁶² For instance, galectin-9 mRNA expression has been reported to be induced in peripheral blood monocyctic cells after allergen stimulation.⁴⁵ Galectin-9 expression is also raised in eosinophils from hyper-eosinophilic patients.⁶³

It has been proposed that galectin-9 may have a complex role in inflammation homeostasis by exerting pro- and anti-inflammatory events depending on either the concentration at sites of inflammation or cell type.⁶⁴ Galectin-9 binding to TIM-3 has been shown to reduce interferon-gamma production by inducing apoptosis of Th-1 cells.³⁰ Conversely, galectin-9 treatment induced tumor necrosis factor-alpha production by mast cells.⁶⁵ Thus, galectin-9/TIM-3 signaling can initiate or terminate the inflammatory response by positively regulating maturation of innate cells, antigen presentation and pathogen clearance, and limiting an excessive immune response, especially that mediated by T cells.⁶⁶

Disruption of galectin-9–TIM-3 interaction is commonly used to examine the mechanisms behind this signaling axis in several models of disease. Figure 6 summarizes the different approaches adopted to block TIM-3 engagement by galectin-9 via N-glycosylation of the receptor. Intriguingly, galectin-9 interaction with a yet unidentified membrane glycoprotein has recently been shown to block Th-17 function via O-glycosylation. In addition to TIM-3, galectin-9 has

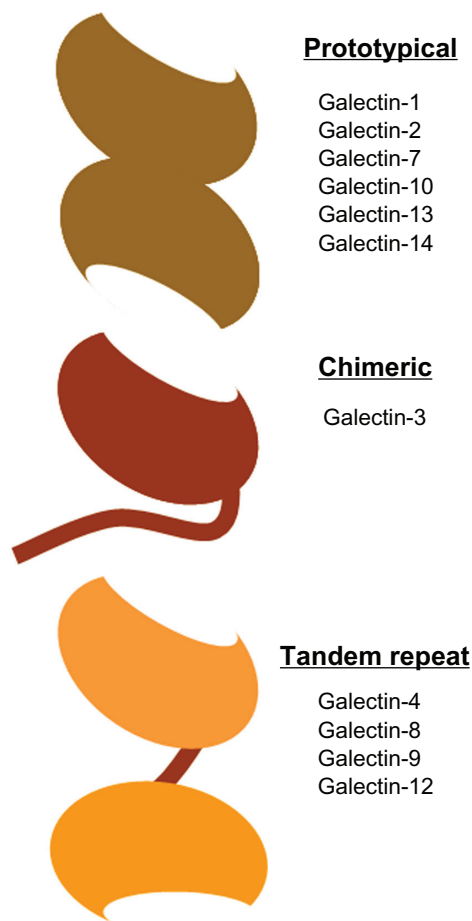


Figure 4 The different types of galectins in humans.

Notes: Human galectins have been classified according to their structure into prototypical, chimeric, and tandem repeat. The oval domain represents the carbohydrate recognition domain (CDR). Prototypical galectins such as galectin-1 contain only one type of CDR, chimeric galectin-3 contains a single CDR attached to a long polypeptide chain, whereas tandem-repeat galectins such as galectin-9 consist of two different CDRs connected by an inter-domain polypeptide linker.

also been reported to bind to other glycoproteins containing β -galactosides in their structure, including CD44, a surface receptor expressed on epithelial cells involved in cell-matrix adhesion and interaction,^{67,68} IgE,⁶⁹ glucose transporter 2,⁷⁰ and Epstein–Barr virus latent membrane protein-1.⁵¹

Galectin-9 isoforms	Full length/linker peptide region (amino acids)	Calculated size (kDa)	
Galectin-9 (s)	NH ₂ —[CRD1]—[CRD2]—COOH	311/14	34.69
Galectin-9 (m)	NH ₂ —[CRD1]—[CRD2]—COOH	323/26	35.89
Galectin-9 (l)	NH ₂ —[CRD1]—[CRD2]—COOH	355/58	39.52
rhgalectin-9 (gal9Null)	NH ₂ —[CRD1]—[CRD2]—COOH	297/2	33.10

Figure 5 Schematic representation of galectin-9 isoforms structure.

Notes: Sequencing of galectin-9 revealed isoforms of different sizes differing only in the linker peptide region length (small [s], medium [m], and long [l]). The structure of proteolytically resistant recombinant galectin-9 (rhgalectin-9 [gal9Null]) is also shown.⁴²
Abbreviation: CRD, carbohydrate recognition domain.

TIM-containing molecules in airway disease

The TIM gene family was located to a section of chromosome 11 in mice syntenic to human chromosome 5q23-35.⁷¹ This region was identified as a novel gene locus for human atopic disease (ie, asthma, allergy, and eczema) in a study comparing congenic mouse strains with different susceptibility to asthma. Despite the initial discovery linking TIM and the airway hyperreactivity regulatory locus (*Tapr*) in mice,⁷¹ there is a paucity of studies on the function of TIM-containing molecules in lung inflammatory disease. Most of the existing studies were carried out in mice models of asthma. More recently, TIM-containing molecules have been implicated in sarcoidosis, pneumonia, tuberculosis, influenza, and cystic fibrosis (Table 1).

TIM-1 in airway disease

Although the role of TIM-1 and TIM-3 in the development of lung allergy has recently been questioned in a study using knockout mice,⁷² TIM-1 expression has been reported to be upregulated in the lung of asthmatic mice.⁷³ Indeed, blockade of mouse TIM-1 decreased the Th-2-type immune response and airway inflammation in a murine model of asthma in a number of studies.^{23,74,75} Blockade of TIM-1 with anti-TIM-1 antibody during initial challenge with antigen prevented air-

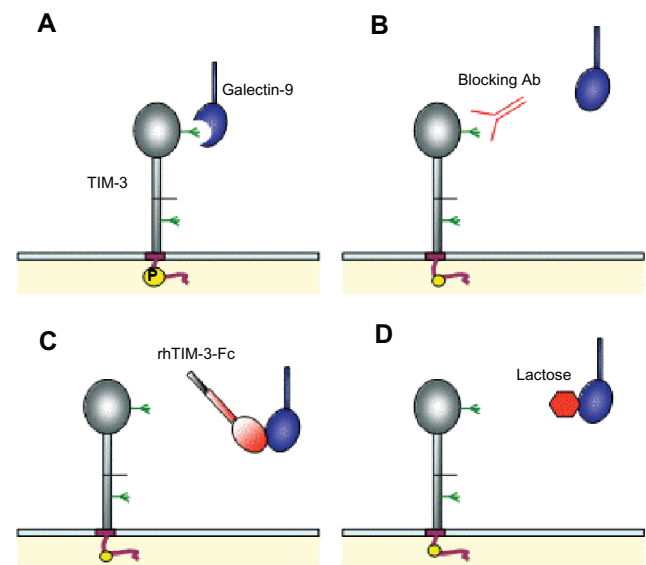


Figure 6 Schematic representations of T cell immunoglobulin and mucin-domain (TIM)-containing molecule-3-galectin-9 interaction disruption strategies. (A) Galectin-9 binds to the TIM-3 immunoglobulin variable domain via carbohydrate interactions.³⁰ (B) Blockade of TIM-3 by anti-TIM-3 antibody (Ab). (C) Soluble human recombinant TIM-3-Fc chimeric protein (rhTIM-3-Fc) acts as a galectin-9 scavenger receptor, preventing TIM-3-galectin-9 interaction. (D) Galectin-9 preferentially binds to excess lactose in solution due to galactosidase affinity.

way hyperresponsiveness in a mouse model.⁷⁴ Using a similar approach, a second group reported that administration of anti-TIM-1 antibody between sensitization and allergen exposure also reduced airway hyperresponsiveness.²³ Interestingly, TIM-1 ligation with monoclonal antibodies induced either positive or negative effects in a mouse allergy model, depending on the antibody. Antibodies recognizing the stalk or IgV domain attenuated inflammation, indicating that ligation of this TIM-1 region promotes inhibitory functions. In contrast, antibodies directed against the mucin-domain activated the inflammatory response.⁷⁵ Therefore, therapeutic use of anti-TIM-1 antibodies in asthma emerges as a promising novel treatment approach, although special attention to the TIM-1 epitope recognized by the antibody is required. Consistent with the previous results, antagonism of human TIM-1 in a humanized murine model of experimental asthma has been shown to have positive therapeutic effects.⁷⁶ In this study, blockade of TIM-1 with A6G2, an antibody against the IgV domain, ameliorated inflammation and airway hyperresponsiveness in severe combined immunodeficiency mice adoptively transferred with peripheral blood monocytic cells from asthmatic patients. The effects of TIM-1 inhibition were exerted via suppression of Th-2 cell proliferation and cytokine production, providing encouraging data in support of the use of TIM-1 targeted antibodies for the treatment of asthma.

Despite the increasing body of work in mouse models of lung disease, there are virtually no studies in humans supporting the role of TIM-1 in airway inflammation (Table 1). Recently, T cells obtained from peripheral blood and bronchoalveolar lavage (BAL) fluid of non-Löfgren sarcoidosis patients exhibited lower TIM-1 expression than those of sarcoidosis patients with Löfgren.⁷⁷ Non-Löfgren patients exhibit a marked Th-1 inflammatory response and often present with a less favorable prognosis than Löfgren patients. Since an imbalance towards a Th-1 phenotype is believed to be the hallmark of airway inflammatory response in sarcoidosis, the study by Idali and colleagues suggested that downregulation of TIM-1 in Th cells was linked to a higher Th-1 response.⁷⁷ However, TIM-1 regulatory properties in airway disease are not limited to T cells, as it has been shown that TIM-1 engagement in natural killer cells exacerbated lung injury in a bleomycin model of pulmonary fibrosis by suppressing interferon-gamma production.⁷⁸

Clearly, a more detailed knowledge of the exact mechanisms underpinning TIM-1 signaling in airway disease is required before expanding the findings obtained from murine models to human studies. Alternatively, a better understanding of the molecular pathways underlying TIM-1 function may open a new area of research involving development of agonistic and/or antagonistic modulators of TIM-1

Table 1 T cell immunoglobulin and mucin-domain (TIM)-containing molecules in airway disease

Disease	TIM protein	Function	References
Asthma	TIM-1 (m)	Blocking TIM-1 antibody reduced Th-2-driven allergic response	23
	TIM-1 (m)	Antagonistic TIM-1 antibody increased Th-2-driven allergic response	74
	TIM-1 (m)	Epitope-dependent effect of TIM-1 antibodies on Th-2 response, causing exacerbation or inhibition of airway inflammation	75
	TIM-3 (m)	Blocking antibody administration reduced Th-2-driven allergic response	86
	TIM-1 (m)	Expression increased in T cells	73
	TIM-3 (m)	Expression increased in T cells	87
	TIM-3 (m, h)	Defective apoptotic clearance induces airway hyperresponsiveness. Dependent on TIM-3 polymorphism	88
	TIM-1 (h)	Antagonistic antibody reduced Th-2 response in a humanized asthma mouse model	76
	TIM-2 (m)	TIM-2-deficient mouse demonstrate exacerbated Th-2 response in asthma model	7
	TIM-1/3 (h)	TIM-3 and TIM-1 are not essential for airway hyperresponsiveness	72
Sarcoidosis	TIM-1 (h)	Reduced expression in T cells of non-Löfgren's patients is in agreement with an exaggerated Th-1 response	77
Sarcoidosis	TIM-3 (h)	Reduced expression in T cell in BAL and blood correlated negatively with CD4/CD8 ratio	77
Tuberculosis	TIM-3 (m/h)	TIM-3 interacted with galectin-9 on macrophages to restrict intracellular bacterial growth	12
Cystic fibrosis	TIM-3 (h)	Constitutive upregulation in bronchial epithelial cells induced IL-8 production	91
Pneumonia	TIM-3 (m)	Galectin-9 intraperitoneal administration reduced IL-17 production and bacterial clearance	89
Pulmonary fibrosis	TIM-1 (m)	Co-stimulation on NKs enhances the production of IL-4 and inhibits production of IFN- γ	78
Influenza	TIM-3 (m)	Blocking TIM-3–galectin-9 interaction resulted in improved immune response against influenza A virus	90

Abbreviations: BAL, bronchoalveolar lavage; h, human; IFN- γ , interferon-gamma; IL, interleukin; m, mouse; NKs, natural killer T cells; Th, T-helper.

function; however, to date, such compounds have not been reported.

A role for TIM-2 in respiratory disease

Given that TIM-2, unlike the other TIM proteins discussed in this review, is not expressed in humans, the potential of this molecule to be a therapeutic target in human lung disease is very limited. Nevertheless, several studies support the role of TIM-2 as a modulator of Th-2 responses in mouse models. Blockade of TIM-2 with recombinant TIM-2 (TIM-2-Ig) was sufficient to mount a Th-2 immune response and ameliorate disease progression in a model of autoimmune encephalitis. Additionally, the TIM-2 knockout murine model displays an exacerbated Th-2-driven response in a model of ovalbumin-induced airway inflammation.⁷ Semaphorin-4A is a recognized TIM-2 ligand,⁷⁹ yet semaphorin-4A-deficient mice develop exaggerated Th-2 phenotypes, supporting TIM-2 as an inhibitor of Th-2 responses.⁸⁰ TIM-2 was also identified as a specific heavy chain ferritin (H-ferritin) receptor leading to endocytosis of extracellular H-ferritin in liver⁸¹ and brain.⁸² H-ferritin has been reported to display immunological properties, mainly as a regulator of proliferation and differentiation of immune cells.⁸³ Interestingly, elevated levels of H-ferritin were found in cystic fibrosis BAL fluid compared with levels found in other inflammatory respiratory conditions.⁸⁴ A link between high levels of ferritin and altered TIM expression has been suggested but not formally demonstrated.⁵ Nevertheless, none of the human TIM receptors appear to bind H-ferritin.²⁷ Since murine TIM-2 does not have a human ortholog,²⁴ whether another human TIM receptor can adopt the Th-2 inhibitory function described in mice⁸⁵ remains to be elucidated.

TIM-3 and airway infection and disease

TIM-3 has also been implicated in the development of asthma (Table 1). Given the role of TIM-3 as a negative regulator of Th-1 mediated immunity, blockade of TIM-3 may prove useful in the treatment of this disease. Indeed, blockade of TIM-3 with a specific antibody reduced airway hyper-reactivity and induced a switch from Th-2- to Th-1-type response in a murine experimental model.⁸⁶ Consistent with these results, expression of TIM-3 in CD4+ cells was increased after ovalbumin challenge in a mouse model of asthma,⁸⁷ further supporting TIM-3 as a negative regulator of Th-1 mediated immunity. In line with the described TIM-3 function, low levels of TIM-3 expression in CD4+ cells from peripheral blood and BAL were correlated with an increased Th-1-type immune response in sarcoidosis,⁷⁷ a prototypical

Th-1 inflammatory disease. Additionally, the role of TIM-3 as a phosphatidylserine receptor has been suggested to be involved in the development of airway hyperresponsiveness, as efficient clearance of apoptotic cells is crucial in preventing development of atopy.⁸⁸ Thus, targeting the TIM-3 inhibitory pathway may represent a novel approach to restore the Th-1/Th-2 response balance in airway disease.

TIM-3 has also been suggested to be involved in the regulation of the inflammatory response to airway infection. In a mouse model of *Klebsiella pneumoniae*-induced pneumonia, administration of galectin-9 induced apoptosis of Th-17 cells and reduction of IL-17 generation, which, in this model, proved crucial for bacterial clearance in the lung. Decreased IL-17 production led to impaired neutrophil recruitment into the airways with subsequent reduced bacterial clearance and higher mortality.⁸⁹ These results suggest an important role for TIM-3/galectin-9 in termination of Th-17-mediated immune responses. Regulation of TIM-3 function in CD8 T cells was shown to be critical to mount an adequate immune response against viral infection.⁹⁰ Blockade of TIM-3–galectin-9 interaction by administration of a recombinant TIM-3 protein improved the immune response in a mouse model of influenza A virus infection.⁹⁰ Moreover, galectin-9 knockout mice were refractory to influenza A virus infection. Taking these results together, the indication is that Tim-3–galectin-9 interaction works to limit the extent and potency of the T cell responses to pathogen infection. Since TIM-3 function has proved to be critical during infection, targeting the TIM-3/galectin-9 pathway may be a viable approach. It is worth noting that the level of inhibition appears to be crucial, as it should effectively limit an exacerbated inflammatory response but should avoid an excessive repression of the immune response that could result in defective pathogen clearance. Thus, the TIM-3 targeting agent should preferably be delivered locally rather than systemically.

TIM-3 and its ligand galectin-9 were found to be constitutively overexpressed in human bronchial epithelium from cystic fibrosis patients.⁹¹ Both ligand and receptor were further expressed following exposure to lipopolysaccharide from *Pseudomonas aeruginosa*, which emphasizes the role of TIM-3 under inflammatory conditions. However, in the cystic fibrosis lung, both TIM-3 and its ligand galectin-9 undergo rapid degradation by neutrophil-derived proteases – in particular, neutrophil elastase and proteinase 3 – potentially contributing to the defective bacterial clearance observed within the cystic fibrosis lung despite the high neutrophilic presence. In line with these observations, in addition to targeted delivery of the TIM-3 effector molecule to the lungs,

this molecule should be designed to be resistant to proteolytic cleavage to be an effective agent.

The role of TIM-3 in pathogen infection goes beyond orchestration of the humoral and cellular immune responses. A novel role for TIM-3 in airway infection was recently revealed:¹² in this study, TIM-3 was shown to act as a ligand and to stimulate galectin-9 expressed on the surface of macrophages. Through unidentified mechanisms, galectin-9 engagement on infected macrophages triggered IL-1 β production and subsequent *Mycobacterium tuberculosis* intracellular clearance. The study, by Jayaraman and colleagues, suggested that TIM-3 expressed on Th-1 cells can modulate macrophage-mediated bacterial killing.¹² This constitutes the first report on the role of TIM-3 as a ligand, in addition to the prototypical role as a Th-1 receptor capable of modulating the inflammatory response. These studies open a new area of TIM-3 research on the role of this molecule in bacterial infection.

Galectin-9 in airway disease

Galectin-9 has been shown to be involved in airway disease via TIM-3-dependent and -independent mechanisms (Table 2). Galectin-9 expression was found to be elevated in lung tissue in animal models of asthma.^{61,92} This overexpression of galectin-9 was found to correlate with an increase in Th-2 cytokines and increased cell counts in the lungs, particularly eosinophils. A similar correlation between elevated galectin-9 and high eosinophil counts was reported in patients with acute and chronic eosinophilic pneumonia.⁹³ Interestingly, administration of recombinant galectin-9 attenuated lung inflammation in a mouse model of asthma, which highlights the use of recombinant galectin-9 as a promising therapeutic agent.⁶⁹ In this study, galectin-9 inhibited mast-cell degranulation by disrupting IgE/antigen complex formation. Recombinant galectin-9 administration also ameliorated lung inflammation in a mouse model of asthma due to inhibition of CD44–hyaluronic acid interaction, which is required for recruitment of leukocytes into the airways.⁶⁷ The study also showed that galectin-9 can induce apoptosis of eosinophils, thereby reducing disease severity in this asthma model.

Galectin-9 has been shown to affect antimicrobial immunity in two distinct manners. First, galectin-9 stimulates immune responses via recruitment of immune cells. Secondly, galectin-9 can limit the adaptive immune response, in particular, the T cell response, while promoting the expansion of regulatory cells.⁹⁴ Of interest, a recent publication reported a novel role for galectin-9 in antimicrobial immunity.¹² This

Table 2 Galectin-9 in airway disease

Disease	Function	References
Asthma		
g	Galectin-9 levels increased in an allergic AHR model and correlated with eosinophil counts	92
m	Galectin-9 levels increased in an allergic AHR model and correlated with eosinophil counts	61
g	Galectin-9 administration attenuated allergic inflammation by binding IgE and reducing mast cell degranulation	69
m	Galectin-9 administration attenuated AHR and allergic inflammation binding to CD44, which reduced T cell migration	67
Lung cancer		
m	Galectin-9 administration reduced tumor cell migration	68
m	Galectin-9 administration prolonged survival in a lung cancer model by induction of macrophage differentiation into dendritic-like cells that promote activation of natural killing cells	95
Acute lung injury		
m	Galectin-9 administration ameliorated acute lung injury expanding regulatory macrophages	94
Tuberculosis		
m/h	TIM-3 interacted with galectin-9 on macrophages to restrict intracellular bacterial growth	12
Cystic fibrosis		
h	Constitutive upregulation in bronchial epithelial cells induced IL-8 production	91
Pneumonia		
m	Galectin-9 intraperitoneal administration reduced IL-17 production and reduced bacterial clearance	89
h	Galectin-9 levels correlated with eosinophil counts in acute and chronic eosinophilic pneumonia	93
Sarcoidosis		
h	Galectin-9 levels not altered in Th cells	77
Influenza		
m	Galectin-9 knockout mice mounted a higher response to influenza A virus infection	90

Abbreviations: AHR, airway hyperresponsiveness; g, guinea pig; h, human; Ig, immunoglobulin; IL, interleukin; m, mouse; Th, T-helper.

latter study described for the first time a reversal of TIM-3/galectin-9 signaling pathway whereby galectin-9 acted as a receptor in infected macrophages and was stimulated via interactions with TIM-3 expressed on adjacent Th-1 cells.

Conclusion

The data described so far emphasize a role for TIM-containing molecules as modulators of the immune response in the airways. TIM-containing molecules emerge as ideal candidates for therapeutic intervention in the lung at several levels. First, TIM-containing molecules may effect the inflammatory response in the airways due to their direct role in promot-

ing generation of pro- and anti-inflammatory mediators. Secondly, TIM-containing molecules may act as regulators of cellular homeostasis in the lung via induction of selective apoptosis of immune cells and preferential expansion of regulatory cells via galectin-9 interactions. Additionally, TIM-containing molecules may also help fight lung infections, as they have been shown to be involved in viral recognition and phagocytic cell function. Therefore, manipulation of the TIM-regulated mechanisms may prove beneficial in human airway disease. TIM pathways may be modulated by specific antibodies directed towards well-defined regions of the receptor, recombinant proteins containing the precise agonist/antagonist motifs, or small-molecule drugs. In addition, treatments aimed at modulating TIM-regulated pathways should consider delivery of newly developed drugs to specific areas in the body for localized treatment.

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Disclosure

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the presented manuscript.

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