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The IFITM protein family in adaptive immunity

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Summary

Interferon-inducible transmembrane (IFITM) proteins are a family of small homologous proteins, localized in the plasma and endolysosomal membranes, which confer cellular resistance to many viruses. In addition, several distinct functions have been associated with different IFITM family members, including germ cell specification (IFITM1–IFITM3), osteoblast function and bone mineralization (IFITM5) and immune functions (IFITM1–3, IFITM6). IFITM1–3 are expressed by T cells and recent experiments have shown that the IFITM proteins are directly involved in adaptive immunity and that they regulate $CD4^+$ T helper cell differentiation in a T-cell-intrinsic manner. Here we review the role of the IFITM proteins in T-cell differentiation and function.

Keywords: differentiation; interferon-inducible transmembrane protein; T cell; T helper type 1; T helper type 2.

Introduction

The IFITM family

The family of interferon-inducible transmembrane (*Ifitm/Fragilis*) genes encode small homologous proteins localized in the plasma and endolysosomal membranes, which can confer cellular resistance to many viruses in both mice and humans.[1–3] The *IFITM* family were first discovered as interferon-induced genes in human neuroblastoma cells and their promoters contain one or more interferon-stimulated response elements, making them responsive to type I and type II interferons.[4–7] However, IFITM expression can be regulated independently of interferon signalling.[7,8] *Ifitm* genes are targets of transcriptional repression by Bcl6, and have been shown to be targets of Wnt/β-catenin and Hedgehog (Hh) signal transduction[9–12]; and in murine T cells IFITM2 and IFITM3 expression is regulated by T-cell receptor (TCR) signal transduction.[7,9,13]

In humans, five *IFITM* genes have been identified, which are located on chromosome 11, whereas in mice there are seven *Ifitm* genes, six of which are located on chromosome 7, and one on chromosome 16 (illustrated in Fig. 1a).[6,14–18] Homologous IFITM family genes are present in many other species, including marsupials, birds, fish and reptiles, suggesting important conserved roles for IFITM proteins.[19]

The topology of the IFITM proteins in the membrane is not certain, several different topologies have been described, which are illustrated in Fig. 1(b).[20–22] All IFITM proteins have two transmembrane or intramembrane regions spanning the membrane bi-layer sandwiched between three external regions. The connecting region is highly conserved and is always intracellular, but the N-terminus and C-terminus have been described to be either intracellular or extracellular (Fig. 1c).

Biological functions of the IFITM proteins

Several distinct functions have been associated with different IFITM family members, including germ cell specification (IFITM1–IFITM3),[14–16,23,24] osteoblast function and bone mineralization (IFITM5),[25–29] and immune functions (IFITM1–3, IFITM6),[7,8,13,30–38] in addition to their roles as virus-restriction factors (IFITM1–3, murine IFITM6). The IFITM proteins have also been described to play a role in cell cycle control and apoptosis and their dysregulated expression, over-expression or mutation can be associated with colon cancers and metabolic dysregulation.[39–43] IFITM10 is highly conserved between species with at least 85% amino acid identity between birds,

Abbreviations: Hh, Hedgehog; IFITM, interferon-inducible transmembrane protein; IFN, interferon; IL, interleukin; LAMP1, lysosomal-associated membrane protein; TCR, T-cell receptor; Th, T helper; WT, wild-type

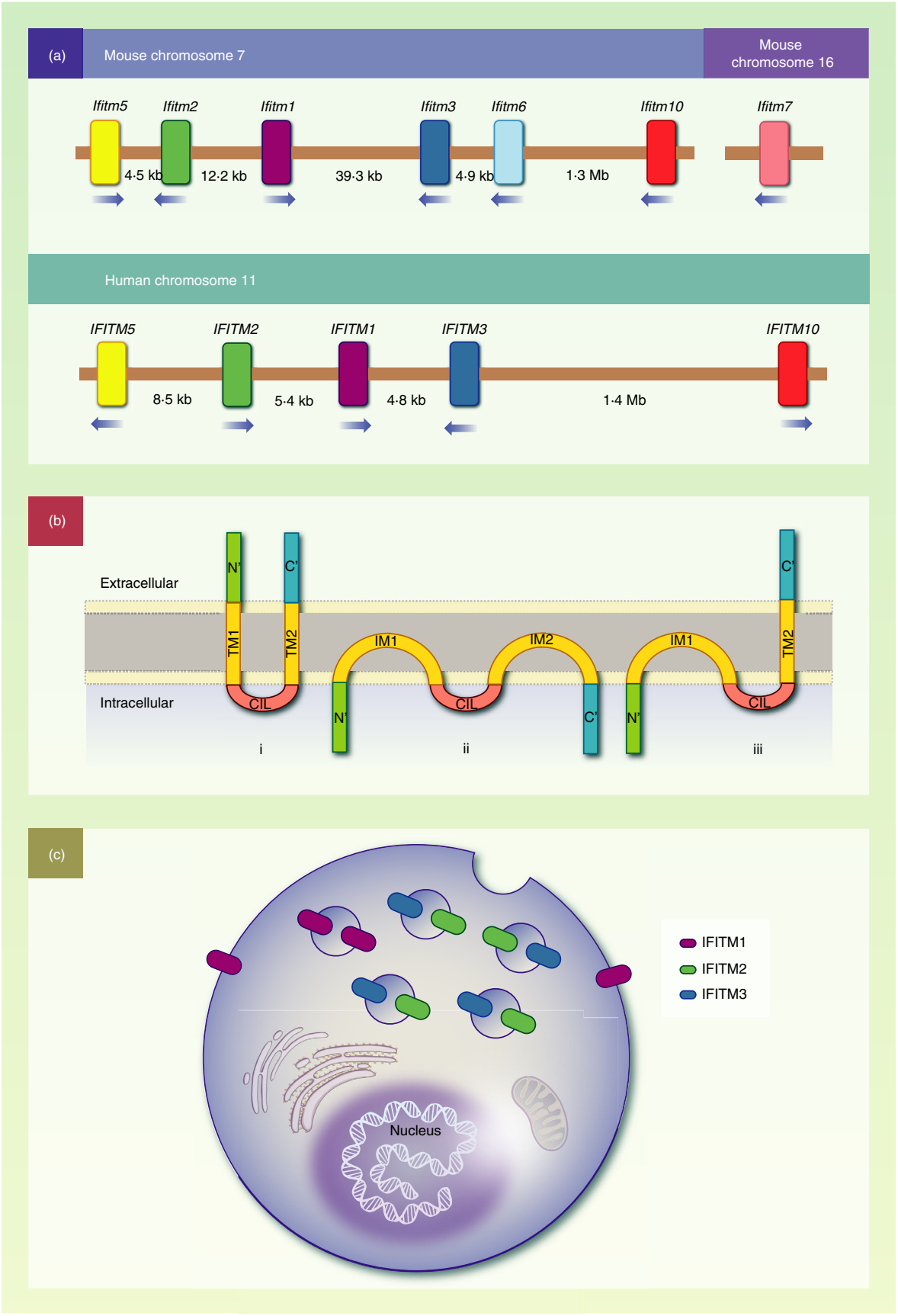

Figure 1. Chromosomal position of interferon-inducible transmembrane (IFITM) genes, IFITM topology and cellular localization. (a) The cartoon illustrates the location and organization of IFITM gene clusters in mouse and human. Introns are represented by a horizontal brown rectangle. Exons are represented by vertical coloured rectangles, arrows below indicate the direction of transcription.[4,18] (b) The cartoon illustrates the proposed models of IFITM protein topology. First model suggests a conserved intracellular loop (CIL) between two transmembrane domains (TM) with extracellular C' and N' terminal domains. Second model shows a CIL between two intramembrane domains (IM) with intracellular C' and N' terminal domains. The third model proposes a CIL between IM and a TM with an intracellular N' and an extracellular C' terminal domain. These three topology models are predominant but alternative models have been proposed for specific IFITM protein topology depending on their function.[1] (c) The cartoon illustrates the cellular localization of IFITM1–3 proteins. IFITM proteins have been shown to span several cellular membranes. IFITM1 is found in different intracellular compartments from IFITM2 and IFITM3 with little overlap.[47,59] IFITM1–3 can all be found on the plasma membrane, but IFITM1 has been shown to be the predominant IFITM associated with the plasma membrane and is also found in early endosomes.[35,60] IFITM2 and IFITM3 are predominately located intracellularly in late endosomes and lysosomes and co-localize with Rab7, CD63 and lysosomal-associated membrane protein (LAMP1).[21] The illustrations in this figure are cartoons that are not drawn to scale.

reptiles and mammals, but its functions have not yet been defined.[19]

IFITM proteins are virus-restriction factors

In tissue-culture experiments, IFITM proteins have been shown to enable cells to resist infection by both enveloped and non-enveloped viruses, including many viruses that affect human health, such as dengue virus, hepatitis C virus, influenza A virus, West Nile virus, human immunodeficiency virus type 1, vesicular stomatitis virus, severe acute respiratory syndrome-related coronavirus, Marburg virus, Ebola virus and Zika virus.[1–3,44–48] Different IFITM proteins specialize in targeting different viruses.[3,20,46] *In vivo* studies have confirmed the importance of IFITM proteins in resistance to viruses. In mice, constitutive deletion of the five-gene cluster of *Ifitm* genes on chromosome 7 (*Ifitm1–3, -5* and *-6*) or of *Ifitm*3 alone (*Ifitm3*$^{-/-}$) rendered animals highly sensitive to influenza infection, and in humans genome-wide association studies and sequencing studies have shown that *IFITM3* restricts influenza *in vivo*.[8,38,49–55]

Distinct mechanisms have been proposed to explain the ability of different IFITM family members to restrict different classes of viruses, including inhibition of viral entry and also entry-independent effects, such as suppression of viral protein synthesis or viral replication.[3,21,44–48,56,57] Differences in the cellular localizations of the IFITM proteins may explain their different activities in the inhibition of entry of diverse viruses at their specific sites of fusion. However, determination of the precise subcellular localization of the different IFITM proteins remains elusive, and their localization within the cell may be dependent on cell type. IFITM1 has been found at the cell surface and also co-localizes with early endolysosomal markers.[30–33,58–60] IFITM2 and IFITM3 also co-localize with endosomal markers, but are found in membranes of different endosomal and lysosomal compartments compared with IFITM1, and co-localize with Rab7, CD63, lysosomal-associated membrane protein[3,47,59,61] (Fig. 1c). The way in which IFITM proteins prevent viral entry at different sites is also unclear, and different experimental systems have provided evidence for many mechanisms, such as by changing membrane fluidity or physical properties of cell membranes, or by changing properties of the cytoplasm or lumina.[22,62–64]

The IFITM proteins in adaptive immunity

IFITM expression in murine T cells

Gene and protein expression studies have demonstrated that IFITM1–3 are expressed in murine CD4$^+$ and CD8$^+$ T cells.[7,11,13,37] Expression of *Ifitm2* and *Ifitm3* are regulated by TCR signalling.[13] In naive CD4$^+$ T cells, RNA sequencing showed that expression of *Ifitm3* was rapidly down-regulated during the first 24 hr after activation by anti-CD3/CD28 ligation in T helper type 0 (Th0), Th1 and Th2 culture conditions, whereas *Ifitm2* was up-regulated and its expression continued to rise for the first 3 days after activation and levels of *Ifitm1* were low and were not changed by TCR signalling.[13]

In contrast, expression analysis of IFITM3 protein by Western blotting on naive CD8$^+$ and CD4$^+$ T cells following anti-CD3/CD28 activation demonstrated that IFITM3 protein is up-regulated by day 3 following T-cell activation.[7] This up-regulation was independent of interferon signalling, as naive T cells purified from mice which constitutively lack interferon-γ (IFN-γ), the IFN-α receptor, or the transcription factors IIrf3 and Irf7, which drive interferon-induced up-regulation of *Ifitm3*, all showed increased expression of IFITM3 protein 2 days after TCR ligation.[7]

The difference in expression patterns of the *Ifitm3* gene and the IFITM3 protein on activation of naive CD4 T cells may reflect differences in the strength of the activation signal given in the two experimental systems, or be due to changes in the rate of turnover and ubiquitination of the IFITM3 protein on TCR activation,[65–67] so that although *Ifitm3* gene expression initially decreases, IFITM3 protein levels rise.

Ifitm3 is also regulated by Hh-mediated transcription in murine $CD4^+$ T cells.[11]

IFITM expression in human T cells

The *IFITM1–3* genes are expressed in human lymphocytes, and *IFITM1* is expressed at their cell surface, where it has been shown to associate with receptor signalling complexes.[12,31–33]

The IFITM family in T-cell differentiation and function

IFITM3 and influenza infection

In mice, IFITM3 protects against influenza, and *Ifitm3*$^{-/-}$ mice die when infected with doses of influenza virus that would not be lethal in wild-type (WT) mice.[49,50] In addition to its protective role in other cell types, such as respiratory epithelial cells and the heart,[49,68] IFITM3 protects cells of the immune system from viral infection, thereby enabling them to mount an effective immune response.[7,8,38] During influenza infection, IFITM3 is up-regulated in dendritic cells in the lung by type I IFN, allowing them to survive and migrate to the draining lymph node in order to present viral antigens.[38] IFITM3 is then rapidly up-regulated on T cells on their activation in the draining lymph nodes, and high IFITM3 expression is maintained because they migrate to sites of viral infection, providing a survival advantage that enables them to carry out their effector functions.[7,8] Interestingly, IFITM3 is also constitutively expressed in tissue-resident T cells in lung and airways, and also spleen, skin and brain, suggesting that it promotes their survival at these sites of potential viral infection.[8,12,13,37,53,69] Hence, several *in vivo* and *in vitro* studies have demonstrated the importance of IFITM3 in immunity to viral infection, by enhancing survival and viral resistance of immune effector populations, but these studies did not demonstrate an additional direct function of the IFITM proteins in the immune function of T cells or dendritic cells.

The IFITM family in mouse $CD4^+$ T-cell differentiation

We investigated the role of IFITM proteins in peripheral $CD4^+$ T-cell function in mice deficient in the five *Ifitm* genes clustered on chromosome 7. These mice are deficient in *Ifitm1–3*, *-5* and *-6* and are referred to as *Ifitm*Del$^{-/-}$.[17] Whole-genome transcriptome analysis of resting $CD4^+$ T cells from spleens of *Ifitm*Del$^{-/-}$ mice showed that these cells had a Th1-like transcriptional signature compared with their WT counterparts.[13] To investigate if this difference was the result of changes in the immune environment of the cells, or the result of a $CD4^+$ T-cell-intrinsic influence on T helper differentiation, we purified naive ($CD62L^+$ $CD44^-$ $CD25^-$) $CD4^+$ T cells from *Ifitm*Del$^{-/-}$ and WT littermates and carried out *in vitro* differentiation experiments in which we activated cells in Th-skewing conditions. These *in vitro* experiments showed a clear bias in differentiation towards Th1.[13] After 3 days in culture, a greater proportion of the *Ifitm*Del$^{-/-}$ $CD4^+$ T cells cultured in Th0 and Th1 conditions expressed Tbet than WT, whereas in Th2 conditions the proportion of $Gata3^+$ cells was reduced. Expression of the Th1-associated molecules Cxcr3 and CD54 and expression of *Tbx21* and *Il27ra* were also increased in the *Ifitm*Del$^{-/-}$ cells cultured in Th1-skewing conditions compared with their WT counterparts. Bias towards Th1 differentiation was also demonstrated by cytokine production: *Ifitm*Del$^{-/-}$ cells produced less interleukin-4 (IL-4) and IL-13 than WT when cultured in Th2-skewing conditions, but more IFN-γ when cultured in Th1-skewing conditions. Hence, the absence of the IFITM family of proteins led to an overall bias towards Th1 differentiation and a reduction in Th2 differentiation when purified naive $CD4^+$ T cells were activated, suggesting that one or all of the IFITM proteins inhibit Th1 differentiation but promote Th2 differentiation in a T-cell-intrinsic manner[13] (illustrated in Fig. 2a). As *Ifitm2* expression rapidly increased on activation, IFITM2 might be the most likely candidate family member for this function.

In support of this, bias towards Th1 differentiation was not observed when purified naive *Ifitm3*$^{-/-}$ $CD4^+$ T cells were activated in Th-skewing conditions *in vitro*,[13] indicating that IFITM3 was not the sole family member responsible for promotion of Th2 differentiation, although a synergistic or additive effect between the IFITM proteins was not excluded.

The IFITM family in allergic and inflammatory disease

The *Ifitm*Del$^{-/-}$ mice also showed reduced Th2 responses and Th2 immunopathology *in vivo*.[13] On induction of allergic airways disease they had less severe disease and a weaker Th2 response, with lower *Il4* expression, cellular infiltration and mucous production in the lung than their WT littermates. In addition to a reduction in eosinophils, myeloid dendritic cells and mast cells, T cells were reduced in the bronchoalveolar lavage and IL-27 secretion was increased but IL-13 production decreased, and the $CD4^+$ population in the mediastinal lymph nodes had a more Th1-like phenotype, with higher cell surface expression of CD27, but lower expression of the Th2-marker T1ST2. Consistent with the *in vitro* cytokine data, lungs from the allergic airways disease-induced *Ifitm*Del$^{-/-}$ mice had higher expression of *Ifng*, suggesting that although interferon-inducible, the IFITM family provide negative feedback on IFN-γ signalling to dampen Th1 immunity in the lung.

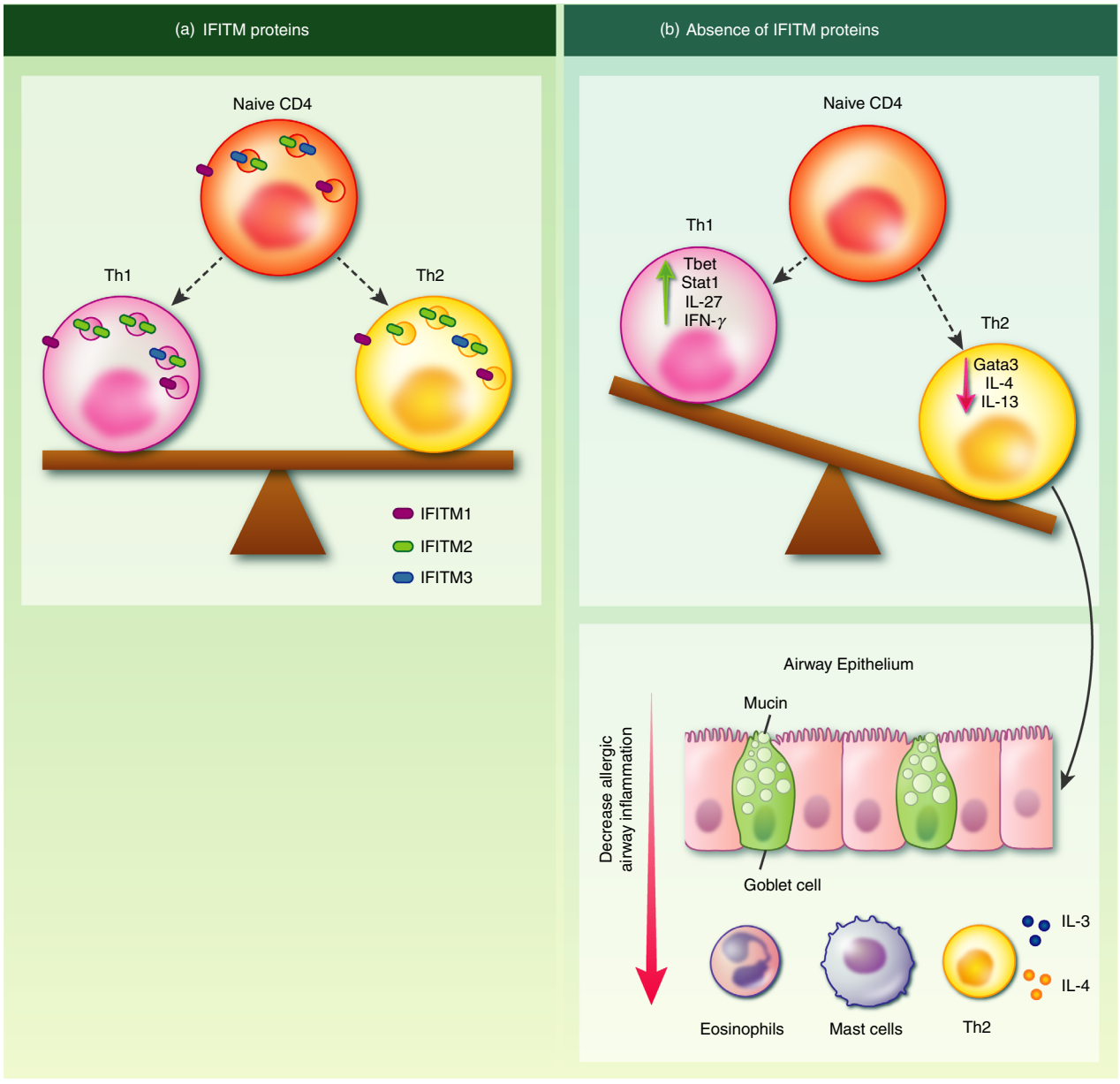

Figure 2. Interferon-inducible transmembrane (IFITM) proteins are involved in T helper type 1 (Th1) and Th2 differentiation. Cartoons show a not-to-scale graphical representation of the role of IFITM proteins in the regulation of Th1/Th2 differentiation.[13] (a) In normal conditions, differential expression of IFITM proteins maintains the normal balance between Th1 and Th2 differentiation on activation of naive $CD4^+$ T cells. (b) Upper panel: In the absence of IFITM proteins, the balance of the Th1/Th2 differentiation is altered on activation of naive $CD4^+$ T cells. Differentiation of Th1 cells is promoted with higher expression of key Th1 regulators, while Th2 differentiation is suppressed. Lower panel: IFITM deficiency decreases allergic airway inflammation, with lower cellular infiltration, mucous secretion, and Th2 response in a mouse model of allergic airway disease (asthma).

On induction of allergic airways disease in *Ifitm3*$^{-/-}$ mice, however, there were no significant changes in eosinophil, mast cell or T-cell infiltration in lung or bronchoalveolar lavage or in T1ST2 expression on T cells compared with WT, although macrophage and neutrophil infiltration was reduced.[13] Therefore, as with the *in vitro* data, deletion of *Ifitm3* alone did not appear to affect the *in vivo* $CD4^+$ Th2 response in lung; although, additive or synergistic effects between IFITM family members were not excluded.

The role of IFITM proteins in human allergic asthma has to our knowledge not been investigated, but genome-wide association studies have linked *IFITM2* and/or *IFITM3* variants to potentially relevant traits, such as the proportion or count of basophils and eosinophils in blood, and lung function.[70,71] Given the link between

IFITM and Hh signalling and the fact that Hh signalling has also been shown to promote Th2 differentiation and exacerbate allergic asthma, it will be important to investigate the interactions between IFITM proteins and the Hh pathway in allergic asthma.[11,72–77]

The IFITM proteins are associated with other atopic and inflammatory diseases. In atopic dermatitis patients, *IFITM1–3* are up-regulated in lesional skin compared with non-lesional skin from the same individuals, although the functional consequences of their increased expression have not been investigated.[78] Likewise, their expression is up-regulated in inflamed mucosa of ulcerative colitis and Crohn's disease patients,[43,79] and polymorphisms in *IFITM1* and *IFITM3* are associated with increased susceptibility to ulcerative colitis.[80,81] In mice, the IFITM family protect against colitis.[82] IFITM3 deficiency led to exacerbation of chemical-induced colitis, with increased infiltration of macrophages and effector T cells to the colon lamina propria, and biased $CD4^+$ Th differentiation to Th17.[82] That this exacerbated colitis was attributable to cells of the haematopoietic system was confirmed in bone marrow transplantation experiments. Interestingly, *Ifitm*$Del^{-/-}$ mice developed spontaneous chronic colitis, indicating that other IFITM proteins are also protective against colitis, and both *Ifitm3*$^{-/-}$ and *Ifitm*$Del^{-/-}$ showed changes in the faecal microbiota.[82]

Mechanisms of action of IFITM proteins in immune cells

Several studies have shown that IFITM proteins enhance immunity to viral disease by inhibiting viral infection of immune effector cells, thereby enhancing their survival and ability to mount an effective immune response,[7,8,12,38] but the way in which IFITM proteins prevent viral infection is unclear and seems dependent on the cell type and the particular IFITM family member and virus interaction, with experimental evidence supporting many possible mechanisms, including inhibition of viral entry, fusion, transcription and translation.[3] Our recent study highlighted a virus-independent T-cell-intrinsic function for IFITM proteins in Th differentiation[13] and although the mechanism for their T-cell-intrinsic influence on Th differentiation is also unknown and requires investigation, several possibilities arise by extrapolation from the virus-restriction studies and from their cellular localization and expression patterns.

First, it is possible that IFITM proteins influence Th differentiation by influencing the molecular order of membranes and membrane fluidity.[62] In human T cells, high membrane order is associated with Th2 differentiation and IL-4 production, and intermediate membrane order is associated with Th1 cells and IFN-γ production.[83] Hence, IFITM deficiency could bias differentiation towards Th1 by reducing membrane order, although how membrane order influences Th differentiation also remains unknown.

Second, it is possible that the IFITM proteins are involved in the regulation of cytokine signalling, and promote IL-4 signal transduction over Th1-cytokine signalling to polarize differentiation. Given their presence in endolysosomal intracellular vesicles, this theory suggests that IFITM proteins are involved in internalization, trafficking or degradation of some cytokine receptors or signalling pathway components, but not others. In support of this, IFN receptor signalling involves internalization by clathrin-dependent endocytosis,[84] so the presence of IFITM2 and IFITM3 in the membrane of late endosomes may modulate IFN signalling.

A third possibility is that the IFITM proteins are involved in enhancing or regulating the transduction of other signalling pathways that regulate Th differentiation. In support of this, they are transcriptional targets of Wnt and Hh signalling,[10,11] and both these pathways promote Th2 differentiation.[72,74,85,86]

Finally, it is possible that the IFITM proteins have unknown direct consequences for Th differentiation through an influence on transcription or translation (as has been described for some viral genes).

Conclusions

Recent studies have identified new roles for the IFITM family in Th differentiation and atopic and inflammatory disease, which are independent of their functions in cellular resistance to viral infection.[13,36,80–82] Mouse studies showed that while IFITM deficiency was protective against induction of Th2 immune pathology and asthma,[13] it exacerbated Th17-driven inflammation in colitis,[82] highlighting the context dependency of their impact on inflammation. Clearly, further studies will be required to investigate the contribution of the different family members to the immune response and inflammation, and the cellular and molecular mechanisms that underlie their functions. It will be important to assess the impact of the IFITM family on the adaptive immune response to infectious disease and cancer.

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Disclosure

The authors have no competing interests.

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