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Invited review

Title: **The IFITM protein family in adaptive immunity**

Short title: **IFITM proteins in adaptive immunity**

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Abbreviations IFITM: interferon-inducible transmembrane protein; Hh: Hedgehog; TCR: T cell receptor; TM: transmembrane; IM: intramembrane; IAV: influenza virus A; GWAS: genome wide association study; lysosomal-associated membrane protein (LAMP1); IFN; interferon; Q RT-PCR: quantitative reverse-transcription polymerase chain reaction; Th: T helper; WT: wild type; BAL: bronchoalveolar lavage; ADD: allergic airways disease; UC: ulcerative colitis.

Abstract

The family of interferon-inducible transmembrane (IFITM) proteins are small homologous proteins, localised 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 mineralisation (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.

Introduction

The IFITM family

The family of interferon-inducible transmembrane (*Ifitm*/*Fragilis*) genes encode small homologous proteins localised 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 shown to be targets of Wnt/beta-catenin and Hedgehog (Hh) signal transduction[9-12]; and in murine T cells IFITM2 and IFITM3 expression is regulated by 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 Figure 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 Figure 1B[20-22]. All IFITM proteins have two transmembrane (TM) or intramembrane (IM) 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 (Figure 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 mineralisation (IFITM5)[25-29] and immune functions (IFITM1-3, IFITM6)[7, 8, 13, 30-38], in addition to their roles as viral 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, reptiles and mammals, but its functions have not yet been defined[19].

IFITM proteins are viral 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 (IAV), West Nile virus, HIV-1, vesicular stomatitis virus, SARS Coronavirus, Marburg virus, Ebolavirus, and Zika virus[1-3, 44-48]. Interestingly, different IFITM proteins specialize in targeting different viruses[3, 20, 46]. In vivo studies have confirmed the importance of IFITM proteins in viral resistance. 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 (*Ifitm*3-/-) rendered animals highly sensitive to influenza infection, and in humans genome wide association studies (GWAS) 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 localisations of the IFITM proteins may explain their different activities in inhibition of entry of diverse viruses at their specific sites of fusion. However, determination of the precise subcellular localisation of the different IFITM proteins remains elusive, and their localisation within the cell may be dependent on cell type. IFITM1 has been found at the cell surface and also colocalises with early endolysosomal markers[30-33, 58-60]. IFITM2 and IFITM3 also colocalise with endosomal markers, but are found in membranes of different endosomal and lysosomal compartments than IFITM1, and colocalise with Rab7, CD63, lysosomal-associated membrane protein (LAMP1)[3, 47, 59, 61] (Figure 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 naïve CD4+ T cells, RNA-sequencing showed that expression of *Ifitm3* was rapidly downregulated during the first 24 hours after activation by anti-CD3/CD28 ligation in Th0, Th1 and Th2 culture conditions, whereas *Ifitm2* was upregulated 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 naïve CD8+ and CD4+ T cells following anti-CD3/CD28 activation demonstrated that IFITM3 protein is upregulated by day 3 following T cell activation[7]. This upregulation was independent of interferon signalling, as naïve T cells purified from mice which constitutively lack IFNγ, the IFNα receptor, or the

transcription factors IIrf3 and Irf7, which drive interferon-induced upregulation of *Ifitm3*, all showed increased expression of IFITM3 protein two days after TCR ligation[7].

The difference in expression patterns of the *Ifitm3* gene and the IFITM3 protein on activation of naïve 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 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 upregulated in dendritic cells in the lung by type I interferon, allowing them to survive and migrate to the draining lymph node in order to present viral antigens[38]. IFITM3 is then rapidly upregulated on T cells on their activation in the draining lymph nodes, and high IFITM3 expression is maintained as they migrate to sites of viral infection, providing a survival advantage which 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]. Thus, 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 5 *Ifitm* genes clustered on chromosome 7. These mice are deficient in *Ifitm*1-3, 5 and 6 and are referred to as *Ifitm*Del-/-[17]. Whole genome transcriptome analysis of resting CD4+ T cells

from spleen of *Ifitm*Del-/- mice showed that these cells had a Th1-like transcriptional signature compared to their WT counterparts[13]. To investigate if this difference was due to changes in the immune environment of the cells, or the result of a CD4+ T cell-intrinsic influence on T helper differentiation, we purified naïve (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 of *Tbx21* and *Il27ra* were also increased in the *Ifitm*Del-/- cells cultured in Th1-skewing condition compared to their WT counterparts. Bias towards Th1 differentiation was also demonstrated by cytokine production: *Ifitm*Del-/- cells produced less IL4 and IL13 than WT when cultured in Th2-skewing conditions, but more IFNγ when cultured in Th1-skewing conditions. Thus, absence of the IFITM family of proteins led to an overall bias towards Th1 differentiation and reduction in Th2 differentiation when purified naïve 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 Figure 2A). As *Ifitm2* expression rapidly increased on activation, IFITM2 might be the most likely candidate family member for this function.

In support if this, bias towards Th1 differentiation was not observed when purified naïve *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 (AAD) 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 (BAL) and IL27 secretion was increased but IL13 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 AAD-induced

*Ifitm*Del-/- mice had higher expression of *Ifng*, suggesting that although interferon-inducible, the IFITM family provide negative feedback on IFNγ signalling to dampen Th1 immunity in the lung. On induction of ADD in *Ifitm3*-/- mice, however, there were no significant changes in eosinophil, mast cell or T cell infiltration in lung or BAL or in T1ST2 expression on T cells compared to WT, although macrophage and neutrophil infiltration were 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 GWAS 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 upregulated in lesional skin compared to non-lesional skin from the same individuals, although the functional consequences of their increased expression have not been investigated[78]. Likewise, their expression is upregulated in inflamed mucosa of ulcerative colitis (UC) and Crohn's disease patients[43, 79], and polymorphisms in *IFITM1* and *IFITM3* are associated with increased susceptibility to UC[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 *Ifitm*3-/- and *Ifitm*Del-/- showed changes in the fecal 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 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 viral-restriction studies and from their cellular localisation 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 IL4 production and intermediate membrane order is associated with Th1 cells and IFNγ production[83]. Thus, 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 IL4 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 internalisation, trafficking or degradation of some cytokine receptors or signalling pathway components, but not others. In support of this, IFN receptor signalling involves internalisation by Clatherin-dependent endocytosis[84], so 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 which 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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Competing Interests

The authors have no competing interests.

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Figure Legends

Figure 1. Chromosomal position of IFITM genes, IFITM topology and cellular localisation.

A. The cartoon illustrates the location and organisation of IFITM gene clusters in mouse and human. Introns are represented by a horizontal brown rectangle. Exons are represented by a 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 localisation 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 colocalise with Rab7, CD63, and lysosomal-associated membrane protein (LAMP1)[21].

The illustrations in this Figure are cartoons which are not drawn to scale.

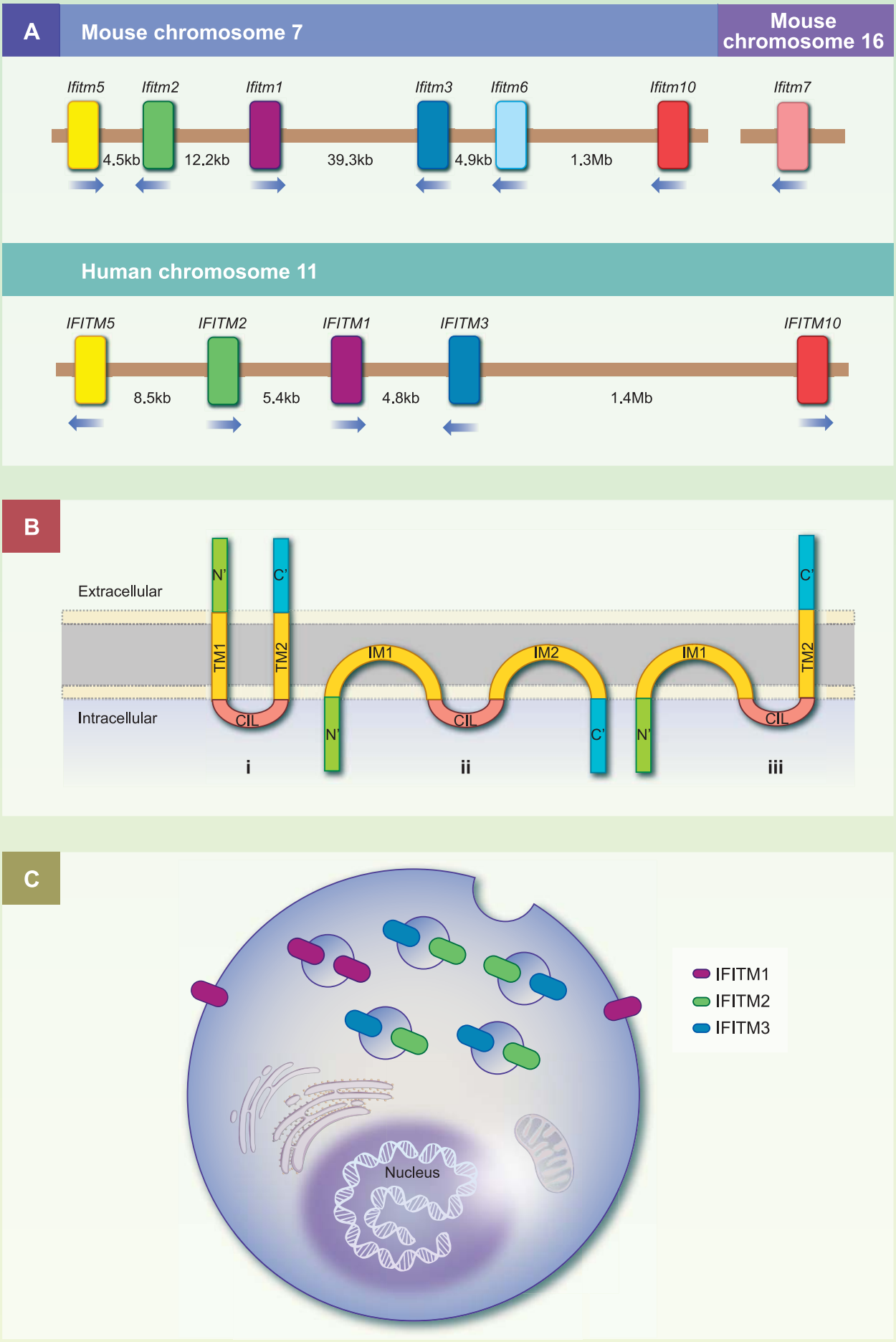
Figure 2. IFITM proteins are involved in Th1 and Th2 differentiation

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 naïve CD4+ T cells.

B. Upper panel: In 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).


A
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1.3Mb
Human chromosome 11
IFITM5
IFITM2
IFITM1
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IFITM10
8.5kb
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N'
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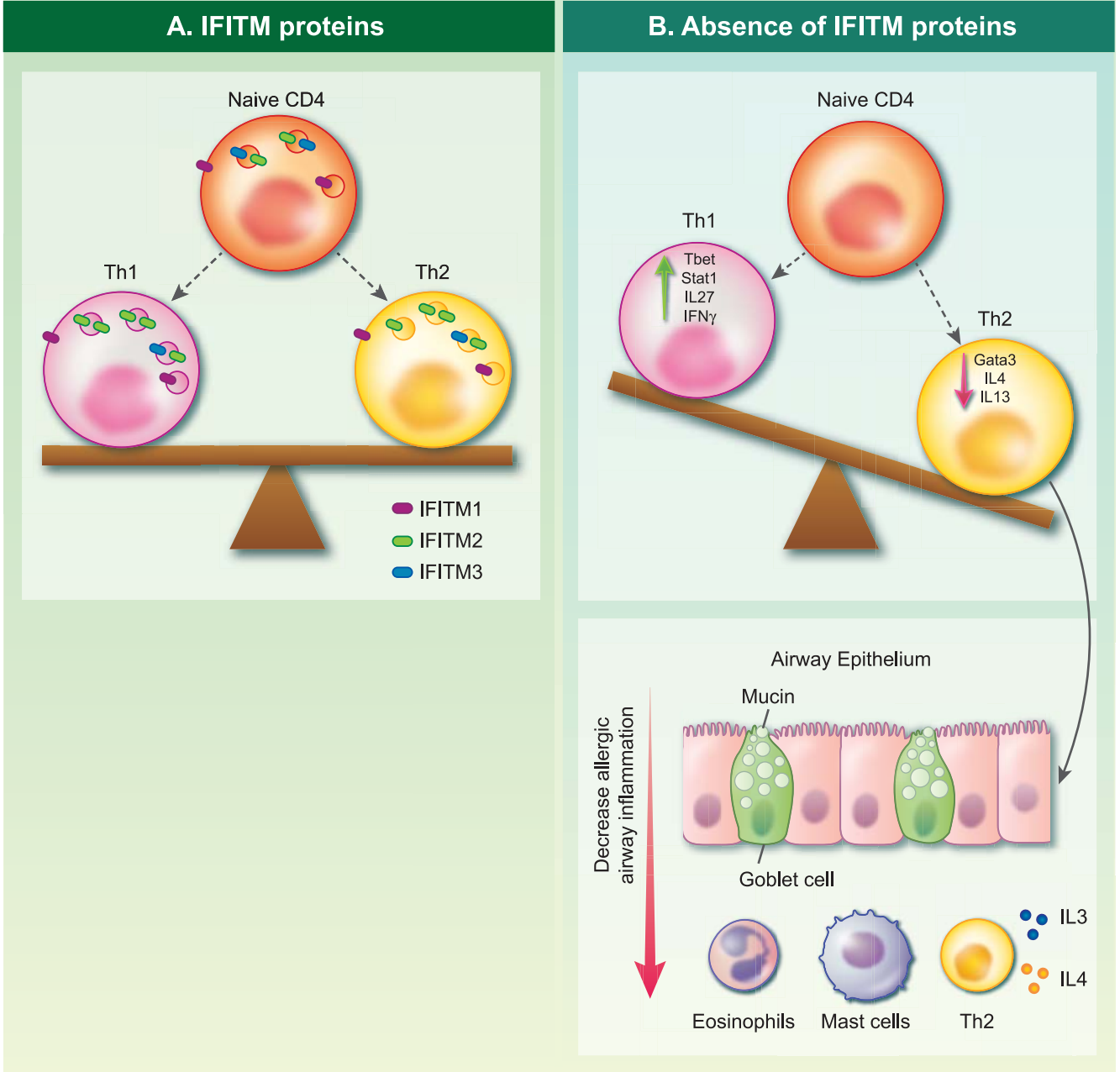
IFITM3


A. IFITM proteins
Naive CD4
Th1
Th2
IFITM1
IFITM2
IFITM3
B. Absence of IFITM proteins
Naive CD4
Th1
Tbet
Stat1
IL27
IFNγ
Th2
Gata3
IL4
IL13
Airway Epithelium
Mucin
Goblet cell
Decrease allergic airway inflammation
IL3
IL4
Eosinophils
Mast cells
Th2