See ARTICLE page 172 (vol. 4, issue 2)
See ARTICLE page 186 (vol. 4, issue 2)

The tale of TL1A in inflammation

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TL1A (also known as TNFSF15) is a tumor necrosis factor (TNF) family member expressed by monocytes, macrophages, dendritic cells (DCs), synovial fibroblasts, and endothelial cells in response to stimulation by cytokines, immune complexes, and microorganisms.^{1–5} Its cell surface receptor, DR3 (also known as TNFSF25, WSL-1, TRAMP, and LARD), is mainly expressed by T cells.⁶

The early reports from *in vitro* functional studies indicated that TL1A can enhance T cell proliferative responses and can augment T-helper 1 (Th1) cytokine production, interferon gamma (IFN γ) in particular.^{1,7,8} Two new reports in the last issue of *Mucosal Immunology* suggest, however, that the role of TL1A *in vivo* may be considerably more extensive than previously assumed.^{9,10} Here, we will review what is now being revealed about the biology of TL1A, and we will attempt to put this information in the context of the role that TL1A may have in driving inflammatory diseases that affect mucosal tissues such as the gut and the lung.

The first surprising observation to arise from the transgenic mice created by Meylan *et al.*⁹ and Taraban *et al.*¹⁰ was the marked elevation of interleukin-13 (IL-13), not IFN γ , in the small intestine. Even though the two transgenic mouse lines are quite distinct—TL1A overexpression in T cells is driven by the CD2 promoter in one line, whereas TL1A expression is driven in DCs by the CD11c promoter in the other—both of these

mouse lines exhibited 20–50-fold higher IL-13 compared with control littermates. Unlike the predominant IFN γ cytokine profile seen in the *in vitro* studies with TL1A, the IFN γ levels in both transgenic mouse lines were largely unchanged. This unexpected finding of a more predominant Th2-type cytokine profile in the TL1A transgenic mice underscores the importance of exploring the biology of novel molecules in the whole organism, rather than relying only on more constricted *in vitro* systems.

Accompanying the grossly elevated levels of IL-13 in the small intestine, both lines of TL1A transgenic mice spontaneously developed intestinal inflammation—regardless of whether the TL1A was overexpressed in T cells or DCs. Disease severity correlated to transgene expression level. Inflammation was largely restricted to the small intestine and was most severe in the terminal ileum, whereas the colon was not affected. The ileitis was characterized by the classical features of distorted villi, an inflammatory cell infiltrate into the lamina propria, and a thickening of the muscularis and

submucosa. A more striking observation was the prominent goblet cell hyperplasia in the inflamed small intestine, most likely due to the excessive amounts of IL-13. Indeed, treatment of the transgenic mice shortly after birth with a neutralizing anti-IL-13 antibody abolished the intestinal inflammation, including goblet cell hyperplasia, whereas anti-IL17 antibody treatment had no effect. These data clearly support the hypothesis that elevation of the Th2 cytokine, IL-13, is the key driver for the intestinal inflammation in TL1A transgenic mice. The predominant inflammation in intestine but not in other tissues is likely due to chronic intestinal immune activation by TL1A and gut microflora. This is consistent with the previous findings of TL1A as a co-stimulatory factor with relative little effect on resting cells.¹¹

The source of excessive IL-13 production in the TL1A transgenic mice likely comes from activated T cells. The frequency of CD69⁺ and CD44^{hi}CD62^{lo} T cell populations was increased in both lines of mice, regardless of which cell type overexpresses TL1A. It is possible that some of the IL-13 could be derived from natural killer T (NKT) cells, as TL1A has been reported to enhance NKT cell proliferation and IL-13 production *in vitro*.¹² However, in the TL1A transgenic mice, the NKT cell numbers are reduced compared with control littermates. The mechanistic basis for this decreased number of NKT cells is not clear, and it remains to be seen whether this could be due to exhaustion of this cell population following prolonged activation by TL1A, or due to direct negative regulation by TL1A itself. A similar intestinal inflammation phenotype was recently reported in another TL1A transgenic mouse line generated by Shih *et al.*¹³ In addition, these mice

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also displayed elevation of IFN γ , intestinal fibrosis, and a low frequency of extra-intestinal pathology, such as ulcerated skin lesions and erythematous swollen joints. The phenotypical variations in these different transgenic mouse lines may be a result of differences in the way in which the transgenic mice were generated and/or housed, e.g., use of different promoter/enhancer, different background strains, and/or different gut microflora between animal facilities.

The phenotype of intestinal inflammation in the TL1A transgenic mice supports the emerging evidence of TL1A polymorphisms being associated with susceptibility to developing inflammatory bowel disease (IBD). The first genome-wide association study (GWAS) in IBD revealed significant linkage of TL1A single-nucleotide polymorphism (SNP) with Japanese and European IBD patient populations.¹⁴ Subsequent additional GWASs extended the association of TL1A polymorphisms to Asian and Caucasian IBD patient populations, and to both adult and pediatric patient populations.¹⁵ Corresponding to the genetic findings, increased TL1A expression has been reported in inflamed Crohn's disease lesions compared with uninvolved areas, and in UC patient serum.^{8,16,17} Although expression is increased, the functional consequence of the various TL1A polymorphisms remains to be fully determined. Faster kinetics and higher levels of TL1A expression following Fc γ R activation with immune complex have been reported in monocytes and T cells from patients carrying the disease-associated TL1A SNPs, supporting the notion that aberrant TL1A expression may be a factor driving IBD development.^{18,19} More extensive TL1A expression analysis in a larger population of IBD patients, including both intestinal tissues, serum and peripheral blood, is going to be necessary for advancing our understanding of how TL1A really contributes to IBD pathogenesis.

Given the mounting evidence that increased expression of TL1A may contribute to IBD disease pathogenesis, there is blossoming interest in the therapeutic potential of TL1A blockade for treating IBD. In mice, neutralizing

anti-TL1A antibody treatment appears to be efficacious in attenuating intestinal inflammation. In chronic dextran sulfate sodium-induced colitis, TL1A and DR3 expression is increased in the colon and the draining mesenteric lymph nodes, and treatment with a hamster-anti-mouse neutralizing anti-TL1A antibody, either prophylactically or therapeutically, attenuated the disease development.²⁰ Anti-TL1A neutralizing antibody treatment was also shown to be effective at attenuating weight loss and intestinal inflammation in the trinitrobenzene sulfonic acid-induced colitis model.⁹ Although the obvious interpretation to these studies is that TL1A neutralization is beneficial for treating mouse models of intestinal inflammation, the antibodies used were both hamster derived. Hamster antibodies are notoriously effective at eliciting antibody-dependent cell-mediated cytotoxicity, and it should be noted that TL1A, like most other TNF family members, is a type II transmembrane protein that can be expressed on cell surface as well as processed into a soluble form. It remains to be determined whether the efficacy observed with anti-TL1A antibody treatment in these IBD models resulted from TL1A blockade or depletion of cells expressing cell-surface TL1A. Evaluation of TL1A blockade vs. depletion in other spontaneous mouse IBD models will be useful in validating the potential of TL1A as a therapeutic target for treating human IBD patients.

As mentioned above, the dramatic induction of IL-13 accompanied by goblet cell hyperplasia in the small intestine of the TL1A transgenic mice revealed an unexpected, but important, role for TL1A in Th2 cell regulation. One might be tempted to predict that these mice might be prone to other inflammatory diseases involving IL-13, goblet cell hyperplasia, and Th2 cells, such as is seen in mouse models of asthma. Somewhat surprisingly, neither of the studies describing the TL1A transgenic mice reports on any spontaneous inflammatory changes in the lung. It will be very interesting, though, to examine whether these TL1A transgenic mice are more susceptible to experimentally induced allergic responses, especially because there is already one

report showing that TL1A blockade can dampen lung inflammatory responses in the ovalbumin-induced asthma model.¹² Moreover, concordant with the pharmacological inhibition of TL1A being beneficial at attenuating lung inflammation, DR3 knockout mice are reported to be resistant to ovalbumin-induced asthma.¹¹

It appears that multiple cell types might contribute to the lung inflammation in the allergic asthma models, including T cells and NKT cells. Adoptive transfer of DR3-deficient T cells or DR3-mutant NKT cells failed to support the inflammatory processes in the lung.^{11,12} It remains to be determined if and how TL1A expression is regulated in the ovalbumin-induced lung asthma model in normal animals. It is possible that local aerosol antigen challenge directly induces TL1A expression in the lung tissues. The expression of local TL1A may subsequently induce secretion of IL-13 directly or may simply promote Th2 effector cell accumulation in the lung. Supporting this hypothesis, the DR3-deficient mice, although resistant to lung inflammation, are still able to launch immune response upon systemic ovalbumin challenge.¹² This begs the question of whether there is a specific requirement for the TL1A–DR3 pathway in the local mucosal inflammatory response but not in the systemic immune response. Perhaps this can be tested via pharmacological inhibition studies.

Despite the increasing number of publications describing a role for TL1A in lung inflammation, there are no reports regarding whether TL1A and/or DR3 expression is aberrant in human asthma patients. Given the recent findings of the impact of TL1A blockade in Th2 responses in preclinical models of asthma and lung inflammation, it might be fruitful to investigate whether there is any association between TL1A polymorphisms and susceptibility to diseases of the human lung. If so, TL1A may prove interesting as a drug target for asthma.

In addition to demonstrating efficacy in preclinical models of IBD and asthma, TL1A blockade has shown efficacy in mouse experimental autoimmune encephalomyelitis and collagen-induced arthritis models.^{21,22} This opens the question of

Table 1 The effects of intervening in the TL1A–DR3 pathway in rodent models of inflammatory disease

Human diseases	Mouse disease models	Means of inhibition	Reference
IBD	Chronic DSS-induced colitis ^a	TL1A antibody	20
	TNBS-induced colitis	TL1A antibody	9
	Gxi2 ^{-/-} T-cell transfer colitis	TL1A antibody	20
Asthma	OVA asthma model ^a	TL1A antibody	12
		DR3 ^{-/-}	11
Multiple sclerosis	MOG _{35–55} EAE model	TL1A ^{-/-}	21
		DR3 ^{-/-}	11
Arthritis	Antigen-induced arthritis	DR3 ^{-/-}	22
	Collagen-induced arthritis	TL1A antibody	22

Abbreviations: DSS, dextran sulfate sodium; EAE, experimental autoimmune encephalomyelitis; IBD, inflammatory bowel disease; OVA, ovalbumin; TNBS, trinitrobenzene sulfonic acid.

^aIndicates efficacy with both prophylactic and therapeutic anti-TL1A antibody treatment.

The table shows the efficacy of inhibition of the TL1A–DR3 pathway by either genetic knockout or treatment with anti-TL1A neutralizing antibody in the indicated rodent disease models.

whether TL1A blockade merely affects IL-13 and IFN γ responses, or whether the impact is broader across the immune universe. Recently, TL1A has been implicated in Th17 cell regulation.^{20,21} Recombinant TL1A protein has been reported to enhance differentiation and proliferation of Th17 cells, although it should be noted that DR3^{-/-} T cells can still be polarized toward the Th17 pathway similar to wild-type T cells. TL1A also appears to modulate regulatory T cell (Treg) responses, although it is not clear whether this regulation is beneficial or detrimental to inflammation. Treatment of Treg cells by either recombinant TL1A or an anti-DR3 agonist antibody has been reported to enhance Treg cell proliferation.^{9,10,23} However, whether the outcome of this Treg expansion by TL1A might be counterbalanced by TL1A suppression of Treg cell induction and attenuation of Treg cell suppressive activity remains to be seen.^{9,10} Indeed, pending on the timing of treatment relative to antigen exposure, both TL1A antagonist antibody and DR3 agonist antibody have been shown to be effective in alleviation of allergic lung inflammation in the ovalbumin asthma model.^{12,23} The emerging role of TL1A in regulating Th17 and Treg cells is largely derived from mouse studies. Further studies, using human cells in particular, will be essential for clarifying what type of regulatory activity TL1A exerts on Th17, Treg, or other immune cell types.

Taken together, TL1A is emerging as an important cytokine involved in a variety of preclinical inflammatory disease models (Table 1), although how it mediates its effects, and which cells are the real targets, remains to be elucidated. Association of TL1A SNPs with human IBD patients strongly supports its potential contribution to human IBD pathogenesis. More extensive human disease association studies, together with better characterization of TL1A blockade in animal disease models, will be important in the evaluation of TL1A as a therapeutic target for IBD, asthma, and other inflammatory diseases.

DISCLOSURE

HH and JLV are employees of Amgen. The authors declared no conflict of interest.

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