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Toll-Interacting Protein in Pulmonary Diseases
Abiding by the Goldilocks Principle

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Abstract

TOLLIP (Toll-interacting protein) is an intracellular adaptor protein with diverse actions throughout the body. In a context- and cell type–specific manner, TOLLIP can function as an inhibitor of inflammation and endoplasmic-reticulum stress, an activator of autophagy, or a critical regulator of intracellular vacuole trafficking. The distinct functions of this protein have been linked to innate immune responses and lung epithelial-cell apoptosis. *TOLLIP* genetic variants have been associated with a variety of chronic lung diseases, including idiopathic pulmonary fibrosis, asthma, and primary graft dysfunction after lung transplantation, and with infections, such as tuberculosis, *Legionella* pneumonia, and respiratory viruses. TOLLIP exists in a delicate homeostatic balance, with both positive and negative effects on the trajectory of pulmonary diseases. This translational review summarizes the genetic and molecular associations that link TOLLIP to the development and progression of noninfectious and infectious pulmonary diseases. We highlight current limitations of *in vitro* and *in vivo* models in assessing the role of TOLLIP in these conditions, and we describe future approaches that will enable a more nuanced exploration of the role of TOLLIP in pulmonary conditions. There has been a surge in recent research evaluating the role of this protein in human diseases, but critical mechanistic pathways require further exploration. By understanding its biologic functions in disease-specific contexts, we will be able to determine whether TOLLIP can be therapeutically modulated to treat pulmonary diseases.

Keywords: TOLLIP protein; lung diseases; communicable diseases; biomarkers; genetic research

Clinical Relevance

TOLLIP (Toll-interacting protein) is a ubiquitous protein whose roles in inflammation, autophagy, and vacuole transport have significant implications for numerous pulmonary and infectious diseases. This review summarizes the genetic associations and molecular mechanisms linking TOLLIP to these conditions and highlights future avenues for exploration that may unveil new therapeutic strategies targeting TOLLIP.

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It has been 20 years since Burns and colleagues first reported TOLLIP (Toll-interacting protein) as a new inhibitory adaptor protein involved in IL-1β signaling (1). In its early history, TOLLIP was viewed primarily as an antiinflammatory protein modulating both acute and chronic inflammatory responses (2). It is now increasingly recognized that TOLLIP's functions are essential to the processes of immune-cell activation, cell survival, pathogen defense, and numerous other biologic processes. TOLLIP executes its inhibitory roles by preventing IRAK-1 (IL-1 receptor–associated kinase-1) autophosphorylation and by promoting receptor degradation, thereby negatively regulating NF-κB activation (1, 3). This critical role in innate immune responses is perhaps most important to the lungs—an organ system with the surface area of a tennis court, which is in continuous contact with the external environment. Cells at the front lines of host defense must respond to invaders and maintain a barrier, and this is where TOLLIP's inflammatory functions are of primary relevance.

TOLLIP also plays a central role in autophagy, in which it binds with the central protein LC3, which complexes with ubiquitinated protein aggregates that are then shuttled via autophagosomes to lysosomes for clearance (4, 5). Through its association with LC3, TOLLIP also promotes autophagosome–lysosome fusion to enable intracellular clearance of damaged mitochondria (6). These processes are especially relevant to pulmonary diseases, given our increasing awareness of the importance of cellular "quality-control" functions such as autophagy in idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), asthma, and various other conditions (7).

The importance of TOLLIP to pulmonary diseases was more clearly recognized in 2013, when several SNPs within the *TOLLIP* locus were found to be associated with IPF susceptibility and prognosis (8). Since this time, other chronic pulmonary and infectious diseases have been linked to dysfunction in pathways where TOLLIP plays an integral role. SNPs within the *TOLLIP* locus have been identified that are associated with susceptibility to other pulmonary diseases. Despite this increasing body of literature, the impact of TOLLIP in these various pulmonary conditions remains unclear, largely as a result of the protein's variable and often conflicting actions both within and between cells.

Two prior reviews about TOLLIP have focused on the protein's role in innate immunity, protein trafficking, and inflammatory responses (2, 9). TOLLIP-related diseases and their associated pathways were systematically summarized in a recent review published by our group (10). This translational review adds to the literature by discussing the protein's molecular regulation, by reviewing *TOLLIP* variants implicated in noninfectious and infectious pulmonary diseases, and by reviewing the mechanistic impacts of TOLLIP dysregulation in these conditions. TOLLIP exists in a delicate balance throughout the body, where too much or too little of it can contribute to disease pathogenesis. In other words, TOLLIP must exist in the "Goldilocks zone" to allow for intracellular inflammatory, autophagy, and vacuole-trafficking processes to function normally. By capitalizing on our knowledge of this complex protein, we may unveil strategies that enable the development of novel therapeutics for pulmonary diseases (11, 12).

Molecular Regulation of *TOLLIP* Expression

Based on the IPF cell atlas (http://www.ipfcellatlas.com/) (13), *TOLLIP* is expressed in all cell types throughout the lungs in both normal states and in patients with IPF. It is most highly expressed in immune cells (macrophages, mast cells, monocytes, T-regulatory cells) and alveolar type 1 epithelial cells. To understand how TOLLIP functions in pulmonary disease states, it is important to first understand transcriptional and post-transcriptional mechanisms that regulate *TOLLIP* expression.

Transcriptional Regulation

Several transcription factors have been reported to modify gene expression at the *TOLLIP* locus. As an example, transcription factor ELF-1 (E74-like ETS transcription factor 1) binds to the −194 to −186 *cis*-acting elements of *TOLLIP*, thereby suppressing *TOLLIP* expression in the human-monocyte cell line THP-1 (14). This has potential implications for obstructive pulmonary diseases, in which ELF-1 is upregulated in total lung-tissue mRNA extracted from patients with COPD (15). In addition, ELF-1 plays a critical role in innate immune responses to viral infections that is distinct from type-1 IFN responses (16), indicating that this transcription factor may contribute to the associations between reduced *TOLLIP* expression and infectious and obstructive pulmonary diseases. Future therapeutic strategies targeting ELF-1 or other transcription factors that regulate *TOLLIP* expression may prove beneficial in preventing the development and/or progression of these conditions.

DNA methylation is one of the most common forms of epigenetic modification regulating genetic transcription. When CpG dinucleotide sites are methylated upstream of the transcriptional start site of a gene, this modification generally serves to suppress gene expression from the downstream locus. Studies have reported that the *TOLLIP* locus is hypermethylated in several different disease states, including IPF (17–19). Reduced expression as a result of hypermethylation at the *TOLLIP* locus may have significant intracellular impacts in several infectious and noninfectious pulmonary diseases in which this protein plays a central role.

TOLLIP expression is also altered through histone modifications, another primary form of epigenetic regulation. The histone lysine methyltransferase Ezh1 can silence *TOLLIP* expression by maintaining H3K27 me3 (trimethylation of histone H3 lysine 27) on the proximal promoter of *TOLLIP* (20). Through this mechanism, Ezh1 promotes TLR (Toll-like receptor) signaling through transcriptional suppression of *TOLLIP* in mouse peritoneal macrophages. Another study demonstrated that the demethylating agent 5′-aza-2′-deoxycytidine and the HDAC inhibitor Trichostatin A were able to induce *TOLLIP* expression (18). Trichostatin A has demonstrated efficacy in abrogating airway hyperresponsiveness in murine models of asthma (21), potentially in part through its actions at the *TOLLIP* locus.

Knowledge of the impact of epigenetic modifications at the *TOLLIP* locus is important for understanding how the gene is regulated in normal and disease states. Traditional therapeutic strategies targeting

disease-specific epigenetic modifications present challenges related to lack of organ and locus specificity (22). Recent advances have demonstrated potential for locus selectivity by combining selective DNA-binding pyrrole–imidazole polyamides with the HDAC inhibitor suberoylanilide hydroxamic acid to create the novel small molecule therapeutics called suberoylanilide hydroxamic acid–pyrrole–imidazole polyamides (23). These therapeutics enable targeted transcriptional activation at genetic loci of interest by preventing histone deacetylation. Ongoing research into the impacts of HDAC inhibitors and DNA methyltransferase inhibitors on *TOLLIP* expression and increased investment in targeted epigenetic therapeutics in pulmonary disease are warranted.

Post-Transcriptional Regulation of TOLLIP

MicroRNA regulation. Several microRNAs (miRNAs) are involved in *TOLLIP* regulation, including miR-31, which commonly functions as a tumor suppressor (24, 25). Small intestinal epithelial cells with higher miR-31 levels repress *TOLLIP* mRNA translation by binding to the region +1876 to +2398 3′ untranslated region of *TOLLIP* (26). The suppression of *TOLLIP* translation by miR-31 may be relevant to pulmonary diseases, in which miR-31 is underexpressed in the sera of patients with IPF (27) and overexpressed in the bronchial brushings of patients with chronic mucus hypersecretion in asthma and COPD (28). miR-291b has been implicated in metabolic homeostasis (29), as well as in endothelial cell apoptosis (30). miR-291b has also been shown to negatively regulate *TOLLIP* by binding to the +458 to +470 3′ untranslated region in primary Kupffer cells from ethanol-fed rats (31). Investigations of these and other miRNAs need to be extended to lung tissues to evaluate whether they modify *TOLLIP* expression in disease-relevant pathways. With tissue-specific drug-delivery options, these miRNAs could prove valuable as therapeutic modulators of TOLLIP functions in pulmonary disease.

Splicing variants. To date, there have been four different human TOLLIP isoforms reported, three of which are found in human peripheral-blood mononuclear cells (PBMCs). The TOLLIP protein contains three domains, including the TBD (Myb [TOM]-binding domain), the C2 (conserved 2) domain, and the CUE (coupling of ubiquitin to endoplasmic-reticulum [ER] degradation) domain (9). Isoform A, the dominant isoform, contains all six exons. Isoform B skips exon 2 and as a result lacks the TBD. Isoform C skips exon 3 and as a result lacks the C2 domain. Isoform D uses an alternative exon, which has not been detected in human PBMCs (32). In mice, there also exist three splicing isoforms of *Tollip*, but there has been little exploration of the impact of these variants on Tollip functions or on disease model phenotype (32). Other uninvestigated splicing variants may play roles in human disease. Knowledge of how TOLLIP splicing variants impact pathophysiology in both humans and mouse models of disease reflects an integral step in increasing our understanding of the functions of this ubiquitous protein.

TOLLIP in Noninfectious Pulmonary Diseases

TOLLIP's functions in the critical intracellular processes of inflammatory regulation, autophagy, and vacuole trafficking make it a prime candidate for investigation in the pathogenesis underlying a number of noninfectious pulmonary diseases. Dysregulation of TOLLIP's effects appears to be of primary relevance to alveolar and airway epithelial cells within the lungs. When cells are stimulated with pathogen-associated molecular patterns and IL-1β, TOLLIP acts to inhibit the MyD88-associated autophosphorylation of IRAK-1 (33). This leads to dampening of NF-κB–mediated acute inflammation and subsequent type-1 IFN release (1, 34, 35). NF-κB signaling may play a critical role in a number of pulmonary diseases, including pulmonary fibrosis (36, 37), obstructive lung diseases (38, 39), and primary graft dysfunction (PGD) in lung-transplant recipients. In addition, TOLLIP's roles in autophagy and regulation of intracellular vacuole trafficking have major implications for the pathogenesis of multiple pulmonary diseases (7). Most importantly, *TOLLIP* has been identified as a key genetic locus involved in a number of infectious and noninfectious pulmonary diseases. Genetic variants in the *TOLLIP* locus that are associated with pulmonary diseases are summarized in Table 1.

TOLLIP in IPF

TOLLIP has been implicated to play a role in susceptibility to and progression of IPF, particularly related to its impacts on autophagy and apoptosis in the early and late stages of the disease (Figure 1). Genome-wide association studies (GWASs) have found three *TOLLIP* SNPs (reference SNP 111521887 [rs111521887], rs5743894, and rs5743890), which are associated with reduced *TOLLIP* expression and IPF susceptibility (8). The SNPs rs111521887 and rs5743894 are in linkage disequilibrium, suggesting that either or both of these SNPs may be indirectly associated with the phenotype of IPF. The SNP rs5743890 appears to be independent of the other two SNPs and may also be directly or indirectly associated with the IPF phenotype. Although *TOLLIP* mRNA levels are decreased in individuals who carry the minor alleles for all three identified SNPs, rs5743890_G minor allele has a clinical effect that is the opposite of the other two. Carrying the minor allele for rs111521887 and rs5743894 is associated with increased susceptibility to IPF, whereas carrying the minor allele rs5743890_G appears to protect against the development of IPF. Despite this protective effect against IPF onset, individuals with IPF who carry the minor allele of rs5743890 actually have an increased risk of mortality (8). These findings indicate that TOLLIP may protect against the development of IPF; however, in later-stage disease, elevated TOLLIP levels may prevent dysfunctional cells from going through appropriate apoptosis. In this late stage of disease, the presence of rs5743890_G may contribute to more rapid disease progression and mortality. The SNPs rs111521887 and rs5743894 are not associated with mortality in IPF (8). Evidence regarding linkage disequilibrium between the *MUC5B*-promoter SNP rs35705950 and *TOLLIP* SNPs are conflicting among GWASs performed in the IPF literature (8, 40, 41). The most recent large-scale GWAS of patients with IPF indicated that the previously reported signals at the *TOLLIP* locus were not independent of the association with the *MUC5B*-promoter variant (40). Despite these conflicting data, the body of literature supporting a pathophysiologic role of

Table 1. TOLLIP SNPs Associated with Pulmonary Disease Development, Progression, or Response to Therapy

SNP on *TOLLIP* Locus	Major/Minor Allele	mRNA	Location	Disease	Case Patients/Control Subjects	Effect Estimate (95% CI)	*P* Value	Subject Cohort	Reference
rs5743899	A/G	Decrease	Intron	Tuberculosis	671/760	Not available	6.97×10^{-7}	Vietnam	Shah *et al.*, 2012 (57)
				Tuberculosis	209/201	1.83 (1.20–2.80)	0.005	Chinese Han	Wu *et al.*, 2018 (58)
				FEV_1/FVC in individuals with asthma	51/55	4.4% lower FEV_1/FVC (9.0% lower, to 0.2% higher)	<0.05	United States	Huang *et al.*, 2016 (52)
rs3750920	C/T	Increase	Exon	Tuberculosis	671/760	Not available	7.03×10^{-16}	Vietnam	Shah *et al.*, 2012 (57)
				Tuberculosis	613/603	0.66 (0.45 to 0.98)	0.04	Chinese Tibetan	Wu *et al.*, 2020 (59)
				Benefit from NAc therapy in IPF	60/54	0.14 (0.02 to 0.83)	0.03	United States	Oldham *et al.*, 2015 (43)
rs5743854	C/G	Decrease	Promoter	Latent tuberculosis infection	143/106	3.26 (1.36 to 7.81)	0.008	United States	Shah *et al.*, 2017 (60)
				Protection against Legionnaires disease	88/309	0.35 (0.16 to 0.76)	0.008	The Netherlands	Shah *et al.*, 2019 (61)
rs3168046	G/A	Unknown	3′UTR	Post–lung transplant PGD	225/503	1.42 (1.11 to 1.83)	0.0061	United States	Cantu *et al.*, 2016 (55)
rs5743867	A/G	Unknown	Intron	Tuberculosis	209/201	1.86 (1.22 to 2.83)	0.004	Chinese Han	Wu *et al.*, 2018 (58)
rs111521887	A/G	Decrease	Intron	IPF	1,501/1,492	1.48 (1.32 to 1.66)	2.20×10^{-12}	United States	Noth *et al.*, 2013 (8)
					602/3,366	1.49 (1.24 to 1.79)	1.60×10^{-5}	United Kingdom	Allen *et al.*, 2017 (79)
					2,127/8,049	1.00 (0.91 to 1.10)*	0.996	United States & United Kingdom	Allen *et al.*, 2020 (40)
rs5743894	A/G	Decrease	Intron	IPF	1,501/1,492	1.49 (1.33 to 1.68)	1.35×10^{-12}	United States	Noth *et al.*, 2013 (8)
				ILAs	1,699/10,274	1.14 (1.02 to 1.28)	0.021	Europe & North America	Hobbs *et al.*, 2019 (80)
rs5743890	A/G	Decrease	Intron	IPF	1,501/1,492	0.61 (0.52 to 0.71)	3.43×10^{-11}	United States	Noth *et al.*, 2013 (8)
					602/3,366	0.79 (0.64 to 0.97)	0.024	United Kingdom	Allen *et al.*, 2017 (79)
					2,127/8,049	0.85 (0.76 to 0.95)*	0.002	United States & United Kingdom	Allen *et al.*, 2020 (40)
				ILAs	1,699/10,274	0.93 (0.82 to 1.06)	0.27	Europe & North America	Hobbs *et al.*, 2019 (80)
rs3829223	C/T	Unknown	Intron	IPF	1,735/1,890	0.78 (0.71 to 0.86)	4.14×10^{-7}	United States	Fingerlin *et al.*, 2013 (81)

Definition of abbreviations: CI = confidence interval; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; ILA = interstitial lung abnormality; IPF = idiopathic pulmonary fibrosis; NAc = *N*-acetylcysteine; PGD = primary graft dysfunction; rs = reference SNP; TOLLIP = Toll-interacting protein; UTR = untranslated region.
*Denotes that this statistical analysis was performed after adjustment for linkage disequilibrium with the *MUC5B* SNP rs35705950.

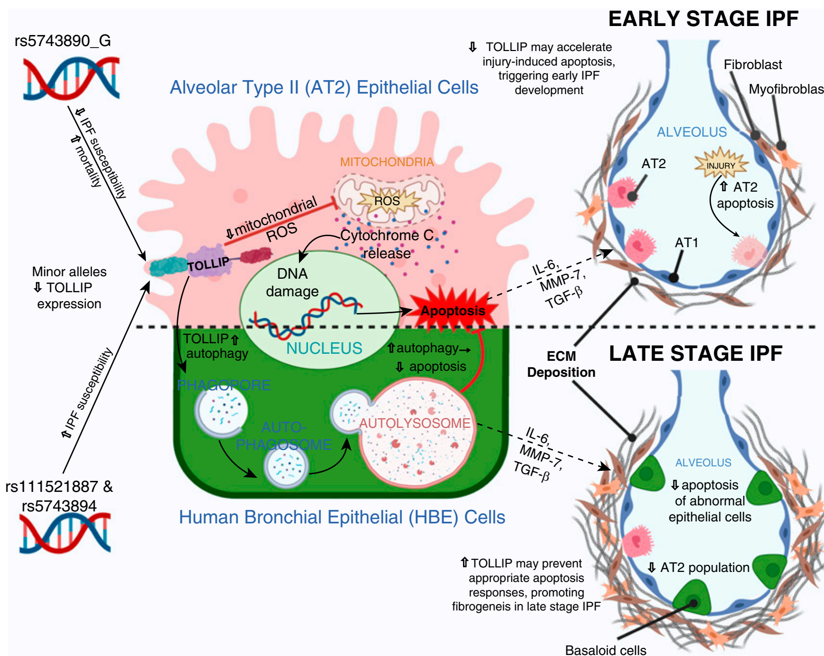

Figure 1. TOLLIP's (Toll-interacting protein's) genetic and molecular involvement in idiopathic pulmonary fibrosis (IPF) pathophysiology. TOLLIP reduces mitochondrial ROS, which suppresses the mitochondrial-apoptosis pathway. TOLLIP increases autophagy, which further inhibits apoptosis. TOLLIP levels in IPF vary on the basis of cell type and disease stage (alveolar type II and bronchial epithelial cells separated by dashed horizontal line), but reduced TOLLIP levels may contribute to early IPF development, whereas elevated levels may contribute to late-stage disease progression. The minor allele G for reference SNP 5743890 (rs5743890) is associated with reduced susceptibility and increased mortality from IPF, whereas rs111521887 and rs5743894 have the opposite effect on IPF susceptibility. Solid black arrows indicate positive effects, red lines with perpendicular bars indicate negative effects, and outlined arrows indicate up or down regulation. AT1 = alveolar type 1; AT2 = alveolar type 2; ECM = extracellular membrane; HBE = human bronchial epithelial; MMP7 = matrix metalloproteinase 7; ROS = reactive oxygen species; TGF-β = transforming growth factor-β.

TOLLIP in IPF warrants ongoing investigation (42).

Patients with IPF who carry the *TOLLIP* rs3750920 TT genotype may benefit from *N*-acetylcysteine treatment, whereas this treatment may be harmful for those with the CC genotype (43). These findings contribute a possible explanation for the wide variation in outcomes reported from *N*-acetylcysteine treatment in patients with IPF (44, 45).

Recently, our group reported that total TOLLIP expression (as indicated by mRNA and protein levels) is decreased in lungs from patients with IPF compared with normal lungs, but some atypical epithelial cells in IPF show strong TOLLIP expression (42). We demonstrated that *in vitro*, TOLLIP protects bronchial epithelial cells from undergoing apoptosis after bleomycin challenge. The antiapoptotic effects of TOLLIP may result from its promotion of mitophagy to rescue the cell from an apoptosis fate. It is plausible that in normal conditions, epithelial cell–specific TOLLIP protects against injury-induced apoptosis. Therefore, the reduced expression of TOLLIP in IPF lungs may contribute to deranged epithelial-cell physiology in early stages of IPF development. In contrast, the higher expression of TOLLIP in atypical epithelial cells (46), a cellular feature of fibrotic IPF lungs, may play a detrimental role in later stages of IPF by protecting these cells. These findings in IPF highlight the Goldilocks principle, whereby tight homeostatic regulation of TOLLIP may play a critical role in preventing the development of pulmonary fibrosis. The cell type– and context-specific alterations in TOLLIP levels in IPF exist alongside other derangements manifested by elevations in molecular mediators, like MMP7 (matrix metalloproteinase 7) (46), TGF-β (transforming growth factor-β) (47), and IL-6 (48), which are secreted from lung epithelial cells as pulmonary fibrosis progresses. Our increasing understanding of the cell-, disease-, and stage-specific variations in *TOLLIP* expression will help to guide future advances in the fight against IPF.

From an epigenetic regulation standpoint, CpG dinucleotides surrounding the *TOLLIP* locus are differentially hypermethylated in lung-tissue samples from patients with IPF in comparison with control subjects (19). This indicates that inherited and environmental factors may modify epigenetic regulation at this locus, which may have important implications for the pathogenesis of IPF. Future therapeutic strategies may consider targeting epigenetic regulation at *TOLLIP* and other IPF-relevant loci.

TOLLIP in Asthma

TOLLIP appears to play a key role in modulating pathogen responses in allergic diseases like asthma (Figure 2). Early studies using mouse models of asthma indicate that TOLLIP expression is increased in house dust mite–sensitized compared with PBS-sensitized mice exposed to *Streptococcus pneumoniae* (49). This upregulation of TOLLIP and other negative regulators of TLRs is associated with impeded neutrophil recruitment to the lungs in the context of bacterial infection, which may contribute to asthma exacerbations. Models using *Tollip*-knockout (KO) mice have also demonstrated that TOLLIP plays an essential role in downregulating IL-13–mediated pulmonary eosinophilia (50). By increasing IL1-R1 (IL-1 receptor 1) internalization and lysosomal degradation (3), TOLLIP may also contribute to reduced airway eosinophilia, given that IL1-R1 signaling leads to eosinophilic airway inflammation in mouse models of mucoobstructive lung disease (51).

In human subjects, patients with asthma who carry the AG or GG genotype for the rs5743899 *TOLLIP* SNP demonstrate increased airflow obstruction in comparison with individuals with asthma with the AA genotype. These minor allele carriers have reduced TOLLIP expression in airway epithelial cells (52). This TOLLIP deficiency promotes airway inflammation while compromising the antiviral mechanisms of airway epithelial cells in an autophagy-dependent manner. These findings indicate that TOLLIP exists in a precarious balance in asthma pathophysiology. Higher TOLLIP expression appears beneficial in preventing allergic inflammatory cascades in response to allergen stimulation; however, the presence of this protein in states of bacterial infection impedes appropriate neutrophil recruitment. Further exploration of the role of TOLLIP in allergic pulmonary diseases is

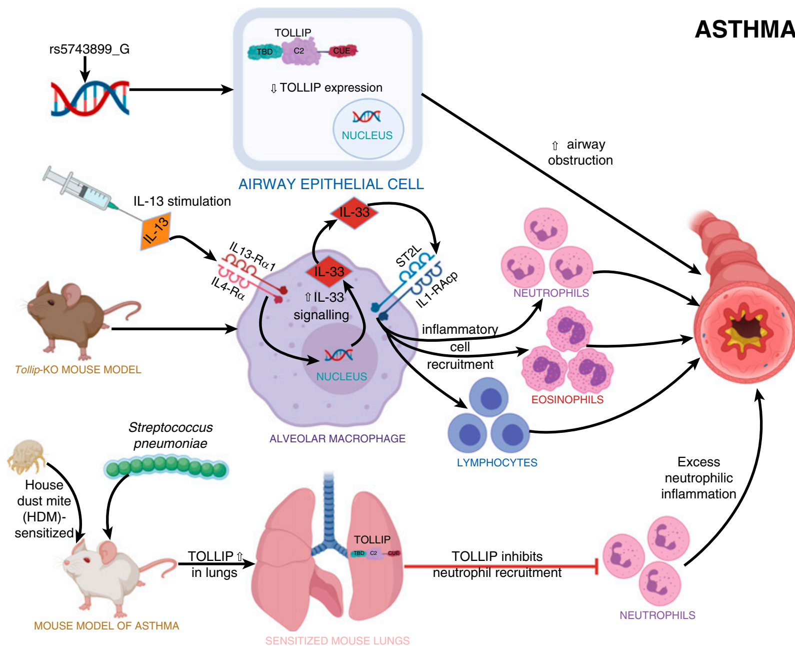

Figure 2. TOLLIP's genetic and molecular involvement in asthma pathophysiology. The minor allele G of rs5743899 is associated with reduced TOLLIP expression and subsequently increased airway obstruction. *Tollip*-KO mice stimulated with IL-13 experience increased IL-33 signaling in alveolar macrophages isolated from *Tollip*-KO mice (indicated by solid black arrow), which leads to recruitment of inflammatory cells (neutrophils, eosinophils, and lymphocytes), which may exacerbate the asthma phenotype (depicted by a narrowed airway). Mice sensitized to HDMs and exposed to *Streptococcus pneumoniae* have elevated levels of Tollip in their lungs, which impedes neutrophil recruitment, thereby potentially contributing to asthma exacerbations. Solid black arrows indicate positive effects, red lines with perpendicular bars indicate negative effects, and outlined arrows indicate up or down regulation. Cytokine receptors are depicted by colored lines with nodes. C2 = conserved 2; CUE = coupling of ubiquitin to endoplasmic reticulum degradation; HDM = house dust mite; IL1RAcP = interleukin 1 receptor accessory protein; IL13-Rα1 = interleukin 13 receptor subunit alpha 1; IL4-Rα = interleukin 4 receptor subunit alpha; KO = knockout; ST2L = ST2 receptor; TBD = Myb (TOM)-binding domain.

warranted, especially given the fact that this protein is central to autophagy, which is a critical process involved in airway remodeling in asthma (53, 54).

TOLLIP in Lung Transplant

The role of TOLLIP in survival after lung transplant was highlighted in one study, which found that carriers of one copy of the minor allele in SNP rs3168046 of *TOLLIP* experienced an 11.7% increased risk of PGD (55). The authors postulate that TOLLIP's involvement in TLR signaling and innate immune responses is key to modifying the risk of PGD after lung transplant. Another study using a porcine model of lung transplantation found that pigs treated with *N*-acetylcysteine were protected from the development of PGD and had higher levels of NF-κB in BAL fluid (56). It is plausible that in humans, variations at the *TOLLIP* rs3750920 SNP may impact responses to *N*-acetylcysteine after lung transplant as it does in patients with IPF and that this effect may be related to TOLLIP's impacts on NF-κB signaling.

TOLLIP in Infectious Pulmonary Diseases

The lung is the initial site of exposure to a multitude of environmental agents and pathogens. As such, innate immune responses within the lungs are critical for preventing the development of pulmonary infections. As an essential regulatory protein in inflammatory responses by both airway epithelial cells and immune cells like monocytes and macrophages, TOLLIP dysregulation contributes to the manifestations of a number of infectious pulmonary diseases, including tuberculosis (TB), *Legionella* pneumonia, and respiratory viral infections.

TOLLIP in TB

TOLLIP's role in modulating inflammatory responses has highlighted it as a protein target of potential pathophysiologic relevance to the development of active pulmonary TB (Figure 3). Several *TOLLIP* SNPs are associated with susceptibility to pulmonary TB, two of which, rs5743899 and rs3750920, are associated with monocyte *TOLLIP* expression levels. Subjects carrying the rs5743899 minor allele have lower *TOLLIP* expression in monocytes and increased susceptibility to pulmonary TB, whereas subjects carrying the rs3750920 minor allele have higher monocyte *TOLLIP* mRNA expression and reduced susceptibility to pulmonary TB (57). Newer studies in Chinese populations have confirmed that the *TOLLIP* rs5743899 minor allele is a risk factor for pulmonary TB, compared with latent TB infection, while also reporting that the rs5743867 minor allele confers increased susceptibility to pulmonary TB and that the rs3750920 minor allele protects against progression to pulmonary TB (58, 59). Recently, the G allele of a promoter SNP, rs5743854, has been associated with a decreased *TOLLIP* mRNA level and an increased frequency of latent TB infection (60).

By analyzing whole blood collected from bacillus Calmette-Guérin (BCG)-vaccinated infants at 10 weeks of age, the *TOLLIP* rs5743854 minor allele was found to be associated with decreased BCG-induced IL-2^+ $CD4^+$ T-cell cytokine responses and proliferation (60), which are critical responses for successful BCG vaccination. This study also found that diminished *Mycobacterium tuberculosis* replication in TOLLIP-deficient THP-1 monocytes resulted from activation of immune-killing mechanisms (60), which would further reduce the duration of efficacy of the BCG vaccine.

From a mechanistic standpoint, peripheral-blood monocytes with the hypofunctional rs5743899_GG genotype produce more of the proinflammatory cytokine IL-6 after stimulation by TLR-2 and -4 ligands or *M. tuberculosis* (57). These findings indicate that TOLLIP negatively regulates TLR signaling in immune cells, thereby helping to prevent the development of active pulmonary TB. As such, therapeutic strategies aimed at increasing *TOLLIP* expression may prove beneficial in this disease.

TOLLIP in Legionnaires Disease

In human subjects, carriers of the rs5743854 minor allele on the *TOLLIP* locus experience

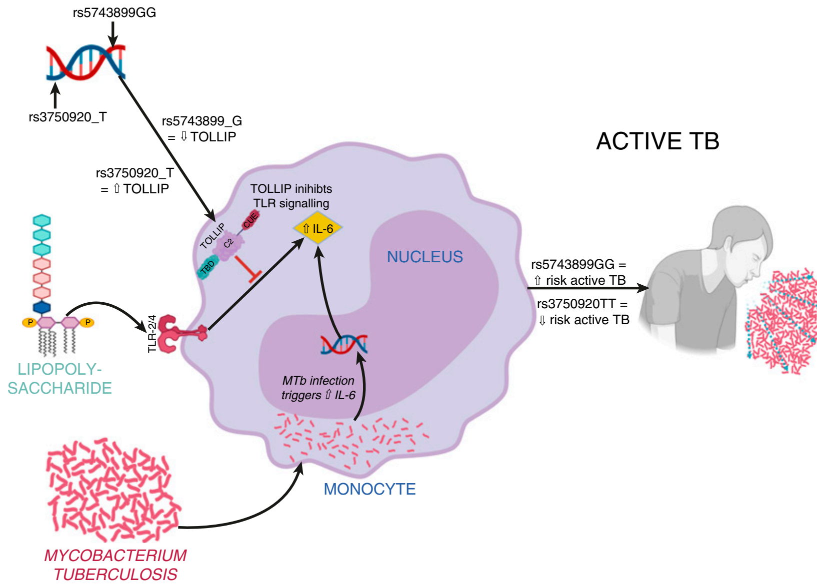

Figure 3. TOLLIP's genetic and molecular involvement in tuberculosis (TB) pathophysiology. The minor allele G of rs5743899 is associated with reduced TOLLIP levels and increased risk of active TB, whereas rs3750920 T allele is associated with increased TOLLIP and reduced risk of active TB. In response to LPS or MTb, monocytes respond with increased IL-6 production and release. TOLLIP inhibits TLR-2/4 signaling, thereby reducing IL-6 inflammatory cascade. Solid black arrows indicate positive effects, red lines with perpendicular bars indicate negative effects, and outlined arrows indicate up or down regulation. C2 = conserved 2; MTb = *Mycobacterium tuberculosis*; P = phosphate; TLR-2/4 = Toll-like receptors 2 and 4.

TOLLIP deficiency and are protected from the development of Legionnaires disease (61). In further *in vivo* analyses, mice deficient in TOLLIP are better able to clear *Legionella pneumophila* infection, experience less polymorphonuclear and monocyte tissue infiltration, and have more proinflammatory cytokine production in bronchoalveolar fluid (61). Peritoneal macrophages from *Tollip*-KO mice induce TNF and IL-1β after activation with TLR or live *L. pneumophila*. Macrophages with Tollip deficiency experience an enhanced inflammatory reaction, which could explain the fewer *Legionella* bacterial colony-forming units observed after infection in *Tollip*-KO mice. *L. pneumophila* intracellular replication is also reduced in *Tollip*-KO macrophages, potentially through autophagy suppression. Lastly, human PBMCs carrying the rs5743854 minor allele experience increased proinflammatory cytokine production after *L. pneumophila* infection (61). These insights indicate that TOLLIP may prove an important target for prevention or management of Legionnaires disease, but this is in a direction that contrasts with its effect in TB. As such, therapeutic strategies aimed at preventing adverse outcomes from *L. pneumophila* should aim to suppress *TOLLIP* expression.

TOLLIP in Respiratory Viral Infections

TOLLIP's role in responses to respiratory viral infections, especially rhinovirus, is becoming increasingly clear (Figure 4). The *TOLLIP* rs5743899 minor allele, which is associated with lower *TOLLIP* expression, is negatively associated with nasal rhinovirus concentration (62). Among individuals not carrying the *TOLLIP* rs5743899 minor allele, an IL-6 SNP associated with a lower IL-6 expression is correlated with higher viral titers. These findings emphasize that TOLLIP inhibits IL-6 production and suggest that TOLLIP may inhibit viral detection and clearance through a TLR2-dependent pathway. In primary human tracheobronchial epithelial cells costimulated with type 2 cytokines (IL-13 and IL-33) and rhinovirus, *TOLLIP* KO increases IL-8 production (63). TOLLIP promotes soluble IL1RL-1 (IL-1 receptor–like 1) production, which is responsible for IL-8 induction. In the context of IL-13 and IL-33 treatment, which is more relevant to asthma, *TOLLIP* KO promotes excessive airway neutrophilic responses to rhinovirus infection (63).

Preliminary data also support the role of TOLLIP in modifying responses to other respiratory viral infections such as influenza. Bioinformatics approaches have demonstrated that influenza targets TOLLIP-associated pathways, which is likely to mediate in part the pulmonary pathogenicity of this virus (64). This is supported by the finding in acute-lung-injury mouse models infected with H9N2 influenza virus that *Tollip* expression is significantly upregulated in response to treatment with epigallocatechin-3-gallate, which inhibits TLR-4 signaling (65). TLR signaling in response to viral pathogen-associated molecular patterns is largely mediated through recruitment of adaptor proteins like MyD88, which trigger downstream NF-κB signaling by inducing IRAK-1 autophosphorylation (66). TOLLIP inhibits IRAK1 autophosphorylation, thereby reducing NF-κB–pathway activation (35). Through this mechanism, TOLLIP may abrogate innate immune responses to respiratory viral infections.

Future Directions for the Investigation of TOLLIP in Pulmonary Diseases

Novel Molecular Insights of TOLLIP Functions

Over the past 20 years, *TOLLIP* variants have been linked to various human lung diseases, and knowledge of TOLLIP-related cellular biology has greatly expanded. Recent evidence suggests that TOLLIP stabilizes the STING (stimulator of IFN genes) protein, which resides on the ER surface (67). STING acts as a gatekeeper on the ER surface, which, once degraded, leads to activation of ER stress pathways within a cell (68). Thus, TOLLIP may play a pivotal role on inhibiting intracellular ER stress, a cellular process implicated in the development of numerous pulmonary diseases. Future work should explore TOLLIP's role in other pulmonary diseases, such as COPD, lung cancers, and acute lung injures, on the basis of its essential role in these basic biologic functions.

TOLLIP exists in a careful homeostatic balance that, when dysregulated in either direction, can contribute to disease pathogenesis (67). For example, TOLLIP's function as a STING stabilizer is compromised when polyglutamine congregates are introduced into the same cell (67). In this case, autophagy pathways

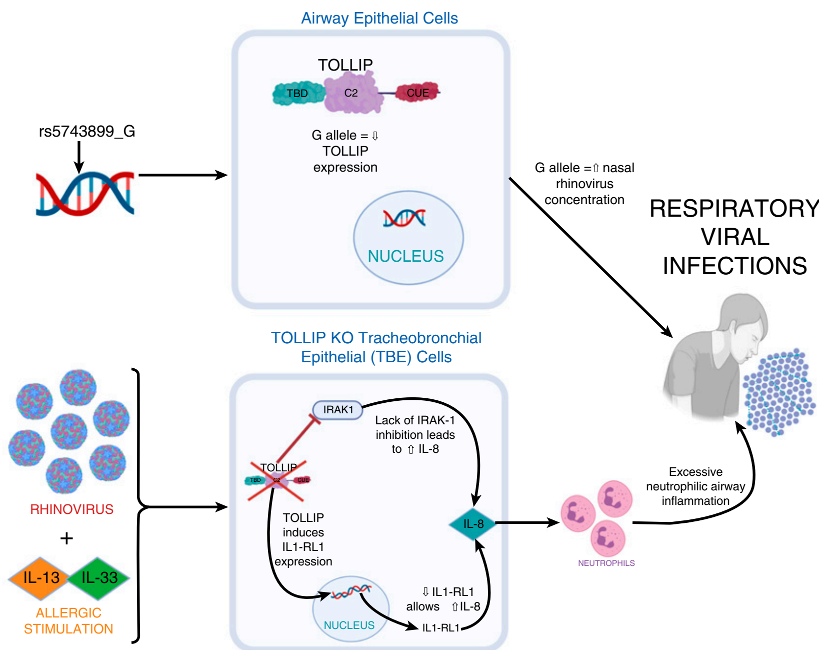

Figure 4. TOLLIP's genetic and molecular involvement in respiratory virus pathophysiology. The minor allele G of rs5743899 is associated with reduced TOLLIP expression and subsequently increased nasal rhinovirus concentration. In KO TBE cells (indicated by a red X) exposed to rhinovirus and the allergic cytokines IL-13 and IL-33, TOLLIP is unable to inhibit IRAK-1 (IL-1 receptor–associated kinase-1), and its absence also results in reduced IL1-RL1 (IL-1 receptor–like 1) expression, both of which result in increased IL-8, which triggers excessive neutrophilic inflammation. Solid black arrows indicate positive effects, red lines with perpendicular bars indicate negative effects, and outlined arrows indicate up or down regulation. TBE = tracheobronchial epithelial.

consume TOLLIP, thereby preventing it from participating in other pathways and leading to potentially pathogenic dysregulation of this protein. Given TOLLIP's ubiquitous nature and its complex intracellular interactions, a comprehensive evaluation of this protein's role in these intertwined biologic pathways is much needed to further unveil its role in the development of complex pulmonary diseases.

Murine Models to Evaluate TOLLIP Functions

Animal models represent a critical step toward fully understanding TOLLIP's role in specific lung diseases. Global-*Tollip*-KO mice were originally generated by replacing exon 1 (including the start codon) with a neomycin cassette (69). Recent evidence in humans indicates that alternative TOLLIP splicing variants exist, which use a different start codon (32). As such, alternative splicing variants may exist in current KO mice, thus potentially allowing altered splicing variants of TOLLIP to remain active within these models. TOLLIP splicing variants may play an important role in disease pathophysiology.

Another important consideration for *Tollip*-KO mouse models resides in the fact that *TOLLIP* expression may be regulated in opposite directions in different cell types relevant to disease. For example, *TOLLIP* is upregulated in basaloid cells in IPF lungs in comparison with control lungs, whereas it is downregulated in IPF macrophage subpopulations in the lungs (42). Thus, the development of cell type–specific KO mice would be invaluable in studying disease pathophysiology as it relates to TOLLIP function.

The single-dose intratracheal bleomycin injury is the most common experimental model of pulmonary fibrosis. Combined with aging, bleomycin-induced fibrosis becomes persistent with the presence of mitochondrial dysfunction and release of reactive oxygen species, which recapitulates some of the processes that occur in humans with IPF (70). Despite these strengths, the bleomycin model presents a number of discrepancies with the pathophysiology of IPF (71, 72). Nonetheless, it remains the most widely employed model, and future studies should explore the impact of *Tollip* KO in this and the combined bleomycin + aging model. Another attractive model of pulmonary fibrosis is the murine gammaherpesvirus 68–infected mouse, which demonstrates epithelial injury and mitochondrial dysfunction resembling human IPF (73). Repeat viral infections likely contribute to the development of human lung fibrosis (74), and given TOLLIP's impact on responses to viral infection, the role of TOLLIP in these viral models of fibrosis warrants future study.

Future Therapeutic Strategies Targeting TOLLIP

Modulation of TOLLIP function and expression should be considered when developing novel therapeutics for pulmonary disease. Given TOLLIP's cell-type specificity and variable impacts in a multitude of diseases, therapeutic approaches that enable finer regulation of TOLLIP's activities throughout the body are required. For example, inhaled therapeutics containing miRNAs such as miR-31 and miR-291b (27–29) could be trialed for efficacy in murine models of pulmonary fibrosis, with close observation of the effects on *Tollip* expression and development of fibrosis. In addition, the impact of *TOLLIP* variants on the efficacy of *N*-acetylcysteine in IPF should be thoroughly evaluated in other pulmonary diseases in which *N*-acetylcysteine has historically had variable effects, including in non–cystic fibrosis bronchiectasis, COPD, and asthma (75, 76).

Lastly, inhibition of the NF-κB inflammatory cascade in response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) appears to result in more severe disease and a worse prognosis from coronavirus disease 2019 (COVID-19) (77). As a central player in the NF-κB–signaling pathway, TOLLIP may represent a novel target for investigation in the fight against COVID-19 (1). TOLLIP reduces downstream NF-κB signaling by inhibiting IRAK-1 autophosphorylation and receptor degradation TLR-2 and -4 and IL-1β signaling through IL1-R1 (35, 78). TOLLIP's activities lead to reduced type 1 IFN release in airway epithelial cells and inflammatory cells. This function could translate to therapeutic benefit if used to prevent the delayed immune responses and cytokine storm seen in patients with severe

COVID-19. Evaluating the downstream implications of TOLLIP inhibition on immune responses to SARS-CoV-2 and other viral infections reflects a prescient area for further investigation that may unveil new therapeutic strategies.

Conclusions

TOLLIP is emerging as an important molecule in pulmonary diseases, in which it exerts context-dependent and often counterbalancing functions. TOLLIP plays an essential role in multiple pathways, including inflammation, cell cycle and apoptosis, mitochondrial homeostasis, autophagy, and ER stress. To preserve normal physiologic functions, TOLLIP must exist in the ideal Goldilocks zone of homeostatic balance. Variants in the *TOLLIP* locus that lead to altered expression of this protein have been implicated in susceptibility to numerous infectious and noninfectious pulmonary diseases. More translational studies are warranted to determine whether TOLLIP represents a viable therapeutic target in specific pulmonary conditions. ■

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