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Review Article

From gene identifications to therapeutic targets for asthma: Focus on great potentials of *TSLP*, *ORMDL3*, and *GSDMB*

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ABSTRACT

Asthma is a chronic respiratory disease, and clinically, asthma exacerbations remain difficult to treat. The disease is caused by combinations of and interactions between genetic and environmental factors. Genomic and genetic approaches identified many novel genes to treat asthma and brought new insights into the disease. The products of the genes have functional roles in regulating physiological or pathophysiological processes in airway structural cells and immune system cells. Genetic factors also interact with environmental factors such as air pollutants, and bacterial and viral infections to trigger the disease. Thymic stromal lymphopoietin (*TSLP*), orosomucoid-like 3 (*ORMDL3*), and gasdermin B (*GSDMB*) are three genes identified by genetic studies to have a great potential as therapeutic targets of asthma. TSLP is an important driver of type 2 inflammation. ORMDL3 mediates cell stress, sphingolipid synthesis, and viral and bacterial infections. GSDMB regulates cell pyroptosis through its N and C terminals and can bind sulfatides to influence inflammatory response. Investigating inhibitors or modulators for these pathways would bring a new landscape for therapeutics of asthma in future.

Introduction

Asthma is a heterogenous respiratory disease and imposes a huge burden in society with enormous annual costs of healthcare and productivity loss. Globally, there were more than 43 million new cases in 2017, and over 272 million prevalent cases and near half million deaths in the same year.[1] By 2025, a further 100 million people are estimated to be affected.[2] It is one of the top causes of chronic respiratory disease-related deaths worldwide.[3] A recent study showed that the annual economic burden of asthma for all ages was more than $81.9 billion.[4] In China, the overall prevalence of asthma was 4.2%, representing 45.7 million Chinese adults.[5]

Asthma is characterized by intermittent inflammation of the small airways of the lungs. The presence of inflammation can lead to irreversible airway scarring and intractable airflow limitation. Immunoglobulin E (IgE) is the major molecule that mediates inhalant allergen exposures for asthma. It underlies type 1 hypersensitivity and raised IgE levels are increasingly prevalent in asthma and other allergic diseases. IgE is produced when B cells are stimulated to undergo immunoglobulin class switch recombination (CSR) in response to antigen stimulation and co-stimulatory signals. The CSR is controlled by many transcriptional factors.[6]

Clinically, asthma is managed by reducing airway inflammation. Inhaled corticosteroids (ICS) are still the mainstay for asthma treatment, and short-acting beta-agonists (SABAs) can also rapidly reduce airway bronchoconstriction for relieving the symptoms. Leukotriene receptor antagonists, therapies against T helper 2 (Th2)-type cytokines, IgE-specific antibodies, therapies against tumor necrosis factor (TNF), vitamin D, probiotics, pathogen-associated molecular patterns (PAMPs) and toll-like receptors (TLRs) agonists, and interferons (IFNs) are all possible treatment means for asthma.[7] Although these advanced biological therapies have reduced the exacerbation rates of moderate to severe asthma by 50%,[8] two-thirds of patients with severe asthma treated with biologics continue to experience uncontrolled disease.[9] Approximately 5% of patients are not controlled even on high doses of ICS.[10] Searching for new therapeutic methods remains a challenging task for controlling asthma.

This review describes the current progress for genetic and genomic approaches of asthma and briefly discusses the interactions between genetic factors and environmental factors in mechanisms of asthma. *TSLP*, *ORMDL3*, and *GSDMB* are three genes identified by genome-wide association studies (GWASs) and are involved in pathways for development of asthma. This review focuses on the research of the three genes and discusses the potential therapeutic targets for asthma in the pathways.

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Genomic and genetic approaches identified many novel asthma genes including *TSLP*, *ORMDL3* and *GSDMB*

It has long been known that asthma runs in families and that children of asthmatic parents are at increased risk of asthma.[11] Hunting asthma genes began in the late 20th century. With the discovery of new polymorphisms in human genomes, the first asthma-associated gene was mapped to human chromosome 11.[12] The β chain of the high-affinity receptor for IgE (FcεRI-β) was subsequently localized to the region. Since then, candidate gene studies, positional cloning approaches, and GWASs identified >100 genes in human genome to be associated with asthma or asthma-related traits.[13] Classic examples of the asthma genes that were identified by using positional cloning are a disintegrin and metalloproteinase 33 (*ADAM33*),[14] PHD finger protein 11 (*PHF11*),[15] and dipeptidyl peptidase like 10 (*DPP10*).[16] ADAM33 is a disintegrin and metalloproteinase glycoprotein and has the functional roles in airway remodeling.[17] PHF11 was found to act as a T-cell regulator in both human and mouse.[18,19] *DPP10* encodes a type 2 membrane protein that has been shown to bind specific voltage-gated potassium channels, altering their expression and biophysical properties.[20] Experimental *Dpp10* point mutation in mouse leads to increased airway responsiveness following allergen challenge.[21]

At the beginning of this century, with the advance in experimental platforms, GWASs emerged as a powerful tool to dissect the genetic causes of complicated diseases. It achieved the objectives by examining the relationship between allele frequencies and disease statuses with a large number of genetic markers across the genome. Until now, more than ten GWASs were performed on asthma worldwide. These studies identified more than 100 genes that were responsible for asthma or asthma-associated traits. In recent data from the UK Biobank and the Trans-National Asthma Genetic Consortium, 66 previously unknown asthma loci were identified.[22] Most identified genes by GWASs play roles in regulation of immune response and lung airway tissue remodeling.[13] The polymorphisms of the identified genes contribute only a little to asthma. Some of the novel genes were not linked to asthma before, and these genes also play roles in other inflammatory diseases.[13] The most identified loci that control IgE levels are in the human leukocyte antigen-DR (HLA-DR) region. Various loci influence asthma in different populations, but *TSLP*, *ORMDL3*, and *GSDMB* genes were identified by most studies.

In 2007, the first GWAS results on asthma were published in *Nature* and identified one locus on human chromosome 17q21 to have great responsibility for asthma, particularly for childhood asthma, and this locus contains two important potential genes *ORMDL3* and *GSDMB*.[23,24] The other most studied gene *TSLP* was first found to have roles in animal models of asthma, and then its polymorphisms were also identified to associate with asthma by GWASs.[25] The three genes now became the hot research fields of asthma as there are novel druggable targets in their signaling pathways. The genetics progress of asthma has been systematically reviewed.[26,27] Table 1 lists the major genes that were identified through the most powerful GWASs on asthma and IgE.

Interactions of *TSLP*, *ORMDL3* and *GSDMB* with environmental factors in asthma

Asthma can be triggered by environmental factors such as allergens, occupational factors, tobacco smoking, and air pollutants to induce airway hyperresponsiveness and inflammation.[28] There is evidence that a single nucleotide polymorphism can be associated with either disease or protection depending on the environment to which a subject is exposed.[29] For example, the increased risk of asthma conferred by 17q21 genetic variants is restricted to early-onset asthma and the risk is further increased by early-life exposure to environmental tobacco smoke.[30] Interactions among soluble cluster of differentiation 14 (sCD14), TLR 2, and chromosome 5 variants, and defined environments such as compounds typically found in farming communities, microbial products from pets and households, and tobacco smoke have been well documented.[29]

Children who grow up on farms have a lower risk of developing asthma. Asthma prevalence increased in urban areas, but growing up on traditional farms offers protection.[31] The asthma-protective effect of farms appears to be associated with rich home dust microbiota.[32,33] The protective effect was independent of bacterial richness and total bacterial load and was associated with reduced pro-inflammatory cytokine responses against bacterial cell wall components *ex vivo*.[34] The phenomenon has been termed the hygiene hypothesis. Repeated exposure to diverse common infections and to environmental microbiota during childhood[32] is strongly associated with a healthy maturation of the immune system and with protection from the development of asthma and allergies later in life.[7] A recent study showed a lack of microbial stimulation during the first year of life can increase their inherited asthma risk. Conversely, adequate maturation of the gut microbiome in this period may protect these pre-disposed children.[35]

Bacteria and viruses are the most extensively studied microorganisms relating to asthma pathogenesis. Other microbes, including fungi and even archaea, can also potently influence airway inflammation.[36] The most studied viruses include respiratory syncytial virus (RSV), human rhinovirus (HRV) and influenza virus. RSV is an important pathogen in young children and accounts for >70% of severe infantile viral bronchiolitis cases.[37] A recent cohort study suggests that lower respiratory infections caused by HRVs are as important as infections caused by RSV in terms of the risk of later asthma development.[38] Influenza virus may also induce asthma through multiple ways.[39] In a mouse model study, influenza infection acutely induced airway hyperreactivity (AHR), a cardinal feature of asthma, independent of T helper type 2 cells and adaptive immunity.[40]

Pathogenic proteobacteria, particularly *Haemophilus*, are more frequent in the bronchi of adults with asthma than in the bronchi of controls. Similar increases in proteobacteria were found in children with asthma. In addition, there was a lack of members of the phylum Bacteroidetes in individuals with asthma when compared with controls, suggesting a potential role for commensals and pathogens in influencing asthma.[41] Invasive pneumococcal infection (IPI), which is caused by the common pathogen *Streptococcus pneumoniae* (*S. pneumoniae*), has also been linked to asthma. Adults with stable asthma have been shown to have a greater incidence of *S. pneumoniae* carriage.[42] Bacterial pathogens such as *Haemophilus influenzae*, *Staphylococcus aureus*, *Moraxella catarrhalis*, and *Pseudomonas aeruginosa* have also been associated with neutrophilic asthma.[7] Filamentous fungal species of *Aspergillus*, *Alternaria*, *Cladosporium*, *Penicillium*, and *Didymella* genera produce spores that may act as allergens and initiate bronchial asthma in atopic individuals.[43] Commensal microbes are necessary for the induction of a balanced, tolerogenic immune system. The identification of commensal bacteria in both gastroenteric and respiratory tracts could be an innovative and important issue.[44]

Allergen exposure, viral infections, microflora, helminth infections, diesel exhaust, cigarette smoke, and chemicals trigger TSLP production, resulting in initiation of the sensitization process and the exacerbation of allergic diseases.[45] Interactions between genetic factors and environmental factors have fruitful results from investigations of *ORMDL3* gene. It provides evidence that *ORMDL3* regulates HRV replication in airway epithelial cells.[46-48] Early symptomatic respiratory virus infection is a risk factor for subsequent asthma, and HRV accounts for nearly two-thirds of childhood asthma exacerbations. Of particular interest is that alleles associated with high levels of *ORMDL3* transcription confer susceptibility to HRV-induced wheezing in children with asthma. How environmental factors interact with GSDMB is less clear, but as *ORMDL3 and GSDMB* are in a very short distance on the genomic location and share the same promoter region, the environmental factors which affect *ORMDL3* expression may also affect *GSDMB* expression in cell defense mechanisms.

Table 1
Genes identified by major GWASs for asthma and IgE.

Trait	Genes	Populations	References
Asthma	*ORMDL3*	British, German	23
	IL1RL1/IL18R1, HLA-DQ, IL33, SMAD3, IL2RB, GSDMB, GSDMA, SLC22AS, IL13, RORA	The GABRIEL Consortium	24
	CRCT1, PYH1N, SRP9, IL1RL1, RTP2, EPHA5, TSLP, GALNT10, FBXO43, IL33, C11orf71, RASSF8, AURKB, GSDMB, C19orf2	North American	25
	IL18R1, CD83, PRPS1L1, ERBB2, STARD3, IL1R1, IL13, FLJ37543, NDFIP1, GCLC, DUSP4, ACO1, OR52E4, ETS1, STAC2, ORMDL3, ZNF665	UK, West Australia	63
	ZPBP2, ORMDL3, GSDMB, IL1RL1, TSLP	Puerto Ricans, North American populations	116
	DENND1, ORMDL3, GSDMB	European, African American	117
	IL6R, IL1R1, WDR36, RAD50, IL33, LRRC32, RORA, SMAD3, ORMDL3, IL2RB	Australian	118
	USP38, TSLP, PBX2, NOTCH4, BTNL2, HLA-D, CDK2, KIKZF4	Japanese	119
	ADAMTS4, CD247, TNFSF4, ADORA1, PEX14, IL1RL1, D2HGDH, LPP, TLR1, TSLP, RAD50, NDFIP1, BACH2, HLA-DQA1, HLA-C/MICA, CDHR3, IL33, LRRC32, STAT6, RAD51B, SMAD3, CLEC16A, ZPBP2	European	120
	IL1RL1, SLC25A46, IL13, NDFIP1, HLA-DRB1, MICB, GPX5, BACH2, TPD52, RANBP6, GATA3, EMSY, STAT6, RORA, SMAD3, CLEC16A, ERBB2, ZNF652	Multi-ethnic populations	65
IgE	*FCER1A, IL13, HLA-DRB1, STAT6, IL4-R/IL21R*	The GABRIEL Consortium	24
	HLA-DQB1, HLA-DRB5, and ZNF365, IL4R	Latino American	121
	FCER1A, TH2LCRR, RAD50	European	122
	FCER1A, STAT6, TH2LCRR, IL13, HLA-H, HCP5B, HLA-A, HLA-W, CADM3-AS1, ACKR1, HLA-DQB3, MTCO3P1, OR10J8P, OR10J7P, LINC00299, IL4R, LPP	European	123
	MTCO3P1, HLA-DQB1, WWP2, HLA-DQB3, MTCO3P1, SUCLG2	African American, Latin American, European	124
	LINC01896, Y_RNA, KAZN, SIPA1L2, NCKAP5, GRXCR2, SLC17A4, TSBP1-AS1, HLA-DRA, RPS4XP8, SCAF8, PDE3A, TMEM132C, RNASE9, RNASE11, LINC00922, RNA5SP428	Peruvian	125

GWASs: Genome-wide association studies; IgE: Immunoglobulin E.

The roles of *TSLP*, *ORMDL3*, and *GSDMB* in asthma

TSLP and asthma

The gene *TSLP* is located on the human chromosome 5q22. It has five exons that encode 159 amino acids. There are three alternative forms for the gene. TSLP was first identified in the supernatant of a mouse thymic stromal cell line, which supports the proliferation and development of immature B cells.[49] Thymic stromal derived lymphopoietin receptor (*TSLPR*) knockout mice have demonstrated resistance to "asthma"[50] and skin specific overexpression of TSLP resulted in an atopic dermatitis-like phenotype in mice expressing an inducible thymic stromal lymphopoietin transgene.[51] *TSLP* messenger RNA (mRNA) levels were higher in the epithelium of human beings who have asthma.[52] The T allele of the rs2289276 single nucleotide polymorphism (SNP) in *TSLP* was associated with lower levels of cockroach allergen-specific IgE and total IgE in girls.[53] It was also found that levels of human *TSLP* mRNA and protein increased in the airways of patients with asthma as compared with controls, and the magnitude of this expression correlates with the severity of disease.[52,54] Several studies have shown an association between an SNP in the human *TSLP* locus and protection from asthma, atopic asthma, and airway hyperresponsiveness, suggesting that differential regulation of *TSLP* expression might influence disease susceptibility. These results also suggest that targeting TSLP may inhibit multiple biological pathways involved in asthma.[55]

The roles of TSLP in asthma were documented well. *TSLP* encodes a hemopoietic cytokine that signals through a heterodimeric receptor complex composed of the TSLPR and the interleukin (IL)-7R alpha chain. TSLP mainly impacts myeloid cells and induces the release of T-cell-attracting chemokines from monocytes and enhances the maturation of CD11c(+) dendritic cells (DCs).[56] It promotes Th2 cell responses in asthma.[57,58] TSLP shorter isoform is an antimicrobial protein, displaying antibacterial and antifungal activities.[59] TSLP is mainly secreted by epithelial cells, airway smooth muscle cells, keratinocytes, stromal cells, fibroblasts, mast cells, macrophages/monocytes, granulocytes, and DCs.[60,61] TSLP signaling pathways that underlie asthma pathophysiology include mitogen-activated protein kinases (MAPKs), nuclear factor kappa light chain enhancer of activated B cells (NF-κB), signal transducer and activator of transcription 3 (STAT3), and signal transducer and activator of transcription 5 (STAT5), which influence cell proliferation, anti-apoptosis, dendritic cells migration, and airway inflammation.[62]

ORMDL3 and asthma

After discovery of the association between the polymorphisms of *ORMDL3* with asthma in 2007, the results have been replicated in many subsequent investigations worldwide,[63,64] including a multi-ancestry global meta-analysis of asthma.[65] The locus is strongly associated with many asthma cases, such as childhood asthma, severe asthma, and asthma exacerbations. The gene is located on the human chromosome 17 and has four exons that encode 153 amino acids. Until now, there is no identification of missense mutation of the gene.

Human ORMDL3 is a trans-membrane protein anchored in the endoplasmic reticulum (ER).[63] The ER is the cytoplasmic membrane system responsible for the storage of calcium, protein folding, and synthesis of lipids. ER stress can reduce the capacity of protein folding, and thereby regulates cellular responses to inflammation. ORMDL3 facilitates the unfolded protein response to cellular stress by influencing ER calcium ATPase and ER-mediated Ca^{2+} flux.[66] It interacts with the serine palmitoyltransferase (SPT) enzyme complex in sphingolipid synthesis to regulate ceramide and sphingosine-1-phosphate (S1P) levels.[67] ORMDL3 could work in multiple pathways in regulating airway inflammation in epithelial cells.[68] ORMDL3 promotes eosinophil trafficking and activation. The overexpression of *ORMDL3* in eosinophils causes increased rolling, distinct cytoskeletal rearrangement, extracellular signal-regulated kinase phosphorylation, and nuclear translocation of NF-κB.[69] ORMDL3 upregulates airway smooth muscle proliferation, contraction, and Ca^{2+} oscillations in asthma airway smooth muscle cells.[70]

ORMDL3 regulates asthma in multiple pathways. ORMDL3 regulates major HRV receptor intercellular adhesion molecule 1 expression. It is

also an important molecule to regulate metabolisms of ceramide and S1P, epithelial cell ER stress, ER-Golgi interface, and glycolysis in the epithelial cells when asthma occurs.[71]

GSDMB and asthma

The human chromosome 17 asthma locus also contains *GSDMB* gene. The risk alleles reside on a common haplotype that harbors three genes encoding *ORMDL3*, gasdermin B (*GSDMB*), and zona pellucida-binding protein 2 (*ZPBP2*). The genetic variants on human chromosome 17q21 drive the association that influences the transcription of these genes, resulting in increased *ORMDL3* and *GSDMB* expression and decreased *ZPBP2* expression.[72] *GSDMB* and *ORMDL3* are in a few kilobase part of positions and have the same orientation for transcription. The two genes may share the same promoter area in the genomic structure. The polymorphisms of *GSDMB* were also found to have been significantly associated with asthma in GWASs.[24,25,63]

The human *GSDMB* gene consists of 12 exons, and four different splice variants have been described, which differ in exons 6 and 7 of the gene.[73] GSDMB is expressed exclusively in the epithelium of the skin and gastrointestinal tract in a highly tissue-specific manner.[74] GSDMB is also expressed in the bronchial epithelium and may be responsible for airway remodeling.[75] Like other gasdermins, GSDMB also contains a cytotoxic N-terminal domain and a C-terminal repressor domain. It is a pore-forming effector protein that causes membrane permeabilization and pyroptosis, a lytic pro-inflammatory type of cell death.[76] Pyroptosis can be mediated by gasdermin D, gsdermin E, Gasdermin C, and GSDMB. There is strong evidence that caspase-1, caspase-11/4/5, caspase-3/7, and caspase-8 are activated during this process.[77]

Proteolytic cleavage between these two domains releases the intramolecular inhibition on the cytotoxic domain, allowing it to insert into cell membranes and form large oligomeric pores, which disrupts ion homeostasis and induces pyroptosis.[78] The pyroptotic activity of the GSDMB protein can be affected by a splice variant rs11078928 that deletes the entire exon 6, which encodes 13 amino acids in the critical N terminus.[79] GSDMB-positive cells can be killed by natural killer cells and cytotoxic lymphocytes through pyroptosis. Interferon-γ (IFN-γ) upregulates GSDMB expression and promotes pyroptosis.[80] All gasdermin N-domains can bind phosphoinositides on the inner plasma membrane leaflet, but only in GSDMB, this binding is not inhibited by its C-terminal domain, and only GSDMB has been shown to bind sulfatides.[81] Polymorphisms in *GSDMB* genes rs2305479A and rs2305480 T could lead to aberrant sulfatide transport, and this perturbation might compromise the integrity of the epithelial cell barrier and/or promote inflammatory processes, and both variants could significantly regulate the expression of other neighboring genes such as *GSDMA* and *ORDML3*.[82]

GSDMB is the most divergent member of the gasdermin family, and it is not present in the mouse and rat genomes, so epithelial cell models have the advantages to investigate its function. GSDMB may be required for lipopolysaccharide (LPS)-induced pyroptosis in epithelial cells. Different *GSDMB* splice variants have been detected in humans, with one transcript encoding a GSDMB with a caspase-1 cleavage site in the interdomain linker (encoded by exon 6). The N-terminal and C-terminal domains of GSDMB have different binding roles compared to other GSDM family proteins. Sulfatide, a component of the apical membrane of epithelial cells, specifically binds to the N-terminal domain of GSDMB. Expression quantitative trait locus (e-QTL) analyses showed that the primary biliary cholangitis (PBC) susceptibility allele of rs12946510 was significantly associated with lower endogenous expression of ORMDL3 and GSDMB in whole blood and the spleen, which indicates that the two genes are involved in the inflammatory response.[83] GSDMB regulates asthma through pyroptosis and inflammatory response as discussed above.

The major pathways of TSLP, ORMDL3, and GSDMB in the development of asthma are shown in Fig. 1.

Potential targets for asthma therapy with pathways of *TSLP*, *ORMDL3*, and *GSDMB*

TSLP, ORMDL3, and GSDMB play multiple roles in cells in stimulating response; therefore, the pathways of signal transduction can provide exciting novel targets for asthma treatment. Clinical trials are ongoing with TSLP inhibitors, and inhibitors and modulators in ORMDL3 and GSDMB pathways are under lab investigation.

Targeting TSLP pathways

As TSLP is an alarmin cytokine in epithelial cells after stimulation, targeting TSLP, and TSLP-mediated signaling can bring new therapeutic means to asthma. The inhibitors and modulators that work in TSLP pathways could be divided into the following categories: antibody to TSLP, antibodies to the TSLPR, fusion antibodies for TSLP and IL-7Rα, and small molecules to inhibit TSLP production.

The most promising results were from tezepelumab (AMG 157), a monoclonal antibody that binds to human TSLP; it prevents interaction with its receptor to inhibit multiple downstream inflammatory pathways. As a promising biological drug, it is a potential treatment of severe asthma.[84] Tezepelumab can attenuate most measures of allergen-induced early and late asthmatic responses. It had significantly decreased the levels of blood and sputum eosinophils before and after the allergen challenge and the fraction of exhaled nitric oxide.[55] ASP7266 is a novel recombinant fully human immunoglobulin G1 (IgG1) monoclonal antibody against the TSLPR. It potently inhibits TSLP-induced cell proliferation and C-C motif chemokine ligand 17 (CCL17) production. It also inhibits TSLP-stimulated $CD4^+$ T-cell differentiation and IL-5 production. In sensitized monkeys, ASP7266 completely suppressed ascaris extract-induced allergic skin reactions.[85] The other effective strategy is combining antibodies to TSLP and other relevant cytokines expressed in the airway epithelium to inhibit the signaling mediated by the two cytokines.[86] Cytokine traps consist of fusions between the constant region of IgG and the extracellular domains of two distinct cytokine receptor components involved in binding the cytokine.[87] The TSLP trap is a fusion protein consisting of the ectodomains of TSLPR, and IL-7Rα, can inhibit TSLP-induced STAT5 and is able to significantly inhibit TSLP-driven DC activation.[62] 2-(4-{2-[(phenylthio)acetyl]carbonohydrazonoyl}phenoxy)acetamide (PA) is a chemical that can inhibit the TSLP mRNA expression and production by blocking the caspase-1 signaling pathways. PA inhibits the TSLP production and improves the activation and phosphorylation of NF-κB as well as the degradation and phosphorylation of inhibitory factor kappaB alpha (IκBα), indicating that PA would be effective in treating inflammatory and atopic disorders through the downregulation of TSLP.[88] The other small molecule that can inhibit TSLP mRNA and binding was baicalein, a major component of *Scutellaria baicalensis*, which was found to be the first small molecule to block TSLP signaling pathways. It inhibited eosinophil infiltration in house dust mite-induced and ovalbumin-challenged mouse models.[89]

Targeting ORMDL3 pathways

A recent finding is that microbial exposure could sharply decrease the incidence of wheezing illnesses in early life in carriers of the susceptibility alleles at the *ORMDL3* locus.[90] As ORMDL3 is a key molecule in the *de novo* pathway of sphingolipids, compounds that can be used include myriocin, which is a potent inhibitor of serine palmitoyltransferase, the first step in sphingosine biosynthesis. Myriocin can decrease *de novo* sphingolipid synthesis and increase bronchial reactivity in the absence of inflammation. It affects intracellular magnesium homeostasis and alters the bronchial sensitivity to magnesium.[91] In another experiment, myriocin enhances allergen-induced Th2 inflammation and airway hyperresponsiveness.[92] The inhibition of *de novo* pathway may cause the ceramide and S1P increments in airways that may be the

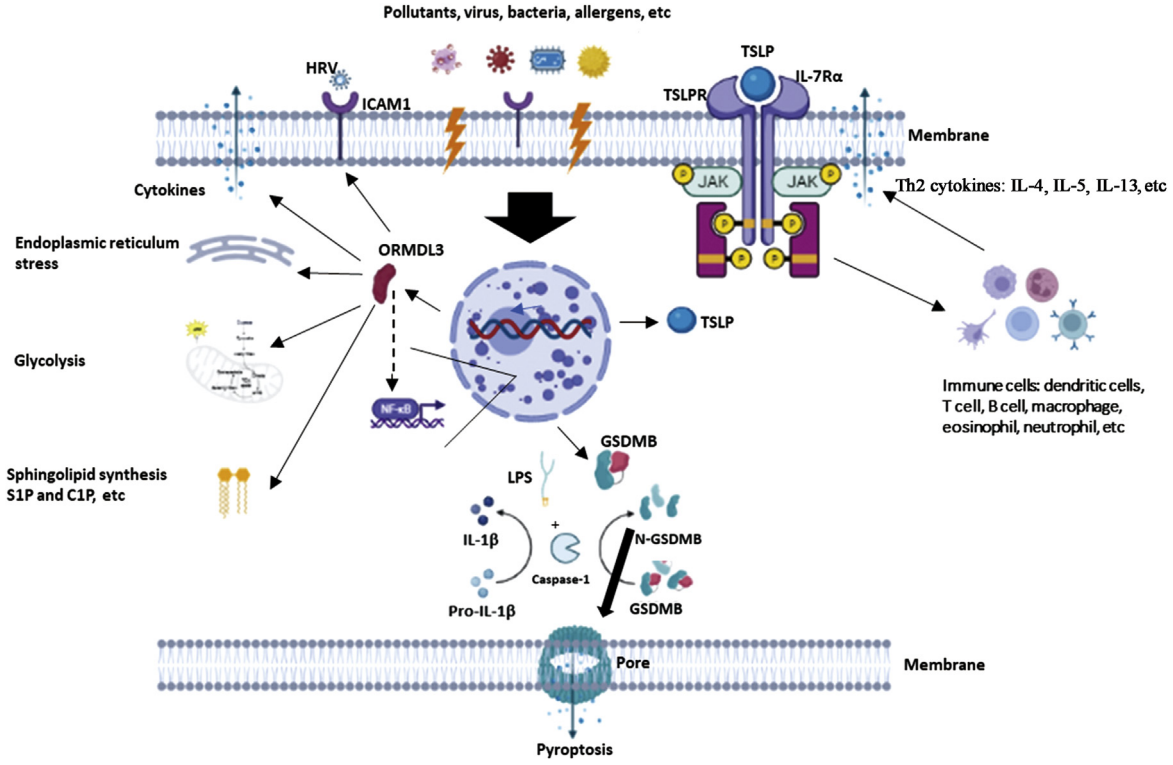

Fig. 1. Major pathways of TSLP, ORMDL3, and GSDMB in the development of asthma. Pollutants, viruses, bacteria, allergens, and other stimulants can induce TSLP, ORMDL3, and GSDMB expressions from epithelial cells. Circulating TSLP binds TSLPR and IL-7Rα to phosphorylate JAK and STATs for initiating pro-inflammatory signaling and then influences immune cells including T cells, B cells, DCs, NKT cells, eosinophils, neutrophils, basophils, monocytes, mast cells, and macrophages to release Th2 cytokines IL-4, IL-5, IL-13, etc. GSDMB may be cleaved by caspase-1 or other caspases with the help of LPS to the N-terminal domain (N-GSDMB). N-GSDMB forms a pore in the membrane for cell pyroptosis. Inflammatory cytokines can be released through pyroptosis. ORMDL3 regulates the sphingolipid level including S1P and C1P for signaling transduction. ORMDL3 facilities ER stress and glycolysis to influence NF-κB for inflammatory genes expression. ORMDL3 also regulates HRV receptor ICAM1 expression, which regulates HRV infection. C1P: Ceramide-1-phosphate; DC: Dendritic cell; ER: Endoplasmic reticulum; GSDMB: Gasdermin B; HRV: Human rhinoviruses; ICAM1: Intercellular adhesion molecule 1; IL: Interleukin; JAK: Janus kinase; LPS: Lipopolysaccharides; NF-κB: Nuclear factor kappa B; NKT: Natural killer T cell; ORMDL3: Orosomucoid-like 3; P: Phosphorylation; S1P: Sphingosine-1-phosphate; STAT: Signal transducer and activator of transcription; Th2: T helper 2; TSLP: Thymic stromal lymphopoietin; TSLPR: Thymic stromal lymphopoietin receptor.

compensating feedback from the salvage pathway for sphingolipids metabolism. The *ORMDL3* knockdown cells showed early higher inflammatory response and the later reduced inflammatory response after stimulation may be the outcomes of interaction between the two pathways. In *ORMDL3* knockdown and knockout models, ceramide levels and other sphingolipid levels were shown to increase.[68,93] In a mouse model, three hours after exposure of the airways to myriocin, a broad range of lung sphingolipids were decreased, including sphinganine, sphingosine, S1P, and ceramides, and airway hyperreactivity was increased. Similar results were obtained with the SPT-deficient mice. Both mouse and human bronchial contractile responses were directly increased by myriocin.[91] Fumonisin B1 is an S1P mimetic that inhibits ceramide synthase and increases sphinganine, sphinganine 1-phosphate, and 1-deoxysphinganine.[94] These are bioactive sphingolipids in the cell-transducing system. Tamoxifen inhibits ceramide glycosylation and hydrolysis by the enzyme acid ceramidase and thereby depresses formation of S1P.[95] S1P is a key molecule in T lymphocyte activation[96] and regulates a diverse range of cellular processes that are important in immunity, inflammation, and inflammatory disorders.[97] FTY720, immunomodulator and functional S1P1 receptor agonist, acts as a high-affinity agonist at the G protein-coupled S1P receptor-1 on thymocytes and lymphocytes, inducing aberrant internalization of the receptor.[98] FTY720 inhibits both T-cell receptor-dependent and -independent activation of primary human T cells.[99] ORMDL3 inhibitors potentially downregulate virus-induced exacerbations (viral entry, replication, and inflammation) and disease development. Until now, no effective ORMDL3 inhibitor has been identified.

Targeting GSDMB pathways

GSDMB can bind to nitrocellulose membranes immobilized with sulfatide.[81] Sulfatides are anionic glycolipids with a sphingosine backbone, to which fatty acyl chains of lengths between 16 and 24 carbon atoms, and galactosyl moieties are added. They are most abundant in the brain, making up 4% of total myelin sheath lipid, contributing to its integrity and homeostasis,[100] but are also expressed in the highly polarized epithelium such as the gastrointestinal tract, kidneys, and islets of Langerhans.[101] Sulfatides are ligands for P- and L-selectins, which are expressed on platelets and leukocytes, respectively. P-selectin binding to sulfatides on cancer cell membranes facilitates metastasis.[102] L-selectin signaling enhances apoptotic body clearance in macrophages,[103] but in neutrophils, sulfatides impede activation and nuclear translocation of 5-lipoxygenase.[104] Finally, sulfatides are ligands for galectin-4, and this interaction is crucial for the targeting of lipid raft components to the apical domains of the polarized epithelium. Lipid rafts are central to cell signaling[104] and secretion of pulmonary surfactants in type 2 alveolar epithelial cells.[105] Sulfatides have anti-inflammatory effects in selectin-dependent acute lung injury,[106] and it can suppress immunogenic maturation of lung DCs to reduce allergic airway inflammation in mouse models of asthma.[107]

Pyroptosis occurrence leads to the release of pro-inflammatory cytokines IL-1β and IL-18 to the extracellular environment, causing inflammatory effects that contribute to diseases.[108] It is reported that some molecules or compounds that block pyroptosis may lead to effective treatments for various inflammatory diseases.[109] For GSDMB-mediated

Table 2
Potential inhibitors and modulators for pathways of TSLP, ORMDL3, and GSDMB.

Proteins	Potential inhibitors and modulators	Possible effect on the pathways	References
TSLP	TSLP antibodies: Tezepelumab (AMG157)	Decreasing allergen-induced response and decreasing eosinophiles	55,84
	TSLPR antibodies: ASP7266; RG7258	Inhibiting naive $CD4^+$ T-cell differentiation and IL-5 production	62,85,86
	Fusion protein: TSLP-trap; TSLPR and IL-7Rα	Inhibiting TSLP-induced STAT5 and is able to significantly inhibit TSLP-driven DC activation	62,85,86
	TSLP mRNA inhibitor: PA	Inhibiting TSLP mRNA expression and production by the blocking of caspase-1 in mast cells	88
	Small molecule inhibitor baicalein	Blocking of caspase-1 signaling pathways	89
ORMDL3	Myriocin	Inhibiting serine palmitoyltransferase but has bronchial reactivity	91,92
	Fumonisin B	S1P mimetic fumonisin B1 is a mycotoxin that inhibits ceramide synthases	94
	Tamoxifen	Inhibiting ceramide glycosylation and depressing formation of S1P	95
	FTY720	S1P1 receptor agonist inhibits activation of primary human T cells	98,99
GSDMB	Sulfatides	Anti-selectin-dependent inflammatory responses; suppressing immunogenic maturation of lung DCs	106,107
	Andrographolide	Amelioration of radiation-induced lung inflammation and fibrosis	110
	MCC950, anakinra, atorvastatin, kanglexin, ethyl pyruvate, Ac-YVAD-CMK, dendrobium alkaloids, resveratrol	Inhibiting caspase-1 pathways	109

DC: Dendritic cell; GSDMB: Gasdermin B; IL: Interleukin; mRNA: Messenger RNA; ORMDL3: Orosomucoid-like 3; PA: 2-(4-{2-[(phenylthio)acetyl]carbonohydrazonoyl}phenoxy)acetamide; S1P: Sphingosine-1-phosphate; STAT: Signal transducer and activator of transcription; TSLP: Thymic stromal lymphopoietin; TSLPR: Thymic stromal lymphopoietin receptor.

pyroptosis, andrographolide has been shown to inhibit pyroptosis and to contribute to amelioration of radiation-induced lung inflammation and fibrosis.[110] MCC950, anakinra, atorvastatin, kanglexin, ethyl pyruvate, Ac-YVAD-CMK, *Dendrobium* alkaloids, and resveratrol are all inhibitors of caspase 1.[109] The effectiveness of these inhibitors in airway disease management has not been studied, and the next stage is to test if these compounds are effective.

The search of the therapeutic means for regulating TSLP, ORMDL3, and GSDMB for asthma has drawn attention, and it will bring fruitful results in near future. The most studied potential inhibitors and modulators of TSLP, ORMDL3, and GSDMB are listed in Table 2.

Future perspectives

Genomic and genetic approaches of asthma identified many novel genes. Many new pathways have been revealed to influence epithelial cells, neutrophils, eosinophils, smooth muscle cells, and lymphocytes in asthma mechanism. Severe asthma occurs in 5–10% of asthma patients and is poorly controlled. A genome-wide association study on moderate-to-severe asthma identified three novel signals in *GATA3*, *KIAA1109*, and *MUC5AC* genes. The other 21 signals had been identified in previous studies on asthma.[111] *TSLP* and chromosome 17q loci were among these signals. Investigating these signals and their influence on asthma, particularly on severe asthma, would be of benefit for the future asthma management.

Clinically, eosinophilic, and neutrophilic asthma can be observed but are not mutually exclusive subtypes of asthma. Neutrophils accumulate in the airways of asthma patients with more severe airflow obstruction, and the cells are prominent in airway secretions during acute severe asthma exacerbations. Eosinophils may also be present in excess. These insights about the relationships between cellular inflammation and phenotypes of asthma can be defined by specific cellular and molecular markers. These markers will ultimately guide personalized treatment programs.[112]

The IL-23/Th17 pathway is a central component of cellular immunity, and IL-17A is a signature cytokine of this pathway.[113] IL-17A is recognized as an inflammatory cytokine and mainly exerts its function on myeloid cells and mesenchymal cells to induce the expression of granulocyte colony-stimulating factor (G-CSF), IL-6, and certain kinds of chemokines, which, in turn, increase granulopoiesis and recruit neutrophils to the infectious site.[114] IL-17A is widely reported to regulate chronic inflammatory diseases, including respiratory diseases such as asthma.[115] Interestingly, chromsome17q21 polymorphisms have been found to have associations with ORMDL3 expression and IL-17 secretion early in life. In *ORMDL3* knockdown epithelial cells, IL-17A-induced inflammatory response was much lower than in control cells,[47] suggesting ORMDL3 may regulate IL-23/Th17 pathways.

The polymorphisms of *TSLP*, *ORMDL3*, and *GSDMB* have strong associations with asthma. As asthma is a polygenetic disease, one gene mutation may only account for a small portion of the asthma population. TSLP, GSDMB, and ORMDL3 are all expressed on the human epithelium. *ORMDL3* and *GSDMB* genes are in a tight linkage disequilibrium block, and the two genes share the same promoter on human chromosome 17q. The polymorphisms have shown the strongest associations with asthma or asthma-related traits in the asthma population worldwide. TSLP works as one of the alarm cytokines in the inflammatory response and targeting TSLP may have effect on multiple pathways of asthma. The novel therapeutic means will provide new treatments not only for asthma but also for other chronic inflammatory diseases.

The next research strategy is to understand how the genetic factors interact with environmental factors such as air pollution, viral, bacterial, and fungal infections, and allergens to influence the asthma pathophysiology. The different genetic backgrounds may have different impact on the lung and gut microbiome, which have been shown to affect the immune response in many inflammatory diseases.

Understanding their mechanisms will provide new insights into the disease and for the management. Our laboratory has initially identified *PHF11*[15] and *DPP10*[16] through positional cloning, and *ORMDL3* and *GSDMB* through GWASs[23] as asthma genes. We applied animal models and

cellular models to investigate functions of these genes in asthma.[19,21,68] We currently use the air–liquid interface (ALI) culture as an efficient tool to study cell–cell interactions following exposure to aerosolized or gaseous forms of air pollutants, bacteria, and viruses.[71]

The future strategy will be to focus on the investigation of potential inhibitors and modulators in the novel pathways. Personalized medicine involving genotypic screening of the risk alleles and pharmacogenetic studies can improve asthma management. Further research on *TSLP*, *ORMDL3*, and *GSDMB* will bring fruitful results in treating asthma, especially severe asthma.

Conflicts of interest

There is no known conflict of interest arising from this review.

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