

Targeting Neutrophils in Severe Asthma via Siglec-9

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Abstract

Severe asthma comprises only 5% of patients with asthma, but the burden it brings to the social health system accounts for more than half of all asthmatics. Clinical evidence shows that severe asthma is often linked to the recruitment and activation of neutrophils in the airways. However, the underlying molecular and immunological mechanisms of neutrophilia in severe asthma are not clear and currently available drugs exert only limited effects on neutrophilic inflammation. Great efforts are underway to address the mystery of neutrophilic inflammation in chronic respiratory disorders. Sialic acid-binding immunoglobulin-like lectins (Siglecs) are members of the immunoglobulin gene family. Of note, Siglec-9 is uniquely expressed by human neutrophils and monocytes, as well as a minor population of natural killer cells. Engaging this structure with antibodies or glycan li-

gands results in programmed cell death in human neutrophils. Intriguingly, the administration of Siglec-E antibody abolished the recruitment of neutrophils in mouse models of neutrophilic pulmonary inflammation *in vivo*. Given that neutrophils are probably a major culprit in the generation and perpetuation of inflammation, targeting Siglec-9 could be beneficial for the treatment of severe asthma, chronic obstructive pulmonary disease, and related pulmonary disorders characteristic of neutrophilia.

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Introduction

Asthma is a chronic respiratory disease that affects more than 235 million people worldwide [1], characterized by variable and recurrent inflammation, reversible obstruction of airflow, and airway hyperreactivity [2]. The typical pathogenesis of airway inflammation in asthma is attributable to the actions of eosinophils, neutrophils, mast cells, T lymphocytes, and dendritic cells [3]. Eosinophils migrate from the peripheral circulation to in-

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flammatory lesions where they release peroxidase, major basic protein, eosinophil cationic protein, and ethylene glycol dinitrate; these mediators appear to contribute to airway inflammation [4, 5]. The recruitment, activation, growth, differentiation, and survival of eosinophils are regulated by interleukin (IL)-5 [6]. However, for patients with severe asthma, treatment with anti-IL-5 monoclonal antibody (mAb) has limited effectiveness [7].

Asthma was recently recognized as a heterogeneous disease. Moreover, neutrophilic, rather than eosinophilic, inflammation of the airway has been implicated in severe asthma [8, 9]. Consequently, neutrophils might be a more valuable target than eosinophils in the management of severe asthmatic patients. A systematic review addressing the role of neutrophils in severe asthma revealed potential targets for anti-neutrophil treatment [10]. Factors that interrupt neutrophil reactivity represent promising therapeutic targets; these include small-molecule compounds and agonist antibodies that recognize neutrophil inhibitory or death receptors. In this approach, the activity and/or function of neutrophils are negatively regulated by the selective ligation of small-molecule compounds or agonist antibodies [10–14].

For decades, investigators have sought to identify novel receptors on the neutrophil cell membrane that would serve as effective, cell type-specific targets for treating neutrophilic airway inflammation. The immunoglobulin superfamily comprises sialic acid-binding immunoglobulin-like lectins (Siglecs) that bind to sialic acid moieties on glycans. Siglec-9 is uniquely expressed by human neutrophils and monocytes and populates a minor subset of natural killer (NK) cells. Ligation of Siglec-9 results in the apoptosis and autophagy of human neutrophils [11, 14, 15].

In this review, we discuss the current understanding of neutrophilia in severe asthma. We describe the immunoregulatory mechanisms of human Siglec-9 in neutrophil apoptosis and autophagy, and Siglec-9 as a potential target for immunotherapy for severe asthma.

Pathogenesis of Severe Neutrophilic Asthma

Both eosinophilic and neutrophilic inflammation are implicated in severe asthma. Wenzel et al. [8] proposed that severe asthma could be separated into 2 inflammatory subgroups: eosinophil⁺/neutrophil⁺ and eosinophil⁻/neutrophil⁺. Results of a study by the European Network for Understanding Mechanisms of Severe Asthma (ENFUMOSA) indicated an increased number of

sputum neutrophils and augmented release of eosinophil-derived mediators for patients with severe asthma [16], indicating as neutrophilic and eosinophilic airway inflammation. In severe asthma, the neutrophil percentages were elevated in the airway, which may contribute to corticosteroid-resistant or refractory asthma [17].

IL-8 is a powerful factor for neutrophil chemotaxis and a crucial inflammatory mediator for patients with neutrophilia [18]. Ligation of IL-8 to its surface receptor changes the morphology of neutrophils and further induces the directional recruitment of neutrophils to the bronchial wall and lung lumen of asthmatic patients [19]. IL-8-positive cells are increased in respiratory bronchial and subepithelial areas in severe asthma [20]. The mechanisms of IL-8 may be involved in upregulation of the Th17 response. Th0 cells are known to secrete IL-6 and TGF- β to activate Th17 cells and the consequent production and release of IL-17 into the airways [21]. There is evidence that IL-17A and IL-17F contribute to the neutrophilic asthma severity and enhanced migration and recruitment of neutrophils [22] via the increase of IL-8, as described above.

Tumor necrosis factor- α (TNF- α) is another proinflammatory cytokine in neutrophil-related asthma generated by airway epithelial cells, macrophages, and mast cells that plays a pivotal role in IL-8-dependent neutrophil recruitment and infiltration into the lung and airspace [23, 24]. The mechanisms of TNF- α -induced neutrophil migration are associated with the upregulation of neutrophil adhesion molecules, such as the intercellular adhesion molecule 1, E-selectin, and β_2 -integrins in endothelial cells.

It is generally recognized that neutrophils do not directly secrete Th2-related cytokines, which enhance the recruitment of eosinophils in severe asthmatic patients. However, they have a strong ability to release various mediators, including reactive oxygen species (ROS), matrix metalloproteinase-9 (MMP-9), neutrophil elastase, leukotriene B₄, and platelet-activating factor into the lung and peripheral blood [25], which are responsible for mucus secretion, basement membrane injury, or adhesion and recruitment of eosinophils in severe asthma (Fig. 1).

Treatment of Neutrophil-Dominant Severe Asthma

Generally, clinical research supports the hypothesis that broad-spectrum antibiotic and anti-inflammatory treatment may be beneficial for patients with severe neutrophilic asthma. Inhalation is the necessary delivery

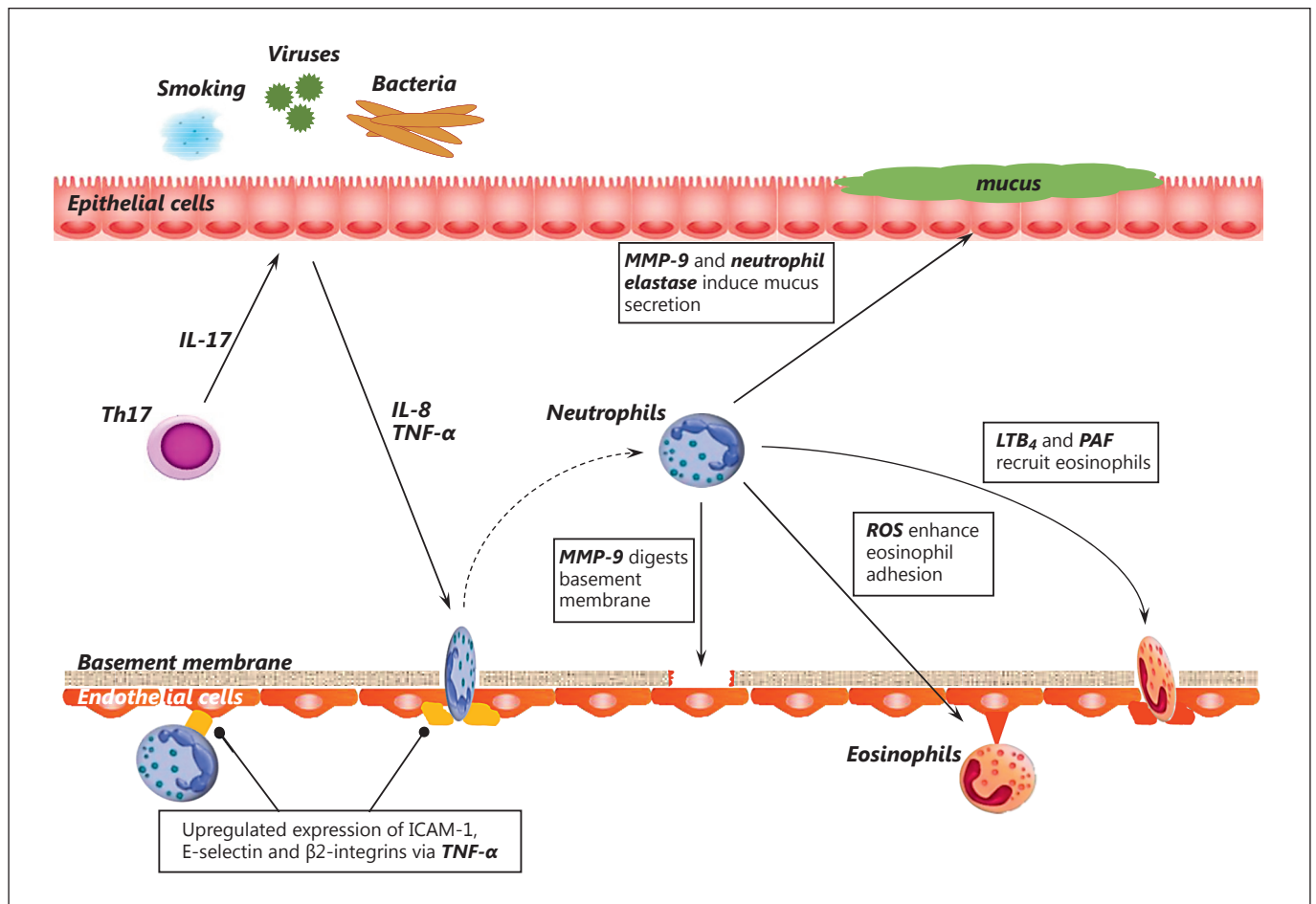


Fig. 1. The role of neutrophils in the pathogenesis of severe asthma. Allergens (e.g., bacteria, viruses, cigarette smoking) induce a Th17 response that overproduces IL-17, which in turn stimulates epithelial cell production and the release of TNF- α and IL-8, leading to the amplification of neutrophilic inflammation in the airways. Neutrophil elastase and MMP-9 stimulate mucus hypersecretion

from goblet cells. Neutrophil-released MMP-9 also contributes to the degradation of basement membrane. On the other hand, in the absence of eosinophil chemoattractants, IL-8-stimulated neutrophils facilitate the adhesion and recruitment of eosinophils in the airways by releasing LTB₄, PAF, and MMP-9.

route for this therapy, owing to adverse effects with oral administration. Macrolide antibiotics, such as clarithromycin, are beneficial for neutrophilic asthmatics who are infected with atypical bacteria [26]. However, treatment with the macrolide clarithromycin does not consistently improve the condition of patients with predominantly neutrophilic severe asthma, although there is a positive effect on airway hyperresponsiveness [27]. In addition, a variety of broad-spectrum anti-inflammatory inhibitors (e.g., kinase inhibitors [28], PDE4 inhibitors [29], CXCR2 antagonists [30]) have been developed in the treatment of severe asthmatic patients. However, most of them are usually with unsatisfactory responses or appear to cause

mechanism-related side-effects, which limit their clinical application [31].

With the recent advances in exploring pathobiological features, especially a further understanding of the mechanisms of molecular and cellular biology in neutrophilic severe asthma, specific targeted treatments may play an important role in efficiently controlling disease progression via underlying immune responses. IL-17 appears to be an ideal therapeutic target in neutrophil-dominant severe asthma, including 2 antagonistic antibodies (secukinumab and brodalumab). Unfortunately, neither of them showed a treatment efficacy in asthmatic patients [32, 33]. On the other hand, 3 different kinds of TNF- α

inhibitors have been undertaken in clinical trials, e.g., anti-TNF- α antibodies (golimumab and infliximab) and TNF- α receptor recombinant protein (etanercept) [25]. However, inactivation of TNF- α may induce the respiratory infections and cancers so that extreme caution needs to be raised when apply these treatment strategies in severe asthma.

Bronchial thermoplasty (BT) is a unique nonpharmacological therapeutic method approved by the US Food and Drug Administration, which may also be selectively beneficial for patients with severe asthma [34]. The targeted radiofrequency energy is delivered to the bronchial lumen to reduce airway narrowing and resistance through ablation of the airway smooth muscle (ASM) hypertrophy [35]. Three large randomized clinical trials have indicated that BT therapy contributes to reducing asthma acute exacerbations and an improvement in the quality of life of subjects with asthma [36–38]. However, the treatment efficacy of BT in neutrophil-dominant asthma is still unknown. Owing to the fact that ASM secretes pro-inflammatory compounds, any effective reduction of ASM by BT would play an immediate role in the down-regulation of those mediators in the airway wall. Disappointingly, current studies have already shown that neutrophilic asthma-related cytokines, including IL-17 and TNF- α , were not significantly changed after BT intervention [39]. Also, no benefits have been found in the percentage of neutrophils in bronchi biopsies with BT treatment [40]. These findings suggest that further studies are necessary in order to determine a more rational application of BT in neutrophilic severe asthma.

Siglecs

Siglecs are transmembrane cell-surface receptors of the immunoglobulin superfamily that comprise 2–17 extracellular domains; an amino-terminal V-set Ig-like domain binds various sialic acids [41]. Most Siglecs, including Siglec-9, have 1 or more immunoreceptor tyrosine-based inhibitory motifs (ITIMs) within their cytoplasmic domains. These ITIMs confer a negative regulatory function via the recruitment and activation of tyrosine phosphatases, including the SH2 domain-containing proteins tyrosine phosphatase (SHP)-1 and SHP-2 [41].

So far, 14 Siglecs in humans and 9 Siglecs in mice have been identified. Siglecs can be classified into 2 categories based on evolutionary conservation and sequence homology. CD33-related Siglecs, such as Siglec-9 and Siglec-E, share approximately 50–80% sequence identity

but have a more rapid rate of evolution, owing to extensive sequence polymorphisms and shuffling or deletion of exons coding for Ig domains [42].

Discovery of Siglec-9

In 2000, Zhang et al. [43] characterized the biochemical features and expression form of Siglec-9. A full-sized cDNA clone encoding human Siglec-9 was isolated from a polymerase chain reaction-based cDNA library of dibutyryl cAMP-exposed HL-60 cells [43, 44]. The results of Western blotting with a specific mAb indicated moderate to high expression of Siglec-9 among human neutrophils, monocytes, plasmablasts, plasma cells, and a minor population of NK cells. A small subcategory of human CD8+ and CD4+ T cells and approximately 50% of human B cells also exhibited weak expression of Siglec-9 [43–45].

Characteristics of Siglec-9

Siglec-9 comprises an N-terminal Ig V-set domain adjoining 2 IgC2-set domains, transmembrane segments, and a cytoplasmic tail with 2 putative ITIMs [43]. The membrane-proximal motif of Siglec-9 satisfies the consensus ITIM of CD-33-related Siglecs: (Lle/Val/Leu/Ser)-Xaa-Yyr-Xaa-Xaa-(Leu/Val). The membrane-distal motif of Siglec-9 violates the consensus [43, 44]. Mutation of the proximal or distal motif combined with in vitro exposure to pervanadate reveals that co-crosslinking of SHP-1 and SHP-2 with the proximal ITIMs of Siglec-9 was naturally occurring and also could be amplified in the laboratory [46]. The distal motif of Siglec-9 is highly conserved, which suggests that signaling factors might interact with this region. These findings indicate that phosphorylated Siglec-9 can recruit and activate SHP-1 and SHP-2, resulting in downregulation or inhibition of intracellular activation signals [47].

Siglec-9 as a Regulator of Neutrophil Apoptosis and Autophagy

Von Gunten et al. [11] demonstrated that Siglec-9 plays a crucial role in triggering apoptotic neutrophil death via a caspase-dependent pathway in the absence of cytokines. This cytotoxicity can be augmented with pro-inflammatory cytokines, including granulocyte macrophage colony-stimulating factor (GM-CSF), interferon

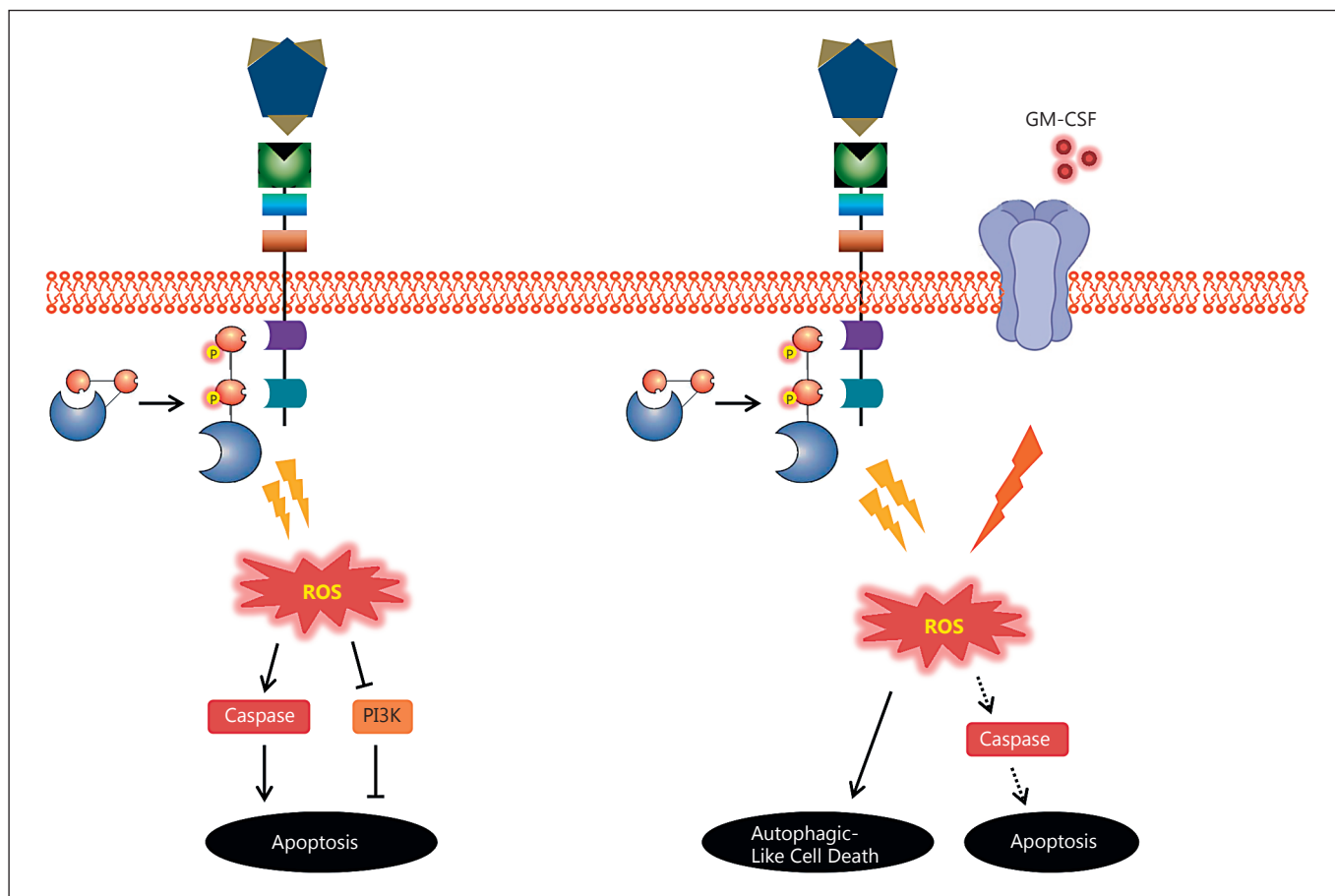


Fig. 2. A model representing how Siglec-9 may contribute to apoptosis and autophagy in neutrophils. Siglec-9 binding to sialic acid-containing glycan ligands leads to the recruitment of SHP-1/2 tyrosine phosphatases to its intracellular ITIMs. Phosphorylation of SHP-1/2 is followed by the generation of ROS in the absence of concurrent survival cytokine stimulation, resulting in neutrophil

apoptosis in a caspase-dependent manner. Meanwhile, PI3K might be inhibited by SHP-1/2 phosphorylation, which blocks the antiapoptotic pathway in neutrophils. Neutrophil death is different in the presence of GM-CSF, which it stimulates in a caspase-independent manner, also designated as an autophagic-like cell death.

(IFN)- α , and IFN- γ (Fig. 2). No ROS was detected with scavengers of ROS or neutrophils, confirming that ROS-mediated neutrophil death is associated with caspase-dependent and independent mechanisms. Other investigators have suggested that ROS are overproduced by mitochondria as a result of cellular stress or death receptor ligation; this process could modulate autophagic cell death in the presence of autophagosomes or autolysosomes [48].

Multiple vacuole-like structures have also been detected in neutrophils by means of transmission electron microscopy, indicating an autophagic cell death process [11, 15]. These neutrophils were observed to enlarge temporarily in size, which is a hallmark of increased autophagic activity [15, 49, 50]. In inflammatory disorders, such as

rheumatoid arthritis or sepsis, neutrophils undergo caspase-independent autophagic cell death via Siglec-9 ligation without cytokine stimulation, which suggests that neutrophils acquired the capacity to inhibit caspase under conditions of cytokine exposure [11]. Under normal conditions, Siglec-9 potentially could induce the apoptosis of neutrophils. However, for microenvironments with hyperinflammation, cytokines appear to switch from inducing apoptosis to autophagy, and this cell-death function may be more effective.

There remains a bottleneck in understanding of the mechanisms by which caspases are activated. Ligation of Siglec-9 could recruit and activate SHP-1 and serve as a negative regulator of cell survival and function. PI3K (phosphoinositide 3 kinase), which participates in anti-

apoptotic signaling in neutrophils [51], would be dephosphorylated by SHP-1 through its association with the regulatory p85 subunit [52]. Moreover, SHP-1 is an important regulator of neutrophil apoptosis [53]. Interaction of SHP-1 with its inhibitory receptor could increase its enzyme activity and further block cytokine-induced signaling in anti-apoptosis and differentiation pathways [54], thereby decreasing the number of neutrophils.

Characterization of Siglec-E

Siglec-E, another CD33-related Siglec, is expressed broadly in murine hematopoietic cells and innate leukocytes, including neutrophils, monocytes, macrophages, NK cells, dendritic cells, and B-1-like lymphocytes [55]. Siglec-E comprises 467 amino acids with 3 extracellular domains, 1 transmembrane segment, and an intracytoplasmic tail with 2 ITIMs [56]. The amino acid sequence of Siglec-E shares extensive similarity with human Siglec-7 and Siglec-9 [56]. Consistent with Siglec-9, the ITIMs of Siglec-E can recruit and activate SHP-1 and SHP-2, suggesting that the Siglec-E receptor exerts negative regulation on hematopoietic cells and the immune system [56]. Laubli et al. [57] found increased tumor cell death *in vivo* in Siglec-E-knockout mice; this condition was completely reversed in Siglec-9 transgenic mice. Therefore, existing evidence supports the hypothesis that murine Siglec-E is a functional ortholog of human Siglec-9 [55, 56].

Siglec-E as an Inhibitor of Neutrophil Recruitment

Neutrophils are among the most abundant leukocytes in the blood and are essential to the innate host response against fungal and bacterial infections. However, the immune system must prevent bystander damage to tissues caused by the excessive recruitment and accumulation of circulating neutrophils. Otherwise, a variety of inflammatory disorders may result, including acute lung injury, acute respiratory distress syndrome, and severe asthma [3, 58, 59].

In a model of lipopolysaccharide (LPS)-induced acute pulmonary inflammation, Siglec-E-deficient mice were found to exhibit an increased recruitment of neutrophils; this process could be reversed upon blockade of the β_2 -integrin CD11b. This finding indicated that Siglec-E is a negative regulator of CD11b-dependent adhesive interactions between neutrophils and the capillary endotheli-

um of the lung [12, 13]. When neutrophils are exposed to fibrinogen, a CD11 ligand, Siglec-E becomes enriched over the cell surface. Moreover, Siglec-E-deficient neutrophils were found to exhibit the CD11b-dependent upregulation of phosphorylation of Syk and p38 mitogen-activated protein kinases (MAPKs). Syk and p38 MAPKs are crucial for recruiting neutrophils into the lung [60, 61]. Given the role of Siglec-E as a negative receptor in neutrophil recruitment, Siglec-9 (the human functional ortholog) has potential implications in the treatment of inflammatory lung disorders.

In addition to a Syk-dependent pathway triggered by CD11b that suppresses neutrophil recruitment, activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex and production of ROS may be involved. NADPH oxidase is a membrane-bound enzyme complex that can be assembled and activated under various stimulatory conditions. Studies addressing genetic deficiencies of NADPH oxidase have demonstrated a critical role of this complex in antimicrobial host defense in humans [62] and mice [63]. The results of other studies involving NADPH oxidase-deficient mice confirmed a phagocyte-derived ROS-mediated anti-inflammatory function, which contributes to the suppression of neutrophil recruitment in the lung [64, 65]. Schwarz et al. [66] speculated that Siglec-E evolved in mammals because it helped control ROS levels during chronic inflammation. However, McMillan et al. [13] demonstrated that Siglec-E affects ROS homeostasis. The results of *in vitro* and *in vivo* studies have shown that β_2 -integrin-dependent production of ROS by neutrophils in response to acute LPS challenge was largely dependent on Siglec-E, which is responsible for the recruitment of neutrophils to the lung. These results support the hypothesis that CD11b-mediated outside-in signaling could be upregulated or downregulated as needed on the basis of Siglec-E recruitment to fibrinogen-coated sites, in turn mediated by sialic acid [12, 13]. Both signaling pathways constitute a coordinated protective response against neutrophil-triggered acute lung injury.

Natural Ligands for Siglec-9 and Siglec-E

Siglec-9 allows interactions between leukocytes and red blood cells via $\alpha_2,3$ -linked or $\alpha_2,6$ -linked sialic acid in a ligand-dependent manner [43, 44]. To further define detailed structures of these high-affinity ligands, various associated glycan structures have been screened. Among them, NeuAc $\alpha_2,3$ Gal $\beta_1,4$ 6-SO₃-N-acetyl-D-glucos-

amine (GlcNAc; this compound is also designated 6-sulfo-sialyl Lewis X) was identified as a high-avidity binding ligand [67, 68]. However, Siglec-E bound to α 2,8-disialic acid more strongly than to α 2,3-sialyllactose and bound weakly to α 2,6-sialyllactose on polyacrylamide [55]. This binding pattern of Siglec-E to glycolipids and gangliosides differs from that of Siglec-9 [69].

In an in vitro study, Jandus et al. [70] identified a subset of Siglec-9-expressing NK cells for which the engagement of Siglec-9 resulted in immune evasion. Laubli et al. [57] found that ligands for Siglec-9 expressed on carcinoma cells can modulate an immune response mediated by myelomonocytic cells. Although further verification is needed, the potential interaction of Siglec-9 expressed on NK cells and myelomonocytic cells with sialylated tumor-associated ligands on tumor cells may play a role in cancer progression. A novel sialylated ligand of Siglec-9-N-glycosylated lectin galactoside-binding soluble 3 binding protein (LGALS3BP) was identified from tumor cell extracts by means of affinity chromatography [71]. LGALS3BP also bound Siglec-E, albeit with lower affinity. LGALS3BP exhibited the functions that would be expected for a Siglec-9 ligand, including inhibition of neutrophil activation. LGALS3BP was also highly expressed in human colorectal [72] and prostate cancers [73], suggesting an underlying mechanism of immune evasion by tumor cells. Thus, this Siglec-9 ligand may serve a novel immunoinhibitory function that facilitates immune evasion by tumor cells during cancer progression.

Mice that lack Siglec-E exhibit acute pulmonary neutrophilic inflammation owing to the significant recruitment of neutrophils to the lung [12, 13]. Similarly, human Siglec-9 ligands have been shown to participate in inflammation. In a study of patients with chronic rhinosinusitis, Jia et al. [74] demonstrated that Siglec-9 ligands were significantly upregulated in a subset of submucosal gland cells, epithelium, and connective tissue of the inferior turbinate. Upregulation of Siglec-9 ligands could, in turn, elevate the expression of mucin 5B in an NF- κ B-dependent pathway. Hence, sialoglycan ligands of Siglec-9 may play an important role in airway inflammation, indicating a novel target for the control of inflammation. Glycosaminoglycans (GAGs) are a group of natural glycans that constitute a major component of vertebrate cells and the extracellular matrix. GAGs function similarly to sialic acid [75]. Hyaluronan (or hyaluronic acid; HA), a class of GAG, occurs as repeating disaccharide units of GlcNAc and D-glucuronic acid (GlcA) linked through β 1–4 and β 1–3 bonds. Among them, HMW-HA (i.e., [GlcNAc β 1–4GlcA β 1–3] $_n$), is recognized as a high-molecular-weight

HA with an apparent molecular weight of >1,000 kD [76]. HMW-HA, via its first Ig-like domain, can bind Siglec-9 in a sialic acid-independent manner and exert immunosuppressive functions on human neutrophils [77]. This interplay between HMW-HA and Siglec-9 may recruit SHP-1 to inhibitory receptors, thereby impairing neutrophil functions (e.g., oxidative burst, neutrophil extracellular trap formation) and inducing apoptosis [77]. This dynamic process is exploited by group A *Streptococcus* by means of an HMW-HA capsule, which enables escape from neutrophil attack [78]. In mouse neutrophils, a weaker interaction was observed between HMW-HA and inhibitory Siglec-E. Dissection of this mechanism should involve the study of wild-type and knockout animal models [77].

Glycotargeting Siglec-9 and Siglec-E

Compared with conventional treatment, targeted drug or gene delivery (TDGD) offers superior efficiency and specificity [79]. Carbohydrate recognition-based TDGD has been applied broadly for decades, and glycotargeting could be a powerful strategy for treating immune disorders [80]. Developments in glycobiology and nanotechnology have facilitated the extensive application of carbohydrate-decorated TDGD systems [81]. Certain lectins, including Siglec-9 and Siglec-E, are ideal candidates for carbohydrate recognition-based TDGD because they allow for selective binding and the internalization of bound carbohydrates. By way of liposomes or other nanoparticles, molecules with high affinity for Siglec-9 can deliver toxic payloads or immunomodulatory factors to targeted cell subsets [67].

The synthetic compound D24 is a competitive ligand for Siglec-9. D24 bound Siglec-9 on Chinese hamster ovary cells, even in the presence of *cis* ligands, and was not recognized by other Siglecs [67, 82]. Siglec-9-targeted liposomes were generated by covalently binding D24 to PEGylated lipids and implanting the complexes into liposomal nanoparticles. Siglec-9-expressing Chinese hamster ovary cells interacted with and proceeded to endocytose these particles [82]. Siglec-9-expressing leukocytes can also be targeted by D24-liposome complexes, with an affinity proportional to the concentration of Siglec-9. On the contrary, no interaction was detected for Siglec-9-negative cells.

Spence et al. [83] described a novel therapeutic platform of targeting Siglec-E and Siglec-9 with di(α 2 $\rightarrow$ 8) N-acetylneuraminic acid-decorated nanoparticles (α 2,8 NANA-

NP) that could inhibit the production of LPS-induced pro-inflammatory cytokines on macrophages and monocytes. IL-10 is one of the few anti-inflammatory cytokines with various effects on macrophages and monocytes [84]. The functional mechanism of α 2,8 NANA-NP is based on the suppression of Siglec-E-dependent inflammation by significantly enhanced levels of IL-10. In contrast, secretion of LPS-induced proinflammatory cytokines, including TNF- α and IL-6, were decreased by the overexpression of Siglec-9 [85] or using anti-Siglec-9 agonist antibodies [86] in mouse RAW and human THP-1 macrophage-like cell lines, and human PBMC-derived macrophages. These observations suggest that Siglec-9 and Siglec-E may restrain the proinflammatory response in macrophages.

There is evidence that Siglec-9 was highly expressed in the monocyte-derived immature dendritic cells (DCs) and was weakly expressed among mature DCs [87]. The ligation of Siglec-9 with specific blocking antibodies [87] or using the Siglec-9 phosphorylation inhibitor dasatinib [88] could significantly decrease the production of IL-12 and enhance the migration of monocyte-derived DCs. It is generally believed that DCs play an important role in T helper immunity and IL-12 is also involved in the differentiation of Th0 cells into Th1 cells [89], suggesting that targeting Siglec-9 has a potential role in improving DC-related immunotherapeutic approaches.

Knowledge about targeting Siglec-9 on a subgroup of NK cells mainly involves a mature phenotype and increased chemotactic potential in tumor immunosurveillance [70]. NK cell cytotoxicity was significantly inhibited by the engagement of Siglec-9 by agonistic antibodies. However, this effect was completely reversed when NK cells were treated with specific blocking Fab fragments, indicating the central role of Siglec-9 in regulating NK cell-dependent tumor immunosurveillance [70].

Anti-Siglec-9 Autoantibodies in Intravenous Immunoglobulins

Human intravenous immunoglobulin (IVIg) has garnered attention as a therapy for autoimmune disease and systemic immune disorders [90]. Human IVIg includes autoantibodies against Fas, which could yield neutrophil death in a caspase-dependent manner [91]. Siglec-9 could also participate in this caspase-dependent cell death process owing to its activation by IVIg [14, 91–93]. Cytokines, such as GM-CSF and IFN- γ , could boost the cytotoxic effects of IVIg via the induction of ROS, resulting in caspase-independent cell death [92]. Hence, caspase-

dependent and caspase-independent neutrophil death could be induced by the naturally occurring anti-Siglec-9 autoantibodies in IVIg.

IVIg has potential clinical applications owing to these autoantibodies. The effectiveness of high-dose systemic administration of IVIg in a cytokine-enriched microenvironment has been attributed to the cytotoxicity of IVIg on local inflammatory cells. Siglec-9-mediated neutrophil cell death may be used to treat sepsis [94]. On the other hand, IVIg-related neutropenia have been observed in some patients [95, 96], which may be in association with natural anti-Siglec-9 autoantibodies [92]. Overall, high-dose IVIg replacement therapy might be of clinical value for the treatment of severe asthma. However, serious concerns should be raised with respect to the safety of IVIg treatment.

Conclusions

Severe asthma is one of the most frequent causes of death in hospitalized patients. At present, the pathogenesis of severe asthma remains elusive and treatment is primarily palliative. Current evidence suggests an essential role for neutrophilic inflammation in the pathogenesis of severe asthma. Human Siglec-9 and its murine functional ortholog Siglec-E are immunomodulatory receptors found predominantly on neutrophils and are important in the induction of neutrophil apoptosis and autophagy. Clinically, high-dose IVIg replacement therapy is widely used for systemic inflammatory and autoimmune disorders. Specific ligands or natural autoantibodies to Siglec-9 have been detected in IVIg, possibly accounting for the initiation of the Siglec-9-induced inhibitory or death signaling in neutrophils in caspase-dependent and independent manners. Hopefully, therapies for efficient anti-neutrophilic inflammation can be developed by using specific ligands or antibodies to Siglec-9 as well as a nanoparticle-based Siglec-targeting platform. Further studies are underway to dissect the tantalizing glycobiology of Siglec-9 and to develop a series of potent, specific ligands, antibodies, and small molecule compounds targeting Siglec-9 for the treatment of neutrophil inflammatory disorders, including severe asthma.

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Disclosure Statement

The authors declare that they have no financial or commercial conflicts of interests.

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