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The role of extracellular traps released by neutrophils, eosinophils, and macrophages in asthma

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Abstract

Extracellular traps (ETs) are a specialized form of innate immune defense in which leukocytes release ETs composed of chromatin and active proteins to eliminate pathogenic microorganisms. In addition to the anti-infection effect of ETs, researchers have also discovered their involvement in the pathogenesis of inflammatory disease, tumors, autoimmune disease, and allergic disease. Asthma is a chronic airway inflammatory disease involving multiple immune cells. The increased level of ETs in asthma patients suggests that ETs play an important role in the pathogenesis of asthma. Here we review the research work on the formation mechanism, roles, and therapeutic strategies of ETs released by neutrophils, eosinophils, and macrophages in asthma.

Keywords Asthma, Extracellular traps, Eosinophils, Neutrophils, Macrophages

Background

Asthma is a common respiratory disease characterized by airway inflammation, mucus overproduction, and airway remodeling [1]. A global survey revealed that there are 358 million asthma patients worldwide [2], with a total of 45.7 million asthma patients in Chinese aged 20 and above [3]. Recent studies have shown an increased production of extracellular traps (ETs) in asthma patients [4], suggesting a potential role of ETs in asthma. ETs are a complex net-like structure composed of DNA and various active proteins. ETs can be produced by activated neutrophils, eosinophils, basophils, macrophages, mast cells, dendritic cells, and monocytes. The release of ETs

is usually accompanied by the lysis of the cell, which is known as extracellular traps cell death (ETosis) [5, 6]. ETs can serve as an immune defense function by binding and killing pathogens such as bacteria, fungi, viruses, and parasites [7], but also participate in disease progression. This review mainly focuses on the role of the ETs derived from neutrophils, eosinophils, and macrophages in asthma.

Neutrophil extracellular traps (NETs)

In 2004, Brinkmann and colleagues first discovered that neutrophils can release a net-like structure composed of chromatin and granule proteins in response to LPS or IL-8 stimulation. They named the net-like structure as neutrophil extracellular traps (NETs) [8]. The formation process of NETs is accompanied by breakdown of nuclear and plasma membranes, and this novel form of rapid cell death was later named as neutrophil extracellular trap cell death (NETosis) [9].

Initially recognized as a tool used by neutrophils to kill pathogenic microorganisms [8], NETs possess an intact

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net-like structure to facilitate pathogen clearance [10, 11]. NETs can also enhance the phagocytic activity and antimicrobial function of macrophages, ultimately boosting their ability in host immune responses [12]. However, excessive NETs can result in a shift from their protective role to a harmful effect [13]. The components of NETs can act as autoantigens and contribute to autoimmune disease by activating immune cells such as B lymphocytes and T lymphocytes and disrupting immune tolerance [14]. NETs also contribute to tissue damage and inflammatory response [15, 16]. It has been demonstrated that NETs are involved in the pathogenesis of respiratory diseases including asthma, chronic obstructive pulmonary disease, acute lung injury, pneumonia, and lung tumors [17].

Composition and structure of NETs

Neutrophils, as the most abundant type of leukocytes and the primary effector cell of the host's first line of immune defense, perform functions such as phagocytosis, degranulation, and releasing NETs [18].

NETs possess a complex three-dimensional spatial structure. Using high-resolution scanning electron microscopy, NETs are composed of smooth fibers with a diameter of 15- to 17-nm and globular domains measuring 25- to 50-nm in diameter [8, 19]. The principal active proteins in NETs are neutrophil elastase (NE), cathepsin G, myeloperoxidase (MPO), citrullinated histone 3 (CitH3), calprotectin, α -defensins, and antimicrobial peptides [8, 20]. Histone is the most abundant protein,

accounting for approximately 70% of all NETs-related proteins, followed by NE [19]. Given the composition of NETs, cell-free DNA(dsDNA), MPO-DNA, and CitH3 can serve as biological markers of NETs [21, 22].

Formation and release of NETs

Various pathogens and inflammatory factors can induce neutrophils to release NETs, including bacteria, fungi, viruses, phorbol-12-myristate-13-acetate (PMA), interleukin-8 (IL-8), tumor necrosis factor- α (TNF- α), lipopolysaccharide (LPS), and granulocyte-macrophage colony-stimulating factor (GM-CSF) [22]. NETs are formed in two pathways, including suicidal NETosis and vital NETosis [23] (Fig. 1). Initially, the formation of NETs was thought as a form of cell death [20], and was termed as suicidal NETosis. Suicidal NETosis is the predominant way of NETs formation and is mainly regulated by nicotinamide adenine dinucleotide phosphate (NADPH) and Raf-MEK-ERK pathways [24]. The process of suicidal NETosis involves several complicated steps, summarized as chromatin decondensation, chromatin swelling into the cytoplasm, disintegration of nuclear and plasma membranes, mixing of cytoplasmic and nuclear contents, and release of the nuclear-cytoplasmic mixture [9, 25]. Different from other forms of cell death including cell necrosis or apoptosis, the prominent morphological features of NETs are the dissolution of neutrophil nuclear and cytoplasmic granular membranes, the fusion of chromatin and cytoplasmic contents, and the disappearance of cytoplasmic organelles [9]. Nevertheless,

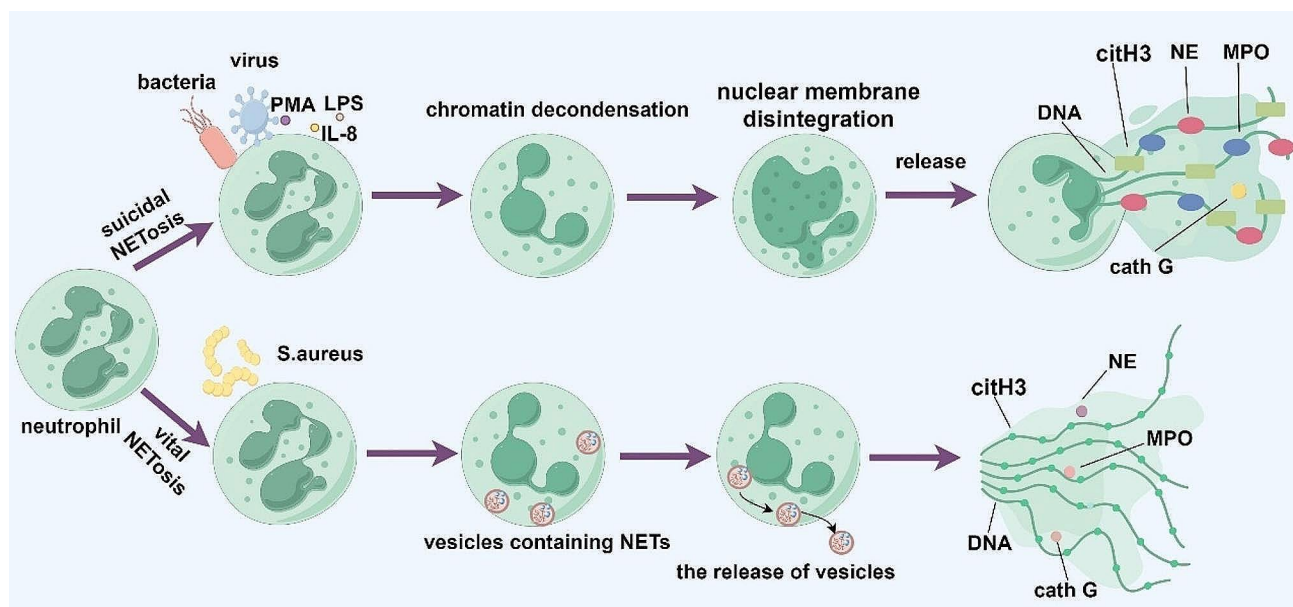


Fig. 1 Schematic diagram of NETs formation. There are two pathways of NETs formation. Suicidal NETosis includes chromatin decondensation, the disintegration of nuclear membranes, and the release of the nuclear-cytoplasmic mixture. Vital NETosis is characterized by releasing eDNA via vesicle budding. PMA, phorbol-12-myristate-13-acetate; IL-8, interleukin-8; LPS, lipopolysaccharide; NE, neutrophil elastase; Cath G, cathepsin G; MPO, myeloperoxidase; CitH3, citrullinated histone 3

there is another pathway for NETs formation with a non-lethal outcome, known as vital NETosis. Vital NETosis is characterized by the release of chromatin and proteases extracellularly via vesicle budding while the plasma membranes remain intact [26].

Suicidal NETosis is NADPH oxidase-dependent whereas vital NETosis is NADPH oxidase-independent. NADPH oxidase activation contributes to the production of reactive oxygen species (ROS), leading to chromatin decondensation and nuclear membrane rupture, thus initiating the release of NETs [27]. Diphenylene iodonium (DPI), an inhibitor of NADPH oxidase, suppresses both ROS and NETs formation in neutrophils [9]. PMA, as the most potent inducer of NETs, enhances NETs release by stimulating ROS production [28]. However, NADPH oxidase is not required for vital NETosis. During the infection of *Staphylococcus aureus*, neutrophils release chromatin and granule proteins into extracellular space via vesicle budding in a NADPH oxidase-independent manner [26].

The common enzymes NE and MPO in NETs contribute to NETosis. NE is translocated from the cytoplasm to the nucleus and binds to chromatin, resulting in chromatin decondensation through histone degradation, thereby facilitating the release of nuclear DNA into the extracellular space [29, 30]. MPO converts hypochlorous acid into hydrogen peroxide which then activates NE. Activated NE can degrade the cytoskeleton and nuclear membrane, leading to the mixing of nuclear and cytoplasmic contents [25, 31].

Peptidyl arginine deiminase 4 (PAD4) is an enzyme responsible for catalyzing the citrullination of histones, a process that has been shown in multiple studies to promote the formation and release of NETs [32, 33]. Histone citrullination affects DNA binding to histones, leading to chromatin decondensation and subsequently resulting in NETs release [34]. Nitric oxide has been implicated in the formation of NETs, potentially through the upregulation of active nitrogen production under oxidative stress conditions, which in turn leads to histone citrullination [35]. Histones, as the most abundant protein in NETs, can activate neutrophils and further promote NETs release in a feedback manner [36]. Another study has identified histamine as an inducer of NETs release in bovine neutrophils through the NADPH oxidase, ERK, and p38 pathways [37]. Autophagy may also play a role in NETs formation and release [27, 38]. The formation of NETs was significantly increased after pretreatment with autophagy inducer trehalose followed by PMA intervention, while the autophagy inhibitor hydroxychloroquine sulfate inhibited NETs formation in cultured human neutrophils. Similar results were observed in OVA-induced mouse model of allergic airway inflammation [39].

Interestingly, gender and age have been associated with differences in NETs formation. Females show a stronger ability for NETs formation compared to males, while elderly individuals exhibit diminished ability of NETs formation in response to inflammatory stimuli compared to younger individuals [40]. Despite extensive research on NETs, the regulatory mechanism of NETs formation remains incompletely understood, including the stimulating factors and underlying signaling pathways.

NETs and asthma

Neutrophils can infiltrate the airway, release inflammatory cytokines, and modulate the function of other cells, thereby playing a crucial role in asthma [41]. Based on the infiltration of inflammatory cells in induced sputum, asthma can be classified into four different inflammatory phenotypes: eosinophilic asthma, neutrophilic asthma, mixed granulocytic asthma, and paucigranulocytic asthma [42]. Neutrophilic asthma accounts for approximately 20–30% of the asthma patients population and is often associated with corticosteroid-resistant severe asthma [43, 44]. NETs may play a crucial role in asthma especially in neutrophilic asthma or severe asthma.

Increased NETs in human asthma

Several studies have shown the level of NETs is significantly increased in clinical specimens from asthma patients, and is positively correlated with the severity of asthma. Dworski et al. performed immunofluorescence staining on bronchial biopsy specimens from patients with neutrophilic asthma and observed the co-localization of dsDNA with NE [45], suggesting the presence of NETs in asthmatic airways. In a study comparing induced sputum samples from healthy individuals and asthma patients, significantly higher levels of extracellular DNA (eDNA), antimicrobial peptide LL-37, α -defensin, and NE were found in the asthma group, especially in those with neutrophilic asthma [46]. Plasma levels of CitH3 and dsDNA were also found to be elevated in asthma patients compared to healthy controls, and a negative correlation was observed between the concentration of CitH3 and forced expiratory volume in one second (FEV₁)/forced vital capacity (FVC) ratio [22]. Additionally, asthma patients with severe respiratory symptoms and impaired lung function tend to have higher plasma NETs levels [47]. Collectively, these findings indicate that increased release of NETs may be involved in the pathogenesis of asthma.

The levels of NETs were also elevated in corticosteroid-resistant severe asthma. It was reported that patients with severe asthma have markedly higher circulating NETs levels compared to mild-to-moderate asthma patients and healthy controls. Besides, the increased NETs levels were negatively correlated with the FEV₁/

FVC ratio in patients with severe asthma [48, 49]. Inhaled corticosteroids (ICS) are the main therapeutic drugs for asthma [50], and patients who regularly use ICS show significantly lower average plasma NETs levels compared to those who do not use or only occasionally use ICS [47]. Furthermore, circulating levels of NETs were significantly higher in patients with corticosteroid-resistant asthma compared to patients with corticosteroid-sensitive asthma [51]. These findings suggest that the abundance of NETs is associated with corticosteroid responsiveness, and could serve as an indicator to predict the effectiveness of ICS in asthma patients.

NETs in mouse models of allergic airway inflammation

NETs release is increased in mouse models of allergic airway inflammation. In a mouse model of neutrophilic airway inflammation, the levels of NETs in bronchoalveolar lavage fluid (BALF) were significantly increased and were associated with the severity of airway inflammation [52]. OVA/LPS-induced neutrophilic airway inflammation mouse model showed a significant release of NETs in lung tissue. Interestingly, clearing NETs or inhibiting the release of NETs can alleviate airway inflammation and hyperresponsiveness [53]. Released NETs contribute to airway inflammation and mucus secretion by enhancing the ability of antigen presenting of dendritic cells and promoting Th2 immune responses in house dust mite (HDM)/LPS-induced mouse model of neutrophilic airway inflammation [54]. In the Sendai virus-induced mouse model, airway NETs release is associated with airway inflammation and hyperresponsiveness [55]. Taken together, animal studies have provided evidence supporting the potential role of NETs in the pathogenesis of asthma.

NETs in the pathogenesis of asthma

NETs can damage airway epithelium. It was shown that NETs leads to the disruption of airway barrier integrity and exacerbation of symptoms in asthma [49]. This occurs through the inducing apoptosis in airway epithelial cells, suppressing tight junction-associated genes including claudins 4, 8, and 11, and promoting degradation of E-cadherin [49, 56, 57]. It was also reported that NETs induced by LPS can damage the tight junction protein occludin in lung epithelium, exacerbating the severity of neutrophilic airway inflammation [58]. The impaired ability of alveolar macrophages to clear NETs leads to the prolonged presence of NETs, contributing to chronic airway inflammation in neutrophilic asthma [59]. In severe asthma, excessive NETs can activate inflammasomes and enhance IL-1 β secretion by monocyte-macrophages [60, 61]. NETs also can induce the release of thymic stromal lymphopoietin (TSLP), IL-33, and IL-25 by epithelial cells, which further activates the

downstream immune responses [62]. Furthermore, NETs can stimulate airway epithelium to secrete cytokines such as IL-1, IL-6, and IL-8, promoting neutrophil infiltration in airway [63]. NETs also induce the secretion of CXCL1, CXCL2, and CXCL8 chemokines by the Toll-like receptor 4 (TLR4) / nuclear factor kappa-B (NF- κ B) pathway in airway epithelial cells, leading to enhanced neutrophil aggregation and release of inflammatory cytokines [64]. NETs can promote the secretion of TNF- α and IL-6 by recruiting and activating CD4⁺ and CD8⁺ T cells, which in turn aggravates airway inflammation [55]. In addition, the proteases from NETs could activate IL-1 and IL-36 in neutrophilic asthma [62]. These findings indicate that NETs play an important role in the pathogenesis of asthmatic airway inflammation (Fig. 2).

NETs are associated with acute exacerbation of asthma and mucus hypersecretion. During asthma exacerbation induced by rhinovirus, IL-33 levels in nasal secretions of asthma patients were found to be associated with the levels of NE and dsDNA. Blocking IL-33 can alleviate rhinovirus-induced neutrophilia and suppress the formation of NETs in a mouse model [65]. This suggests that IL-33 is not only induced by NETs, but also promotes the NETs formation. Excessive mucus secretion in the airways is a hallmark of asthma. NETs can induce airway mucus hypersecretion by promoting MUC5AC gene expression [66]. Additionally, NETs can activate the TLR4/NF- κ B signaling pathway, leading to airway mucus secretion [67].

Both asthma patients and mouse models showed increased formation of NETs, which were associated with airway inflammation, hyperresponsiveness, mucus secretion, and lung function. It is clear that NETs play a critical role in the pathogenesis of asthma, but the underlying molecular mechanisms remain to be further studied.

NETs as a therapeutic target for asthma

The significant correlation between elevated NETs and the severity of asthma suggests inhibiting the formation of NETs or promoting their degradation may represent promising therapeutic approaches for asthma.

Intraperitoneal injection of Nec-1, an inhibitor of NETs formation, decreased MPO activity and inflammatory cytokine levels, and alleviated airway inflammation in mouse model of allergic airway inflammation [52]. BB-Cl-amidine, an inhibitor of PAD4, has been reported to effectively alleviates airway inflammatory cell infiltration in mice [68]. In Sendai virus-induced mouse model, the release of NETs was increased during the early stage of viral infection. Intraperitoneal injection of Cl-amidine effectively suppressed the recruitment of inflammatory cells and the secretion of inflammatory cytokines [55].

The degradation of NETs also represents a potential therapy for asthma. In a rhinovirus-induced mouse

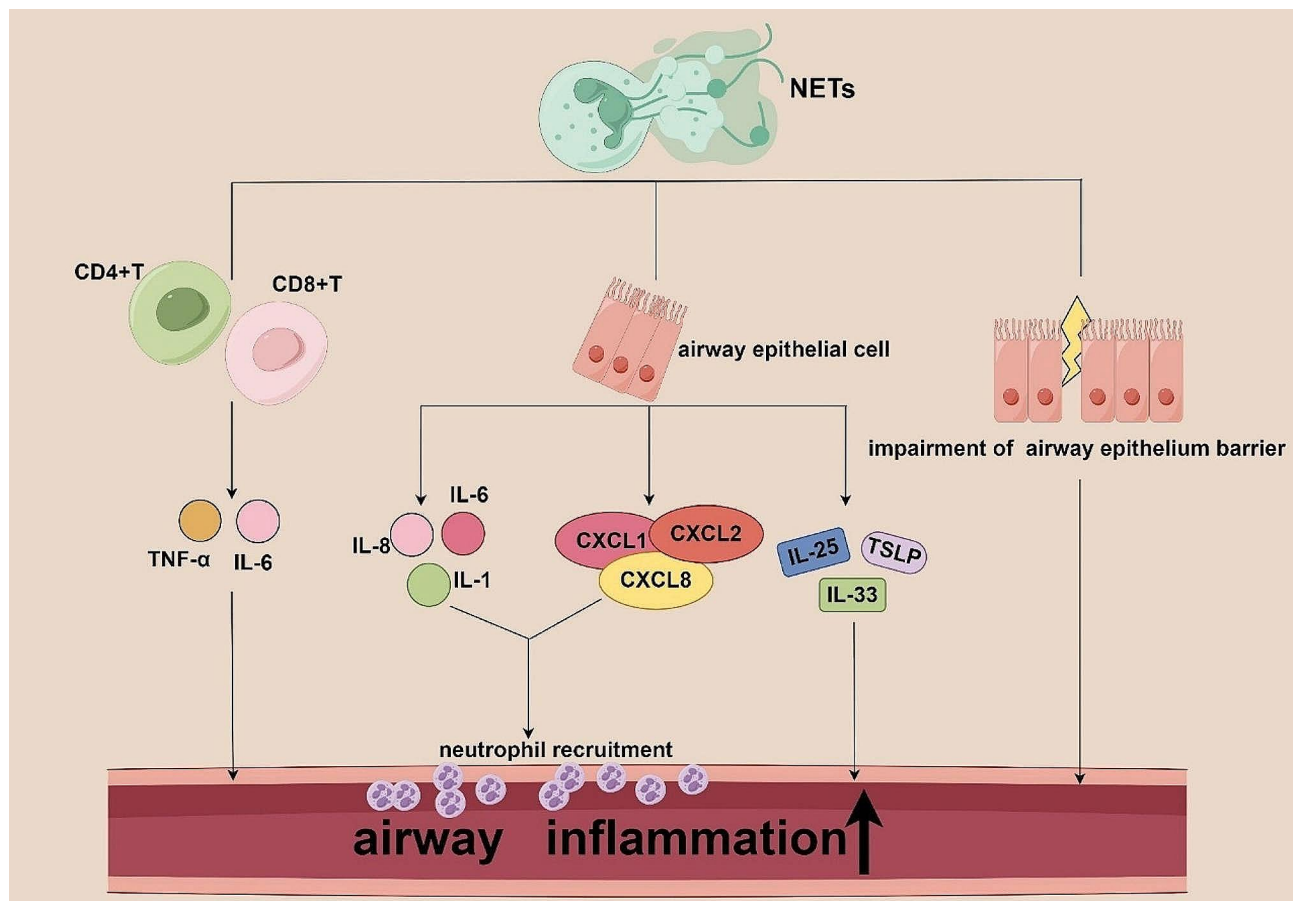


Fig. 2 The role of NETs in asthmatic airway inflammation. NETs are involved in asthmatic airway inflammation by mediating direct airway epithelial barrier injury, promoting the secretion of cytokines from epithelial cells, enhancing the recruitment of T cells and the secretion of TNF- α and IL-6 by T cells. TNF- α , tumor necrosis factor- α ; IL-1/6/8/25/33, interleukin-1/6/8/25/33; TSLP, thymic stromal lymphopoietin; CXCL1/2/8, CXC motif chemokine ligand 1/2/8

model with increased release of NETs and airway inflammation, treatment with deoxyribonuclease (DNase) to degrade NETs alleviated airway inflammation and mucus secretion [69]. In toluene diisocyanate-induced mouse model of neutrophilic airway inflammation, administration of DNase I also ameliorated airway inflammation by degrading NETs [70].

In human, as a mucolytic agent that can reduce the viscosity of mucus by degrading DNA in sputum, recombinant human deoxyribonuclease (rhDNase) is widely used in cystic fibrosis therapy [71, 72]. Several clinical trials have evaluated the therapeutic effect of rhDNase on asthma. It is reported that treatment with rhDNase can rapidly alleviate airway mucus obstruction in asthma [73]. Additionally, rhDNase can improve respiratory symptoms and lung ventilation in severely ill, non-intubated adult asthmatics refractory to bronchodilators [74, 75]. However, nebulized rhDNase treatment did not provide significant benefits for children with acute asthma [76] or asthmatic children with persistent small airway obstruction [77]. One possibility is that rhDNase can alleviate airway obstruction caused by mucus plug, but

it can not improve airway obstruction caused by smooth muscle contraction. Besides the beneficial effect on airway mucus obstruction, rhDNase may also ameliorate airway inflammation by degrading NETs as suggested by the findings from mouse experiments. The therapeutic effects of azithromycin in severe asthma have been reported [78, 79]. Several studies have concluded that the administration of macrolide antibiotics, particularly azithromycin, can ameliorate asthma symptoms by inhibiting NETs formation [80, 81].

Eosinophil extracellular traps (EETs)

EETs are a part of the innate immune response and can be present in various infectious, allergic, and autoimmune diseases [45, 82, 83]. Similar to NETs, EETs can resist pathogen invasion by capturing and eliminating pathogens, particularly when the epithelial barrier is impaired [84, 85].

The composition of EETs

Simon and colleagues observed that eosinophils release mitochondrial DNA (mtDNA) into the extracellular

space, and first proposed the concept of EETs in 2008 [84]. Multiple factors including IL-5, LPS, IgG, IgA, platelet-activating factors, calcium ionophores, PMA, and sorbitol acetate are involved in cell lysis and the release of DNA during the formation of EETs [86–88].

EETs are composed of 25–35 nm chromatin fibers which are thicker than the fibers in NETs (15- to 17-nm). In contrast to neutrophil lysis and release of protease in neutrophil, intact eosinophil granules composed of eosinophilic cationic protein, eosinophil peroxidase and major basic protein are released [45, 84, 86]. Regarding the source of eDNA in EETs, multiple studies showed that the eDNA in EETs mainly originates from nuclear DNA [86, 89–93], and one study revealed that mtDNA constitutes $8.72 \pm 1.99\%$ of the eDNA of EETs [89]. It is reported that fast-forming EETs consist of mitochondrial DNA and cationic proteins derived from cytoplasmic secondary granules, whereas slow-forming EETs predominantly consist of nuclear DNA without cationic proteins [94]. Compared to NETs, EETs have less protease content and are less susceptible to degradation. Therefore, EETs exhibit more stable and concentrated chromatin structures when compared with NETs [90, 95, 96]. Due to their stability and resistance to degradation, EETs can function in the body for a longer time than NETs [97].

Factors involved in EETs formation and release

The process of EETs formation is termed as EETosis, a form of eosinophil death [98]. Multiple studies demonstrated that nuclear and plasma membrane rupture and disintegration occur during EETosis, ultimately leading to cell death [90, 92, 99, 100]. However, several studies showed that eosinophils remain alive after the release of EETs without significant cell necrosis or apoptosis [84, 101]. One study demonstrated that the fate of eosinophil after the release of EETs may depend on the stimulation factor [102]. It has been reported that IL-5, LPS, ROS, autophagy, bacteria, and lysophosphatidylserine (LysoPS) can trigger the release of EETs [84, 92, 103]. Toll-like receptors and adhesion receptors are involved in the release of EETs [104]. TSLP can induce eosinophils to release EETs and contributes to the pathogenesis of asthma [89, 105, 106].

Eosinophils release EETs through a NADPH oxidase-dependent pathway, which is associated with ROS production [90]. In mouse model of allergic airway inflammation, inhibition of NADPH oxidase by DPI reduced ROS and EETs formation [107]. These observations suggest that NADPH oxidase is essential for EETs formation, but alternative NADPH oxidase-independent mechanisms exist. For instance, LysoPS-induced EETs formation is PAD4-dependent, rather than NADPH oxidase-dependent [92]. Inhibition of PAD4 in human eosinophils led to a decrease in histone citrullination and

EETs formation [89]. This indicates that PAD4 also plays a crucial role in regulating EETs formation.

The formation of EETs is generally believed to be similar to that of NETs. However, autophagy promotes human neutrophils to release NETs [27, 49, 108] but reduces the release of EETs by eosinophils. Adhesion-induced eosinophil cytolysis mediated by the activation of receptor-interacting protein kinase 3 - mixed lineage kinase-like signaling pathway could be inhibited by rapamycin, an inducer of autophagy [109]. Consistent with this, autophagy may confer protection against eosinophil cytolysis and the release of EETs [110]. However, inhibiting autophagy with 3-methyladenine (3-MA) can reduce the release of EETs in a mouse model of allergic airway inflammation [103]. However, it is reported that 3-MA inhibits the release of EETs in eosinophils by suppressing ROS production, rather than blocking autophagy. Besides, the release of EETs of eosinophils from autophagy-related gene 5 knockout mice remains unaffected [111]. Therefore, the role of autophagy in EETs requires further study.

EETs in asthma

Eosinophils are the core inflammatory cells in asthma [112, 113]. Elevated eosinophil count in the blood or airways is not only associated with the frequency of asthma attacks but also indicates the severity of asthma [114]. EETs are implicated in the pathogenesis of asthma. Analysis of airway biopsies and BALF samples from asthma patients revealed the presence of DNA colocalized with major basic protein, which is associated with eosinophilic infiltration [45]. In BALF samples from OVA-induced mouse model, a significant increase in eosinophil counts and eDNA content was observed. Immunofluorescence staining further revealed co-localization of eDNA with eosinophil peroxidase [89, 115]. The existence of EETs in both asthmatic patients and mouse model suggests a role of EETs in asthma pathogenesis.

EETs contribute to the airway inflammation of asthma (Fig. 3). Eosinophil cationic protein in EETs can directly damage the airway epithelium, thus worsening airway inflammation [88]. EETs had the potential to stimulate the secretion of neurotransmitters and neuropeptides from pulmonary neuroendocrine cells, resulting in the recruitment of inflammatory cells and the release of inflammatory cytokines in the airway [89]. Several studies demonstrated that elevated levels of EETs could interact with type 2 immune cells to enhance Th2 immune responses in severe eosinophilic asthma [88, 116, 117]. In a mouse model of allergic airway inflammation, exogenous injection of EETs induced the expression of airway epithelial-derived cytokines including IL-1 α , IL-1 β , IL-33, and TSLP in BALF, and increased the counts of type 2 innate lymphoid cells (ILC2) in lung tissue [118].

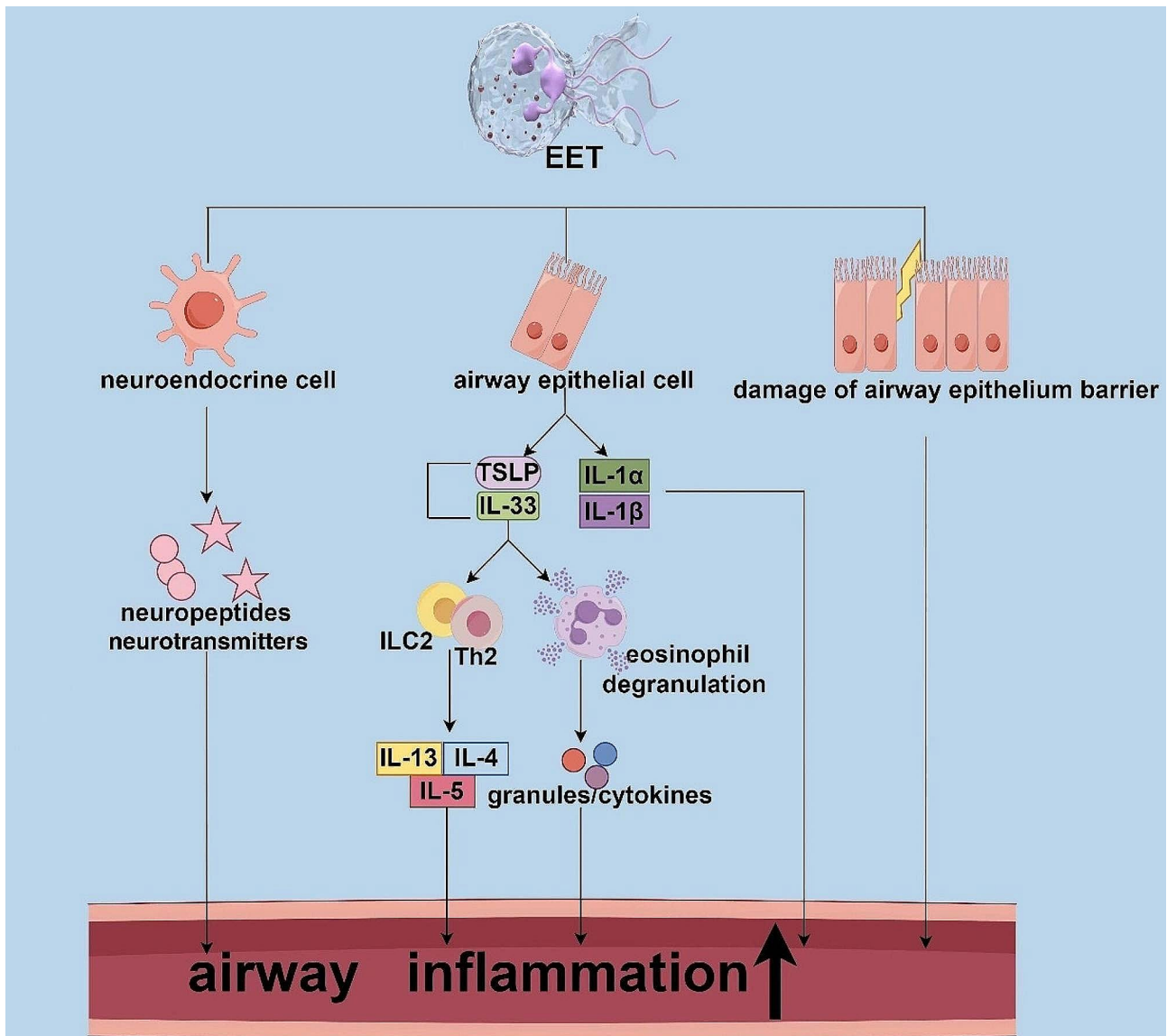


Fig. 3 The role of EETs in asthmatic airway inflammation. EETs promotes airway inflammation in asthma through several mechanisms, including mediating damage to the airway epithelial barrier, activating the secretion of neurotransmitters and neuropeptides in neuroendocrine cells, stimulating the secretion of airway epithelial-derived cytokines (TSLP, IL-33, IL-1 α , IL-1 β) to induce eosinophil degranulation and activate type 2 immune cells. ILC2, type 2 innate lymphoid cells; IL-1 α /1 β , interleukin-1 α /1 β ; IL-13/4/5/33, interleukin-13/4/5/33; TSLP, thymic stromal lymphopoietin

On the other hand, EETs trigger the airway epithelium to release IL-33 and TSLP which in turn induce ILC2 activation as well as eosinophil degranulation, thus aggravating airway inflammation [119].

EETs play a role in the acute exacerbation of asthma. Respiratory syncytial virus is a common trigger of asthma exacerbation and it can stimulate the release of EETs [120]. Moreover, elevated release of EETs could result in airway obstruction and impairment of lung function by increasing mucus viscosity in asthma [107]. Although the precise mechanism underlying EETs' contribution to asthma remains elusive, the role of EETs in asthma should not be underestimated.

Significance of EETs as a target for asthma therapy

EETs as a potential therapeutic target for asthma can be explored from two perspectives: inhibiting EETs formation and eliminating components of EETs. So far, there has been limited research in this field.

DNase has been shown to cleave eDNA and degrade EETs [115, 121]. Treatment with rhDNase resulted in a significant decrease in EETs formation in BALF and lung tissue and improvement in airway resistance and lung function in OVA-induced mouse model [122]. Surfactant protein-D (SP-D), a product of airway epithelial cells, can bind directly to the membrane of eosinophils to inhibit EETs formation, thus reducing the exacerbation of

asthma [123]. TSLP secreted by epithelial cells can regulate immune cell activation and the release of type 2 cytokines, contributing to airway inflammation in asthma [124]. Anti-TSLP antibodies effectively inhibit the release of EETs, thereby alleviating airway hyperresponsiveness [118].

Macrophage extracellular traps (METs)

Macrophages have diverse functions including support for development, maintenance of homeostasis, immune surveillance, and regulation of tissue repair [125]. Macrophages are also capable of releasing a net-like structure known as METs to capture and eliminate microorganisms [126].

Definition of METs

In 2010, Chow and colleagues first reported that RAW264.7 cells can release DNA to form METs when stimulated by PMA [127]. Similar to NETs and EETs, METs are composed of eDNA and active proteins including histones, elastase, and MPO [126, 128–130]. However, METs released by THP-1 cells lack protease when stimulated by TNF- α , hypochlorous acid, and nigerin [131]. As the central scaffold of METs, the eDNA of METs mainly originates from nuclear DNA [129, 132–135]. However, several studies suggest that the origin of eDNA in METs is from mitochondria [131, 136].

Formation and release of METs

The formation of METs is generally accompanied by macrophages death, a process known as METosis [127, 134, 137, 138]. Pathogenic microorganisms, hypochlorous acid, PMA, IL-8, and TNF- α can induce macrophages to release METs [134, 139]. The studies on the mechanisms underlying METs formation are currently limited. Similar to NETs, METs formation can be classified into NADPH oxidase-dependent and NADPH oxidase-independent pathway. Inhibitors of NADPH oxidase, such as DPI or apocynin, can decrease METs formation by inhibiting ROS production [132, 140, 141]. Exposure of alveolar macrophages to cigarette smoke extracts could increase the production of ROS and METs, suggesting a positive role for ROS in METs formation [142]. Human alveolar macrophages producing METs showed higher ROS fluorescence intensity compared to non-producing macrophages, and NADPH oxidase inhibitors significantly inhibit the release of METs [141]. However, the formation of METs induced by some pathogens is independent of NADPH oxidase [129, 133, 135, 136].

PAD enzymes are responsible for catalyzing the citrullination of histones, a process critical for the formation of EETs [143]. The release of METs was associated with the citrullination and partial cleavage of histone H3 [144]. The citrullination of histones in METs may involve the

activation of either PAD4 or PAD2. The release of METs was accompanied by the upregulation of PAD4 gene, and knockdown of PAD4 with small interfering RNA could inhibit METs release in THP-1 cells [145]. Citrullination of histone H4 is dependent on PAD2 in METs induced by TNF- α [137].

The difference in METs release is associated with the type of stimulus and the polarization state of the macrophages [146, 147]. For instance, M1 macrophages induced by interferon- γ and LPS released METs, while M2 macrophages induced by IL-4 did not release METs [145]. Treating bovine macrophages with 100 μ M PMA could induce METs formation [132], whereas there was no MET release in murine J774A.1 macrophages stimulated by 100nM PMA [136]. Regarding pathogenic microbial stimulation, bacteria, parasites, fungi, and acid-fast bacilli all can induce METs release [148]. In the formation of METs induced by pathogens, the proportion of macrophages that release METs increases with the diversity of infectious pathogens [149].

METs in asthma

Macrophages, as the most abundant immune cells in the airway, play a critical role in chronic airway inflammation and remodeling in asthma [150]. However, there is limited research on METs in asthma. Using LPS/IFN γ to stimulate the macrophages isolated from the peripheral blood of asthma patients, the level of M1-like macrophage EETs (M1EETs) in the severe asthma is significantly higher than those in the non-severe asthma group, and the level of M1EETs is negatively correlated with FEV₁% pred. M1EETs was shown to activate airway epithelial cells, neutrophils, ILC1, and ILC3 to promote the migration of inflammatory cells and the release of inflammatory cytokines, thereby enhancing airway inflammation [151]. This suggests that METs are involved in the airway inflammation of severe asthma.

Limited research work on the role of METs in asthma makes it hard to evaluate their potential as a novel target for asthma therapy. DNase I can effectively disrupt the main structure of METs by digesting the eDNA strands [142]. However, DNase is unable to eliminate histones and pro-inflammatory proteins in METs [146]. PAD mediates histone citrullination during the formation of METs, indicating that PAD inhibitors may have therapeutic effect for asthma [152].

Conclusion

Extracellular traps play a beneficial role in innate immune defense against pathogens, but their enzymes and active proteins can also contribute to tissue damage and inflammatory response, making them a double-edged sword. The elevated production of NETs, EETs, and METs in asthma patients suggests their potential role in regulating

pathological processes including airway inflammation, airway hyperreactivity, and mucus secretion.

Most of the studies focused on the basic structure and classical formation pathways of ETs. The specific mechanisms of ETs in asthma remain largely unexplored, posing significant challenges for the development of drugs targeting ETs in asthma. Currently, there are some unresolved questions about NETs, EETs, and METs that require further research. For example, what is the threshold level of ETs release when it becomes pathogenic? What are the roles of active proteins and granzymes from ETs in asthma? What are the mechanisms underlying the increased formation of NETs, EETs, and METs in asthma patients? Deeper insights into ETs and their formation mechanisms are necessary to address these questions. As the studies of ETs in asthma continue to grow, NETs, EETs, and METs may emerge as effective therapeutic targets for asthma in the future.

Abbreviations

ETs	Extracellular traps
NETs	Neutrophil extracellular traps
NE	Neutrophil elastase
MPO	Myeloperoxidase
CitH3	Citrullinated histone 3
dsDNA	Double-stranded DNA
PMA	Phorbol-12-myristate-13-acetate
IL-8	Interleukin-8
TNF- α	Tumor necrosis factor α
LPS	Lipopolysaccharide
GM-CSF	Granulocyte/macrophage colony-stimulating factor
NADPH	Nicotinamide adenine dinucleotide phosphate
ROS	Reactive oxygen species
PAD4	Peptidyl arginine deiminase 4
EA	Eosinophilic asthma
NA	Neutrophilic asthma
eDNA	Extracellular DNA
ICS	Inhaled corticosteroids
BALF	Bronchoalveolar lavage fluid
HDM	House dust mite
TLR4	Toll-like receptor 4
NF- κ B	Nuclear factor kappa-B
TSLP	Thymic stromal lymphopoietin
DNase	Deoxyribonuclease
rhDNase	Recombinant human deoxyribonuclease
EETs	Eosinophil extracellular traps
mtDNA	Mitochondrial DNA
LyoPS	Lysophosphatidylserine
3-MA	3-Methyladenine
ILC2	Type 2 innate lymphoid cells
RSV	Respiratory syncytial virus
SP-D	Surfactant protein-D
METs	Macrophage extracellular traps
M1ETs	M1-like macrophage extracellular traps

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Author contributions

W.G. and G.Z. designed the research. G.C. and L.Z. collected the data. H.J. and Z.L. prepared the materials. G.C., C.H., W.G., and G.Z. wrote the manuscript. All authors read and reviewed the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

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Competing interests

The authors declare no competing interests.

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