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Review

Role of Neutrophils on the Ocular Surface

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Abstract: The ocular surface is a gateway that contacts the outside and receives stimulation from the outside. The corneal innate immune system is composed of many types of cells, including epithelial cells, fibroblasts, natural killer cells, macrophages, neutrophils, dendritic cells, mast cells, basophils, eosinophils, mucin, and lysozyme. Neutrophil infiltration and degranulation occur on the ocular surface. Degranulation, neutrophil extracellular traps formation, called NETosis, and autophagy in neutrophils are involved in the pathogenesis of ocular surface diseases. It is necessary to understand the role of neutrophils on the ocular surface. Furthermore, there is a need for research on therapeutic agents targeting neutrophils and neutrophil extracellular trap formation for ocular surface diseases.

Keywords: ocular surface disease; dry eye syndrome; neutrophil; neutrophil extracellular trap

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1. Introduction

The ocular surface of the eyeball is the part of the eye in contact with the outside world, serving as a primary barrier against external substances and pathogens [1]. The cornea is a transparent tissue that refracts light entering the eye, focusing it on the retina and acting as a barrier against the outside [2]. The conjunctiva is a mucous membrane that attaches to the cornea and becomes the surface surrounding the eyeball [3]. It forms a conjunctival sac surrounding the inner eyelid and connecting to the eyelid [2]. In addition, the conjunctiva is connected to the nasal mucosa and supplied with tears from the lacrimal gland through the lacrimal duct [2]. The mucous membrane of the conjunctiva has many blood vessels and produces a large amount of mucus from goblet cells [3]. The subconjunctival tissue contains many lymphoid tissues and the immune system [3]. The ocular surface immune system can be divided into innate and adaptive immune systems [4]. The innate immune system includes basophils, dendritic cells, eosinophils, Langerhans cells, mast cells, monocytes and macrophages, neutrophils, and natural killer cells, whereas the adaptive immune system includes T and B lymphocytes [5].

Neutrophils are members of the innate immune system and are at the forefront against infection, but they are involved in adaptive immunity through interactions with T and B cells [6]. They have been reported to play an essential role in autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and anti-neutrophil cytoplasmic antibodies-associated vasculitis (AAV) [7]. Although the ocular surface is in contact with external pathogens, both innate and adaptive immunity are involved in the pathogenesis of dry eye syndrome, characterized by tear instability and inflammation of the ocular surface [8]. Therefore, the role of neutrophils in the ocular surface is discussed in this article.

2. Methods

A systematic literature search was performed on PubMed and Medline for papers published before 30 August 2021. The following combined search terms were used: "neutrophil," "neutrophil extracellular traps," "dry eye," "ocular surface," and "NETosis." Both

human and animal studies were included in the outcome evaluation. Correspondences, notes, and editorials were excluded. Neither language filter nor limitation of publication time was applied during the literature search. References of the retrieved studies were also reviewed manually to identify relevant articles.

A review of the literature in the PubMed database identified 175 articles. After extensive study, 15 articles were included (Figure 1).

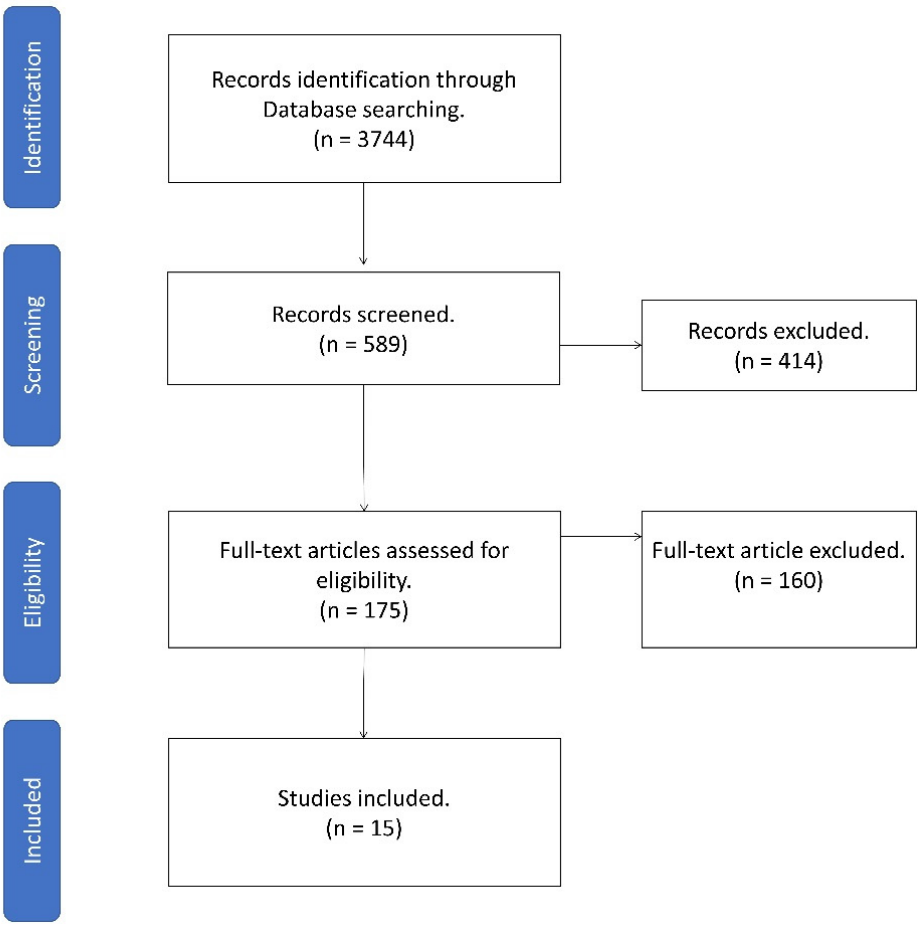

Figure 1. Flowchart of the study screening process for original studies in neutrophils for the ocular surface.

3. Neutrophils in Immunity

Neutrophils are considered short-term and terminally differentiated phagocytes with no significant gene expression or regulatory role in adaptive immunity [6]. However, in recent years, opinions on the role of neutrophils have been developing. Neutrophils are primarily short-term polymorphonuclear cells (PMNs) related with the first line combatant to pathogens, which can phagocytose potentially harmful antigens and trigger strong antimicrobial defenses, including the release of reactive oxygen species (ROS) such as superoxide and neutrophil extracellular traps [9]. Neutrophils extrude their nucleus or mitochondrial DNA to form neutrophil extracellular traps, called "beneficial suicide" [10]. In addition, neutrophils can act as the myeloid-derived suppressor cells (MDSCs) to inhibit adaptive T cell functions such as the expansion of T cells, the activation of other immune cells, or the secretion of cytokines [11].

In response to pathogen exposure, the promptest action of neutrophils is phagocytosis, which arises within minutes [12]. Phagocytosis and opsonization are primarily associated with the ingestion of small microbes, while large-sized bacteria and fungi induce the extrusion of granules from the neutrophils [13]. The primary and secondary granules, which contain cationic defensins, myeloperoxidase (MPO), neutrophil elastase, iron chelators, lactoferrin, human neutrophil lipocalin, and several metalloproteinases, are released primarily in regards to harsh sterile or infectious stimuli, releasing adenosine monophosphate (AMP) and proteases that can efficiently break down bacterial and fungal proteins [14]. Tertiary granules comprising matrix metalloproteinases and gelatinases are released to facilitate their migration through the extracellular matrix [15,16].

Neutrophil extracellular traps have been described as one of the ways in which neutrophils remove microbes [17,18]. Neutrophils release a chromatin network adorned with granular-derived antibacterial peptides and active enzymes, including cathepsin G, MPO, and neutrophil elastase [19]. Neutrophil extracellular trap formation, known as NETosis, was initially reported as an extracellular antibacterial form resisting microbe. However, recently it has been reported that neutrophil extracellular trap formation is involved in autoimmune/rheumatic and auto-inflammatory disease states beyond microbial death [20]. Neutrophil extracellular traps are released after infection with Gram (+) and (-) bacteria, especially by large-sized microbes [21]. Neutrophils generally kill small microbes through phagocytosis, but larger microbes that are not easily digested release cytoplasmic granules and promote nuclear translocation of neutrophil elastases to form neutrophil extracellular traps [17]. In addition, neutrophil extracellular trap formation is induced by nitric oxide, autoantibodies, cytokines such as interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α, hydrogen peroxide, lipopolysaccharides, phorbol-12-myristate-13-acetate, ionophores for calcium ion, and the interaction with activated platelets or vascular endothelial cells [22,23].

The three main signaling pathways of neutrophil extracellular trap formation have been discussed. First, after phorbol-12-myristate-13-acetate stimulates neutrophils through the protein kinase C (PKC) and Raf-mitogen-activated protein kinase (MEK) extracellular signal-regulated kinase signaling pathways, it induces the activation of nicotinamide adenine dinucleotide phosphate oxidase 2, triggering the associated signaling cascade and neutrophil extracellular trap formation through the production of ROS [20,24]. ROS formation promotes the migration of two key enzymes, MPO and neutrophil elastase, stored in the neutrophil granules, to the nucleus and induces chromatin decondensation, leading to the release of nuclear neutrophil extracellular traps [20]. Hydrogen peroxide is converted to hypochlorous acid by MPO, which activates neutrophil elastase to break down the cytoskeleton and nuclear membrane, allowing neutrophil extracellular trap excretion [25]. Second, the increase in intracellular calcium levels activates the peptidylarginine deiminase 4 (PAD4) enzyme, which moves to the nucleus, leading to histone citrullination and chromatin decondensation [26]. This mechanism is independent of nicotinamide adenine dinucleotide phosphate oxidase 2 [27]. Third, another form of neutrophil extracellular trap formation is the mitoNET formation [28]. Mitochondria are degraded and release the oxidized mitochondrial DNA into the extracellular space by mitochondrial ROS production or the stimulation of toll-like receptor 4 or complement factor 5a receptor [28,29]. Neutrophil extracellular trap formation induced by nitric oxide and phorbol myristate acetate induces both nuclear and mitochondrial neutrophil extracellular trap formation [23].

Neutrophil extracellular trap is thought to be enrolled in the onset of autoimmune and autoinflammatory diseases [30]. Autoantibodies to neutrophil extracellular trap components, including the citrullinated histones with DNA, MPO-DNA complexes, and neutrophil elastase-DNA complexes, are common in several systemic autoimmune diseases [31]. Defects in the process of neutrophil extracellular trap formation, excessive neutrophil extracellular trap formation, and delayed neutrophil extracellular trap formation clearance are all associated with autoimmunity [31]. Neutrophil extracellular traps have been suggested to play a pivotal role in various autoimmune diseases, including

systemic lupus erythematosus, vasculitis, rheumatoid arthritis, and chronic inflammatory bowel diseases such as Crohn's disease and ulcerative colitis [32–35]. Circulating and synovial neutrophils in patients with rheumatoid arthritis are more prone to forming neutrophil extracellular traps than in healthy controls [36,37]. Neutrophil extracellular trap formation is a source of autoantibody and stimulates inflammatory responses in rheumatoid arthritis [37]. In rheumatoid arthritis, anti-citrullinated protein antibodies are formed, associated with neutrophil extracellular trap formation and neutrophil count [38]. Neutrophil extracellular trap formation can also be provoked by neutrophil binding of anti-neutrophil cytoplasmic antibodies and anti-ribonuclear protein (RNP) antibodies [39]. As an extracellular bactericidal mechanism used by neutrophils, neutrophil extracellular traps go through steps that include ROS production, PAD4 activation, granule formation, chromatin decondensation, and active release of DNA/histone/cathelicidin antimicrobial peptide cocktail into the extracellular space [27].

Peptidyl-arginine deiminase 2 (PAD2) and PAD4 are the posttranslational modification enzymes converting protein arginine or mono-methylarginine to citrulline [40]. PAD2 and PAD4 are implicated in the pathogenesis of several autoimmune diseases [41]. Histone citrullination by PAD2 and PAD4 is essential for neutrophil extracellular trap formation [31,42,43]. Hypercitrullination in synovial fluid and anti-citrullinated protein antibodies in plasma are found in rheumatoid arthritis [44], suggesting that the hypercitrullinated molecules may serve as autoantigen. PAD2 and PAD4 are potential biomarkers and therapeutic targets of sepsis [45]. PAD4 inhibitor block neutrophil extracellular trap formation [46], reducing bleomycin fibrosis [47,48]. Simultaneous inhibition of PAD2 and PAD4 ameliorates neutrophil extracellular trap formation and reduces inflammatory cytokine production [49].

Neutrophil elastase is a proteolytic enzyme belonging to the chymotrypsin-like family of serine-proteolytic enzymes, a protein packaged in cytoplasmic neutrophil granules of neutrophil granulocytes [50]. Neutrophil elastase is unnecessary for neutrophil extracellular trap formation with non-infectious stimuli [51], but degrades the extracellular matrix including elastin, collagens, proteoglycan, fibronectin, immunoglobulins, and surfactant proteins and stimulates the pro-inflammatory cytokines to contribute to inflammation [52]. Neutrophil elastase reduces the secretion of secretory leukoproteinase inhibitor (SLPI) by lung epithelial cells [53]. The role of neutrophils in the immune system is summarized in Table 1 and Figure 2.

Table 1. Role of neutrophil in immunity.

References	Findings
Richards and Endres 2014. [12]	Phagocytosis of neutrophil against pathogen
Brinkmann et al. 2004, Brinkmann and Zychlinsky 2012, Keshari et al. 2012, Fonseca et al. 2018 [17,18]	Neutrophil extracellular trap formation of neutrophil against pathogen
Angelidou et al. 2018, Frizinsky et al. 2019, Tsourouktsoglou et al. 2020, Bach et al. 2020, Fatemi et al. 2021 [30–32,34,35]	Neutrophil extracellular trap formation contributing to autoimmune diseases
Liu et al. 2018, Li et al. 2020. [41,46]	PAD2/PAD4 activation in autoimmune diseases
Dunlevy et al. 2012, Martinod et al. 2016 [51,52]	Neutrophil elastase released from neutrophil causes inflammation
Puga et al. 2011, Cerutti et al. 2013, Governa et al. 2017, Vlkova et al., 2019 [54–57]	Interaction of neutrophil and adaptive immune response including T cells and B cells
Hosoki et al. 2016, Arebro et al. 2017, Polak et al. 2019 [58–60]	Neutrophils contribute to allergic response
Jones et al. 2010, Glenn et al. 2016, Yıldızet al. 2021, Liu et al. 2021 [61–64]	Neutrophils contribute to immunological rejection in transplanted organ

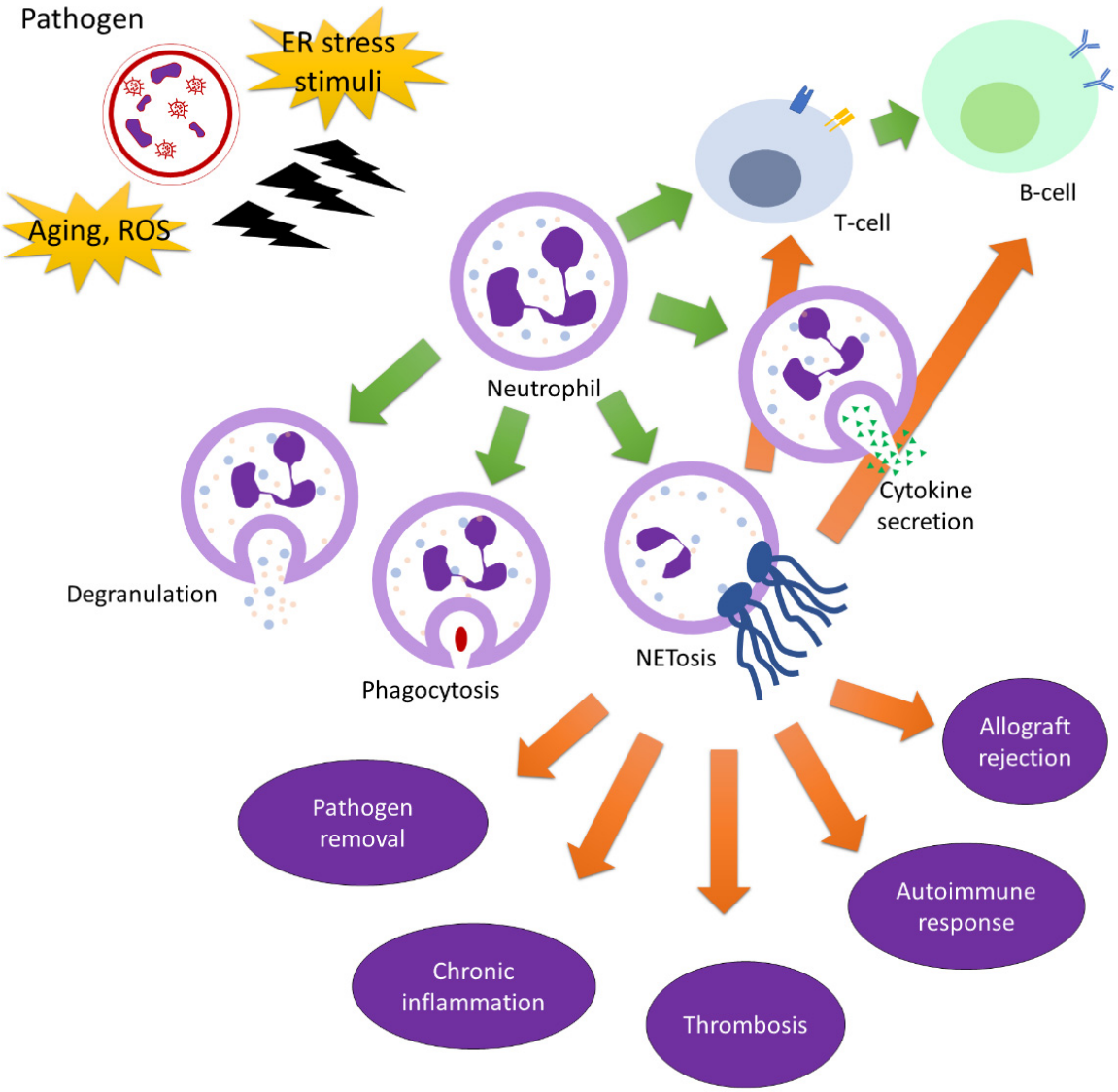

Figure 2. Neutrophils in immunity.

4. Neutrophils in Adaptive Immunity

Neutrophils have been suggested to modulate adaptive immunity, although they have been thought to be a significant member of innate immunity [65]. They regulate T cell proliferation and cytokine production [66,67]. Neutrophil extracellular trap activates dendritic cells, causing Th1 polarization to produce cytokines from T cells [67]. Neutrophils directly regulate T cells by engaging with antigen-presenting cells [68–70] and suppress the cytotoxic activity of innate and adaptive killer cells in cancer [71]. Further, they enhance the responsiveness of CD8+ T cells to T-cell receptor triggering signals [54], whereas neutrophils from common variable immunodeficiency patients actively inhibit T cell activation and secretion of IFN-γ via the ROS formation [55]. Contact between T cells and neutrophil extracellular trap enhances T cell responses to specific antigens [72]. Programmed cell death protein 1 (PD-1) axis, expressed on the surface of activated T-cells promoting apoptosis, blocks neutrophil cytotoxicity in cancer [73]. T cells enhance neutrophil function in host resistance in candida infection [74]. The T cells promote phagocytosis and chemotaxis of neutrophils through C-C motif chemokine ligand 8 [75], whereas the B cells secrete antibodies in response to antigen [76]. Neutrophils help B cell activation to produce antibodies in the spleen through IL-10 and IL-21 [56,57] and

destruct the pathogens by opsonization [77,78]. Interaction between neutrophils and B cells leads to B cell differentiation and activation and neutrophil infiltration through C-C motif chemokine ligand 1/C-C motif chemokine ligand 2 [79]. Furthermore, the allergic response has been described to be associated with the role of neutrophils [58]. Elevation of neutrophil-attracting chemokine IL-8 and IL-17 was found in allergic disease, and neutrophils were infiltrated into the tissues in a toll-like receptor 4-, myeloid differentiation protein-2-, and C-X-C motif chemokine receptor 2-dependent manner to sensitize the allergic response [58,59]. Activated neutrophil promotes T cell activation in allergic disease [59] and contributes to IgE production in allergen-specific B cells through presenting antigen [60].

During immunologic rejection of organ transplantation, neutrophils are the first immune cells to infiltrate in the transplanted organs [80]. Neutrophils are essential in promoting alloimmune responses and immunological rejection is ameliorated by inhibiting neutrophil extracellular traps [61–63]. However, deficient neutrophil extracellular trap formation has been reported in patients undergoing bone marrow transplantation [64].

5. Role of Autophagy in Neutrophils

Autophagy is a critical mechanism in cell biology that allows cells to maintain nutrient and energy homeostasis by removing damaged or harmful intracellular components and is involved in cell survival and death depending on cell type and stress conditions [81]. Autophagy with impaired control has been linked to various diseases, including neurodegenerative diseases, inflammatory diseases, and cancer [82]. During autophagy, cytoplasmic components are surrounded by double-membrane vesicles known as autophagosomes, delivered to lysosomes for degradation (autologous lysosomes) [83]. In this process, damaged cellular elements or intracellular pathogens are detected and removed to protect cells and nourish them by recycling cytoplasmic macromolecules and organelles [83].

Autophagy is required to regulate inflammation by modulating pathogen removal, antigen presentation, cytokine production, and immune response and is a regulator of neutrophil function [84]. Autophagy is required to develop long-term survival subsets of neutrophil-derived granulocytes positive for CD15, CD66b, CD63, CD11b, MPO, and neutrophil elastase [85]. Autophagy is an important modulator of neutrophil extracellular trap formation through mTOR-dependent pathways [86]. Autophagy positively modulates neutrophil extracellular trap formation, and thus, diminishing autophagy is associated with decreased neutrophil extracellular trap formation [87]. Autophagy activation through the inhibition of the mammalian target of rapamycin (mTOR) by rapamycin promotes neutrophil extracellular trap formation, whereas autophagy inhibition by wortmannin suppresses neutrophil extracellular traps release [87]. Phosphoinositide 3-kinases (PI3K)–AKT–mTOR axis links autophagy and neutrophil extracellular trap induction and significantly impacts both processes [88]. In infection with invasive bacteria, autophagy in neutrophils precedes neutrophil extracellular trap formation, and autophagy-related 5 knockdown blocks neutrophil extracellular trap formation [89]. In infection with invasive *E. coli*, autophagy in neutrophils precedes neutrophil extracellular trap formation, and autophagy-related 5 silencing completely blocks neutrophil extracellular trap formation [89]. Neutrophils isolated from aged mice defective in autophagy-related 5 showed a reduction of neutrophil extracellular trap release [90].

In recent years, the role of autophagy in neutrophil-mediated inflammation and autoimmune diseases has been described [91]. Neutrophils move to the site of inflammation as the frontline of innate immunity [92]. Autophagy is a protective mechanism for neutrophil-mediated inflammation, and inhibiting autophagy can lead to uncontrolled inflammation [93]. Autophagy inhibits degranulation and affects nicotinamide adenine dinucleotide phosphate oxidase-mediated ROS production, down-regulating apoptosis and affecting neutrophil tissue invasion [94]. Knockdown of autophagy-related 5 and autophagy-related 7 reduces the inflammatory function of neutrophils by inhibiting ROS production and degranulation [95]. In the inflammatory process, endoplasmic reticulum stress can provoke neutrophil autophagy, and autophagy can suppress endoplasmic

reticulum stress [96,97]. Inhibition of autophagy through the knockdown of nucleotide oligomerization domain (NOD)-like receptor pyrin domain-containing protein 3 (NLRP3) or inhibition of NLRP3 inflammasome promotes neutrophil recruitment and phagocytosis, thereby enhancing pathogen removal and improving the survival of septic mice [98].

Autophagy is required in the production and extrusion of several neutrophil cytokines [87,99]. The release of IL-1β, one of the pro-inflammatory cytokines secreted from neutrophils, was suppressed by autophagy inhibitors or autophagy-related 5 silencing. Inhibition of autophagy may be an effective treatment in neutrophil-mediated inflammatory diseases [35].

6. Endoplasmic Reticulum Stress in Neutrophils

Endoplasmic reticulum stress is involved in the pathogenesis of many diseases such as dry eye, rheumatoid arthritis, diabetes, dementia, and cancers [100–103]. It is linked to cellular dysfunction, inflammation, oxidative stress, apoptosis, and autophagy. Mitochondrial activity and endoplasmic reticulum stress are required for neutrophil differentiation [104]. Endoplasmic reticulum stress reduces during both neutrophil and macrophage differentiations, and the activities of protein kinase R-like endoplasmic reticulum kinase and activating transcription factor 6 were decreased, and that of inositol-requiring enzyme 1-α is enhanced during neutrophil differentiation [104]. The role of endoplasmic reticulum stress of neutrophils was investigated in acute lung injury [105]. Elevated endoplasmic reticulum stress levels were observed in infiltrated neutrophils in the acute lung injury mice model [105]. Sensors for endoplasmic reticulum stress, including protein kinase R-like endoplasmic reticulum kinase, activating transcription factor 6, and inositol-requiring kinase 1, were enhanced in neutrophil in acute lung injury [105]. Suppression of endoplasmic reticulum stress inhibited the inflammation [105]. Inositol-requiring enzyme 1-α is a crucial regulator of neutrophil extracellular traps through ROS generation and caspase-2 activation [106]. Endoplasmic reticulum calcium level is increased in the neutrophils in cystic fibrosis in response to endoplasmic reticulum stress response, which exaggerates the inflammation [107]. Tunicamycin-induced endoplasmic reticulum stress signaling (protein kinase R-like endoplasmic reticulum kinase/activating transcription factor 4/CCAAT-enhancer-binding protein homologous protein signaling) aggravates airway inflammation via elevation of inflammatory cytokines (IL-6, IL-8, and TNF-α) in a murine model of neutrophilic asthma [108]. Endoplasmic reticulum stress/X-box-binding protein 1 enhances mucin secretion through the influence of neutrophil elastase [109]. Neutrophil induces apoptosis in cancer cells through an endoplasmic reticulum stress pathway [110].

7. Neutrophils in Aging

Neutrophil extracellular traps remove the old vessels to promote remodeling [111]. However, aging drives neutrophils to be pathogenic, contributing to vascular diseases [112,113]. The intestinal microtome regulates neutrophil aging by enhancing C-X-C motif chemokine receptor 4 and reducing L-selectin [114]. Interaction between neutrophils and the microbiome contributes to the maturation of the immune system and the pathogenesis of immune-mediated diseases and cardiovascular diseases [115,116]. Aged neutrophils are characterized by altered expression of surface molecules such as lymphocyte function-associated antigen-1, macrophage-1 antigen, toll-like receptor-4, platelet endothelial cell adhesion molecule-1, and higher oxidative stress levels [117]. In addition, neutrophil extracellular traps are more prone to be formed in aged neutrophils [117]. Neutrophil extracellular traps accumulation compromises organ functions and impairs revascularization and vascular repair after ischemic injuries [118]. Delayed clearance of neutrophil extracellular traps facilitates autoimmune reactivity [119].

Neutrophil aging induces chronic inflammation of vessels, affecting lacrimal glands and ocular surfaces. Since neutrophil extracellular trap formation is also easily activated on the ocular surface in the elderly, it may be one of the pathogenic mechanisms of dry eyes in elderly patients.

8. Human Factors Affecting Neutrophils

Smoking has been reported to elevate the neutrophil count in blood [120,121] and to increase neutrophil elastase-induced inflammation through the elevation of IL-8 production and proteinase-activated receptor-2 [122]. Neutrophils are stimulated to produce C-X-C chemokine ligand 8 through toll-like receptor-9 receptor activation, inducing chronic inflammation [123]. Air pollution, including particulate matter 2.5 (PM2.5) and particulate matter 10 (PM10), causes inflammation, where neutrophils are involved [124,125]. Smoking enhances the impairment of neutrophil function by air pollution [126]. Chronic alcohol drinking impairs normal neutrophil extracellular trap formation and phagocytosis [127,128], although single alcohol drinking exaggerates the neutrophil response to the microbiome [129]. Neutrophil activation and functions are suppressed by alcohol consumption through C-X-C chemokine ligand 1/C-X-C chemokine receptor type 2 [130]. Vitamin D has been reported to have an immunomodulatory function [131]. Vitamin D enhances the production of IL-8 in neutrophils, although it does not affect the neutrophil phagocytic capacity in response to lipopolysaccharide [132]. Vitamin D deficiency is associated with high blood neutrophil count and neutrophil reactive oxygen species levels [133,134].

Hyperlipidemia is associated with blood neutrophil count [135] and increases leukotriene B4 production in neutrophils by increasing the nuclear translocation of 5-lipoxygenase, which initiates the synthesis of leukotrienes from arachidonic acid [136]. Atherosclerosis is facilitated by hyperlipidemia-induced elevation of blood neutrophil count [137]. In diabetes, high blood glucose level affects the metabolism of neutrophils [138] and enhances transient neutrophil activation followed by the inhibition of cell activity [139]. Hyperglycemic condition primes neutrophils to produce more superoxide and cytokines and to form more neutrophil extracellular traps than in normoglycemic conditions [140].

9. Neutrophils in COVID-19

Coronavirus disease (COVID-19) is an infectious disease which is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [141,142] and involves the lungs accompanying the associated systemic complications [143,144]. In COVID-19, neutrophils are heavily infiltrated into tissues and play an important role in the pathogenesis of complications [145]. SARS-CoV-2 directly stimulates neutrophil extracellular trap formation [146], inducing epithelial cell death [146] and circulates in the blood vessels to contribute to immunothrombosis by secreting IL-1α and cathepsin G [147,148]. The presence of circulating neutrophil extracellular traps may be a prognostic factor for COVID-19 because they block blood vessels and increase mortality [144,149–151]. Targeting neutrophil extracellular trap in COVID-19 may improve the prognosis and reduce the complications by preventing neutrophil extracellular trap-induced thrombosis [152].

10. Neutrophils on the Ocular Surface

Neutrophil extracellular trap formation by neutrophils has been reported to protect against corneal infection by Pseudomonas and Aspergillus on the ocular surface [153–155]. Infectious keratitis is a severe disease that can threaten vision. Neutrophils kill the pathogen by phagocytosis, degranulation, and neutrophil extracellular trap formation as the front line against pathogen [156–158]. In *Pseudomonas keratitis*, neutrophils infiltrate and form neutrophil extracellular traps to kill the pathogens [159], but it can cause corneal damage [160]. Killing Pseudomonas with inhibition of neutrophil extracellular traps may be a useful way to reduce corneal damage and improve clinical prognosis [160]. Bacterial biofilms are difficult to treat once they are formed, but in this particular situation, neutrophil extracellular traps confine the pathogen and prevent it from spreading to surrounding tissues [153].

Fungus, such as aspergillus or candida, is too large to be removed by phagocytosis, and thus, it is removed by neutrophil extracellular trap formation [161,162]. Inhibition of neutrophil extracellular trap exacerbates fungal keratitis [161]. Viral keratitis, such as herpes virus or adenovirus, is accompanied by infiltration of neutrophils [163–165] through secretion of cytokines or chemokines including IL-6, IL-17, or C-X-C motif chemokine ligand 1/keratinocytes-derived chemokine [165,166], which can lead to corneal damage [167]. In ocular surface burns, neutrophils first appear in the ocular tissue, remove the dead tissue, and trigger an inflammatory and fibrotic reaction [168]. Excessive neutrophil infiltration appears in severe eye burns and is known as an indicator of poor corneal prognosis [169]. Inhibition of neutrophil extracellular trap formation has been reported to increase the rate of corneal wound healing in burns through inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells activation [170]. Dry eye disease is characterized by tear instability, hyperosmolarity, and ocular surface inflammation and is associated with ocular discomforts [171]. Neutrophil infiltration and degranulation occur in patients with dry eye disease [172,173]. Our previous study revealed that systemic endoplasmic reticulum stress induced the neutrophil infiltration in lacrimal glands, which provoked ocular surface inflammation in the dry eye model [97]. It has been reported that neutrophil extracellular trap formation markers, such as neutrophil elastase, MPO, and citrullinated histone H3, exist on the ocular surface [174]; extracellular DNA production and clearance mechanisms are dysregulated in dry eye disease, which results in ocular surface inflammation [175]. Hyperosmolarity promotes neutrophil extracellular trap formation, which was inhibited by anti-inflammatory/proapoptotic agents [176]. Meibomian glands dysfunction, which is associated with blepharitis, is a common cause of evaporative dry eye disease [177]. Neutrophil extracellular trap formation orchestrates the inflammation and occludes the ducts of exocrine glands and the blood vessels [178]. Neutrophil extracellular trap obstructs meibomian glands and cause meibomian glands duct dilation and acinar atrophy [179].

Neutrophils affect the ocular surface in ways other than neutrophil extracellular trap formation. Systemic immune-inflammation index (SII) levels, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio were higher in patients with dry eye disease [180]. The neutrophil-to-lymphocyte ratio is calculated as neutrophil count divided by the lymphocyte count [181] and may be useful to estimate the activity of autoimmune and inflammatory diseases [182]. Further, the neutrophil-to-lymphocyte ratio increases in patients with non-Sjögren dry eye disease [183], suggesting that non-Sjögren dry eye disease may be associated with systemic inflammation [183]. The platelet-to-lymphocyte ratio is a novel inflammatory marker, which may be used in many diseases for predicting inflammation and mortality [184].

In dry eye disease, the secretion of lipoxin A4 from neutrophils is regulated by dietary $\omega-3$ docosahexaenoic acid (DHA) [185]. Elevated lipoxin A4 levels in ocular tissue contribute to the severity of dry eye disease by affecting Treg, TH1, and TH17 effector cells [186]. Sjögren's syndrome (SS) is an autoimmune disease involving lacrimal and salivary glands [187]. Autoantibodies to Sjögren's syndrome antigen B (SSB), a ribonucleoprotein, have been frequently reported in SS [188]. It is unclear what role neutrophils play in SS, but SSB activates mitogen-activated protein kinase (MAPK) pathway and nuclear factor kappa-light-chain-enhancer of activated B cells signaling to induce IL-8 release from neutrophils [189]. There is a need for research on therapeutic agents targeting neutrophils and neutrophil extracellular trap formation for ocular surface diseases. The role of neutrophils in dry eye disease is summarized in Table 2 and Figure 3.

Table 2. Role of neutrophils on the ocular surface.

References	Mode of Action or Mechanism	Organ
Cho et al. 2019 [97]	Neutrophil inflammation	Lacrimal glands
Sonawane et al. 2012, Barliya et al. 2017, Mahajan et al. 2021 [174,175,179]	Neutrophil extracellular formation	Ocular surface and meibomian glands
Ozarslan et al. 2020 [180]	Increased neutrophil-to-lymphocyte ratio	Blood
Gao et al. 2018 [186]	Lipoxin A4 amplification	Ocular surface
Wan et al. 2020 [170]	Wound healing of cornea	Cornea and ocular surface
Tibrewal et al. 2014 [176]	Hyperosmolarity of tear film promotes neutrophil extracellular traps formation	Ocular surface

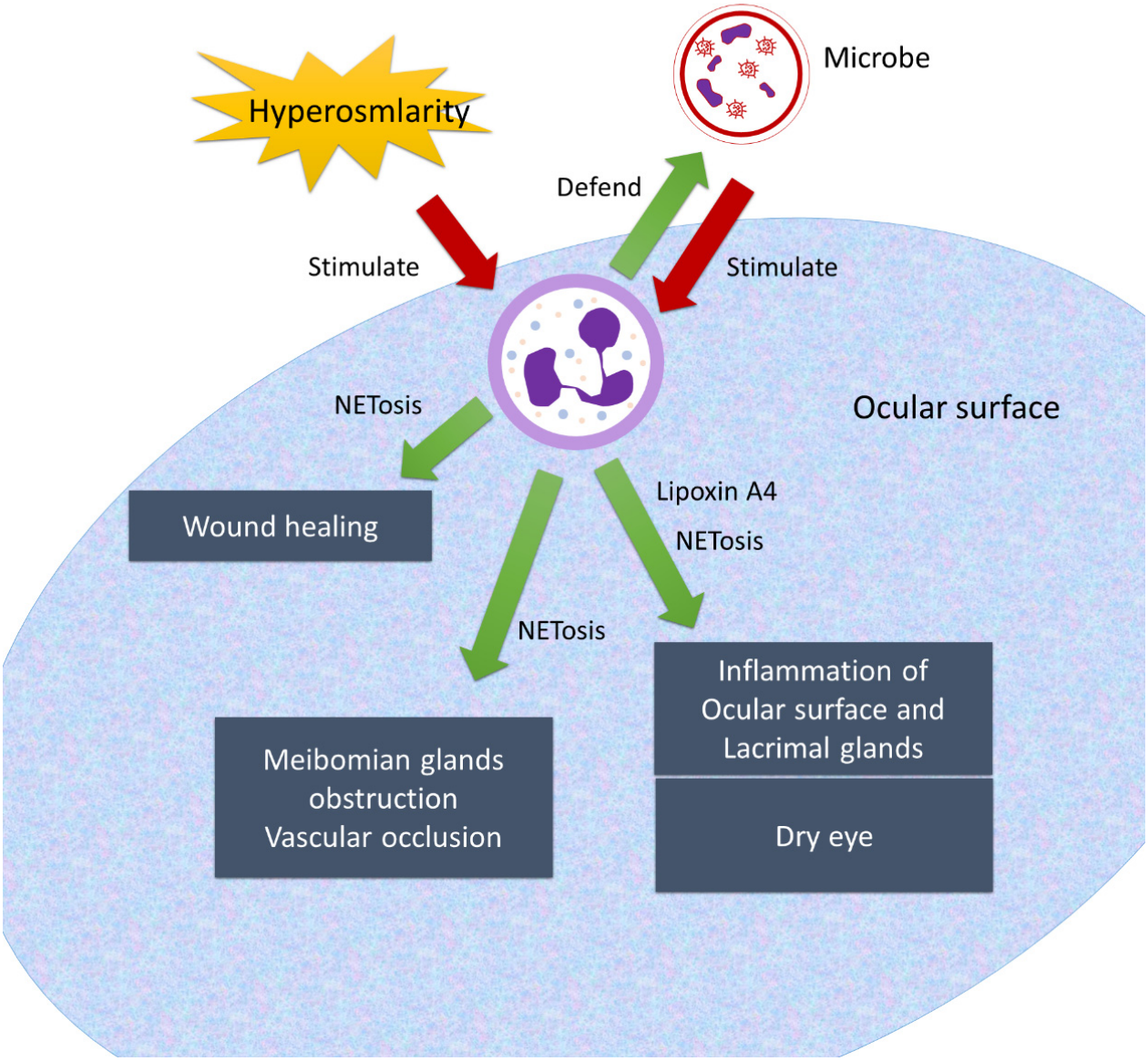

Figure 3. Neutrophils on the ocular surface.

11. Drug Development

Inhibition of neutrophil extracellular trap formation may reduce the inflammation and inflammation-associated damages on the organ, which can serve as a treatment option for dry eye disease or other autoimmune diseases [190,191]. The neutrophil inhibitor has been reported to decrease neutrophil-mediated lung damage in patients with acute respiratory distress syndrome and suggested to modulate the tissue destruction and the disease course [192].

Several neutrophil elastase inhibitors have been developed. Sivelestat, a selective neutrophil elastase inhibitor, prevented phorbol myristate acetate-induced acute lung injury [193,194], enhanced coronary blood flow, and ameliorated myocardial damage after myocardial arrest [195]. Furthermore, it has shown its protective effect in neuromyelitis optica [196], refractory Kawasaki disease [197], knee osteoarthritis [198], steatohepatitis [199], and systemic inflammation such as burn [200]. BAY 85-8501, another selective and potent neutrophil elastase inhibitor, has been revealed to reduce pulmonary disease inflammation [201]. DX-890, a small-protein neutrophil elastase inhibitor, showed anti-inflammatory effects through reducing neutrophil trans-epithelial migration, releasing activity, and neutrophil elastase-induced cytokine expression in airway epithelial cells [52]. MPH-966, neutrophil elastase inhibitor, attenuated intestinal injury and ameliorated intestinal microbiome [202].

PAD2 inhibitor improved survival from endotoxemia induced by lipopolysaccharide through inhibiting neutrophil extracellular trap formation and secretion of pro-inflammatory cytokines [49,203]. PAD4 inhibitors regulate the neutrophils by preventing active nicotinamide adenine dinucleotide phosphate oxidase complex and an oxidative burst in neutrophils [204].

Neutrophil inhibition can be a promising treatment option in dry eye disease. Neutrophil extracellular trap formation inhibition by acetylsalicylic acid and dexamethasone promotes corneal epithelial cell migration in corneal alkali burns through modulating nuclear factor kappa-light-chain-enhancer of activated B cells signaling [170].

12. Conclusions

The ocular surface is a gateway that contacts the outside and receives stimulation from the outside. Neutrophil infiltration and degranulation occur on the ocular surface. Degranulation, phagocytosis, neutrophil extracellular trap formation, called NETosis, and autophagy in neutrophils are involved in the pathogenesis of ocular surface diseases. It is necessary to understand the role of neutrophils in the ocular surface. Furthermore, there is a need for research on therapeutic agents targeting neutrophils and neutrophil extracellular trap formation for ocular surface diseases.

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Abbreviations

SS	Sjögren's syndrome
TNF-α	Tumor necrosis factor-α
NETosis	Neutrophil extracellular trap formation

PAD2	Peptidyl-arginine deiminase 2
PAD4	Peptidyl-arginine deiminase 4
PMN	Polymorphonuclear cells
ROS	Reactive oxygen species
MDSCs	Myeloid-derived suppressor cells
MPO	Myeloperoxidase
AMP	A denosine monophosphate
NLRP3	Nucleotide oligomerization domain (NOD)-like receptor pyrin domain-containing protein 3

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