Neutrophils: A Critical Participator in Common Diseases of Ruminants

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Abstract

Neutrophils, pivotal effector cells involved in innate immunity, play a central role in various infectious and inflammatory diseases. Using a powerful phagocytic killing mechanism, these cells protect the host by destroying the invading pathogens. However, these cells can also cause varying degrees of tissue damage if their activation is not finely controlled. In recent years, the involvement of neutrophils in human diseases has been extensively studied, while their roles in ruminant diseases have rarely been investigated. In the present review, we mainly summarize current knowledge regarding the characteristics and functions of neutrophils in ruminants such as goats and cattle. We emphasize the involvement of these cells in several common diseases such as mastitis, Brucellosis, *Mycoplasma bovis* infection and parasitic infections, among others. We also focus on discussing the relevant mechanisms and signaling pathways underlying these observations. In addition, we compare the phenotypes and functions of neutrophils of different ruminant species. The studies about ruminant neutrophils should help elucidate the pathogenesis of many ruminant diseases and ultimately shed light on the development of novel therapeutics for these diseases.

Keywords: Neutrophil, Protective immunity, Tissue damage, Ruminant disease, Immunotherapy

Nötrofiller: Ruminantların Yaygın Hastalıklarında Kritik Katılımcı

Öz

Doğal bağışıklıkla ilişkili önemli efektör hücreler olan nötrofiller, çeşitli bulaşıcı ve inflamatuvar hastalıklarda temel rol oynarlar. Bu hücreler güçlü bir fagositik öldürme mekanizması kullanarak istilacı patojenleri yok eder ve konağı korur. Bununla birlikte, aktivasyonları iyi kontrol edilmezse değişen derecelerde doku hasarına neden olabilirler. Son yıllarda, nötrofillerin insan hastalıkları ile ilişkisi kapsamlı bir şekilde incelenirken, ruminant hastalıklarındaki rolleri nadiren araştırılmıştır. Bu derlemede esas olarak keçi ve sığır gibi ruminantlarda nötrofillerin özellikleri ve fonksiyonları ile ilgili güncel bilgileri özetlenmiştir. Bu hücrelerin mastitis, Bruselloz, *Mikoplazma bovis* enfeksiyonu ve paraziter enfeksiyonlar gibi yaygın görülen çeşitli hastalıklardaki önemi vurgulanmıştır. Ayrıca, ilgili mekanizmalar tartışılmış ve bu gözlemlerin altında yatan yollara işaret edilmiştir. Ek olarak, farklı ruminant türlerin nötrofillerinin fenotipleri ve işlevleri karşılaştırılmıştır. Ruminant nötrofilleri ile ilgili çalışmalar, birçok ruminant hastalığının patogenezinin aydınlatılmasına yardımcı olmalı ve sonuç olarak bu hastalıklar için yeni terapötiklerin geliştirilmesine ışık tutmalıdır.

Anahtar sözcükler: Nötrofil, Koruyucu bağışıklık, Doku hasarı, Ruminant hastalığı, İmmünoterapi

INTRODUCTION

Neutrophils are polymorphonuclear cells that act as the first line of defense against invading pathogens [1]. By rapidly combating intrusive microorganisms, they limit infections during the initiation stage of an immune response. Neutrophils are the most abundant leukocytes in the blood, where they complete their maturation after migration

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from the bone marrow to the vasculature [2]. Although the lifespan of most neutrophils is very short, with a circulating half-life of only 6-8 h, their overall number is stably maintained due to the dynamic balancing of production, retention, mobilization, margination and clearance [1]. Unlike most other immune cells, senescent neutrophils are an exceptionally long-lived population that may play an important role in maintaining neutrophil heterogeneity and homeostasis. They can be identified by their surface antigenic profile of CXCR4^{high} CD11b^{high} CD62L^{low} and their uniquely small size and excessive nuclear lobulation [3].

Once neutrophils receive signals related to pathogen invasion and inflammation, they immediately migrate to sites where they are needed [1]. Neutrophils have long been considered the terminal effector cells of acute inflammatory response since they basically devour invading pathogens. In addition, numerous studies have shown that neutrophils also release effector molecules including various cytokines, extracellular traps and other effector mediators [2]. These effector molecules are closely tied to the activation, regulation and effector functions of both innate and adaptive immune cells [1]. Additionally, neutrophils can release granules containing highly toxic molecules, products of reactive oxygen species (ROS) and inflammatory cytokines, or may undergo NETosis to trap microbial invaders [4]. Consequently, if neutrophil-mediated immune responses are not strictly regulated, detrimental inflammations and host tissue damages can be caused as a result [5].

In recent years, ruminant farming has been threatened and impacted partly due to a lack of knowledge regarding invading pathogens and host responses. Because neutrophils are central to disease control and prevention, understanding their roles should be a priority. What has been known is that the morphology, function and quantity of neutrophils vary among different ruminant species [6]. Moreover, among healthy dairy goats or cattle, differences in the viability and morphology of blood and milk neutrophils have been reported [7].

In this review, we mainly describe the basic characteristics of neutrophils and review the specific roles of neutrophils in ruminants. We also highlight and summarize the roles of reciprocal communication pathways linking neutrophils with common ruminant diseases.

CHARACTERISTICS AND FUNCTIONS OF NEUTROPHILS

Neutrophil Chemotaxis

Neutrophils are the first immune cells to reach sites of inflammation and infection. They are recruited to such sites via chemotaxis, a cellular process depending on the extracellular chemoattractant gradient [8]. Neutrophils

undergoing chemotaxis are polarized, whereby actin filament (F-actin)-based protuberances on their leading edges moving in a synchronized fashion with cytoplasmic contractions and the trailing edge myosin-based movements to move the entire cell forward. Neutrophil movement within a chemoattractant gradient relies on the action of G protein-coupled receptor (GPCR) signaling pathways employing formylated peptide receptors (FPR1/2/3), classical chemoattractant receptors (BLT1/2, PAFR and C5aR) and chemokine receptors (CXCR1/2 and CCR1/2) [1]. After a chemical attractant binds to a GPCR, a receptor conformational change occurs that results in the activation of downstream signaling pathways, including the phospho-lipase C (PLC) pathway. The PLC pathway activation triggers the production of diacylglycerol (DAG) and inositol triphosphate (IP3) that activate protein kinase C (PKC) and protein kinase D (PKD), which ultimately induce an increase in intracellular calcium level. PKC has multiple isoforms that interact with different participants to promote F-actin activity and regulate cofilin activity [9]. PKD, a direct effector of PLC/PKC axis proteins, can phosphorylate the cofilin phosphatase SSH2 to ultimately regulate downstream cofilin activity during GPCR-mediated neutrophil chemotaxis [10].

Cytotoxic Function

After neutrophils are recruited to sites of infection, they recognize and devour microbes. During this process, their cytotoxic function plays an important role in pathogen killing [8]. This function is made possible during neutrophil differentiation within the bone marrow whereby three types of granule proteins are formed in a stepwise fashion and assembled during maturation into a powerful pathogen-killing weapon. Meanwhile, within each neutrophil cytoplasm, numerous secretory vesicles are present that contain various types of plasma membrane receptors such as receptors for lipopolysaccharide (CD14), complement (CR1 and CR3/Mac-1), urokinase-type plasminogen activator, immune complex and chemoattractant (formyl peptide) [11]. At the infection site, neutrophil extracellular traps (NETs) also function as critical cytotoxins to promote "neutrophil apoptosis" through the process of "NETosis" to achieve extracellular entrapment of pathogens [4].

SPECIFIC FEATURES OF NEUTROPHILS IN RUMINANTS

Dairy Goat Neutrophils

During peak lactation, neutrophils in the blood and milk of dairy goats differ greatly in their morphological features and functions. Milk neutrophils are derived from migrating blood neutrophils that settle in the mammary gland, where they function to combat invading microbes that penetrate the physiological barriers of the papillary duct [12]. Occasionally, neutrophil band cells are found among blood neutrophils, but never among milk neutrophils. Notably,

milk neutrophils generally appear to be more mature than their blood counterparts. However, compared with blood neutrophils, milk neutrophils have impaired phagocytosis and oxidative burst functionality and lower viability, which may be due to spontaneous aging, interactions with milk components and/or diapedesis-based effects. Morphologically, milk neutrophils have a more ruffled appearance and possess a multi-lobed nucleus instead of the 2- to 3-lobed nucleus observed in the blood. In addition, milk neutrophils exhibit relatively lower ability to release gelatinase compared to blood neutrophils under both PMA stimulation or non-stimulation conditions [13].

In the 1960s, Paape introduced the term "somatic cells" (SCs) to refer to various types of cells found in mammalian milk [14]. As is well known, SCs are a handful of host cells found in animal milk that are predominantly leukocytes, including macrophages, neutrophils and lymphocytes, with a few epithelial cells. Thus, based on their origins, SCs can be categorized into two groups, blood-derived SCs and epithelial SCs [15]. The somatic cell count (SCC) in the milk of healthy dairy goats differs from that of cows, partially reflecting the differences in host processes that control normal mammary epithelial cell shedding and renewal [16]. Studies have shown that during mastitis, distinct neutrophil changes occur that differ between animal species, with neutrophils significantly outnumbering macrophages in the milk of mastitic sheep and goat, while macrophages predominate in healthy milk [17].

Bovine Neutrophils

Bovine neutrophils in the milk, similar to their goat counterparts, migrate from the blood to the mammary gland to provide the first line of defense against invading pathogens [7]. Although neutrophils newly migrating into the mammary gland are active phagocytic cells, they are continuously exposed to inhibitors in milk, such as fat globules and casein, resulting in decreased phagocytic capacity accompanied by morphological alteration [18]. Milk neutrophils possess large phagocytic vacuoles containing previously engulfed casein micelles with smooth surfaces and spherical shapes. These micelles are formed during loss of pseudopods by the internalization of pseudopod membrane material that results in milk fat globule formation.

Unlike other animal species in which neutrophils account for multitudinous blood leukocytes, bovine neutrophils make up only 25% of total blood leukocyte numbers. However, mature lactating Holstein cows have a potential pool of more than 100 billion circulating neutrophils that appear to be supplemented by a marginal pool of mature neutrophils adhering to vessel walls [19]. The makeup of neutrophil populations which respond to a particular mammary stimulus depends on the intensity of the stimulus and the strength of the chemotactic agent, with the number of neutrophils in bovine SCs determined by indirect immunofluorescence to be $3\times10^4\sim3\times10^6$ cells/mL [20].

Akin to goat neutrophils, bovine neutrophils are also involved in the inflammatory process of mastitis. In fact, a wide variety of neutrophil β-defensins have been isolated that combat invasive pathogens [21]. However, these anti-microbial weapons also damage the fragile inner layer of the mammary gland and lead to permanent scar formation and decreased mammary epithelial cell participation in lactation. Interestingly, co-culture of neutrophils with cold-pressed terpeneless Valencia orange oil (TCO) has been shown to increase the chemotaxis of these cells *in vitro* without altering their phagocytic ability. Indeed, genes reflecting the pro-inflammatory immune response are generally down-regulated in the presence of TCO, resulting in the inhibition of bacterial growth without negatively altering neutrophil function [22], suggesting that TCO may serve as an effective therapy for mastitis.

Similarly, butyric acid, a short-chain fatty acid that can exert potent anti-inflammatory effects both *in vitro* and *in vivo*, could also serve as a mastitis treatment. Butyric acid acts via several mechanisms to regulate the innate immune response of ruminants: by activating neutrophils, inducing platelet activating factor (PAF), increasing CD63 expression, inducing the release of matrix metalloproteinase-9 (MMP-9) and lactoferrin, inducing NETs formation and through short-chain fatty acids (SCFA)-based pathways [23]. Previous studies have reported that increased production of SCFA is involved in subacute rumen acidosis and the activation of the inflammatory response [24]. In humans and rodents, SCFAs regulate the inflammatory response in the gut through free fatty acid receptor 2 (FFA2), which is activated by butyric acid in cattle. Researchers have found that butyrate activates bovine neutrophils to induce two second messenger events, Ca^{2+} influx and phosphorylation of mitogen-activated protein kinase (MAPK) that are involved in FFA2 activation. Butyric acid-induced Ca^{2+} influx is dependent on extracellular and intracellular Ca^{2+} sources and PLC activation. Therefore, butyric acid appears to be involved in SCFA regulation of inflammation through its effects on neutrophil activation [23].

RELATIONSHIP BETWEEN NEUTROPHILS AND COMMON DISEASES OF RUMINANTS

Role of Neutrophil Leucocyte in Cows and Goats During Mastitis

Mastitis is considered as one of the most complex diseases impacting dairy farming. The disease is caused by inflammation of the mammary gland initiated during several types of bacterial infections, among which *E. coli* infections, *M. agalactiae* infections and *S. aureus* infections are the most common ones [25]. Such infections cause damage or even necrosis of the mammary gland that eventually leads to low milk production and even the
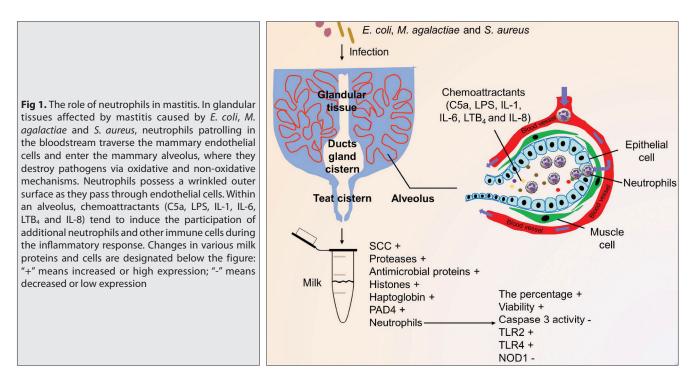
removal of dairy production, seriously hindering the development of the livestock industry and imposing a great economic burden on farmers.

Studies about mastitis have shown that neutrophils can efficiently mount defenses against invading pathogens by migrating from the blood to the mammary gland, where they deploy a cascade of oxidative and non-oxidative response mechanisms to destroy pathogens [26]. In cows with mastitis, both SCC and milk neutrophil percentages are significantly increased while the viability of neutrophils is relatively low [27]. This phenomenon may result from pathogen-based signals that trigger both the release of neutrophils to blood from bone marrow and neutrophil migration from blood through the endothelial cell layer to the mammary gland, ultimately the increase of milk SCC. During this process, chemokine-mediated stimulation is a key determinant of SCC influx. Several potent chemoattractants that recruit milk neutrophils include C5a as well as LPS, interleukin-6 (IL-6), IL-17 and IL-8 [26,28]. Although they are present in increased numbers, milk neutrophils possess decreased viability in mastitis. In a previous study, it was suggested that improving the viability of milk neutrophils might prevent or reduce the severity of *E. coli* mastitis in dairy cows [29]. In fact, the phagocytic activity of milk neutrophils is higher than blood neutrophils during subclinical and clinical mastitis, with the opposite observed in healthy cows [27]. However, when compared to healthy cows, blood and milk neutrophils from cows with clinical mastitis showed a significantly reduced phagocytic activity. These observations imply that the defense mechanism against invading pathogens of the mammary gland greatly depends on the rate at which neutrophils enter the infection site, their ability to produce reactive oxygen intermediates (ROI) and the number of circulating neutrophils at the infection site [30]. In healthy cows, milk neutrophils could be considered inactive cells when compared to circulating cells, since the phagocytic capacity of milk neutrophils is regulated by ROS production and milk neutrophils presumably undergo apoptosis to reduce ROI production after diapedesis. Nevertheless, the study found that immunosuppression always relied on cortisol, not on apoptosis, regardless of the physiological state of the cows [31] *(Fig. 1)*.

Blood neutrophils change their shape as they pass through the mammary epithelial barrier by becoming spherical. Upon entering the mammary gland, they become irregular milk neutrophils with wrinkled outer surfaces. The latter state not only helps to better internalize the cell membrane during phagosome formation, but also increases their surface areas for optimal phagocytosis [30] *(Fig. 1)*.

Bacteria have been shown to undermine neutrophil functions at each of these steps. In mastitis caused by *S. aureus*, IFN-γ serves as a neutrophil priming agent by acting as a primary agonist to influence the early effector arm of the neutrophil response and modulate post-response trafficking [32]. However, several researchers have found that *S. aureus* produces membrane-damaging peptides such as α, β, γ, δ-hemolysins, phenol soluble modulins (PSMs) and bi-component leukocidins that directly punch holes in immune cell membranes to lyse the cells [33]. Moreover, *S. aureus* employs numerous virulence factors to restrain neutrophil activation, chemotaxis and phagocytosis and target key host effector proteins. For example, extracellular fibrinogen-binding (Efb) protein and staphylococcal complement inhibitor (SCIN) proteins

Fig 1. The role of neutrophils in mastitis. In glandular tissues affected by mastitis caused by *E. coli*, *M. agalactiae* and *S. aureus*, neutrophils patrolling in the bloodstream traverse the mammary endothelial cells and enter the mammary alveolus, where they destroy pathogens via oxidative and non-oxidative mechanisms. Neutrophils possess a wrinkled outer surface as they pass through endothelial cells. Within an alveolus, chemoattractants (C5a, LPS, IL-1, IL-6, LTB₄ and IL-8) tend to induce the participation of additional neutrophils and other immune cells during the inflammatory response. Changes in various milk proteins and cells are designated below the figure: "+" means increased or high expression; "-" means decreased or low expression

can target complement protein C3 convertase to prevent the formation of C3a and C3b, resulting in bacterial resistance to neutrophil phagocytosis [34]. Meanwhile, the S. aureus chemotaxis inhibitory protein (CHIPS) inhibits neutrophil migration and activation, preventing neutrophils from responding to both host- and bacterial-derived chemoattractants. Although toll-like receptor 2 (TLR2)-expressing cells can recognize staphylococcal glycan-related lipoproteins such as staphylococcal iron transporter C (SitC), S. aureus produces staphylococcus superantigen-like 3 (SSL3) protein that binds to TLR2 and inhibits the activation of neutrophils and other cells expressing TLR2. Crystal structure-based analysis demonstrates that the binding of SSL3 to TLR2 reduces the size of the available lipopeptide binding pocket by 50%, explaining the observed binding inhibition of TLR2 agonist Pam_2CSK_4. Moreover, S. aureus produces capsular polysaccharides and micro-capsules that may serve as yet another phagocytic escape strategy [35].

Abnormal apoptosis may also be a mechanism involved in neutrophil-based tissue damage in mastitis. Indeed, in the blood and milk of cows with mastitis, neutrophil caspase 3 activity is reduced, which may reflect reduced neutrophil apoptosis that promotes heightened neutrophil activity, ultimately causing tissue damage. Alternatively, the control of inflammation may involve extending neutrophil lifespan via the inhibition of NETosis [4]. In yet another possible scenario, higher neutrophil surface expression of TLR2 and TLR4, but not of TLR9, in cows with mastitis might enhance neutrophil pathogen recognition and immune responses, with crosstalk between C5a and neutrophil TLR4 signaling supporting a positive feedback loop that leads to severe mastitis responses (Fig. 1). In this scenario, the onset of inflammatory reactions would somehow be linked to inefficient LPS detoxification that activates C5 cleavage to form C5a; if a large amount of C5a was produced, neutrophils would be activated via the C5a-C5aR pathway, a possible therapeutic target for mastitis treatment [36]. Another potential pathway in mastitis involves the interaction of neutrophils with the nucleotide-binding oligomerization domain-1 (NOD1), a key factor involved in the sensing of conserved bacterial peptidoglycan motifs that initiates pro-inflammatory and antimicrobial responses. In perinatal cows, neutrophil expression of NOD1 is decreased, which results in the inhibition of NOD1/NF-κB signaling, reduced neutrophil migration to E. coli-infected sites and impaired phagocytosis [37] (Fig. 1). The NOD1/NF-κB pathway modulates neutrophil responses to reduce both neutrophil-mediated killing of bacteria and ROS production that in turn may control early inflammatory responses. Consequently, artificial restoration of neutrophil NOD1 function might be employed to either prevent or treat E. coli-induced mastitis.

Role of Neutrophil Leucocytes in Brucellosis

Brucellosis, caused by the genus Brucella, is a chronic global zoonosis [38]. Within infected macrophages and dendritic cells, pathogenic Brucella ca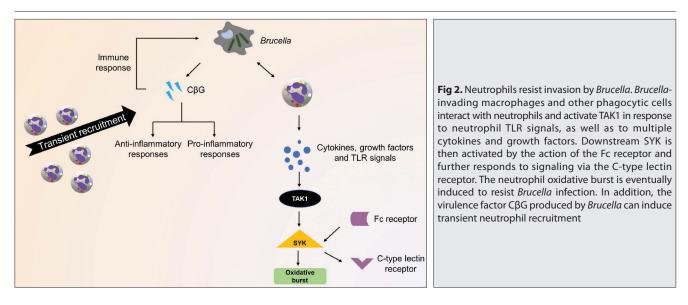n replicate efficiently in the endoplasmic reticulum, a safe intracellular niche at the crossroads of many important host cell functions. There are many subspecies of Brucella, of which B. melitensis, B. abortus and B. suis are the three most common pathogens in humans and livestock [38]. Although Brucella spp. are closely related to each other genetically, they infect a broad range of livestock hosts including goats, cattle, camels, sheep, pigs and even wild animals such as bison, elk and feral swine [39]. Common symptoms of Brucellosis include high abortion rate, high mortality, infertility, low milk yield and a long interval between calving. The ability of Brucella spp. to evade the host immune system determines pathogen virulence, with one demonstrated mechanism involving escape from phagocytic killing. Thus, the mechanism by which neutrophils respond to pathogenic Brucella is a rather interesting and worthwhile research area.

Seminal studies have demonstrated that virulent smooth B. abortus, B. melitensis, B. suis and rough B. canis strains are resistant to neutrophil killing, regardless of whether they are resting or IFN-γ-activated. These results suggest that smooth strains may often be more virulent than rough strains. Bovine neutrophils counter infection by both smooth and rough Brucella spp. using oxidative burst-based killing [39]. This mechanism involves the inflammatory signaling regulator TAK1, a MAP3 kinase that is activated in response to cytokines, growth factors and TLR signals [39]. SYK kinase also plays a key role in the response of neutrophils to inflammations and is activated by Fc receptor binding. TAK1 is one of the major regulators of multiple kinase activation downstream of SYK in response to C-type lectin receptor stimulation. By inhibiting TAK1 or SYK, the degree of oxidative burst of neutrophils infected by B. abortus will be reduced, indicating a role of C-type lectin receptor in the response of bovine neutrophils to B. abortus infection [39] (Fig. 2).
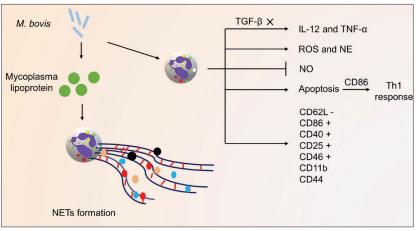
A Brucella virulence factor, β cyclic glucan (CβG), which has no toxicity for cells or animals, can induce dual pro-inflammatory and anti-inflammatory responses leading to transient neutrophil recruitment [40] (Fig. 2). Notably, β-glucan is a polysaccharide of β-D-glucose extracted from cell walls of mushrooms, yeast, oats, barley, seaweed, algae and bacteria. Many researches and clinical studies have suggested that β-glucan acts as a biological response modifier that exhibits anti-tumor and anti-inflammatory properties. It is recognized by various pattern recognition receptors (PRR) expressed on dendritic cells, macrophages and neutrophils. In addition, complement receptor-3 (CR3), lactosylceramides, scavenger receptor and dectin-1 are also involved in β-glucan recognition, the outcome of which can trigger a series of signaling events that regulate the innate and the adaptive immune responses [41].

Effects of Mycoplasma bovis and Mycoplasma Lipoproteins on Neutrophils

Mycoplasma bovis (M. bovis) is the smallest bacterium

Fig 2. Neutrophils resist invasion by *Brucella*. *Brucella*-invading macrophages and other phagocytic cells interact with neutrophils and activate TAK1 in response to neutrophil TLR signals, as well as to multiple cytokines and growth factors. Downstream SYK is then activated by the action of the Fc receptor and further responds to signaling via the C-type lectin receptor. The neutrophil oxidative burst is eventually induced to resist *Brucella* infection. In addition, the virulence factor CβG produced by *Brucella* can induce transient neutrophil recruitment

Fig 3. Effects of *M. bovis* and Mycoplasma lipoproteins on neutrophils. *M. bovis* invades the body and evades the immune response by accelerating neutrophil apoptosis and inducing ROS and NE production while inhibiting NO production. Subsequently, the Th1 response is induced by CD86-mediated signaling. During *M. bovis* infection, changes in molecules expressed on the neutrophil surface occur: "+" means increased or high expression; "-" means decreased or low expression; no mark means no change. Furthermore, neutrophils co-cultured with *M. bovis* could secrete IL-12 and TNF-α in the absence of TGF-β, while pathogen lipoproteins could induce the formation of neutrophil NETs

lacking a cell wall and often causes bovine chronic pneumonia and polyarthritis syndrome, conjunctivitis, otitis media, meningitis and mastitis [42]. These organisms require relatively rigorous cultural conditions for growth, posing a serious hindrance to vaccine preparation and disease prevention. Indeed, the lack of a vaccine has greatly influenced the health, welfare and productivity of dairy and beef cattle.

It has been shown that neutrophils exposed to *M. bovis* exhibit altered bactericidal function *in vitro* and that *M. bovis* inhibits the production of IFN-γ and TNF-α, but not IL-10 [43]. While little is known about how *M. bovis* evades host innate immunity, *M. bovis* has been shown to infect and persist within all PBMC subpopulations and erythrocytes [44]. Importantly, bacterial survival relies on the triggering of a series of pathogenic responses that delay the apoptosis of host cells. Such responses are carried out through the interaction of bacterial components with multiple proteases to activate cell survival pathways and prevent cytochrome C release. Conversely, *M. bovis* evades immune recognition by accelerating neutrophil apoptosis and inducing ROS production, while *in vitro* experiments have shown that the bacteria can also inhibit

NO production, which has a dual biological role as a signaling molecule and cytotoxin. Meanwhile, IL-12 and TNF-α production in the absence of TGF-β has been observed when bovine neutrophils are infected with *M. bovis in vitro*. This observation suggests that an activation state is created based on inflammatory cytokines that may enhance the biological response of bovine neutrophils to *M. bovis* infection. In addition, host NE production is also important for the elimination of *M. bovis (Fig. 3)* [44].

As a unique survival mechanism during infection, *M. bovis* may drive neutrophils to a state of incompetence, as indicated by a decrease in CD62L expression with the up-regulation of CD86, CD40, CD25 and CD46 [44]. This mechanism appears to help *M. bovis* escape the host immune response and survive *in vitro* [44]. In this scenario, increased neutrophil apoptosis following *M. bovis* stimulation results in the activation of type 1 helper T cell responses, with increased expression of CD86 that is involved in antigen presentation to naive T cells [44] *(Fig. 3)*.

As another unique mechanism, mycoplasma lipoprotein, the most abundant component of the mycoplasma membrane, interacts with the host to promote cell

adhesion, determine strain virulence and induce NETosis. It is important that only fat-soluble mycoplasma proteins effectively induce NETs formation as an explanation for *M. agalactiae* evasion from NETs both in cultured sheep neutrophils and in mastitic mammary glands [45]. Using a different mechanism, *S. aureus* has been shown to induce macrophage apoptosis by disassembling NETs and converting them to deoxyadenosine, which induces caspase 3-mediated immune cell apoptosis [46]. In contrast, mycoplasma digests NETs DNA scaffolds, a mechanism requiring live bacteria that prevents the transmission of overlapping signals associated with neutrophil DNA and *M. agalactiae* within the mammary gland [45] *(Fig. 3)*.

Role of Neutrophil Leucocyte in Parasitic Infections

Parasitic infections are an enormous hazard to cattle and goat farming. Many studies have shown that NETs act as a novel effector mechanism in innate immunity against parasitic infections such as *Besnoitia besnoiti (B. besnoiti)*, *Eimeria arloingi (E. arloingi)* and *Cryptosporidium parvum (C. parvum)* infections in cattle and goats [47].

Cattle infected by *B. besnoiti* exhibit clinical symptoms such as systemic dermatitis, orchitis and vulvitis. *In vitro*, bovine neutrophils interact with *B. besnoiti* tachyzoites to induce rapid formation of NETs, which can be eliminated by DNase treatment or reduced by pre-incubation with NADPH oxidase inhibitors, neutrophil elastase (NE) and MPOs. It appears that neutrophils can immobilize parasites by forming an embedded structure so that other immune cells can be recruited quickly to synergistically kill the pathogen. *E. arloingi* coccidiosis in goats is mainly characterized by severe enteritis of 4- to 10-week-old goats, with infection rates as high as 100%. Once neutrophils contact *E. arloingi*, they form NETs during sporozoite or oocyst stages, whereby NETs effectively capture 72% of the sporozoites, greatly reducing the early infection rate [48]. *C. parvum* causes severe enteritis in neonatal livestock as well, triggering the formation of NETs in a time-dependent manner, whereby sporozoite-triggered NETs depend on intracellular Ca^{2+} concentration and ERK 1/2- and p38 MAPK-mediated signaling pathways. In fact, about 15% of parasites formed by *C. parvum* are immobilized in NETs [49].

Taken together, as the first line of immune defense, neutrophils play a unique role in parasitic infections. Neutrophils capture such pathogens mainly through the formation of NETs, delaying pathogen spread and promoting further elimination of invading parasites, although the detailed mechanisms are still unclear.

Other Modulators of Neutrophil Responses

In addition to diseases mentioned above, neutrophils also participate in other inflammatory ruminant diseases including bovine leukemia virus infection, *Pasteurella haemolytica* pneumonia, bovine respiratory disease, lung inflammation induced with *Mannheimia hemolytica*, pulmonary and systemic inflammation of fetal sheep and *Histophilus somni* infection, among others.

Host stress responses and viral invasion can adversely impact neutrophil responses and most typically affect neutrophil number without altering their recruitment, leukotoxin sensitivity or responses to bacterial infections. For example, non-cytogenetic bovine viral diarrhea virus infection leads to reduced production of neutrophils in the bone marrow and ultimately persistent neutropenia [50]. When occurring during abrupt weaning stress with increased neutrophil numbers, virus infections can recruits abundant neutrophils to sites of inflammation. However, the virus-triggered type I interferon response limits the production of CXC chemokines, leaving the host vulnerable to deadly secondary infections. Such infections include pulmonary *Streptococcus pneumoniae* or BRSV infections, the latter of which is characterized by neutrophil airway and alveolar infiltration with lower MPO levels and functionally immaturity [50].

Other factors that modulate neutrophil function include β-hydroxybutyrate, a mediator that can attenuate neutrophil phagocytosis and induce the formation of NETs [23]. Meanwhile, glucocorticoids can increase the survival and recruitment of neutrophils [50]. *H. somni* is able to inhibit an oxidative burst in both neutrophils and alveolar macrophages, while also inducing NETs production in a dose- and time-dependent manner that is not associated with lactate dehydrogenase release [50]. Taken together, both host and pathogen factors can significantly influence neutrophil function, with pathogens employing numerous mechanisms to escape killing by neutrophils and other cells of the immune system.

CONCLUSIONS AND PROSTECTS

We have discussed current knowledge regarding neutrophil chemotaxis and cytotoxic functions. We have also discussed specific features of ruminant neutrophils and the involvement of these cells in common diseases of ruminants. In recent years, studies revealing neutrophil origins, heterogeneity, circadian rhythms and epigenetic control of neutrophil activities have been widely discussed. However, most of these studies are based on humans or mice, researches on ruminant neutrophils are still lacking. Considering that neutrophils are actively involved in various ruminant diseases, further studies are required to explain and understand how neutrophils function during the progression of these diseases. In addition, how to better harness neutrophil activities during disease progression and how to optimally exploit these cells are also needed to explore in the future.

CONFLICT OF INTEREST

The authors declare that no conflict of interest exists.

AUTHOR CONTRIBUTIONS

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