Inflammation, Vol. 40, No. 1, February 2017 (© 2016)
DOI: 10.1007/s10753-016-0458-4

REVIEW

The Diverse Biological Functions of Neutrophils, Beyond the Defense Against Infections

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Abstract—Polymorphonuclear neutrophils are among the first defense against infection and closely involved in the initiation of inflammatory response. It is well recognized that this function of neutrophils was mainly mediated by phagocytosis, intracellular degradation, releasing of granules, and formation of neutrophil extracellular traps after sensing dangerous stress. However, accumulating data showed that neutrophils had a variety of important biological functions in both innate and adaptive immunities, far beyond cytotoxicity against pathogens. Neutrophils can differentially switch phenotypes and display distinct subpopulations under different microenvironments. Neutrophils can produce a large variety of cytokines and chemokines upon stimulation. Furthermore, neutrophils directly interact with dendritic cells (DCs), macrophages, natural killer cells, T cells, and B cells so as to either potentiate or down-modulate both innate and adaptive immunity. In the present review, we summarize the recent progress on the functional plasticity and the regulatory ability on immunity of neutrophils in physiological and pathological situations.

KEY WORDS: neutrophils; immune cell subsets; myeloid-derived suppressive cells; cytokines; inflammation; cell polarization.

INTRODUCTION

The physiological function of the immune system is to protect the host from infectious microbes. Defense against microbes is mediated by early response of innate immunity and later adaptive immunity with different cell types and molecules. In some situations, this process can also damage tissues and cause disease. Polymorphonuclear neutrophils are one of the earliest immune cells recruited to the locations of microorganism infection and inflammatory response [1]. They are the most abundant immune cell population in human blood. Diseases like neutropenia or disorders of neutrophil function show that they are essential for controlling bacterial and fungal infections [2]. Neutrophils develop in the bone marrow from common myeloid progenitors. Granulocyte colony-stimulating factor (G-CSF) is the predominant factor regulating neutrophil proliferation, differentiation, and mobilization. Recent study showed that lifespan of circulatory neutrophils under steady-state conditions is up to 12.5 h for mouse and 5.4 days for human neutrophils [3]. During infection, neutrophils increase their longevity for sevenfold by activation [4]. It is well recognized and emphasized that after sensing conserved molecular signatures associated with microbes and tissue stress, neutrophils quickly activate defensive

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programs promoting phagocytosis and intracellular degradation, production and release of granules that are fully equipped with molecules with active microbicidal activity, generation and release of oxidative bursts, and the formation of neutrophil extracellular traps [5–7]. However, the long-held view that neutrophils function exclusively in the innate phase of the immune response has been greatly challenged by recent studies. Accumulating data showed that neutrophils had a variety of important biological functions in both innate and adaptive immunities, far beyond cytotoxicity against invading pathogens (Fig. 1). Neutrophils can switch phenotypes and display functionally distinctive subpopulations [8]. Neutrophils can interact with dendritic cells (DCs), macrophages, natural killer cells, T cells, and B cells so as to either potentiate or downmodulate both innate and adaptive immunity in a context-dependent manner [9–12]. In the mucosa, neutrophils signal the initiation of inflammatory resolution [13]. Overall, a large number of studies about additional novel and unexpected functions of these cells emerged in recent years. To better understand the roles of neutrophils in immune response regulation and how they participate in the process of diseases, we will summarize the recent progress and our understanding on the functional plasticity and the regulatory ability on immunity of neutrophils in physiological and pathological situations in the present review.

THE PLASTICITY AND SUBPOPULATIONS OF NEUTROPHILS

The strong link between neutrophils and type 1 pro-inflammatory processes has been long recognized [6]. The recent observations showing the close association of neutrophils with type 2 immunity likely reveal our ignorance of the full capacity of neutrophils beyond their well-known roles in inflammatory tissue damage and protective effects against bacterial infections [6, 14]. Recent studies indicated that neutrophils exhibit considerable plasticity. With T/B cell-deficient SCIDbg mice with mild or severe systemic inflammatory response syndrome induced by two different severities of thermal injuries, Tsuda *et al.* identified two distinct neutrophil subsets with different cytokine and chemokine expressions (Table 1), akin to macrophage functional polarization [15]. The defined type 1 neutrophils (N1) mainly express IL-12 and CCL3, and display $CD49d^{+}CD11b^{-}$ phenotype, while type 2 neutrophils (N2) mainly produce IL-10 and CCL2, and show $CD49d^{-}CD11b^{+}$ phenotype [15]. The resting neutrophils have no significant cytokine/chemokine production and are $CD49d^{-}CD11b^{-}$ [15]. Tumor-associated neutrophils (TANs) have been proposed as key mediators of malignant transformation, tumor progression and angiogenesis and in the modulation of the antitumor immunity [1]. TANs were proposed to be polarized between anti-tumorigenic N1 and pro-tumorigenic N2 phenotypes. The antitumor activities

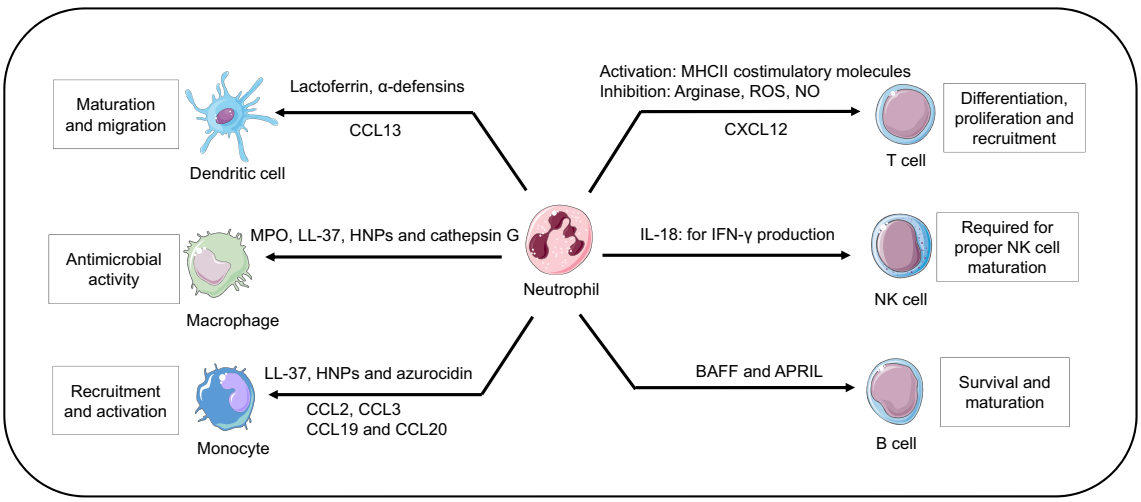

Fig. 1. Neutrophils crosstalk with other immune cells. Under homeostatic and inflammatory conditions, neutrophils can crosstalk with both many types of resident and recruited immune cells, through an array of cytokines, chemokines, granule proteins, and cell surface molecules. *HNPs* human neutrophil peptides, *IFN-γ* interferon γ, *ROS* reactive oxygen species, *MPO* myeloperoxidase, *BAFF* B-cell activating factor, *APRIL* a proliferation-inducing ligand.

Table 1. The Characterics of Differentially Polarized Neutrophils

Parameters	Neutrophils		
	Resting	N1	N 2
Nucleus	Round	Multilobular	Ring
Cytokines			
IL-1β	+	+	+
IL-4	−	−	+
IL-10	−	−	+
IL-12	−	+	−
TNF-α	+	+	+
Chemokines			
CCL2	−	−	+
CCL3	−	+	−
CCL5	Low	Low	High
CXCL1	Low	High	High
Effector molecules			
ROS	Low	High	High
Myeloperoxidase	Low	High	Low
Alkaline phosphatase (ALP)	+	+	+
Arginase	Low	Low	High
Surface molecules			
CD11b	−	−	+
CD49d	−	+	−
ICAM1	−	+	−
Induction of macrophages			
M1	−	+	−
M2	−	−	+
Biofunction			
Anti-bacteria	–	Enhanced	Decreased
Tumor	–	Anti-tumor	Pro-tumor

of N1 neutrophils include high expression of inflammatory cytokines and chemokines, low level of arginase, and strong capability of killing tumor cells *in vitro* [16]. In contrast, N2 neutrophils driven by TGF-β in the tumor acquire a protumoral phenotype [16]. Thus, blockade of TGF-β favors the accumulation of antitumor N1 neutrophils in the tumor to inhibit the tumor growth. These observations are further supported by the presence of N2 neutrophils in the lung tissues of mice during *Nippostrongylus brasiliensis* infection [17]. Sorted lung neutrophils from mice inoculated with *N. brasiliensis* showed unique upregulation of IL-13, IL-33, Igf1, Retnla (RELMα), and Chi3l3 (Ym1), whereas those from LPS-inoculated mice showed distinct upregulation of IL-6 and IL-12β as determined by real-time PCR [17]. The elegant studies performed by Chen *et al.* further demonstrated that these induced N2 neutrophils during primary *N. brasiliensis* infection "instruct" macrophages to gain ability to mediate parasite damage and clearance during secondary infection [17]. The discovery of N2 neutrophils during type 2 immunity strongly supports the functional diversity and different polarization neutrophils in various microenvironments. The inducing factors, intracellular signals, and the contribution to the pathogenesis of N2 neutrophils need to be explored in the future. The relevant studies may provide novel insights to the causes of pathogenesis involving dysregulated type 2 immunity as well as provide opportunities for intervention of new therapeutic approaches to treat severe type 2 immunity-related disorders. The plasticity of neutrophils was further confirmed by the study of Sagiv *et al.* in cancers; three distinct populations of circulating neutrophils were found, in which the mature low-density neutrophils (LDNs) display impaired neutrophil function and immunosuppressive properties in contrast to those of mature, high-density neutrophils (HDNs). Dynamic change of these neutrophil subsets occurred with disease course in cancer and inflammation resolution. Transition of HDNs to LDNs is evident only in blood drawn from mice at the late-stage of cancer, suggesting that it may be mediated via a change in the cytokine/chemokine milieu that is associated with advanced disease [18]. More recently, an anti-inflammatory subset of neutrophils was found at days 5 and 7 after myocardial infarction in a myocardial ischemia-reperfusion injury model in contrast to their pro-inflammatory counterpart in circulation. The number of these cells gradually increased as repair process initiated from day 1 to 7 post-myocardial infarction [19]. It should be noted that neutrophils carry many functional cytokine receptors. The multiple expressions of cytokine receptors support the concept of neutrophil polarization by a particular cytokine milieu, which needs to be addressed in the future.

Poga *et al.* nicely showed that neutrophils colonized peri-marginal zone (MZ) areas but not follicular areas of the spleen in the absence of infection and that neutrophils colonized MZ areas became more prominent and also invade splenic follicular areas after systemtic infection [8]. Splenic neutrophils (B cell-helper neutrophils, termed as to N_{BH} cells) had a phenotype distinct from that of circulating neutrophils (conventional neutrophils, called N_C cells). N_{BH} cells were further divided into two distinct subsets, $N_{BH}1$ and $N_{BH}2$ cells, on the basis of various parameters including the expression of CD15 and CD16. The properties of these subsets of neutrophils in humans are summarized in Table 2. Resting N_C cells highly express CD15 and CD16 on cell surface, but $N_{BH}1$ cells had intermediate expression of CD15 and CD16 and $N_{BH}2$ cells had low expression of CD15 and CD16 [8]. Compared with N_C cells, $N_{BH}1$ and $N_{BH}2$ cells expressed more CD11b, CD24, CD27, CD40L, CD86, CD95, human

Table 2. The Characteristics of Neutrophil Subsets in Human Spleens and Blood

Parameters	Neutrophils		
	N_C	$N_{BH}1$	$N_{BH}2$
Location	Blood	Peri-MZ	Peri-MZ
Surface molecules			
CD11b	+	++	++
CD15	+++	++	+
CD16 (FcγR)	+++	++	+
CD24	+	++	+++
CD27	+/−	+++	+/−
CD40L	+/−	+++	+/−
CD54 (ICAM-1)	+	+	+
CD62L (L-selectin)	+	−	+
CD62P (P-selectin)	+	+/−	+/−
CD86 (B7-2)	−	+	−
CD95 (Fas)	+	++	+/−
CD102 (ICAM-2)	+++	+	++
HLA-I	+	+	++
HLA-II	−	+	+/−
Cytokines/chemokines			
IL-1β	+/−	++	++
IL-4	+/−	++	+
IL-6	+/−	++	++
IL-8	+/−	+	+
IL-10	+/−	++	+
IL-12	+/−	+	+
IL-21	+/−	++	++
TNF-α	+/−	+	+
BAFF	+/−	+	+
APRIL	+/−	+	+
CXCL12	+/−	++	+
CXCL13	+/−	++	+
Functional molecules			
Arginase 1	+/−	+	+
RALDH1	+/−	+	+
iNOS	+/−	+	+
IDO	+/−	+	+
SOCS1	+/−	++	+
Bcl-2	+/−	+	+/−
Bcl-xL	+/−	++	+
Mcl-1	+/−	+	+
Bad	+	−	−
Bak1	+	−	−
Activating MZ B cells	−	+	+

leukocyte antigen I (HLA-I), and HLA-II, but $N_{BH}1$ and $N_{BH}2$ cells had lower expression of the adhesion molecules CD54, CD62L, CD62P, and CD102 [8]. Compared with $N_{BH}2$ cells, $N_{BH}1$ cells had higher expression of CD27, CD40L, CD86, CD95, and HLA-II but lower CD24 expression. Microarray data showed that $N_{BH}1$ and $N_{BH}2$ cells may have more mRNA expressions of B-cell activating factor (BAFF, also known as BlyS), a proliferation-inducing ligand (APRIL), IL-1β, IL-6, IL-8, IL-10, IL-12, IL-21, tumor necrosis factor (TNF)-α, chemokine (C-X-C motif) ligand (CXCL)12, CXCL13, CD40L, arginase 1, RALDH1, iNOS, IDO, SOCS1, Bcl-2, Bcl-xL, and mcl-1 but less for Bad and Bak1, compared with NC cells. Importantly, these N_{BH} cells have the ability to promote immunoglobulin class switching, somatic hypermutation, and antibody production by activating MZ B cells in BAFF-, APRIL-, and IL-21-dependent manners [8]. Circulating neutrophils can acquire N_{BH} function after exposure to splenic sinusoidal endothelial cells dependently on the transcription factor STAT3. Thus, neutrophils may undergo N_{BH} reprogramming in the spleen to assistant MZ B cells to generate antimicrobial immunoglobulin.

Put all together, these studies strongly support the presence of neutrophil functional plasticity and polarization, which we previously neglected. The inducing factors, the regulatory molecular networks, the phenotype characteristics, and the biological significance of the differentially polarized neutrophils urgently need to be clarified. We believe that the relevant studies may provide new insights into the contribution of neutrophils to the pathogenesis caused by inflammatory and immune disorders and may provide new therapeutic approaches to treat neutrophils-related diseases.

THE CYTOKINES AND CHEMOKINES RELEASED BY NEUTROPHILS

Besides their involvement in primary defense against infections, neutrophils produce and release a large variety of cytokines and chemokines either constitutively or upon microenvironmental stimulation [20, 21] (Table 3). It should be noted that some differences of cytokine and chemokine productions by neutrophils exist between humans and mice, in spite of a substantial conservation of genomes between the two species. Interestingly, neutrophils constitutively store some cytokines and chemokines like BAFF, TNF-related apoptosis-inducing ligand (TRAIL), CXCL8, CCL20, and IL-1R antagonist (IL-1Ra) within intracellular pools in significant amounts [22–24]. The *in vivo* and the *in vitro* experiments have shown that mouse neutrophils secrete a large amount of IFN-γ in response to pathogens, like *Nocardia asteroides* and *Listeria monocytogenes* [25–27]. Neutrophils have been found to be an important source of IFN-γ upon *Toxoplasma gondii* infection in rag2/IL-2Rγc-deficient mice, which lack all lymphoid cells [28]. IFN-γ production by neutrophils in this model is likely dependent on Nox2

Table 3. Cytokines and Chemokines Produced by Neutrophils

	Neutrophils	
	Mice	Humans
Cytokines		
IL-1α	+	+
IL-1β	+	+
IL-1Ra	+	+
IL-4	+	+
IL-6	+	+
IL-10	+	+
IL-12	+	+
IL-17	+	+
IL-18	+	+
IL-21	+	+
IL-22	+	+
IL-23	+	+
IL-27	–	+
IFN-β	+	+
IFN-γ	+	+
TGF-β	+	+
G-CSF	+	+
M-CSF	+	+
TNF-α	+	+
APRIL	+	+
BAFF	+	+
MIF	+	–
TSLP	–	+
SCF	–	+
Chemokines		
CCL2	+	+
CCL3	+	+
CCL4	+	+
CCL5	+	–
CCL17	+	+
CCL18	–	+
CCL19	–	+
CCL20	+	+
CCL22	–	+
CXCL1	+	+
CXCL2	+	+
CXCL3	–	+
CXCL5	–	+
CXCL6	–	+
CXCL8	–	+
CXCL9	+	+
CXCL10	+	+
CXCL11	–	+
MIP2	+	–

[29]. IL-23-activated neutrophils have been found to be the predominant source of IL-17 in a mouse model of kidney ischemia-reperfusion injury [30]. In a recent study, IL-23-producing neutrophils were found to infiltrate and accumulate in inflamed colon tissue of patients with IBD and represent the main source of IL-23 participating in disease progression [31]. A population of mouse bone marrow neutrophils constitutively expresses the transcription factor RORγt and rapidly produces IL-17 in a IL-23 and IL-6-dependent manner [32]. IL-10-producing neutrophils were found *in vivo* in patients with periodontal abscess induced by Gram-negative bacteria [33]. Endogenous TNF-α induces human neutrophils to generate IL-6 by prolonging the synthesis of the IκBζ co-activator and sustaining C/EBPβ recruitment and histone acetylation at IL-6 regulatory regions [34]. The wide range of pro-inflammatory, immunoregulatory and anti-inflammatory cytokines, and chemokines produced by neutrophils support the potential diverse and multiple biological significances of neutrophils in physical and pathological situations such as hematopoiesis, angiogenesis, wound healing, and immune disorders [35, 36]. The chemokines produced by activated neutrophils are primarily chemotactic for neutrophils, monocytes/macrophages, DCs, NK cells, and certain T cell subsets, supporting the important roles for neutrophils in amplifying their own arrival and in orchestrating the sequential recruitment and activation of innate and adaptive immune cells in the inflammatory sites [37, 38].

Neutrophils express many pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), Fcγ receptors, complement receptors, and cytokine/chemokine receptors, which can induce cytokine production after activation [39, 40]. Many cytokines like IFN-γ, IL-4, IL-10, and TGF-β can modulate the cytokine and chemokine productions in neutrophils [41, 42]. The cytokine and chemokine productions by neutrophils caused by receptor activation are fairly and kinetically controlled at the transcriptional and/or post-transcriptional levels. microRNA9 regulates cytokine response in activated human neutrophils by directly inhibiting NFKB1/p50 transcripts [43]. microRNA223 negatively controls the productions of CXCL2, CCL3, and IL-6 by neutrophils in mice [44]. Dysregulation of cytokine and chemokine mRNA expression in neutrophils results in many immune-related diseases [45]. Thus, activated neutrophils release a large number of cytokines under fine control which need to be emphasized.

THE PHYSIOLOGICAL ROLES OF NEUTROPHILS VIA CYTOKINES AND CHEMOKINES

Neutrophils can establish the cross-talk with many other immune cells like DCs, macrophages, NK cells,

and lymphocytes through an array of cytokines and cell surface molecules (Fig. 1). Neutrophils can recruit DCs and promote the later maturation as well through lactoferrin, α-defensins, and chemokines (such as CCL3) productions and direct cellular interaction [46, 47]. Upon IFN-γ stimulation, neutrophils express low levels of MHC II and co-stimulatory molecules, which can facilitate T cell activation, Th1 and Th17 cell differentiation, and deliver antigens to DCs [48, 49]. As one of the earliest immune cells recruited into influenza-infected trachea, neutrophils leave long-lasting trails enriched in the chemokine CXCL12 responsible for guiding activated effector T cells to infection sites [50]. Neutrophils can also have immunosuppressive effects. They can inhibit the proliferation of IFN-γ-producing T cells through an NO-dependent mechanism or through depleting extracellular l-arginine by arginase [51–55]. A subset of neutrophils was shown to inhibit T cell proliferation by releasing ROS in the immunological synapse [56]. Neutrophils are the major source of BAFF and APRIL, two TNF members that are critical for B cell maturation, function, and survival [57, 58]. Recently identified N_{BH} neutrophils have the capability to stimulate immunoglobulin diversification and production by splenic MZ B cells mainly through BAFF and APRIL [8]. Neutrophils can secrete IL-22, which critically contributes to the maintenance of intestinal epithelium integrity, protecting against barrier breakdown and generation of a protective response against extracellular pathogenic bacteria [59]. Transfer of IL-22-competent neutrophils to IL-22-deficient animals protected them from dextran-induced colitis and induced the production of antimicrobial peptides [59]. Neutrophils regulate terminal NK cell maturation under steady-state conditions in mice and humans [10]. Recruited neutrophils contribute to monocyte influx at sites of inflammation mainly by secreting chemokines like CCL2, CCL3, CCL19, and CCL20. Neutrophil primary granule proteins also enhance the antimicrobial activity of macrophages by increasing their ability to phagocytose and elaborate cytokines [60]. Therefore, neutrophils participate in keeping immune homeostasis and shaping innate and adaptive immunities through their antigen-presenting ability and cytokine productions.

It is now appreciated that the resolution of inflammation was a biosynthetically proactive process [61, 62]. Uncontrolled inflammation is a unifying component in many diseases [63]. The depletion of circulating neutrophils using anti-Gr1 antibodies resulted in more severe symptoms in a number of different mouse colitis models, strongly implicating neutrophils as a central protective factor in ongoing inflammation and the contribution of neutrophils to the resolution process [64]. Studies with $Gp91^{phox-/-}$ mice which lack a respiratory burst, and neutrophils depletion in acute colitis mouse models revealed that transmigrating neutrophils rapidly deplete the microenvironment of molecular oxygen in an NADPH-oxidase-dependent manner, which subsequently promotes effective hypoxia-inducible factor (HIF)-dependent inflammatory resolution [65]. Indeed, $Gp91^{phox-/-}$ mice developed highly accentuated colitis compared with WT controls [65]. On the other hand, neutrophils serve as a prominent reservoir for adenosine precursors. Adenosine and its analogs can ameliorate the course of a variety of inflammatory diseases [66, 67]. It is reported that adenosine released by neutrophils could stabilize HIF-α and inhibit NF-κB pathways [68–70]. Infiltrated neutrophils in the inflammatory mucosa may represent an important initiation signal or a critical determinant for inflammatory resolution through multiple pathways. During the resolution phase of inflammation, uptake of apoptotic neutrophils by macrophages leads to a decreased IL-23 production by macrophages, which diminishes IL-17 secretion by T cells and hence reduces G-CSF production and neutrophil differentiation. Phagocytosis of apoptotic neutrophils by macrophages stimulates the macrophages to be an M2-like phenotype, producing more IL-10 and less IL-12 to promote tissue repair during resolution of inflammation [71].

THE CYTOKINE/CHEMOKINE-MEDIATED ROLES OF NEUTROPHILS IN DISEASES

Neutrophils are highly involved in the pathogenesis of many diseases such as infections, autoimmune diseases, tumors and graft rejection in a positive or negative manner through multiple pathways. The roles of cytokines and chemokines released by activated neutrophils in the pathogenesis process are briefly summarized in the following part.

Infections. The recent studies showed that human neutrophils possess intracellular sensor systems allowing the recognition of foreign and potentially dangerous RNA and DNA [72–74]. Thus, neutrophils may have the potential ability to act at the front-line of immunity toward extracellular and intracellular microorganisms via cytokine and chemokine release. Neutrophils may carry viral antigens from the skin directly to the bone marrow to promote establishment of $CD8^+$ memory T cells [75]. During viral infections, migrating neutrophils leave behind long-lasting trails enriched in the chemokine CXCL12 is critical for

virus-specific $CD8^+$ T cell recruitment and effector functions [50]. In a *Staphylococcus aureus* infection model, neutrophils are efficiently recruited to sites of infection and subsequent to draining lymph nodes where they augment lymphocyte proliferation in a microbe-dependent manner [76]. Neutrophil-delivered IL-17 in a mouse model of kidney ischemia-reperfusion injury has been shown to regulate NKT cell activation, IFN-γ production, neutrophil infiltration, ultimately inflammation, and tissue injury [30]. Autocrine activity of IL-17 on mouse $ROR\gamma T^+$ neutrophils has been shown not only to induce reactive oxygen species (ROS), but also to increase neutrophil-mediated fungal killing *in vitro* and in a mouse model of *Aspergillus fumigatus*-induced keratitis [32, 77]. IL-17 produced by neutrophils is important in providing protection against early pneumonic plague infection in mice through orchestrating the IFN-γ-mediated programming of M1 pro-inflammatory macrophages [78]. Neutrophil-derived TRAIL induces apoptosis of $DR5^+$ macrophages, thus promoting early bacterial killing in mice of pneumococcal pneumonia [79]. Neutrophils instruct skin Langerhans cells to prime antiviral immune responses which is mainly dependent on TNF-α secreted from macrophages and neutrophils recruited to local tissues [80]. The antiviral effector properties of infiltrating neutrophils were directly related with the TRAIL production of neutrophils in a mouse model of cytomegalovirus infection [81]. Neutrophil-derived IL-18 is critically required for IFN-γ production by NK cells during Legionella infection in mice [82]. Altogether, these data underline the importance of the neutrophil responses in the resistance to intracellular pathogens through cytokine production.

Tumors. The importance of TANs in the establishment, progression, and metastasis of cancers is increasingly appreciated. The accumulating studies reveal a dual role for neutrophils in tumor biology [83, 84]. TAN can promote malignancy in certain situations, e.g., by releasing growth-stimulating signals, matrix-degrading proteases, as well as mediators of angiogenesis. Infiltration of neutrophils is seen in more aggressive types of tumors like pancreatic adenocarcinomas, but a high neutrophil count is associated with a favorable prognosis in patients with a gastric cancer [85, 86]. The ratio of neutrophils and lymphocytes is used as a prognostic factor in patients with colorectal cancer or non-small-cell lung cancer [87, 88]. Under the influence of tumor cells, TANs will differentially polarized and acquire various phenotypes and functions [16, 89]. TANs in turn influence the tumor niche, local microenvironments, and recruiting other immune cells through the release of cytokines like TNF-α, IL-1β, and IL-12, chemokines, ROS, and others [90, 91]. N1-polarized neutrophils express more TNF-α and have the ability to activate $CD8^+$ T cells, whereas N2-polarized neutrophils express more CCL2 and CCL5, and have the ability to inhibit effector T cells [16]. TANs have the ability to chemo-attractant Treg cells in a mouse model of cancer mainly via CCL17, indicating a clear link between TANs and Treg cells [92]. Meanwhile, Treg cells can release CXCL8 (IL-8) to recruit neutrophils [93]. Thus, neutrophils and Treg cells form a recruiting loop to localize and act together. Neutrophils stimulate melanoma cells to migrate toward endothelial cells and metastasize to the lungs in a mouse model of primary cutaneous melanoma caused by ultraviolet exposure [94]. It is reported that human breast cancer cells can stimulate neutrophils to produce oncostatin-M, which in turn increases secretion of vascular endothelial growth factor by tumor cells, promoting angiogenesis and neovascularization [95], establishing an additional cross-talk between neutrophils and tumor cells. In a mouse model of mammary adenocarcinoma, $CD11b^+Gr1^+$ granulocytic cells significantly increased in the lungs prior to tumor arrival [96]. Depletion of neutrophils significantly improved the immune response of the host and inhibited lung metastasis. Neutrophils in the lungs decreased IFN-γ production and produced large amounts of MMP9 and proinflammatory cytokines [96], which together created a microenvironment permissive to the tumor cell metastasis. Using a multistage mouse model of breast cancer, it was also found that T cell-suppressive neutrophils occurs during early tumor progression and preferentially accumulate in peripheral tissues but not in the primary tumor. Tumor-derived G-CSF directs expansion and differentiation of hematopoietic stem cells to skew hematopoiesis toward the myeloid lineage in bone marrow and prolonged G-CSF may be responsible for both the development and activity of immunosuppressive neutrophils [97]. More recently, it is found that tumor-secreted IL-1β stimulates the release of IL-17α from γδ T cells, resulting in systemic, G-CSF-dependent expansion and polarization of neutrophils in mice bearing mammary tumors. The induced immune suppressive phenotype of neutrophils can suppress $CD8^+$ cytotoxic T cells and directly support metastatic spread [98]. Myeloid-derived suppressor cells (MDSCs) comprise a heterogeneous group of cells of myeloid origin, including neutrophils [51, 99]. The levels of MDSCs in peripheral blood are positively correlated with tumor burden and prognosis in cancer patients [100]. Granulocytic MDSCs (G-MDSCs) suppress adaptive antitumor responses in several cancer mouse models, in some cases, through producing large amounts of ROS [101].

In contrast, neutrophils might have anti-tumor activity associated with inhibition of the tumor growth and the establishment of metastatic foci. Proto-oncogene Met is required for neutrophil chemoattraction and cytotoxicity in response to its ligand hepatocyte growth factor (HGF) for iNOS production to release nitric oxide for killing cancer cells. So, Met deletion in mouse neutrophils enhances tumor growth and metastasis [102]. Neutrophil depletion of tumor-bearing animals increases the activation of $CD8^+$ T cells, supporting the idea that N2 neutrophils act in an immune-suppressive fashion [16]. Neutrophils in the lungs of mice with renal carcinoma were highly cytotoxic and create an immunological, anti-metastatic barrier preventing the establishment and growth of metastatic cells [103]. It is reported that neutrophils accumulate in the lungs prior to the arrival of metastatic cells in a mouse model of breast cancer [104]. These neutrophils in the lung inhibit metastatic seeding in the lungs by generating H_2O_2 to eliminate metastatic tumor cells [104]. This type of neutrophils presents in the peripheral blood of breast cancer patients prior to surgical resection but not in healthy individuals, indicating a potential neutrophil-mediated inhibitory process at the metastatic site [104]. It is hypothesized that IFN-β drives neutrophils to be an anti-tumoral N1 phenotype with the ability to restrict tumor angiogenesis and that lack of IFN-β leads to induction of protumoral N2 neutrophils enhancing angiogenesis [105]. Thus, neutrophils acquire either pro- or anti-tumorigenic roles in different tumors or different stages and locations. A better understanding of the mechanisms by which neutrophils act to promote or inhibit primary and secondary tumor growth will significantly help us to develop efficient therapeutic strategies based on stimulation of antitumor immune responses.

Autoimmune diseases. Neutrophils play a significant role in activating DCs through LL-37 peptides and IFN-α in autoimmune diseases, such as systemic lupus erythematosus (SLE) and diabetes [106]. A pattern of demethylation in interferon-signature genes of lupus neutrophils was identified from patients with SLE, supporting a pathogenic role for neutrophils in lupus [107]. The expressions of MHC II and co-stimulatory molecules on neutrophils, which can facilitate T cell activation and deliver antigens to DCs [48, 49], have been detected in patients with active Wegener's granulomatosis and RA [108, 109]. Neutrophils from the blood of rheumatoid arthritis (RA) patients are primed to secrete high levels of ROS and cytokines [110]. Their migration into joints in RA was mainly dependent on the expression of CCR2 [111]. In addition to the release of ROS, granule contents, and NET, as well as the increased antigen presentation ability [112], these activated neutrophils can also secrete high levels of receptor activator of nuclear factor kappa-B ligand and BAFF, which activate osteoclasts and B cells, respectively [113, 114]. In a mouse model of experimental autoimmune encephalomyelitis, neutrophils are the first inflammatory cells to appear in the central nervous system [115]. The infiltrated neutrophils secrete CCL2 and CCL20 to recruit Th17 lymphocytes, which in turn activate neutrophils to produce cytokines and ROS, as well as upregulate MHC-II, and degranulation [116]. The chemokine-dependent reciprocal crosstalk between human neutrophils and Th17 cells will accelerate pathogenesis [116]. A recent study showed that splenic neutrophils help to shape $CD4^+$ T cell responses via producing BAFF, which then contributes to the production of pathogenic autoantibodies and the pathogenesis of mouse lupus [117]. IL-17B was most strongly expressed in human neutrophils in RA patients, which may contribute to the pathogenesis of RA [118]. Neutrophils play an important role in the pathogenesis of inflammatory bowel disease partially by ROS production. Mice lacking the $gp91^{phox}$ subunit of NADPH oxidase are protected from experimental colitis [119]. An increasing numbers of reports showed that MDSCs including G-MDSCs played crucial roles in the regulation of autoimmune diseases. G-MDSCs in synovial fluid from mice with proteoglycan-induced arthritis significantly suppressed autoreactive T cell expansion and DC maturation [120]. G-MDSCs in the synovial fluid from RA patients have the ability to inhibit the cell proliferation of joint-infiltrating T cells [121].

On the other hand, depletion of neutrophils significantly protects mice from the development of contact dermatitis, suggesting the importance of neutrophils in facilitating the development of allergen-specific T cell responses [122]. Ragweed pollen extract-challenge in naive mice induces CXCR2 and TLR4-dependent recruitment of neutrophils into the airways, which is a critical rate-limiting event that stimulates induction of allergic sensitization and airway inflammation. This study reveals the role of neutrophils in airway allergic sensitization induction [123]. Neutrophil-derived histamine is one of the major contributors to pulmonary allergic inflammation in chronic mycoplasma infection [124]. Thus, neutrophils are closely involved in the autoimmune diseases and allergic inflammation.

Graft rejection. Neutrophils accumulate in grafts in large numbers within hours after transplantation and are an important source of chemokines and other inflammatory mediators [125]. Short-term neutrophil depletion or

restraining granulopoiesis in the bone marrow reduces tissue damage associated with transplant-mediated ischemia-reperfusion injury, but alone does not prolong allograft survival to a great degree [126]. Heart allografts survive longer when recipients lack expression of CXCR2, a receptor for neutrophil chemokines. Patients receiving the CXCR1/2 inhibitor reparixin had improved transplant outcomes with glycemic control and decreased insulin requirement [127]. In lung transplant patients, increased neutrophilia is positively associated with the presence of donor-specific antibodies which correlates inversely with allograft survival [128]. However, neutrophil depletion or blocking neutrophil migration synergizes with co-stimulation blockade to induce long-term allograft survival in a heart transplant model [129]. The decrease in neutrophil recruitment to the graft significantly decreased pro-inflammatory chemokine and cytokine expressions so as to subsequently delay T cell infiltration and the adaptive alloimmune response. In allogeneic hematopoietic cell transplantation, neutrophils can cause more aggressive acute graft-versus-host disease (GVHD) mainly through release of ROS by encountering intestinal local microbial flora [130]. Several mechanisms may be involved in which neutrophil-enhanced graft rejection:

1. Causing tissue inflammation that increases effector and memory T cell infiltration into the graft.
2. Producing cytokines and expressing costimulatory molecules that influence T cell priming or antigen presentation [12, 131]. TNF-α produced by neutrophils stimulates DCs to produce IL-12, which skews T cell differentiation into Th1 cells and causes graft rejection [132]. Infiltrated neutrophils in lung allografts express B7 and provide costimulation to alloreactive $CD4^+$ T cells [131].
3. NETs promote graft injury in a platelet-dependent manner [133].

Although granulocytes are generally associated with poor outcomes following transplantation, the protective roles of granulocytes in graft rejection were also recognized in certain conditions. It is reported that a distinct subset of neutrophils with a $CD11b^+Gr\text{-}1^+CXCR4^{high}$ phenotype express high levels of MMP-9 and play an important role in the optimal vascularization of transplanted pancreatic islets in response to vascular endothelial growth factor-A [134]. MDSCs including G-MDSCs play a critical role in transplantation tolerance induction in certain protocols [99]. Wu *et al.* report that Smad3-deficient myeloid cells have immunosuppressive ability in a transplant model [135]. Smad3 decreased the production of iNOS and thus counteracted the suppressive effects of myeloid-derived suppressive cells on alloreactive T cells. Therefore, Smad3-deficient mice had a prolonged allo-skin graft survival compared with WT mice [135]. Thus, neutrophils play a dual role in graft rejection and transplantation tolerance through multiple approaches, which needs to be investigated systemically.

CONCLUSIONS

Neutrophils can differentially switch phenotypes and display distinct subpopulations such as N1 and N2 neutrophils under different microenvironments. Neutrophils can produce a large variety of cytokines and chemokines either constitutively or upon microenvironmental stimulations. Furthermore, neutrophils can directly interact with DCs, macrophages, natural killer cells, T cells, and B cells so as to either potentiate or down-modulate both innate and adaptive immunities. The inducing factors, the regulatory molecular networks, the phenotype characteristics, and the biological significance of the differentially polarized neutrophils urgently need to be clarified. We believe that the relevant studies may provide new insights into the contribution of neutrophils to the pathogenesis caused by inflammatory and immune disorders. Understanding on the functional plasticity and the regulatory ability on immunity of neutrophils in pathological situations may provide new therapeutic approaches to treat the related diseases in clinics in the future.

ACKNOWLEDGMENTS

The authors wish to thank Drs. Yuzhu Hou and Tingting Wu for their kind review of the manuscript. This work was supported by grants from the National Basic Research Program of China (2014ZX10002002-001-002, J.L., Y.Z.; 2011CB710903, Y.Z.), the National Natural Science Foundation of China for General and Key Programs (81130055, C81072396, 31470860, 81530049, Y.Z.), Knowledge Innovation Program of Chinese Academy of Sciences (XDA04020202-19, Y.Z.), the CAS/SAFEA International Partnership Program for Creative Research Teams (Y.Z.), "215" high-level health technology project (2011-J.L.) and National Science and Technology Major Project "Prevention and Treatment of AIDS and Virus Hepatitis" (2014ZX10002002-001-002, J.L.)

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

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