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REVIEW

Neutrophils: Neglected Players in Viral Diseases

Christelle Gabriel,[1] Zhisheng Her,[1] and Lisa F.P. Ng[1,2]

Increasing evidence has shown that neutrophils are able to crosstalk with various immune cells and have paradoxical roles in pathogen infections. However, the role of neutrophils in viral infections remains poorly defined. Here, the various roles that neutrophils play in viral infections and in host immunity are discussed. Various activation mechanisms of neutrophils following virus interactions and the consequences that affect disease pathogenesis are also addressed. Such knowledge not only could be used in the development of tools for clinical management but would also value-add to the current understanding of innate immunity in viral infections and disease pathogenesis.

Introduction

Neutrophils are highly motile phagocytic cells that contribute to the early immune response against invading pathogens. They were first shown by Élie Metchnikoff to participate in the host immune defense following the discovery of a population of "mobile" cells that accumulated and engulfed rose thorns that were introduced into transparent starfish larvae (Metchnikoff, 1883). Following infection, neutrophils are among the first immune cells to infiltrate the site of infection and protect against invading pathogens (Tumpey *et al.*, 2005; Fujisawa, 2008), or to cause disease exacerbation (Sakai *et al.*, 2000; Bradley *et al.*, 2012). These contrasting roles have been demonstrated in human chronic granulomatous disease and in human severe congenital neutropenia (SCN) where individuals who present defective neutrophil functions suffered from recurrent or severe bacterial and fungal infections (Quie *et al.*, 1967; Welte *et al.*, 2006; van den Berg *et al.*, 2009; Beaute *et al.*, 2011). Excessive neutrophil infiltration into tissues has also been shown to lead to tissue damage (Tanaka *et al.*, 1991; Sakai *et al.*, 2000; Ayala *et al.*, 2002; Koedel *et al.*, 2009; Narasaraju *et al.*, 2011; Bradley *et al.*, 2012; Seki *et al.*, 2012). These observations illustrate the importance of balancing the number of neutrophils in regulating their functions during the early infection phase.

Although the functions of neutrophils have been well described in bacterial infections, their roles in viral infections remain poorly characterized despite previous studies on viral respiratory infections (Larson *et al.*, 1977; Ruutu *et al.*, 1977; Rouse *et al.*, 1978; Debets-Ossenkopp *et al.*, 1980; Larson *et al.*, 1980; Mills *et al.*, 1981; Abramson *et al.*, 1982a, 1982b). Here, we present the current knowledge about the roles of neutrophils in viral infections and how understanding the immunobiology of neutrophils could be developed as prognostic tools.

Evidence of Neutrophil Interactions with Viruses

Infiltration of neutrophils at the site of virus infection

Being one of the first responders against invading pathogens, neutrophils are highly motile and can congregate massively at the site of infection; large numbers of neutrophils (up to 80% of infiltrated leucocytes) have been detected in the airways and the lungs during human respiratory syncytial virus (RSV) (Smith *et al.*, 2001; Wojtasiak *et al.*, 2010; Halfhide *et al.*, 2011), herpes simplex virus (HSV) (Wojtasiak *et al.*, 2010), and influenza virus infections (Everard *et al.*, 1994; Kim *et al.*, 2000; Inoue *et al.*, 2001; Perrone *et al.*, 2008). The magnitude of neutrophil infiltration depends directly on the dose (Bradley *et al.*, 2012) and on the strain of inoculated viruses (Perrone *et al.*, 2008; Tate *et al.*, 2011). These phenomena were clearly demonstrated in influenza virus infections where mice inoculated with a high dose of virus exhibited more severe disease symptoms than mice that were inoculated with low doses of virus (Bradley *et al.*, 2012). In addition, mice inoculated with the highly pathogenic influenza virus strains (Thai/16 H5N1, 1918 H1N1, and PR8 H1N1) had more neutrophil infiltration into the lungs and airways compared with mice that were inoculated either with the intermediate pathogenic virus strain (HKx31 H3N2) or the lower pathogenic virus strains (TX/91 H1N1, SP/83 H5N1, and BJx109 H3N2) (Perrone *et al.*, 2008; Tate *et al.*, 2011).

A direct correlation between the number of neutrophils recruited after influenza infection and the levels of neutrophil chemoattractants (MIP-1α, IFN-γ, KC, and MIP-2), and also between viral load in the lungs and disease severity has

[1]Laboratory of Chikungunya Virus Immunity, Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Biopolis, Singapore.
[2]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.

been demonstrated (Perrone *et al.*, 2008; Tate *et al.*, 2011; Bradley *et al.*, 2012), indicating the significant contribution of neutrophils to the pathology of influenza virus infection.

Neutrophils undergo a series of events that include rolling, arrest, adhesion, and extravasation to reach the infected tissues from the circulatory system (Fig. 1a, b). Migration of neutrophils takes place under the influence of key chemoattractants such as interleukin 8 (IL-8) (Kownatzki *et al.*, 1986; Endo *et al.*, 1991; Miller *et al.*, 1992; Mulligan *et al.*, 1993; Broaddus *et al.*, 1994). Despite the absence of IL-8 expression in mice (Modi and Yoshimura, 1999), functional murine IL-8 homologues were identified. Among these homologues, the chemokines KC (CXCL-1) and MIP-2 (CXCL-2) that were initially cloned and isolated, respectively, from murine activated 3T3 cells (Oquendo *et al.*, 1989) and RAW 264.7 cells (Tekamp-Olson *et al.*, 1990) were shown to trigger a massive neutrophil recruitment to the tissues (Zhang *et al.*, 2001).

Experiments performed either *in vitro* with the human airway epithelial cell line A549 (Fiedler *et al.*, 1995) or human primary neutrophils (Arnold *et al.*, 1994; Konig *et al.*, 1996), or in mouse models (Yan *et al.*, 1998; Haeberle *et al.*, 2001; Wareing *et al.*, 2004; Perrone *et al.*, 2008) have demonstrated that IL-8 and its murine homologues are inducible upon viral infections (Fig. 1c, d). IL-8 induction has also been observed in RSV-infected human neutrophils (Konig *et al.*, 1996), in HSV-infected cornea (Yan *et al.*, 1998), in RSV- (Haeberle *et al.*, 2001) and influenza-virus-infected lungs (Wareing *et al.*, 2004; Perrone *et al.*, 2008), and also in bronchoalveolar lavage fluid obtained from influenza-virus-infected mice (Tate *et al.*, 2011). Further, IL-8 was induced through activation of the

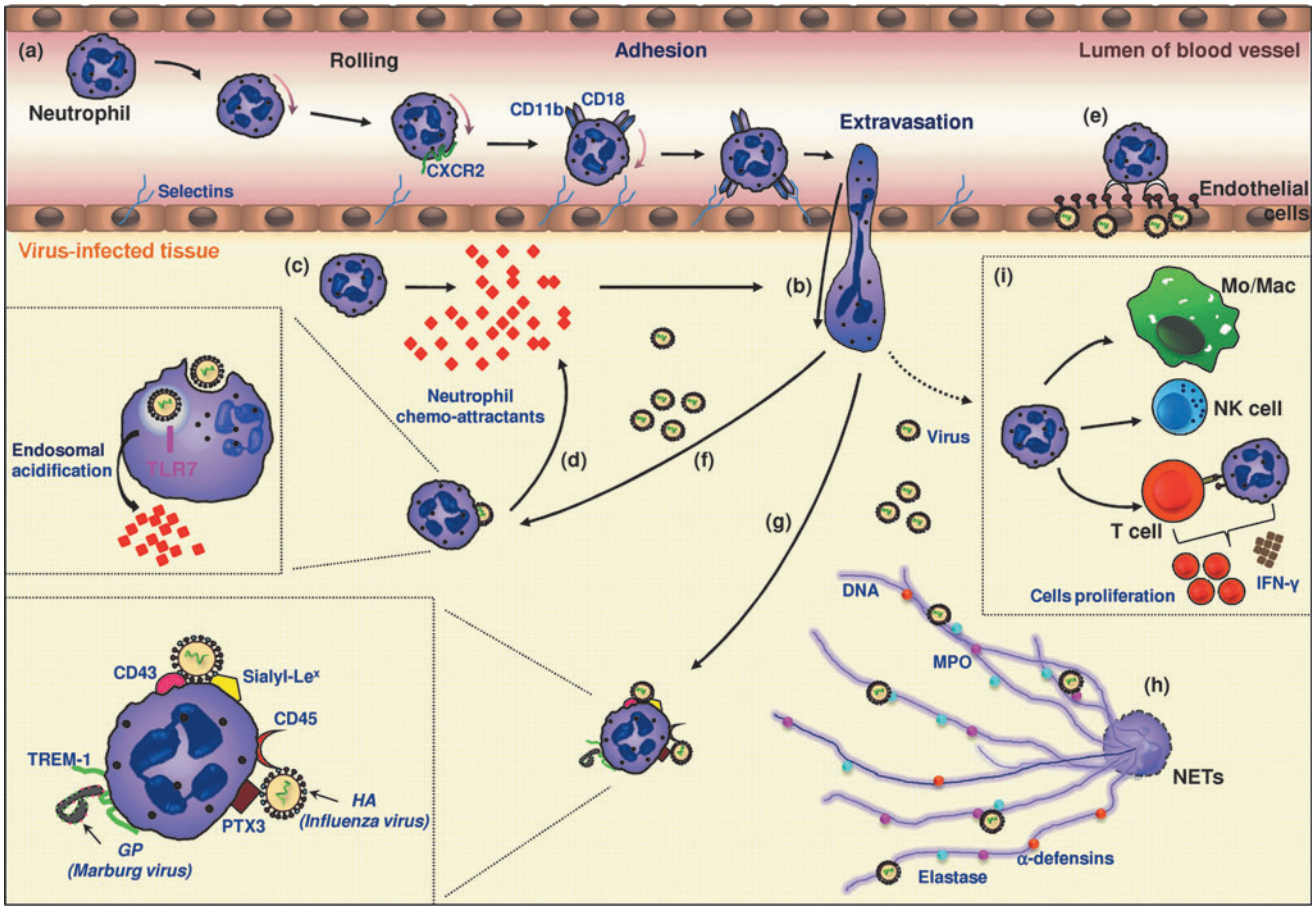

FIG. 1. Fate of neutrophils after virus infections. (a) To infiltrate virus-infected tissues, circulating neutrophils interact with endothelial cells that express various selectins on their surface. First, neutrophils express receptor molecules with low affinity for selectins (e.g., CXCR2) that slow down their progression in the blood vessel and allow them to roll along the endothelial wall. Neutrophils then firmly adhere to endothelial cells by CD11b and CD18 before they (b) extravasate to the infected tissue. Neutrophils migrate under the influence of neutrophil chemoattractant molecules, such as IL-8 (humans), KC, and MIP-2 (mice), that are released by (c) resting neutrophils, and also by (d) virus-infected neutrophils through the activation of TLR7-mediated pathway and endosomal acidification. Neutrophils can interact with either (e) virus-infected cells that present viral antigens, or (f) directly with viral particles. (g) This can be mediated by concomitant interactions between neutrophil surface proteins: CD43, CD45, pentraxin 3 (PTX3), triggering receptors expressed in myeloid cells-1 (TREM-1), proteins bearing specific sialic acid motifs (e.g., sialyl-Le^x), and viral glycoproteins. (h) Neutrophils also interact with invading viruses though the release of neutrophil extracellular traps (NETs) that are constituted by DNA and neutrophil-derived proteins, such as myeloperoxidase (MPO), elastase, and α-defensins. (i) Neutrophils can influence the antiviral response by interacting with other immune subsets, such as monocytes/macrophages (Mo/Mac), NK cells, and T cells, that proliferate and produce IFN-γ after viral antigen presentation.

TLR7 signaling pathway and endosomal acidification (Fig. 1d) (Wang *et al.*, 2008) in a time- and dose-dependent manner (Arnold *et al.*, 1994; Yan *et al.*, 1998; Sakai *et al.*, 2000; Tumpey *et al.*, 2005).

In addition, matrix metalloprotease MMP-9 (Bradley *et al.*, 2012), IL-10 (Mutnal *et al.*, 2010), IL-6 (Fenton *et al.*, 2002; Dienz *et al.*, 2012), and IL-1 (Schmitz *et al.*, 2005) were also implicated in the regulation of neutrophil migration upon viral infections.

Virus interaction

Neutrophils could interact with virus-infected cells and/or virions once infiltrated (Fig. 1e–h). Neutrophils were shown to interact specifically with RSV-infected fibroblasts (Van Strijp *et al.*, 1989), and in influenza-virus-infected epithelial cells that presented the influenza haemagglutinin glycoprotein (HA) on their surface (Ratcliffe *et al.*, 1988). While neutrophil surface molecule CD43 (also known as sialophorin and Ly48) was later identified to bind specifically to influenza virus (Rothwell and Wright, 1994; Abramson and Hudnor, 1995), further investigations using phorbol myristate acetate (PMA) or neutrophil elastase (treatments that cleave CD43 from neutrophil surface) indicated that CD43 was not the unique binding molecule.

To identify novel surface binding molecules, treatment with blocking antibodies that target against a diverse set of surface molecules was done on neutrophils to examine the interaction between neutrophils and influenza virus (Hartshorn *et al.*, 1995). Interestingly, the interaction between neutrophils and influenza virus was shown to mediate through concomitant interactions involving CD43, sialic acid bearing cellular proteins (e.g., sialyl-Le^x antigen), CD45, and influenza HA protein (Fig. 1g).

TREM-1 (triggering receptors expressed in myeloid cells-1), a neutrophil surface protein (Bouchon *et al.*, 2000), has been reported to bind to the surface glycoprotein (GP) expressed by the filovirus Marburg virus (MARV) (Fig. 1g), where TREM-1 recombinant protein was shown to interact with Vero cells that express MARV GP (Mohamadzadeh *et al.*, 2006).

Pentraxin 3 (PTX3), also known as TNF-stimulated gene 14 (TSG-14), was another molecule suggested to be involved in neutrophil–virus interactions as direct interaction was shown with the influenza HA protein (Reading *et al.*, 2008) (Fig. 1g). Moreover, neutrophils also express PTX3 on neutrophil extracellular traps (NETs) (Lominadze *et al.*, 2005; Jaillon *et al.*, 2007) that are networks of fibers released by activated neutrophils (Brinkmann *et al.*, 2004; Yipp *et al.*, 2012).

Virus internalization and replication

The ability of neutrophils to internalize viruses was investigated by confocal microscopy (Mohamadzadeh *et al.*, 2006), PCR (Larochelle *et al.*, 1998; Gerna *et al.*, 2000; Zhao *et al.*, 2008), flow cytometry (Hufford *et al.*, 2012), and immunochemistry (Zhao *et al.*, 2008) to detect the presence of viral-associated molecules and particles (Hackemann *et al.*, 1974; Larochelle *et al.*, 1998). The development of green fluorescent protein–tagged viruses also allowed the visualization of internalized viruses (Duffy *et al.*, 2012; Hufford *et al.*, 2012).

While murine neutrophils are not susceptible to influenza virus (Tate *et al.*, 2011) and alphavirus (Levitt *et al.*, 1979) infections, human neutrophils are susceptible to these viruses (Abramson *et al.*, 1982b, 1986; Zhao *et al.*, 2008) and others, such as MARV (Mohamadzadeh *et al.*, 2006), Epstein-Barr virus (EBV) (Larochelle *et al.*, 1998), and RSV (Halfhide *et al.*, 2011). Viruses may enter human neutrophils by endocytosis or phagocytosis. However, with the exception of MARV (Mohamadzadeh *et al.*, 2006), active replication for EBV (Larochelle *et al.*, 1998), RSV, and influenza virus (Cassidy *et al.*, 1988; Zhao *et al.*, 2008) was presented. Therefore, virus infection in neutrophils could either facilitate virus clearance and control disease progression, or it may exacerbate disease manifestations.

Dual Function of Neutrophils in Viral Infections

A wide variety of neutrophil-deficient mouse models have been successfully used to assess the functional roles of neutrophils in viral infections. For example, neutrophils can be ablated in mice by various methods including exposure to gamma rays (Tsuru *et al.*, 1987); administration of antibodies directed against Gr1 (RB6-8C5), Ly6G (1A8) (Tate *et al.*, 2009; Tate *et al.*, 2011; Bradley *et al.*, 2012), and MIP-2 (Sakai *et al.*, 2000); or through the depletion of neutrophil chemoattractant molecules, such as IL-17RA (Crowe *et al.*, 2009), MMP-9 (Bradley *et al.*, 2012), IL-10 (Mutnal *et al.*, 2010), and CXCR2 (Wareing *et al.*, 2007). Collectively, these studies revealed that neutrophils could either limit or exacerbate disease progression (Fig. 2).

Neutrophils control viral infections through the release of reactive oxygen species and granular proteins

Neutrophils have been demonstrated to eliminate invading pathogens via various mechanisms: oxygen-independent (Belaaouaj *et al.*, 2000; Standish and Weiser, 2009) and oxygen-dependent (Reeves *et al.*, 2002; Ellson *et al.*, 2006) pathways, and NETosis (Brinkmann *et al.*, 2004).

The oxygen-independent pathway is mediated by antimicrobial proteins contained in neutrophil granules that include small cationic α-defensin proteins. Four α-defensins (HNP1–4) have been identified in human neutrophils (Ganz *et al.*, 1985; Wilde *et al.*, 1989; Date *et al.*, 1994), and they constitute up to 50% of neutrophil proteins that are stored in azurophilic granules (Ganz *et al.*, 1985; Rice *et al.*, 1987). These are released in large amounts when stimulated (Ganz, 1987) in response to MIP-1α, MIP-1β, RANTES (Jan *et al.*, 2006), and also during microbial infections (Panyutich *et al.*, 1993; Ihi *et al.*, 1997; Maffei *et al.*, 1999; Ashitani *et al.*, 2002).

Despite the lack of α-defensins in murine neutrophils (Eisenhauer and Lehrer, 1992), the antiviral ability of HNP1–4 was demonstrated (Ganz *et al.*, 1985; Daher *et al.*, 1986; Mackewicz *et al.*, 2003; Chang *et al.*, 2005; Wu *et al.*, 2005; Demirkhanyan *et al.*, 2012). Using recombinant and purified human α-defensins, HNP1–4 inhibited HIV-1 infectivity (Mackewicz *et al.*, 2003; Chang *et al.*, 2005; Demirkhanyan *et al.*, 2012), HSV-1 (Ganz *et al.*, 1985; Daher *et al.*, 1986), and influenza A/WSN virus (Daher *et al.*, 1986). Further characterization was performed in HIV-1 infections where direct interactions between α-defensins and both host receptor and viral envelope proteins were detected (Furci *et al.*, 2007; Demirkhanyan *et al.*, 2012).

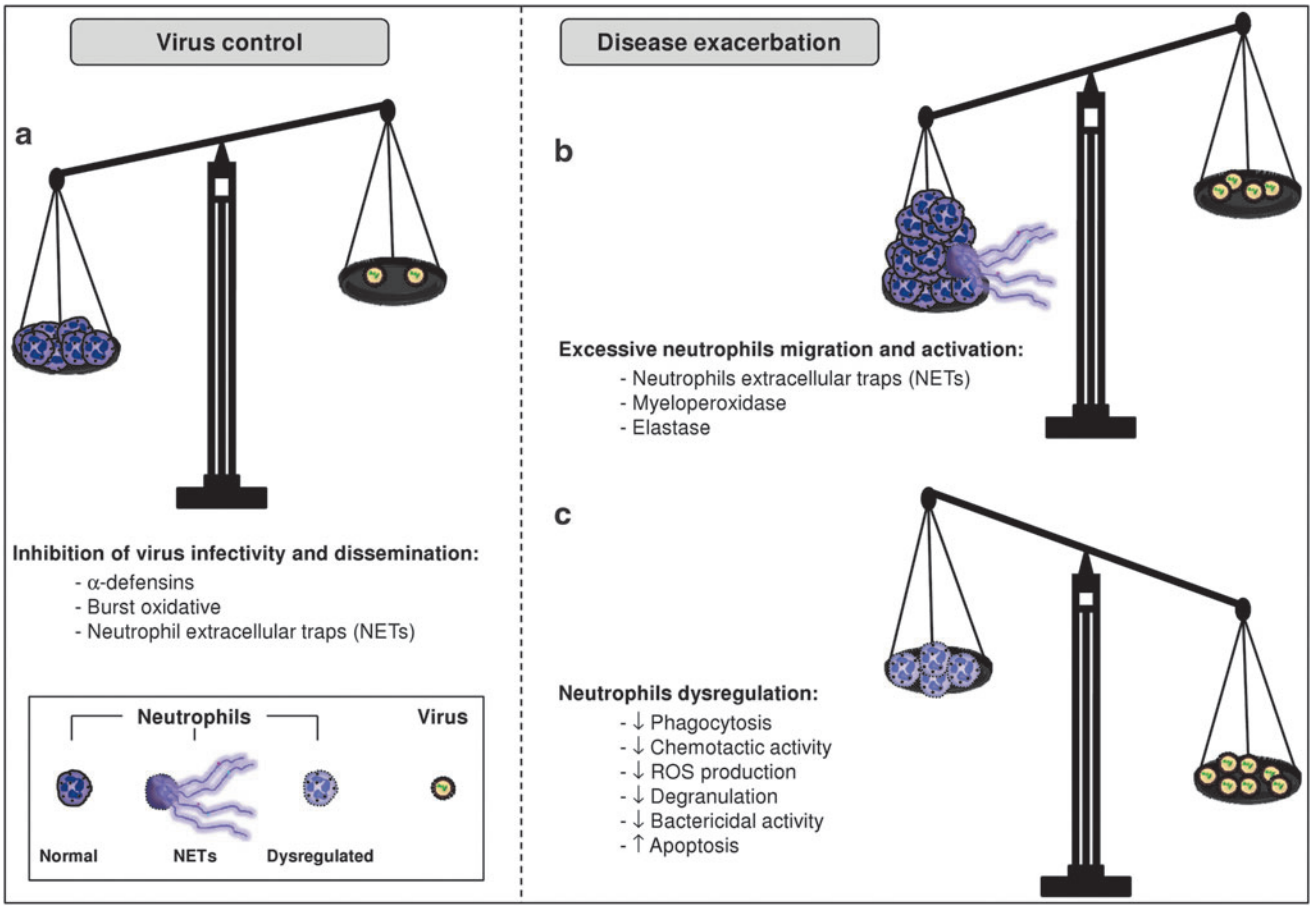

FIG. 2. Contradictory roles of neutrophils. Neutrophils can (a) control viral infections by releasing antimicrobial components, such as α-defensins and reactive oxygen species (ROS), and produce NETs. Such events can lead to the inhibition of virus infectivity, viral RNA degradation, and restrict virus dissemination. However, (b) excessive migration and overactivation of neutrophils can be detrimental to the host and exacerbate disease manifestation. Two neutrophil-related proteins, myeloperoxidase and elastase, are known to cause tissue damage. (c) On the other hand, viruses can dysregulate neutrophil functions and alter phagocytosis, migration, ROS release, and degranulation. These often lead to secondary bacterial infections. Viruses can also induce apoptosis in neutrophils.

Although little is known about the interactions between NETs and viruses, few evidences demonstrated their protective role in viral infections (Saitoh *et al.*, 2012; Jenne *et al.*, 2013). Using microscopy, LPS- and PMA-induced NETs were shown to capture Myxoma virus and HIV-1 to prevent virus dissemination into host tissues. Moreover, myeloperoxidase (MPO; another neutrophil granular protein) and α-defensins, which are associated to NETs, could inhibit HIV-1 infectivity (Saitoh *et al.*, 2012) (Fig. 1h).

The oxygen-dependent pathway (also called burst oxidative) that requires NADPH and MPO enzymes to catalyze the conversion of O_2 into reactive oxygen species (ROS) (e.g., O_2- and H_2O_2) was triggered in neutrophils when incubated with Japanese encephalitis virus (JEV) (Srivastava *et al.*, 1999). Although neutrophils were described to release H_2O_2 and degrade viral RNA, a direct correlation between ROS and killing of JEV has yet to be established.

More recently, neutrophils have been shown to disseminate virus from the skin to the bone marrow and lymph nodes (Duffy *et al.*, 2012). Further, neutrophil depletion with 1A8 antibody unveiled neutrophils' ability to present viral antigens to CD8+ T cells located in the bone marrow (Duffy *et al.*, 2012) and in the lungs (Hufford *et al.*, 2012) to induce CD8+ T cell proliferation (Duffy *et al.*, 2012) and IFN-γ secretion (Hufford *et al.*, 2012) (Fig. 1i).

Dysregulation of neutrophils functions and migration can lead to disease severity

Viruses have evolved several antiviral evasion mechanisms in order to establish long-term virus production in the infected host. Such mechanisms have been described for influenza virus, cytomegalovirus, and EBV infections where neutrophils were altered in their capacity to produce ROS (Abramson *et al.*, 1982b, 1984; Hartshorn *et al.*, 1995; Cooper *et al.*, 1996). In addition, they have been shown to impair the migratory (Ruutu *et al.*, 1977; Larson *et al.*, 1980; Debets-Ossenkopp *et al.*, 1982; Abramson *et al.*, 1984; Cooper *et al.*, 1996) and adhesive properties of neutrophils (Abramson *et al.*, 1984), as well as in the release of effector proteins by degranulation (Abramson *et al.*, 1984; Pang *et al.*, 2000). Moreover, EBV has been reported to induce neutrophil apoptosis (Larochelle *et al.*, 1998). Complementing these observations, the nucleoprotein (Cooper *et al.*, 1996) and neuraminidase (Debets-Ossenkopp *et al.*, 1982), proteins

purified from influenza virus, were shown to alter neutrophil functions.

Influenza virus infections are often followed by secondary bacterial infections in the respiratory tract (Morens *et al.*, 2008). Several studies demonstrated that neutrophils were unable to eliminate bacteria efficiently after influenza virus infection because of the inability to degranulate (Abramson *et al.*, 1982a, 1982b; Debets-Ossenkopp *et al.*, 1982; Pang *et al.*, 2000; Seki *et al.*, 2004).

Such impairments in neutrophil functions were observed in HIV infection where neutrophils of HIV-infected individuals were defective in ROS production (Elbim *et al.*, 1994; Salmen *et al.*, 2012), expression of adhesion molecules (Elbim *et al.*, 1994; Moore *et al.*, 1998), migration (Tufail *et al.*, 2000), antibacterial activity (Ellis *et al.*, 1988), and enhanced apoptosis (Ellis *et al.*, 1988; Salmen *et al.*, 2004).

Neutropenia is a clinical parameter commonly associated with disease severity in HIV-positive patients (Hermans *et al.*, 1996; Moore *et al.*, 2001; Babadoko *et al.*, 2008). While the etiology of neutropenia is multifactorial and often related to the use of myelosuppressive drugs (Sawka *et al.*, 1992), future studies to investigate whether neutropenia is a consequence of enhanced spontaneous apoptosis will be insightful.

Secondary bacterial infections were frequently observed in HIV-infected patients (Krumholz *et al.*, 1989; Fichtenbaum *et al.*, 1994), and neutropenia severity has been demonstrated to correlate with the incidence of bacterial infections (Farber *et al.*, 1991; Keiser *et al.*, 1996; Jacobson *et al.*, 1997). Moreover, treatment of HIV-infected patients using filgrastim (an analogue of G-CSF that reverses neutropenia) was shown to decrease the incidence of bacterial infections (Kuritzkes *et al.*, 1998).

Excessive migration or overactivation of neutrophils to the sites of infection could worsen the severity of disease manifestations (Sakai *et al.*, 2000; Seki *et al.*, 2010; Narasaraju *et al.*, 2011; Bradley *et al.*, 2012; Sugamata *et al.*, 2012). This has been illustrated in influenza virus infection where a reduction of neutrophil infiltration into the lungs was associated with attenuation of tissue damage (Sakai *et al.*, 2000; Seki *et al.*, 2010; Bradley *et al.*, 2012; Sugamata *et al.*, 2012). Moreover, neutrophil-derived proteins, such as MPO (Seki *et al.*, 2010; Sugamata *et al.*, 2012) and elastase, (Seki *et al.*, 2010) are involved in tissue injury.

Finally, the release of NETs into virus-infected lungs could explain the contribution of neutrophils in disease severity. Using super resolution structured illumination microscopy (SR-SIM) and scanning electron microscopy (SEM), the interaction between NETs and HIV-1 was visualized (Narasaraju *et al.*, 2011; Saitoh *et al.*, 2012); HIV-1 was eliminated through the action of MPO and α-defensins present on NETs (Saitoh *et al.*, 2012). However, HIV-1 could also inhibit NET formation by inducing C-type lectin-dependent production of IL-10 by dendritic cells (Saitoh *et al.*, 2012). The relevance of NET-mediated antiviral response in the disease pathology would need to be further validated.

Crosstalk Between Neutrophils and Other Immune Cells

As part of the cellular immune response, neutrophils interact and crosstalk with various immune subsets such as monocytes/macrophages (Mo/Macs) and natural killer cells (NK cells) that both display functional features in viral infections (Fig. 1i).

Crosstalk between neutrophils and Mo/Macs

Mo/Macs are important effectors in viral infections as shown for dengue virus (Fink *et al.*, 2009), influenza virus (van Riel *et al.*, 2011), and chikungunya virus (Her *et al.*, 2010). They contribute to the host antiviral response through the production of cytokines and chemokines (Molina *et al.*, 1990; Fauriat *et al.*, 2010). Neutrophils and macrophages are derived from a common hematopoietic cell progenitor (Inaba *et al.*, 1993) and share several common features in phagocytosis, antigen presentation (Pfeifer *et al.*, 1993; Berg *et al.*, 1994; Kovacsovics-Bankowski and Rock, 1995; Beauvillain *et al.*, 2007; Abi Abdallah *et al.*, 2011; Ostanin *et al.*, 2012), and immunoregulation. During inflammation, neutrophils promote Mo/Mac migration (Taekema-Roelvink *et al.*, 2001; Janardhan *et al.*, 2006; Soehnlein *et al.*, 2008) and extravasation (Chertov *et al.*, 1997; Soehnlein *et al.*, 2008) to the site of infection. However, neutrophils could also be used by pathogens as "Trojan horses" as shown in *Leishmania* infection where apoptotic *Leishmania*-infected neutrophils were internalized by macrophages (Peters *et al.*, 2008). Therefore, Mo/Macs and neutrophils could influence the antiviral response and control disease pathology.

Crosstalk between neutrophils and NK cells

Crosstalk events between neutrophils and NK cells have also been reported as NK cells and neutrophils locate in close proximity in the lymph nodes and spleen to make conjugates (Jaeger *et al.*, 2012). Neutrophils also regulate NK cell functions (Seaman *et al.*, 1982; Dallegri *et al.*, 1985; Shau and Kim, 1988; Shau and Golub, 1989; Gabrilovich *et al.*, 1993; Sporri *et al.*, 2008), maturation (Jaeger *et al.*, 2012), and homeostasis (Jaeger *et al.*, 2012). Recent evidences revealed that NK cells were less responsive, more proliferative, and less mature in patients suffering from SCN (Jaeger *et al.*, 2012). This was demonstrated in the *Genista* mouse model with defective neutrophils and a novel form of neutropenia due to a point mutation in the transcriptional repressor growth factor independence 1 (Gfi1) (Jaeger *et al.*, 2012). This was further substantiated in two other studies where neutrophils were demonstrated to be key activators of NK cells (Sporri *et al.*, 2008; Costantini *et al.*, 2011). Although human neutrophils were demonstrated to increase NK-derived IFN-γ production via CD18- and ICAM-3-dependent pathways (Costantini *et al.*, 2011), GM-CSF and/or IFN-γ released from activated NK cells regulate neutrophil survival and trigger activation by increasing CD64 (FcγRI) and CD11b expression on neutrophils (Costantini *et al.*, 2010).

Neutrophils as a Tool to Distinguish Between Bacterial and Viral Infections

Neutrophils have been used as diagnostic tools in clinical settings: neutrophil absolute count (Wile *et al.*, 2001; Pratt and Attia, 2007; Bressan *et al.*, 2010; Manzano *et al.*, 2011), enumeration of band neutrophils (band count) (Seebach *et al.*, 1997; Al-Gwaiz and Babay, 2007), and the expression levels of neutrophil surface proteins (Nupponen *et al.*, 2001; Livaditi *et al.*, 2006; Adib *et al.*, 2007; Nuutila *et al.*, 2007;

Rudensky *et al.*, 2008; Dilli *et al.*, 2010; Genel *et al.*, 2012; Jia *et al.*, 2013) to predict bacterial infections and severity outcomes. Due to the increasing evidence of neutrophils being involved in mediating viral diseases (Tumpey *et al.*, 2005; Duffy *et al.*, 2012), it is now possible to develop new diagnostic tools capable of distinguishing between viral and bacterial diseases. Some of these tools include the analysis of neutrophil activation markers, such as CD64 (FcγRI) and CD35 (also known as complement receptor 1, CR1), which are expressed at different levels in patients infected with bacteria or viruses (Jalava-Karvinen *et al.*, 2009). This method has been used to distinguish between bacteria- and virus-induced arthritis (Mokuda *et al.*, 2012), and between dsDNA and ssRNA virus infections (Nuutila *et al.*, 2008). Moreover, bacterial and viral infections could also be distinguished by a lumino-dependent chemiluminescence assay that measures the amount of ROS produced by neutrophils upon activation (Prilutsky *et al.*, 2011). As new knowledge continue to unravel the behavior of neutrophils in virus infections, it is conceivable to suggest that future tools should be developed to predict disease severity and efficacy of antiviral treatments in the clinics.

Perspectives

As new evidences unfold, neutrophils are now recognized for their complexity in various infectious and inflammatory diseases. However, their contrasting roles in influencing the outcome of disease pathogenesis create a challenge. Nonetheless, neutrophils remain one of the most important cells for early first-line innate defense, and serve as an important conduit in the cross-roads of adaptive immunity. These previously negleted players should be considered as interesting targets for antimicrobial treatments in clinical settings.

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Address correspondence to:
Lisa F.P. Ng, PhD
Laboratory of Chikungunya Virus Immunity
Singapore Immunology Network (SIgN)
*Agency for Science, Technology and Research (A*STAR)*
8A Biomedical Grove
#04-06
Immunos, Biopolis 138648
Singapore

E-mail: lisa_ng@immunol.a-star.edu.sg

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