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The lymph node neutrophil

Henry R. Hampton[a,b], Tatyana Chtanova[a,b,*]

[a] Immunology Division, The Garvan Institute of Medical Research, Sydney, Australia
[b] St Vincent's Clinical School, Faculty of Medicine, University of New South Wales, Sydney, Australia

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ABSTRACT

Secondary lymphoid organs provide a specialized microenvironment tailored to foster communication between cells of the innate and adaptive immune systems. These interactions allow immune cells to coordinate multilayered defense against pathogens. Until recently dendritic cells and macrophages were thought to comprise the main innate immune cell subsets responsible for delivering signals that drive the adaptive immune response, while the function of neutrophils was largely confined to the innate immune system. However, the discovery of neutrophils in lymph nodes has raised the question of whether neutrophils might play a more extensive role not only in innate immunity *per se*, but also in coordinating the interactions between innate and adaptive immune responses. In this review we discuss the mechanisms and consequences of neutrophil recruitment to lymph nodes and how this recruitment influences subsequent immune responses both *in situ* and at distant sites.

1. Introduction

The immune system of vertebrates consists of two interrelated layers of defense against infection controlled by innate (non-antigen specific) and adaptive (antigen-specific) immune cells respectively [1]. Among the former are professional phagocytes like macrophages, neutrophils and dendritic cells. Innate immune cells provide the first line of defense by trapping and killing pathogens, which they identify by expression of evolutionary-conserved structures, known as pathogen associated molecular patterns (PAMPs) [2]. These include bacterial and fungal cell wall components such as LPS and β-glucans [2]. The host cell surface receptor that senses a PAMP is known as a Pattern Recognition Receptor (PRR) and is not antigen-specific [2]. In the case of neutrophils, their critical role in bacterial clearance, inflammation and wound healing is well recognized and leads to serious deleterious effects for the host when neutrophil development, migration or function is defective [3]. However, in addition to direct pathogen killing, innate immune cells including neutrophils play an important role in initiating and modulating subsequent adaptive immune responses [2,4].

The second layer of immune defense, adaptive immunity, is mediated by antigen-specific T and B lymphocytes present in secondary lymphoid organs such as lymph nodes and spleen [5]. Here, antigens together with co-stimulatory signals are delivered by innate immune cells to the rare antigen-specific T and B cells [6]. The resulting immune response leads to the generation of two classes of activated cells, namely effector cells including a range of killer cells [7] and antibody-secreting cells [8] as well as memory cells [9] responsible for long-term protection against reinfection. In practical terms, rapid cellular communication between the site of microbial invasion and regional lymphoid tissues like the draining lymph node is therefore essential for successful immune responses against pathogens.

Dendritic cells, together with macrophages, were thought to be primarily responsible for cellular transport of antigens from the site of infection into regional lymph nodes [6]. However, according to recent studies in mice, neutrophils also rapidly enter draining nodes following a variety of challenges at peripheral sites [10–15]. Furthermore, they are the first cells to migrate to secondary lymphoid organs carrying bacterial particles [10,15], which can be processed and presented to T and B lymphocytes. This places neutrophils in a unique position to participate in the very early stages of both innate and adaptive immune responses in draining lymph nodes.

Several *in vitro* studies have characterized the cross talk between human neutrophils and cells of the innate and adaptive immune systems [16,17] and have been reviewed elsewhere [18,19]. Here we will focus on how the rapid dissemination of murine neutrophils from the site of inflammation to draining

* Corresponding author at: Immunology Division, The Garvan Institute of Medical Research, 384 Victoria St, Darlinghurst NSW 2010, Sydney, Australia.
E-mail address: t.chtanova@garvan.org.au (T. Chtanova).

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lymph nodes shapes subsequent immune responses, what stimuli drive this process and the molecules are responsible for neutrophil recruitment *in vivo*. Although many differences exist between human and murine neutrophils, detailed analysis of the mouse lymph node neutrophil may provide additional insight into neutrophil functions in human lymph nodes.

2. Induction of neutrophil recruitment to lymph nodes

Neutrophils are rapidly recruited to peripheral inflammatory sites [20]. Likewise, neutrophils can be found in the draining lymph node within 15 min of adjuvant injection [11]. Neutrophil accumulation in draining lymph nodes has also been observed following injection of bacteria such as *Staphylococcus aureus* [15,21], *Pseudomonas aeruginosa* [22], *Listeria monocytogenes* [13], *Yersinia pestis* [23] or BCG [10], infections with the protozoan parasites *Toxoplasma gondii* [13] and *Leishmania major* [24] and intradermal administration of viruses like modified Vaccinia virus Ankara [25,26].

Furthermore, neutrophil recruitment to lymph nodes has been observed following lysis of a tumor grown in murine ears [27]. However, neutrophil influx following induction of a sterile lesion with a needle scratch to ear skin was confined to the scratch site, suggesting that some sterile stimuli induce localized recruitment without subsequent neutrophil relocalisation to lymph nodes [15]. Collectively, these observations show that a wide variety of microbial, viral and even some sterile stimuli can induce neutrophil recruitment to draining lymph nodes.

Neutrophil entry into tissues is guided by inflammatory cytokines such as IL-1β and TNF-α derived from tissue resident macrophages [28]. Similarly, macrophage-induced IL-1β together with IL-1R signaling in neutrophils was required for neutrophil entry into the popliteal lymph node after a footpad injection of *P. aeruginosa* [29]. This combination of IL-1β/IL1-R was also required for lymph node recruitment of neutrophils following an injection of modified Vaccinia virus Ankara [25] and local tumor lysis [27]. In line with these observations, neutrophil recruitment to lymph nodes in response to *T. gondii* was reduced in mice lacking the TLR/IL-1R adaptor protein MyD88 [13].

The pro-inflammatory cytokine IL-17A recruits neutrophils to inflammatory foci [30]. In mice lacking the IL-17A receptor (IL-17RA) fewer neutrophils were recruited to the draining lymph node following lysis of a tumor grown in murine ears [27]. In the same model, an intravenous injection of an IL-17A blocking antibody had a similar inhibitory effect [27]. These results suggest that at least some of the molecules responsible for neutrophil homing to inflamed tissues also regulate neutrophil recruitment to lymph nodes. Further research is needed to identify other possibly unique signals involved in controlling neutrophil influx to lymph nodes.

3. Neutrophil localization and dynamics within draining lymph nodes

The localization of different immune cells within secondary lymphoid organs is tightly linked to their particular functions. Therefore, analysis of neutrophil positioning within the lymph node (Fig. 1), which is influenced by pathogen localization as well as the mode of neutrophil recruitment, may shed greater light on lymph node-specific neutrophil functions.

Several studies including ours have reported rapid neutrophil accumulation in the subcapsular sinus of draining lymph nodes [11,13,29]. This region (Fig. 1), located just below the lymph node capsule, is the site of entry for pathogens and innate immune cells including neutrophils [10,11,13,29] located in afferent lymphatic vessels. In addition, neutrophils arriving in lymph nodes from the bloodstream can move to the subcapsular sinus [21], possibly in response to signals from antigen-capturing cells such as subcapsular sinus macrophages that reside in this area.

In vivo imaging has shed new light on neutrophil dynamics in inflamed tissues including lymph nodes [13,31–33]. Neutrophils, recruited to lymph nodes in response to infection with the protozoan parasite *T. gondii,* were observed to migrate rapidly in coordinated 'swarms' which coincided in space and time with removal of the underlying tissue suggesting that swarm formation may provide a mechanism for large scale tissue remodeling [13]. Furthermore, in the draining lymph node, these neutrophils swarmed around and destroyed infected subcapsular sinus macrophages [13], which sample antigen from the lymph and can present it to B [34] and T [35] lymphocytes. At the same time two-photon microscopy has shown that neutrophils can themselves phagocytose *S. aureus* in the subcapsular sinus [21]. Rapid localization of neutrophils to the subcapsular sinus therefore limits further pathogen spread via killing of pathogens and destroying infected cells.

Neutrophils have also been observed in the interfollicular zone of the lymph node following challenges with *P. aeruginosa, S. aureus* and *Salmonella enterica* [21,29,36]. In this region, located directly below the subcapsular sinus, neutrophils are positioned in close proximity to T and B cells as well as innate-like lymphocytes such as γδ T, NK and NKT cells [21,29]. The innate-like lymphocytes found in the interfollicular zone secrete IFN-γ, which enhances the anti-pathogen activities of adjacent subcapsular sinus macrophages [29]. Interfollicular neutrophils may act to amplify the anti-pathogen activities of the innate-like leukocytes found in this region. For example, a recent study demonstrated that murine neutrophils could form cell-to-cell conjugates with NK cells *in vitro* and were required for optimal NK cell activity [37]. Likewise, human neutrophils interacted with NK cells *in vitro* to enhance IFN-γ production by NK cells in a cell-contact dependent manner [16].

A small number of neutrophils have been detected in the T cell zone following an injection of *S. aureus* [21] and after administration of BCG [10]. These may represent neutrophils recruited to lymph nodes via the HEVs located throughout the T cell zone [38]. It is not yet clear whether neutrophils found in this region are simply in transit on their way to pathogen-rich regions of the lymph node or are involved in lasting interactions with T cells and dendritic cells. Some studies have shown that neutrophils can present antigen to T cells *in vitro* [39]. However, it is not known whether lymph node neutrophils can present antigen to T cells *in vivo*.

Following microbial challenge, neutrophils have also been observed in the medullary regions of lymph nodes [21,29]. In addition to containing macrophages that phagocytose pathogens, this region supports lymphocyte egress from the lymph node and aids in plasma cell development [40]. Little is known about the precise role of neutrophils in this anatomical location, but they could be positioned to prevent pathogen spread via the efferent lymphatic vessels. Neutrophils have also been observed to interact with medullary plasma cells following a *S. aureus* challenge [21]. However, the functional consequences of neutrophil-medullary plasma cell interactions are yet to be identified.

Interestingly, neutrophils appear to be excluded from B cell follicles after a bacterial challenge [21]. However, neutrophils already present within lymph nodes can enter B cell follicles following a sterile injury to this site [21]. This observation suggests that neutrophils can be recruited to B cell follicles when inflammatory signals are present there at sufficient levels.

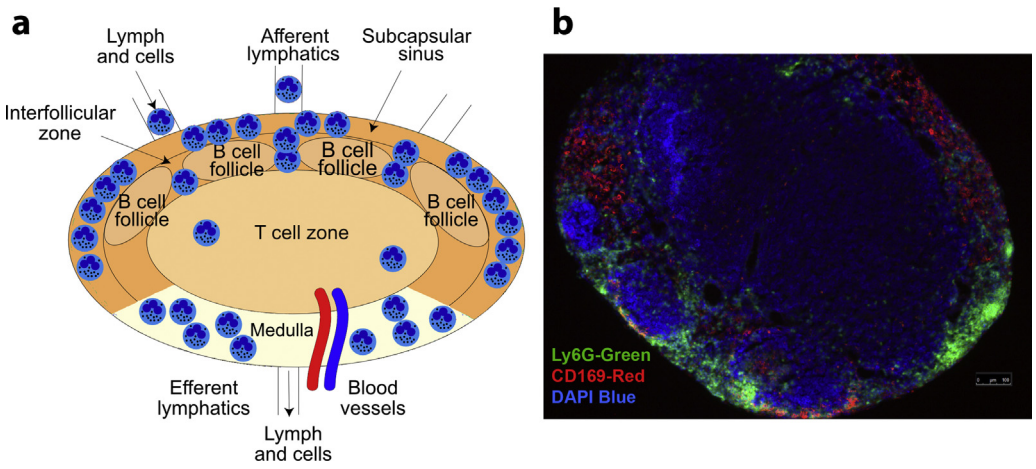

Fig. 1. Neutrophil localization within lymph nodes. (A) Diagram showing locations of lymph node neutrophils recruited in response to microbial challenges. Neutrophils have been observed in the subcapsular sinus, medullary region, interfollicular zone and T cell zone. (B) Immunofluorescence microscopy image of a frozen section of a draining lymph node 8 h after an intradermal injection of *S. aureus*. The lymph node was fixed in formalin and then frozen in OCT. DAPI (blue), Ly6G (neutrophils, green), CD169 (subcapsular sinus macrophages, red). Scale bar represents 100 μm.

4. Mechanisms of neutrophil recruitment to lymph nodes

Neutrophils are the first immune cells to be recruited in response to infection or tissue damage. Intravital microscopy has provided some key insights into the multistep cascade that governs neutrophil migration out of the blood vessels towards inflammatory stimuli [41,42]. Briefly, neutrophils first tether to the activated endothelium expressing E- and P-selectins [43]. This step is followed by rolling and then firm adherence which is mediated by binding of activated β2 and α4 integrins to ICAM-1 and VCAM-1 on endothelial cells [41]. Neutrophils leave the vasculature via transmigration, after which they follow chemoattractant gradients to the site of inflammation [44]. Although neutrophil recruitment into inflamed tissue sites has been extensively studied, the mechanism of recruitment to lymph nodes is less well characterized.

4.1. Lymph node entry via the HEVs

Neutrophils, like T and B cells, can enter lymph nodes from the bloodstream by crossing high endothelial venules (HEVs). The transmigration of neutrophils across HEVs has been observed following an intradermal injection of *S. aureus* [21] and CFA [45] as well as after *in situ* lysis of tumors grown in murine ears [27]. While the molecules required for lymphocyte migration across HEVs are well-defined, and include L-selectin, LFA-1, VLA-4 and CCR7 [38], less is known about the mechanism of neutrophil entry into lymph nodes via the HEVs (Table 1). Administration of an L-selectin blocking antibody reduced neutrophil recruitment to lymph nodes following injections of *S. aureus* [15] or CFA [45] and tumor cell lysis [27]. Additionally, PSGL-1, which can bind to all members of the selectin family [46], was required for immune complex-stimulated entry of neutrophils into lymph nodes [45]. Blockade of the β2 integrin LFA-1 prevented neutrophil entry into lymph nodes in response to killed *S. aureus* [15] and immune complexes [45]. The chemokine receptor CXCR2 is expressed by neutrophils and is required for neutrophil entry into various tumors and inflammatory lesions [47]. CXCR2-deficient mice showed reduced neutrophil accumulation in draining lymph nodes in a tumor lysis model. Consistent with this finding, blocking the CXCR2 ligand, CXCL2, in the same experimental system resulted in a reduction in neutrophil influx to lymph nodes [27]. By contrast, CXCR2 was dispensable for *S. aureus*-induced neutrophil recruitment to lymph nodes [15], suggesting that certain stimuli can over-ride the requirement for this receptor.

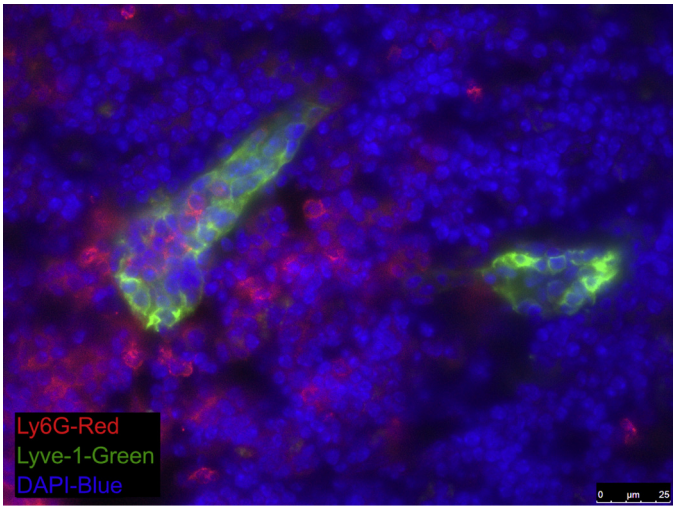

Fig. 2. Neutrophils enter the lymph node via lymphatic vessels. Frozen section of a draining lymph node from a C57BL/6J mouse 8 h following ear skin injection of *S. aureus*. Ten micron sections of the draining lymph node were cut and immunofluorescence microscopy was used to investigate neutrophil location in the draining lymph node. DAPI (blue), Ly6G (neutrophils, green), Lyve-1 (lymphatic vessels, green). Scale bar represents 25 μm.

4.2. Lymph node entry via lymphatic vessels

The long-held view that neutrophils are excluded from lymphatic vessels has been challenged by several studies analyzing neutrophil localization in murine lymph nodes [10,11,13] as well as by analysis of afferent lymph in sheep [48–50]. Intravital microscopy of inflamed skin showed neutrophils migrating within lymphatic vessels [15]. This was followed by relocalisation of neutrophils to draining lymph nodes in response to microbial injection [15]. Moreover, neutrophils could be seen inside lymphatic vessels within lymph nodes (Fig. 2). Lymphatic migration has also been observed following an injection of Complete Freund's Adjuvant (CFA) into the footpad of mice [11,45]. Furthermore, neu-

Table 1
Cell surface molecules possibly required for neutrophil migration into lymph nodes and inflamed tissues.

Molecule	**Required for lymph node entry via HEVs**	**Required for lymph node entry via afferent lymphatics**	**Required for entry into inflamed tissues**
CD62L (L-Selectin)	Yes [15,27,45]	No [15,45]	Peritoneum [56]
CD62P (P-Selectin)	Yes [45]	Not tested	Peritoneum [57]
CD62E (E-Selectin)	Not tested	Not tested	Peritoneum [57]
CD162 (P-selectin glycoprotein ligand-1)	Yes [45]	No [45]	Lung [58]
LFA-1 (CD11a)	Yes [15,45]	Required only in some situations [15,45]	Brain [59], mesentery [60]
Mac-1 (CD11b)	Yes [15,45]	Yes [15,45,54]	Glomerulus [42], liver [44], mesentery [60]
VLA-4	Not tested	Not tested	Lung [61], brain [62], mesentery [60]
ICAM-1	Yes [15]	Required only in some situations [15,54]	Liver [44]
ICAM-2	Not tested	Not tested	Peritoneum [63], cremaster muscle [63]
CXCR2	Required only in some situations [15,27]	No [15]	Liver [44], lungs [64]
CXCR4	Yes [45]	Yes [15,45]	Bone marrow [65]
CCR7	No [15]	Required only in some situations [14,15]	Not known
CD44	Not tested	Not tested	Liver sinusoids [66]

trophil migration from an inflamed lesion to lymph nodes has been observed following lysis of a tumor grown in murine skin [27].

The mechanisms guiding neutrophil migration via lymphatic vessels are still being defined. Dendritic cell migration via this route requires the chemokine receptors CCR7 [51] and CXCR4 [52]. For neutrophils, CXCR4 is also required for migration from inflamed skin to lymph nodes [15,45]. The ligand for CXCR4, CXCL12, is expressed by lymphatic endothelial cells [52]; therefore, it is possible that blocking CXCR4 can reduce neutrophil entry into lymphatic vessels. By contrast, the role of CCR7 in entry of neutrophils into the lymph nodes is controversial. Although CCR7 was required for lymph node recruitment of neutrophils in response to adjuvant [14], a recent study from our laboratory demonstrated that, neutrophil migration from inflamed skin to draining lymph nodes following *S. aureus* challenge can occur independently of CCR7 [15]. Moreover, another study noted that only a small fraction of lymph node neutrophils expressed CCR7 [45]. Thus, CCR7 appears to be dispensable for at least some modes of neutrophil recruitment to lymph nodes. This may be due to the notion that different waves of neutrophil recruitment are governed by distinct mechanisms since $G_{\alpha i}$ signaling, which is downstream of many chemokine receptors, was not required for neutrophil entry into lymph nodes immediately after immunization despite being essential for neutrophil recruitment into draining lymph nodes 72 h later [53].

Another molecule required for neutrophil migration to lymph nodes via lymphatic vessels is the β2 integrin CD11b [15,45]. Confocal microscopy of mouse skin after an injection of *Mycobacterium bovis* demonstrated that blocking CD11b resulted in the accumulation of neutrophils in the space around lymphatic vessels [54], suggesting that this molecule may be required for neutrophil entry into lymphatics. However, since CD11b has over 40 known ligands, the ligand or combination of ligands required for CD11b-dependent neutrophil migration from inflamed tissues to lymph nodes is yet to be determined [55].

5. Neutrophil survival in lymph nodes

Neutrophils have long been considered to be short-lived cells with a half-life of about ten hours in resting mice [20,67,68]. However, a study in 2011 showed that neutrophils could survive for extended periods of time in *S. aureus*-infected wounds [69]. Additionally, at least *in vitro,* inflammatory molecules such as LPS, TNF-α and IL-1β have been found to delay neutrophil apoptosis [70]. Thus, it is possible that neutrophil life-span might be influenced by environmental cues.

Following immunization with killed bacteria and various adjuvants neutrophils are rapidly recruited to lymph nodes but their numbers return to baseline levels within 24–48 h [11,15,21,53]. While this reduction could be due to emigration from lymph nodes, it appears that the majority of lymph node neutrophils are dying *in situ* by apoptosis [15]. Interestingly, following an intradermal injection of killed *S. aureus* fewer neutrophils in the skin were undergoing apoptosis compared to draining lymph node neutrophils [15]. The mechanisms underlying differential survival of neutrophils at the two distinct locations are yet to be determined; however, a shorter life-span of lymph node neutrophils makes sense as it would limit collateral damage to lymphoid tissues associated with neutrophil-mediated tissue injury and remodeling. By contrast, when live rather than dead *S. aureus* was used, neutrophils could be found in the lymph node for at least seven days [21], due either to prolonged survival or to continued neutrophil recruitment to draining lymph nodes.

6. Functions of neutrophils in lymph nodes

6.1. Pathogen killing

Neutrophils are best known for their phagocytic and microbicidal properties and have at their disposal a vast arsenal of enzymes, matrix metalloproteases, reactive oxygen species (ROS) and tissue-remodeling factors which together act to limit pathogen spread [20]. Some of these defense systems first described for neutrophils in inflamed tissues, are also employed by lymph node neutrophils. For example, they can phagocytose *S. aureus* [15,21], foreign proteins [11], viruses [26,71] and fungal cell wall components [72] when present in lymph nodes. Their capacity to mount a protective response in lymph nodes is well illustrated by the demonstration that administration of a neutrophil-depleting antibody following an injection of *P. aeruginosa* into the footpad leads to systemic spread of the bacteria from the popliteal node via the lymphatic system [29].

6.2. Antigen transport

Dendritic cell migration from inflamed tissues to draining lymph nodes is well recognized as an important step in the initiation of adaptive immune responses [6]. However, analysis of migration of

neutrophils in response to *S. aureus* challenge showed that they, in fact, are the first immune cell subset to reach lymph nodes from the site of inflammation [15]. In addition, they can shuttle *S. aureus* particles to lymph nodes [15], and in a canine model, transported fluorescent microspheres from the lung to the tracheobronchial lymph node [73]. Neutrophils containing BCG have been observed in dermal lymphatic vessels [10]. Moreover, neutrophils associated with *S. aureus* and zymosan, a fungal cell wall component, have been found in murine lymphatics [15,72], while ovine neutrophils carrying foreign material were found in afferent lymphatic vessels following an injection of alum [50] or *Salmonella abortusovis* [48].

The consequences of neutrophil-mediated antigen transport can be two-fold. On the one hand neutrophil-associated antigen may stimulate adaptive immunity. Consistent with this function is the finding that prevention of entry of *S. aureus*-pulsed neutrophils into draining lymph nodes reduced T cell proliferation [15]. On the other hand, neutrophils carrying pathogens could promote immune evasion leading to pathogen spread as was shown for *Leishmania major*-containing neutrophils [74]. Similarly two-photon imaging has demonstrated that neutrophils can facilitate the spread of *T. gondii* throughout the intestine [75].

6.3. Innate immune cell recruitment and removal

Lymph node neutrophils can regulate leukocyte recruitment to lymph nodes. For instance, neutrophil-secreted CCL3 (MIP1-α) promoted the recruitment of dendritic cells to the skin following an injection of *L. major* [76]. Likewise neutrophils enhanced dendritic cell migration from the skin to draining lymph nodes during the initiation phase of a contact hypersensitivity reaction [77]. Additionally, they have been shown to promote the migration of dendritic cells from the lung to the mediastinal lymph node following infection with the fungal pathogen *Aspergillus fumigatus* [78]. In some cases, however, neutrophils can prevent rather than enhance the recruitment of other innate immune cells to lymph nodes. For instance, they inhibited the migration of monocyte-derived dendritic cells to lymph nodes induced by injection of *Leishmania mexicana* into the skin [79]. Furthermore, two-photon microscopy observations of neutrophil behavior have demonstrated that they can remove innate immune cells such as subcapsular sinus macrophages during tissue remodeling [13]. Thus neutrophils can downregulate as well as amplify innate immune responses.

6.4. Regulation of adaptive immune responses

Until recently neutrophils have been considered to be terminally differentiated cells destined to die at the site of infection and thus thought to function largely in the innate immune response. However, as mentioned above, neutrophils are the first cells to arrive in draining lymph nodes from the periphery after antigenic challenge [11,15], carry antigens [10,15,72,73], upregulate molecules associated with antigen presentation and co-stimulation [15], produce molecules that recruit and activate other cells—properties consistent with the notion that, in addition to direct pathogen destruction, neutrophils could influence adaptive immune responses via both soluble mediators and cell-to-cell contact.

Many instances of neutrophils shaping adaptive as well as innate immune responses have been documented [11,15,21,53]. For example, two-photon microscopy of the draining lymph node has demonstrated that neutrophils can form stable conjugates with B and T cells [21], while functional studies have revealed that neutrophils can both stimulate and suppress T cell dependent immunity. Thus neutrophils promoted lymphocyte proliferation measured 72 h after immunization with killed *S. aureus* [15], whereas they were responsible at a later time point for a decrease in the serum levels of T cell-dependent IgG antibodies [21]. Other reports point to a role for neutrophils in presenting antigen to CD4 T cells either directly [39] or by fusing with dendritic cells to form a hybrid cell population [80–82]. In addition they have been shown to activate CD8 T cells by cross-presenting exogenous antigens *in vivo* [83] and to prime them in the bone marrow in the case of Ankara Virus infection [71].

A suppressive role for neutrophils has been demonstrated in tumor and acute inflammation settings in humans [84–86]. Neutrophil-produced hydrogen peroxide inhibited T cell proliferation and pro-inflammatory cytokine production [84,85]. Likewise, human neutrophils directly suppressed T cell proliferation [87] and function [88] in a contact dependent manner via neutrophil-expressed PD-L1 (Programmed death ligand 1). Finally, neutrophil depletion led to greater CD8+ T cell activation and was associated with slower tumor growth [89]. However, the mechanism that neutrophils employed to regulate CD8+ T cell activation in this model is yet to be elucidated.

Neutrophils can modulate adaptive immunity by regulating T cell polarization. Antibody-mediated neutrophil depletion results in the development of a Th1 response, rather than a Th2 response, in Balb/c mice infected with the protozoan parasite *L. major* [24]. Additionally, in a model of contact hypersensitivity, depletion of neutrophils reduced the production of IFN-γ by T cells in the draining lymph node [77].

Neutrophils can affect T cell function directly by releasing soluble mediators that regulate T cell priming and lymph node egress [53]. Furthermore, depleting neutrophils was found to result in longer and more frequent dendritic cell-T cell interactions, even though neutrophils were not observed near these interacting cells [11]. Alternatively they can influence T cell responses indirectly by acting upon dendritic cells, either promoting or suppressing T cell responses depending on the context [90–92].

A role for neutrophils in B cell immunity has also been reported. In a recent study of the function of human splenic neutrophils, a role for these cells in amplifying class-switching, immunoglobulin production and somatic hypermutation was demonstrated [17], probably due to secretion of the B cell stimulating factors BAFF and APRIL [93,94]. Another study found that splenic neutrophils, activated by innate lymphoid cells via GM-CSF, stimulated production of IgA, IgG and IgM by marginal zone B cells [95]. On the other hand, a suppressive role for neutrophils in the modulation of B cell responses has been suggested by the finding that neutrophil depletion resulted in higher concentrations of IgA antibodies [96]. Collectively, a growing body of evidence suggests that neutrophils can influence isotype switching and antibody production in B cells, however, these functions are yet to be demonstrated specifically for lymph node neutrophils.

Neutrophils can also influence adaptive immune responses by varying the level of antigen available to other innate immune cells. For example, following an injection of hen egg lysozyme together with CFA, neutrophil phagocytosis reduced antigen acquisition by dendritic cells and macrophages [11]. This led to decreased antigen presentation to T cells and B cells and to a reduction in serum levels of anti-HEL two weeks after immunization [11]. This observation suggests that neutrophils can compete with other APCs for antigen.

Finally, neutrophils may serve to confine the adaptive immune response to the specific draining lymph node [53]. In mice unable to make neutrophils or mice depleted of neutrophils, there were larger T cell responses in distal lymph nodes following immunization with CFA, a phenomenon dependent upon neutrophil-mediated production of the lipid Thromboxane A_2 [53]. Further research is required to determine the specific mechanism by which neutrophils prevent the spread of the adaptive immune response and if this is a com-

mon function of neutrophils following their recruitment to lymph nodes.

7. Concluding remarks

Recent research has shown that neutrophils are recruited to draining lymph nodes in response to a wide variety of stimuli. Due to their rapid recruitment, ability to shuttle antigen and proximity to other immune cells, lymph node neutrophils have the capacity to influence early immune responses. Despite an increasing body of knowledge about the functions and consequences of neutrophil recruitment to lymph nodes, many aspects of lymph node neutrophil behavior remain to be investigated.

While it is becoming clear that neutrophils can shape adaptive immunity in a number of ways, the mechanisms by which they do so in the lymph node microenvironment are becoming increasingly complex. Thus, it is now apparent that neutrophils have antigen presenting machinery to provide the necessary signals to drive antigen-specific T cell responses via direct engagement [15]. Furthermore, neutrophil conjugates with T and B cells have been documented [21]. However, further studies are needed to determine whether direct antigen presentation by lymph node neutrophils plays a significant role in adaptive responses.

A greater understanding of the unique molecular mechanisms that control neutrophil recruitment to lymph nodes via lymph and blood would provide insight into possible therapeutic avenues based on regulating neutrophil migration. This could be used either to promote neutrophil recruitment to lymph nodes with a view to manipulating adaptive immune responses or inhibit neutrophil recruitment in instances where neutrophils may be acting as a "Trojan horse" to further pathogen spread, as has been proposed in the case of infection with *L. major* [97].

The importance of NETosis, a process whereby neutrophils expel chromatin into the extracellular space to trap and destroy bacteria [98], is still emerging *in vivo* with NETs observed in the joints of gout patients [99], in the liver of mice following infection with myxoma virus [32] and in the skin of *S. aureus*-infected mice [31]. Whether there is a possible role for NETosis in lymph nodes is unknown but this would be an interesting area of investigation given the important part played by NETosis in host-defense and the potential for NETs to display of autoantigens such as citrullinated histones [100].

Our report that the half-life and the expression of apoptotic molecules differs between lymph node neutrophils and those recruited to inflamed skin in response to the same pathogen [15] highlights that the paucity of information on how neutrophil lifespan is regulated within distinct microenvironments. Furthermore, the cell types responsible for ingesting apoptotic neutrophils in draining lymph nodes are unknown. Neutrophil apoptosis and subsequent engulfment by lymph node leukocytes may regulate cytokine production in the lymph node, which in turn may influence the adaptive immune response.

Several recent studies have suggested that neutrophils may develop into distinct functional and phenotypic subsets. This idea has been explored in a cancer model [89], a transplantation model [101] and infections with *S. aureus* [102] and the nematode parasite *Nippostrongylus brasiliensis* [103]. Whether lymph node neutrophils can differentiate into different subsets as has been suggested by their distinct phenotypes in different microenvironments [15] is currently unknown.

To date neutrophils have been identified in the afferent lymphatic vessels of sheep [49,50], in the lymph nodes draining canine lungs [73] and in murine lymph nodes [10,13,15]. These observations suggest that neutrophil recruitment to draining lymph nodes in response to inflammation is common to vertebrates. However, there is currently a lack of knowledge about neutrophil migration and function in human lymph nodes. Given the wide variety of roles that neutrophils play in murine lymph nodes this area of study is of substantial importance to medical research.

While one of the primary functions of lymph node neutrophils is limiting pathogen spread via direct killing, the unique microenvironment of secondary lymphoid organs brings neutrophils in to close proximity with many other cells of the innate and adaptive immune systems. Thus, lymph node neutrophils may emerge as one of the key regulators of vertebrate immunity.

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