The role of lymph node sinus macrophages in host defense

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Strategically positioned along lymphatic vessels, lymph nodes act as filter stations preventing systemic pathogen dissemination; they are primary sites of innate immune responses and provide the staging grounds for the generation of adaptive immunity. Critical mediators of these lymph node functions are two phenotypically and functionally distinct subsets of macrophages: the subcapsular sinus macrophages and the medullary macrophages. This review focuses on the phenotype and function of these lymph node sinus-resident macrophages and summarizes methods for their proper identification and experimental manipulation.

Keywords: lymph node; subcapsular sinus; medullary sinus; macrophages; CD169; antiviral immunity

Introduction

The term *macrophage* was coined at the end of the 19th century by Metchnikoff, a pioneer in the study of phagocytosis, to designate a population of amoeboid leukocytes that was able to ingurgitate bacteria.[1–3] Metchnikoff observed phagocytic cells in both primitive and highly organized organisms and suggested that these evolutionary conserved cells function by scavenging dead cells and other debris in order to defend the organism from danger. Research performed in the following century confirmed the existence of phagocytic macrophages from the earliest stages of embryonic development throughout the whole adult life.[4] The ability of macrophages to phagocytose endows them with a variety of tissue-specific trophic roles. Osteoclasts, for example, are bone-resident macrophages that destroy senescent bone cells and, as such, regulate bone morphogenesis.[5] Metchnikoff also performed the first intravital imaging of macrophages,[1] taking advantage of the transparency of starfish larvae. He observed that these phagocytic amoeboid cells accumulated around a rose thorn introduced into the larvae and covered it completely. This process, known today as *inflammation*, was triggered not only by physical damage but also by microbial stimuli, leading to the concept that macrophages participate in host defense. As foreseen by Metchnikoff, orchestration of immune responses is today known to be a major function of macrophages.

Based on their anatomical location, functional specialization, and expression of surface markers, macrophages are characterized by a high degree of heterogeneity.[4,6–9] Notably, macrophage heterogeneity is very high even within the same tissue. For instance, many different subsets of macrophages populate lymph nodes (LNs), under both steady-state and inflammatory conditions. LN macrophages can be divided into two broad categories: sinus-resident macrophages, which are bathed in lymph, and parenchymal macrophages. Tingible body macrophages in B cell follicles and the recently described medullary cord macrophages belong to the latter group.[10–13]

In this review, we will focus on the phenotypic and functional characterization of murine LN sinus macrophages and on their emerging role as central mediators of antimicrobial immune responses in LNs.

LN sinus macrophages

Beneath the collagen-rich capsule, two main regions can be identified in the LN: the cortex and the medulla (Fig. 1). The cortex consists of superficial B cell follicles and a deeper T cell area. The
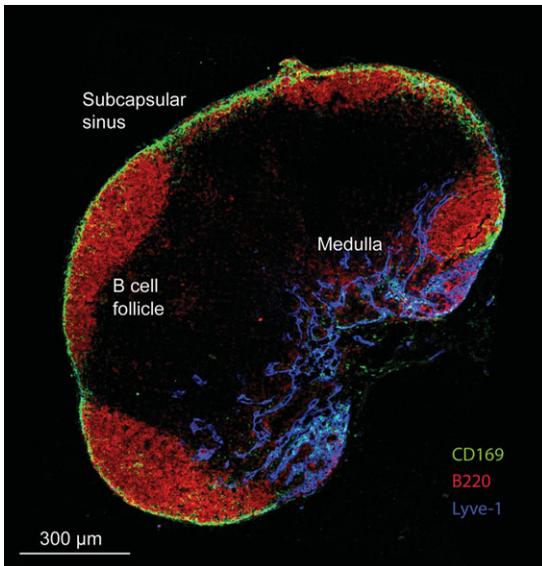
Figure 1. Confocal micrograph of a mouse popliteal lymph node cross-section. CD169^{+} macrophages are highlighted in green, B220^{+} B cells in red, and Lyve-1^{+} lymphatic vessels in blue. Scale bar represents 300 μm.

medulla contains a multitude of lymph-draining sinuses separated by medullary cords.[14]

Upon breaching the LN capsule, afferent lymphatics release their content into the subcapsular sinus (SCS), a region contained between the capsule and the cortex.[14] The SCS wall is lined with sinus-lining cells and contains peripheral nerves that span across the SCS where they are exposed to flowing lymph.[15] After transitioning through the SCS, the lymph percolates through trabecular and cortical sinuses into medullary sinuses and eventually collects into an efferent lymph vessel that leaves the LN.

Two subsets of macrophages populate LN sinuses and are in direct contact with flowing lymph: SCS macrophages and medullary macrophages (Fig. 2). Although both populations express common markers (such as CD11b, CD11c, and CD169), they can be distinguished phenotypically. CD169, originally identified as the sheep erythrocyte receptor (SER),[16] is expressed at higher levels on SCS macrophages than on medullary macrophages.[15,17,18] Moreover, medullary macrophages, but not SCS macrophages, express other markers, such as F4/80, the scavenger receptor MARCO, the C-type lectin SIGN-R1, and the mannose receptor.[15,17–23] By contrast,

SCS macrophages uniquely express ligands for the cysteine-rich (CR) domain of the mannose receptor, which can be labeled using a CR–Fc fusion protein.[23–25] In addition, the expression of Lyve-1, a marker of lymphatic endothelial cells, is more prominent in the medullary region, rendering the histological distinction between SCS and medulla easier (Fig. 1).[18,26,27] Although these markers allow the prompt *in situ* identification of LN macrophage subsets in sections,[15,17,18,20,24,27–30] they cannot be reliably used for flow cytometry, as current LN digestion techniques do not allow for the isolation of pure macrophage subsets.[31]

LN sinus macrophage heterogeneity is reflected in their different developmental requirements. Indeed, the development and functional differentiation of SCS macrophages, but not of medullary ones, critically require macrophage colony stimulating factor (M-CSF or CSF-1) and lymphotoxin (LT) α1β2 and its receptor LTβR.[17,18,32–37]

To study the function of LN macrophages, a number of methods aiming at their depletion have been used. The earliest approaches took advantage of the phagocytic nature of macrophages and of their anatomical location. Bathed in lymph, sinus macrophages are the first cells encountering and phagocytosing material carried by the afferent lymphatics. When injected subcutaneously, compounds such as carrageenan, dextran sulfate, silica, or bisphosphonates are taken up by macrophages in LNs draining the injection site, ultimately reaching cytotoxic intracellular concentrations.[38–46] A subsequent approach made use of liposomes as vehicles to encapsulate cytotoxic drugs, such as the bisphosphonate clodronate.[47] Clodronate-encapsulated liposomes that reach draining LNs upon peripheral inoculation are rapidly phagocytosed by sinus-resident macrophages and, therefore, excluded from the LN parenchyma; clodronate is then released intracellularly and induces macrophage apoptosis.[48] Although clodronate liposome–mediated macrophage depletion has been successfully and extensively used in the last three decades,[49,50] this treatment carries some off-target effects. For instance, it was reported that treatment with clodronate liposomes induces LN hypertrophy (mostly due to B cell expansion) and a myeloid infiltrate at the injection site.[15,30] Moreover, bisphosphonates, regardless of whether they are encapsulated in liposomes or not, were recently
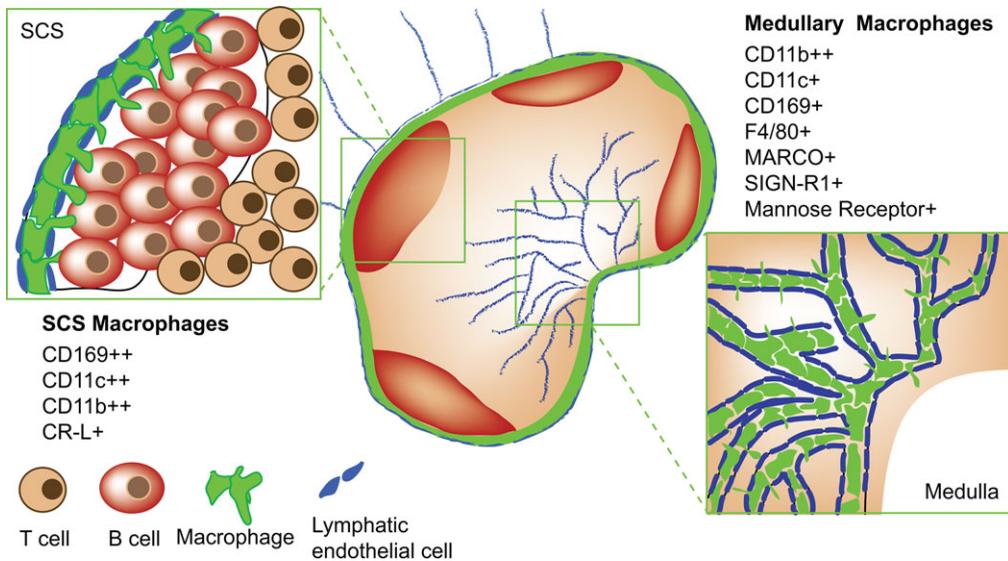
Figure 2. Schematic diagram showing lymph node sinus-resident macrophage populations along with the surface markers they express. Subcapsular sinus (SCS) macrophages are either completely contained within the SCS or they reside in B cell follicles but extend cellular protrusions into the SCS lumen. Medullary macrophages lie within medullary sinuses.

shown to directly target B cells to enhance humoral immune responses upon subsequent antigen challenge.[46] These off-target effects should be taken into account when interpreting experimental results that make use of bisphosphonate-based preparations to study macrophage function.

An alternative approach for macrophage depletion uses injection of diphtheria toxin (DT) in transgenic mouse strains that express the DT receptor (DTR) under the control of macrophage promoters. Systemic injection of DT into CD11c-DTR or CD11b-DTR mice, for example, induces macrophage depletion in several organs, including spleen and LNs.[15,51–55] However, because CD11c and CD11b are also expressed by dendritic cells and monocytes, depletion of only macrophages does not occur. Yet, by taking advantage of the fact that LN sinus macrophages have a high accessibility to lymph-derived materials and a relatively slow turnover rate, it was recently shown that subcutaneous injection of DT in CD11c-DTR mice caused an efficient depletion of LN sinus macrophages while leaving dendritic cells intact.[15] Alternatively, a more specific ablation of LN macrophages has been demonstrated with the use of CD169-DTR mice.[56,57]

Experimental macrophage depletion through the above-mentioned approaches in mice has been instrumental to the understanding of LN sinus macrophage biology. Below we will summarize our current knowledge on the role of these sessile LN-resident macrophages in host defense.

Cellular "flypaper"

When pathogens enter an organism by breaching through the skin or mucosal membranes, innate responses usually eliminate or contain the infectious agents at the entry site. However, some pathogens can escape this initial attack and threaten to spread systemically by accessing tissue-draining lymph vessels that transport interstitial fluid to the venous circulation. Before reaching the blood, however, the tissue-derived lymph must first pass through one or more draining LNs. These organs act as filter stations, preventing systemic pathogen dissemination; due to their strategic position, LN sinus macrophages play a key role in this process.

Early reports showed that lymph-borne antigens arrive in LNs only a few minutes after subcutaneous injection.[11,58,59] The presence of edema at the injection site can increase the interstitial pressure and improve the efficiency of free antigen arrival in draining LNs.[60] Interestingly, a minimum threshold of edema size is required to see the effect on antigen delivery,[60] a consideration that should guide the choice of experimental injection volume.

Antigens whose molecular radius is smaller than ~4 nm can reach the LN cortex through a system of conduits that originate in the SCS and penetrate the LN parenchyma.[61,62] By contrast, the SCS floor is impermeable to larger particles, such as viruses[63]; within minutes upon peripheral inoculation viral particles are captured by both SCS and medullary macrophages.[15,30,64] Selective depletion of these macrophages compromises local viral retention and leads to systemic pathogen spread.[15,30] Even though both SCS and medullary macrophages prevent systemic viral dissemination, these two subsets differ in their ability to capture lymph-borne particulates. Medullary macrophages are poorly selective in their filtering ability and avidly bind any lymph-borne particulate (e.g., bacteria, nanoparticles, apoptotic cells); by contrast SCS macrophages are less efficient at binding particulate antigens, with the significant exception of viruses and immune complexes.[10]

The molecular mechanisms by which LN sinus macrophages capture pathogens are still incompletely understood. Viral capture does not require natural antibodies, complement, or glycosylation of viral glycoproteins.[30] Capture of influenza virus by LN sinus macrophages was shown to be dependent upon lymph-borne mannose-binding lectin.[64] Whether capture of other viruses by LN sinus macrophages requires opsonization by mannose-binding lectin remains, however, to be determined.

The fate of particulates differs whether they are captured by SCS or medullary macrophages. Whereas medullary macrophages are highly phagocytic and rapidly clear pathogens, SCS macrophages are less endocytic and are able to retain viral particles on their surface for at least several hours after capture.[17,30,35,36] As we will describe in the following paragraphs, this last characteristic has important implications for the generation of adaptive immune responses.

In conclusion, our current view establishes LN sinus macrophages as efficient cellular "flypaper" that rapidly captures lymph-borne pathogens, thus preventing their systemic dissemination.

Jump-starting innate immunity

Although both SCS and medullary macrophages capture viruses and help prevent systemic spread, they differ in susceptibility to infection by certain viruses.[10] Perhaps the most striking and best studied example is that of vesicular stomatitis virus (VSV), an arthropod-borne rhabdovirus that causes fatal paralytic disease in mammals, including mice.[65] Upon arrival in LNs, VSV readily replicates in SCS, but not medullary, macrophages.[15,18,30,66]

Several phenotypic and functional features of SCS and medullary macrophages could explain their different permissivity to VSV infection. First, when compared to SCS macrophages, medullary macrophages are more phagocytic and express higher levels of endosomal degradative enzymes.[17,67,68] Second, the two subsets of macrophages differ in expression of antiviral sensing receptors. Although the data should be taken with caution (because of potential contamination of sorted macrophages with other cell types[31]), genome-wide mRNA profile analyses find that medullary macrophages express higher levels of VSV-recognizing TLR4 and TLR13,[17,69,70] while SCS macrophages express more RIG-I, which allows for intracellular VSV recognition[71,72]; the data suggest that medullary macrophages detect and destroy extracellular virus, whereas SCS macrophages are relatively inefficient at eliminating surface-bound virions, which may facilitate productive VSV infection. Third, SCS macrophages may be less responsive to IFN-αβ than medullary macrophages. Recently, Honke *et al.* reported that CD169^{+} metallophilic macrophages—the splenic functional equivalent of LN SCS macrophages—express Usp18, a potent inhibitor of IFN-αβ signaling cascade, thereby allowing locally restricted replication of virus.[73] Whether a similar mechanism is operative in LN SCS macrophages remains to be determined. Of note, it was recently reported that SCS macrophage susceptibility to VSV, and the ensuing host-protective IFN-αβ response, are critically dependent upon LTα1β2.[18] In the absence of B cell–expressed LTα1β2, SCS macrophages assume a medullary-like phenotype and no longer support VSV replication.[17,18] A possible explanation for this observation is that tonic exposure to LTα1β2 on follicular B cells attenuates macrophage responsiveness to autocrine IFN-αβ; without LTβR signaling, macrophages may gain IFN-αβ responsiveness and VSV resistance. Whether LTβR signaling affects *Usp18* expression, or whether there are additional mechanisms by which B cell–derived LTα1β2 renders SCS macrophages physiologically susceptible to VSV infection, is currently not known.
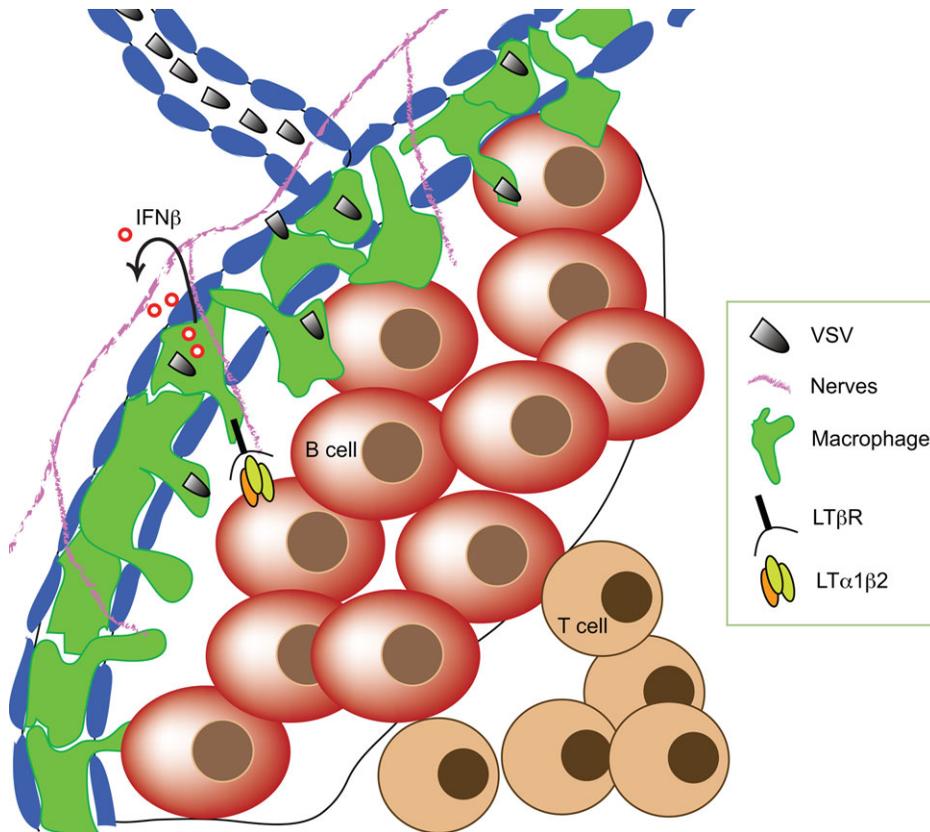
Figure 3. Schematic representation of the early events during lymph-borne VSV infection. Protection against VSV infection is provided by SCS macrophage–derived type I interferon, which prevent VSV from invading peripheral nerves within LNs. Constitutive exposure to B cell–derived LTα1β2 maintains the protective SCS macrophage phenotype.

Enforced VSV replication in SCS macrophages is critical for host survival.[15,18] Before being eventually killed by the cytopathic VSV, SCS macrophages secrete large quantities of IFN-αβ that establishes a local antiviral state, which prevents further viral replication. In the case of the neurotropic VSV, this localized production of IFN-αβ is required to protect intranodal nerves from serving as viral conduits to the central nervous system, thus protecting the host from central ascending nervous system paralysis and death (Fig. 3).[15] It seems paradoxical that this defense mechanism should rely upon the amplification of a potentially lethal virus, but, beyond jump-starting the protective IFN-αβ response, increased viral antigen availability may promote adaptive immunity.[66,73] Moreover, SCS macrophage–derived IFN-αβ induces CXCR3 ligands that recruit antiviral natural killer (NK) cells to the SCS.[74–78] A similar mechanism may underlie the recruitment of plasmacytoid dendritic cells to LN sinuses upon viral infection.[15,79,80]

SCS macrophages were also recently shown to play a critical role in host defense against bacterial infections[81]; they are able to sense bacteria via one or more NLR-based inflammasomes and, in turn, generate active IL-1β and IL-18.[81] The former promotes neutrophil recruitment to handle extracellular bacteria, while the latter leads to IFN-γ secretion by a network of innate lymphoid cells (NK cells, γδ T cells, NK T cells, and innate-like CD8$^+$ T cells) that are spatially prepositioned close to SCS macrophages.[81] IFN-γ, in turn, fosters SCS macrophage antimicrobial resistance.[81]

Together, these data indicate that, besides physically trapping pathogens, LNs are prime sites of innate immune responses that are, either directly or indirectly, exerted by sinus macrophages and function to limit early pathogen spread.

Orchestrating adaptive immune responses

It has been long known that LNs play a critical role in naive B and T cell priming upon viral infection.[82] A number of studies have highlighted the role of LN sinus macrophages in this process, and they have been the subject of comprehensive reviews.[10,37,83–89] Herein, we will limit ourselves to discussing several recent studies that have shed additional light into how LN sinus macrophages coordinate B and CD8^{+} memory T cell responses to viral infection.

Owing to strategic positioning across the SCS floor,[90] SCS macrophages provide the functional link between lymph-borne particulate antigens and motile parenchymal B cells.[17,27,30,91] SCS macrophages can translocate surface-bound viral particles across the SCS floor and present them to migrating B cells in underlying follicles.[30] Particulate antigens trapped by SCS macrophages are also exposed to lymph proteases that can generate smaller proteins, which can penetrate the conduit network.[92] The net contribution of SCS macrophages to the generation of high-affinity neutralizing antibodies is, however, unclear. Indeed, although SCS macrophage depletion by clodronate liposomes reduces early B cell activation,[30] it eventually leads to higher antibody titers in response to subcutaneous antigen challenge.[15,64,93] The interpretation of these results is complicated by the observation that macrophage-depleting formulations that include bisphosphonates (such as clodronate liposomes) exert a direct adjuvant effect on B cells enhancing antibody responses upon subsequent antigen challenge.[46] Indeed, LN sinus macrophage depletion by alternative methods (silica, carrageenan, dextran sulfate, or subcutaneous DT injection in CD11c-DTR transgenic mice) does not have a significant effect on humoral immunity to VSV.[46] Whether antigen presentation by SCS macrophages to B cells has an impact on antibody responses in situations of limiting antigen amount, low B cell precursor frequency, or low B cell receptor affinity remains to be determined and has important implications for vaccine design.

As mentioned earlier, SCS macrophages are permissive to certain viral infections[10] and can therefore potentially present pathogen-derived antigens to CD8^{+} T cells in the context of major histocompatibility complex (MHC) class I molecules. While SCS macrophages seem to be incapable of directly priming naive CD8^{+} T cells,[66] they play a critical role in the rapid and efficient activation of virus-specific central memory CD8^{+} T cells.[94,95] The accelerated response of central memory CD8^{+} T cells to virus infection depends upon a cascade of cytokines and chemokines, particularly ligands for CXCR3, which selectively guide central memory CD8^{+} T cell migration toward infected cells.[94,95] Importantly, when directed migration of central memory CD8^{+} T cells toward infected SCS macrophages is disrupted, viral clearance is compromised.[95] This observation, together with what has been described earlier for the innate immune response, indicates that the ability of SCS macrophages to orchestrate the rapid recruitment of several innate and adaptive immune cells to the site of pathogen replication is critical for host defense.

Conclusions

While LNs have long been known as filter stations and as sites where adaptive immunity develops, recent data in mice underline that these secondary lymphoid organs are also prime sites for effector responses that limit pathogen spread and ensure host survival. LN sinus macrophages, particularly SCS macrophages, play a key role in this process. By being permissive to pathogen replication, they not only perform essential antiviral function, but also coordinate the recruitment of several distinct innate and adaptive immune cells. While it is not clear whether murine LN sinus macrophages have human functional counterparts, it is of note that CD169 is expressed by perifollicular sinus macrophages in human LNs.[96] Although studies on the role of human sinus macrophages during infection are missing, it has been recently reported that the number of CD169^{+} macrophages in regional LNs directly correlates with a favorable prognosis in patients with colorectal carcinoma, raising the question of whether these cells play a role in antitumoral immunity.[97]

Despite recent progress in our understanding of murine LN sinus macrophage biology, several questions remain unanswered: What are the mechanisms by which LN sinus macrophages capture pathogens? Does antigen targeting to SCS macrophages increase humoral immune responses? Do pathogens interfere with macrophage function? What are the phenotype and function of sinus macrophages in LNs

draining tissues other than the skin (e.g., intestine-draining mesenteric LNs)? The answer to these and other questions will require the development of novel tools for the improved identification, isolation, and experimental manipulation of LN sinus-resident macrophage populations.

Acknowledgments

Conflicts of interest

The authors declare no conflict of interest.

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