and protecting it from destruction in the lysosomes, a property ascribed to the human relative of SIGN-R1, DC-SIGN.[19]

In a striking observation, the DCs that capture PR8 are found to increase their migration velocity.[1] Some of the PR8$^+$ DCs migrate in the direction of distant follicular DC (FDC)-containing areas, although their displacement during the imaging period is small and it is not yet clear how close they travel to follicles or FDCs. Further analysis of DC distribution over time by a combination of multiphoton and standard fixed tissue analysis will help address this point. Even if the cells do not travel to follicles, positioning near high endothelial venules or the follicle border could facilitate B-cell antigen encounter.[16] Ablation of CD11c$^+$ DCs using the CD11cDTR system is found to reduce the day 10 antibody response by several fold, consistent with a direct role of DCs in antigen display to B cells. However, although there is a notable T-independent IgM response to influenza virus, the response is at least 10-fold stronger in the presence of T-cell help,[20] leaving open the possibility that the reduced antibody response observed after DC ablation is due to reduced T-cell help.

The findings of Gonzalez *et al.*[1] are significant in providing the first glimpse of how vaccine-type influenza virus is handled on arrival in the draining LN. The study provides some of the first information on a mechanism of antigen capture by SCS macrophages and it demonstrates the ability of DCs situated near the medullary sinuses to take up viral particles and to become motile. Future experiments that track the interaction dynamics of influenza-specific B cells and T cells with influenza-bearing DCs will further refine our understanding of the roles of DCs in promoting the influenza virus-specific antibody response. When taken together with other recent studies on how soluble and particulate antigens reach B-cell follicles for induction of antibody responses,[2,4,21,22] a picture emerges of many overlapping mechanisms at play, perhaps making it difficult for pathogens to outmaneuver this crucial first step of the humoral immune response.

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Lymphocyte activation

Border patrol: SCS macrophages activate iNKT cells too

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The staging of the immune response in secondary lymphoid organs is a major logistical challenge.[1,2] Rare antigen-specific cells must be mobilized and microbial antigens must be retained at specific locations and presented in the appropriate context to enable lymphocyte activation (Figure 1). This involves, among other things, a complex interplay between sessile tissue-resident cells that sense the danger and alert motile

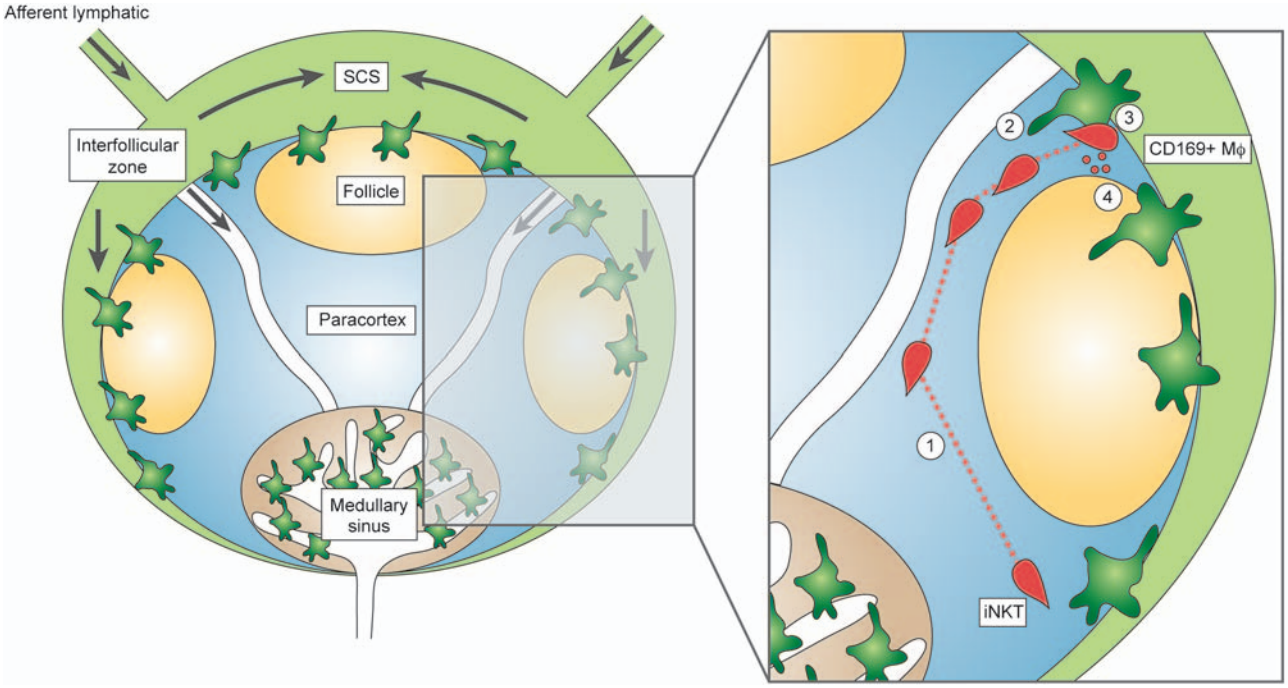

Figure 1 Lymphocyte activation by CD169$^+$ macrophages in the lymph node. Schematic of the lymph node showing pattern of lymph flow and antigen trafficking (arrows). CD169$^+$ macrophages (green) line the subcapsular sinus (SCS) and medullary sinuses. B-cell follicles (orange) are positioned beneath the floor of the SCS and T cells are positioned in the interfollicular zone and paracortex (blue). Earlier studies have shown that CD169$^+$ macrophages capture and present antigen to B cells in the underlying follicle to initiate the antibody response. These macrophages can also activate CD8$^+$ T cells in the interfollicular zone in response to viruses and protozoan parasites. A new study (inset on right) has now identified a role for CD169$^+$ macrophages in the activation of iNKT cells (red). By two-photon microscopy, Batista, Cerundolo and colleagues[4] found iNKT cells patrolled the paracortical T zone, interfollicular and subcapsular regions of resting lymph nodes in a random walk (1). In response to lipid antigen, iNKT cells slowed down (2) and migrated toward the SCS where they arrested on CD169$^+$ macrophages (3) and became activated to secrete cytokines (4). Thus, CD169$^+$ macrophages at the lymph-tissue interface are involved in rapid initiation of immune responses by activating iNKT, B and CD8$^+$ T cells.

lymphocytes to respond to it. The dynamic and relatively short-lived nature of these interactions has only recently been appreciated by direct visualization with two-photon microscopy.[3] Now, in an article recently published in *Nature Immunology*, Batista, Cerundolo and colleagues have used two-photon microscopy to visualize the early steps in the activation of invariant natural killer T (iNKT) cells in intact lymph nodes.[4] In mice, iNKT cells express the invariant TCR α-chain $V_\alpha14$-$J_\alpha18$ as well as NK cell receptors such as NK1.1. These cells recognize lipid antigen presented by the nonpolymorphic major histocompatibility complex class I-like molecule CD1d and rapidly produce large quantities of cytokines on activation.[5] The iNKT cells develop in the thymus in a CD1d-dependent manner and are usually found in the liver and spleen but can also be found in the lymph node at very low frequencies. So what is the function of these rare cells in the lymph node? Batista, Cerundolo and colleagues[4] report that iNKT cells are rapidly activated after challenge with lipid antigens by CD169$^+$ subcapsular sinus (SCS) macrophages and suggest that this may be important in the initiation of the immune response. SCS macrophages line the floor of the SCS and are strategically placed to sample the lymph for incoming pathogens.[2] Until recently, the role of these sessile cells in lymphocyte activation has been understudied because of their low frequencies and difficulties in isolating them.[6] The study[4] by Batista, Cerundolo and colleagues, therefore, is remarkable for their ability to directly visualize interactions between these two rare cell populations and highlights the power of two-photon microscopy to provide additional insights to those obtained by conventional techniques.

The authors purified iNKT cells by two methods and showed that they had similar migration and homing properties following adoptive transfer into recipient mice. Thus, both CD1d-tetramer $^+$TCRβ^+B220$^-$ cells sorted from lymph nodes and TCRβ^+ NK1.1$^+$B220$^-$ cells sorted from liver and spleen were distributed predominantly in the paracortical T-cell zone and patrolled the resting lymph node in a random walk that occasionally took them to the interfollicular regions and outer edges of the follicle beneath the SCS. Notably, iNKT cells were not seen in the B-cell follicle proper. Upon challenge with lipid antigens, iNKT cells upregulated CD25 and CD69 after 6 h and had detectable levels of IFN-γ after 12 h. Two-photon microscopic analysis showed that iNKT cells slowed down and migrated toward the SCS where they arrested on CD169$^+$ macrophages in response to lipid antigen. When all lymph node macrophage populations were ablated with liposomal clodronate, iNKT cell activation and proliferation was impaired. However, the clodronate also induced changes in the size and cellularity of the lymph node.

SCS macrophages have recently emerged to be critical in the initiation of adaptive immune responses in lymph nodes. They protrude their 'heads' through the lymphatic vascular endothelial cell layer into the lumen of the SCS and extend 'tails' into the underlying parenchyma.[7] Thus, SCS macrophages have been shown to activate B cells on challenge with viruses,[8] virus-like particles[9] and immune complexes,[7,10] and to activate CD8$^+$ T cells in response to viruses[11] and protozoan parasites.[12] Despite their

importance, relatively little is known about the biology of this enigmatic cell population other than their tissue distribution and cell-surface marker expression. The field is therefore still in its infancy compared to, for example, the wealth of data on the many dendritic cell subsets and their role in $CD4^+$ and $CD8^+$ T-cell activation. This is partly due to the difficulties associated with purifying these infrequent tissue-resident cells from their native environment and the paucity of reagents to perturb them selectively without causing inflammatory changes to the lymph node. In this regard, a recent study that phenotyped the cells by flow cytometry as $CD169^{hi}CD11c^{int}F4/80^-$ showed that they were less prone to rapidly degrade immune complexes and were dependent on B-cell-derived lymphotoxin signals for their development.[6] In this study, Batista and colleagues used similar cell surface markers to purify SCS macrophages and showed that they were indeed able to internalize, process and present α-galactosidase-dependent lipid antigens via CD1d to activate iNKT cells to secrete IL-2 and proliferate marking them as bona fide antigen-presenting cells.

These new findings[4] raise some interesting questions about the regulation of iNKT cell trafficking in resting and inflamed lymph nodes and the functional consequences of their activation by SCS macrophages. In particular, how iNKT cells arrive at the SCS (for example, through the lymph or high endothelial venules) and their intranodal migration pattern remain to be fully elucidated. Intravital microscopy of lymph node preparations with intact lymphatic vessels and blood flow will help resolve this. Another intriguing question arising from this study is whether SCS macrophages release specific factors to recruit iNKT cells to the SCS and if they can discriminate between lipid and non-lipid antigens. In terms of function, the timing of iNKT cell activation by SCS macrophages and cytokine secretion appears out of synchrony with the timing of B-cell and $CD8^+$ T cell activation reported in earlier studies. For example, at 16 h iNKT cells are arrested on SCS macrophages by which time antigen has already been transported into the follicle and activated B cells have migrated away from the SCS toward the T-B border.[2] Similarly, $CD8^+$ T cell arrest on SCS macrophages after challenge with virus[11] and protozoan parasites[12] occurred after 2–6 h whereas intracellular cytokines were detectable in iNKT cells after 12 h. The observed asynchrony may be due to differences in the antigen systems and experimental models used by these studies. Further two-photon experiments to visualize the coordinate activation of iNKT and B and T cells in the same experimental model will be able to answer these and other questions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Human $CD4^+$ T cell subsets

Human Th9 cells: inflammatory cytokines modulate IL-9 production through the induction of IL-21

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The T helper (Th)1-Th2 hypothesis was first described in 1986.[1,2] It proposed that a naive $CD4^+$ T cell had the potential to differentiate into distinct effector subsets that were specialized to protect against infection by specific pathogens.[1] As such, interferon-γ (IFN-γ)-producing Th1 cells were implicated in immunity against viruses and intracellular pathogens, whereas IL-4-producing Th2 cells were proposed to have a function in immunity against extracellular pathogens and mediating humoral immune responses.[2] Subsequent studies revealed that the cytokine environment at the time of $CD4^+$ T cell activation was the key determinant in generating these effector subsets. This was on account of the ability of a particular cytokine to activate specific transcription factors required for the differentiation of the Th subsets. In the case of Th1 and Th2 cells, this process was dependent on IL-12 acting on *T-bet* and IL-4 acting on *Gata3*, respectively.[3–5] Applying this same concept, whereby a specific cytokine exerts its effect on a transcription factor to mediate lymphocyte differentiation, has led to the identification of other Th subsets believed to have distinct functions in the immune response, namely regulatory T cells (Tregs), Th17 cells and follicular helper T cells.[5–7]

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