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NEWS AND COMMENTARY

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# To the lymph node and beyond: migratory ILC3s regulate innate and adaptive immune responses

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An important characteristic of immune cells is their ability to circulate through the body scouting for pathogens, transformed cells or other potential insults. However, helper-like innate lymphoid cells (ILCs) have been identified as primarily tissue resident. Helper-like ILCs are a recently described set of cell populations composed of three functionally diverse subgroups: ILC1s, ILC2s and ILC3s.[1] ILCs exhibit striking functional similarities to adaptive T cells including expression of subgroup-specific signature cytokines and transcription factors. However, ILCs do not express rearranged antigen receptors and are activated in an antigen-independent manner by cytokines, neuropeptides, leukotrienes and other immunomodulators. With their strategic positioning at barrier surfaces and their immediate way of initiating effector mechanisms, ILCs are able to rapidly shape their tissue microenvironment and orchestrate innate as well as adaptive immune responses. Importantly, ILCs themselves are imprinted by local environmental cues and adopt tissue-specific phenotypes that allow them to tailor their functional capacities to the anatomical niche they reside in. While considered mainly tissue resident, ILC progenitors as well as mature ILCs exhibit limited migratory potential to home to their respective organ during development, to strategically position themselves within an organ or to replenish the exhausted ILC tissue pool. In addition, interorgan trafficking of ILCs has been described.[2] Moreover, identification of human circulating ILC progenitors has led to further discussion about ILC motility.[3] Increasing evidence is also emerging that ILCs are able to directly or indirectly trigger adaptive immune responses, which could be promoted by an ILC migration potential. Whereas T-cell trafficking is well documented, the understanding of ILC motility remains incompletely understood.

ILCs represent a rare cell population and thus addressing their migration is experimentally extremely challenging. In a recent issue of *Mucosal Immunology*, Kästele *et al.*[4] studied ILC migration by using Kaede photoconvertible mice. Kaede mice are genetically manipulated transgenic mice, which express Kaede protein. In Kaede mice photoconversion takes place upon exposure to low-intensity violet light and red labelled cells can be identified as resident cells by the Kaede red protein, whereas migrating and thus not photoconverted cells are identified by the Kaede green protein. Kaede mice are therefore important *in vivo* imaging models to monitor cellular motility within an organ or between different organs. Strikingly, all ILCs within Kaede mice can actively migrate to lymph nodes, a fundamental cellular process previously unknown.

However, the extent of motility by the ILC groups is different depending on the health status. It has been previously shown that ILCs can be found in the lymph. The lymph and the lymphatics build an important network and connect different organs, yet determining the origin of cells in the lymph remains highly elusive. Kästele *et al.* applied an advanced technique by cannulating the thoracic duct and harvesting migrating cells, enabling lymph to be collected from the efferent lymphatics. Lymph was also collected after removal of the mesenteric lymph node, mimicking pseudo-afferent lymphatics. This elegant method enabled cells entering the lymphatics from the tissue or the lymph node to be distinguished, which has never been investigated before. Applying these novel models, a significant population of migratory ILCs could be identified in the lymph node, even if at a lower frequency compared with T cells, their adaptive counterpart. With these elegant and novel techniques, the researchers could not only investigate the

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motility of ILCs but also encourage further research into cell migratory patterns between as well as within organs.

The number of migrating ILCs during infection or conditions of inflammation remains poorly understood, as does a complete understanding of migration potential of all ILC populations. Kästele *et al.* quantitatively addressed which intestinal ILC populations are migrating at steady state as well as under acute inflammatory conditions, demonstrating for the first time that all ILC subsets could migrate under homeostasis. ILC1s represented the main ILC population trafficking at steady state, confirming previous data, with most migrating from the mesenteric lymph nodes.[5] Interestingly, ILC2s, ILC3s and T-bet$^{+}$ ILC3s were also trafficking to a similar extent from the mesenteric lymph nodes. During infection, interferon-γ (IFN-γ) responses are crucial in the defense against *Salmonella*.[6] Indeed, Kästele *et al.* identified that migratory ILCs increased expression of several IFN regulatory factors, indicating ILCs may contribute to the resolution of an intestinal bacterial infection through their IFN signature. Whereas the immune activation phenotype of migrating ILCs at steady state and upon infection remained similar, IFN-γ and granulocyte–macrophage colony-stimulating factor (GM-CSF) coexpression were more pronounced upon infection in the draining lymph node, suggesting that ILCs are actively participating in creating a microenvironment in the lymph node to trigger immune responses and resolution of an intestinal bacterial infection. Altogether, the research team has provided a dynamic overview and thus key insights into spatial–temporal patterns of trafficking of ILCs as rare cell populations, which were typically considered tissue resident only (Figure 1).

The work by Kästele *et al.* revealed for the first time that ILCs, albeit at small numbers, are entering the lymph and can migrate to the draining lymph node of the intestine under homeostatic conditions. Importantly, the ability of ILCs to traffic to the mesenteric lymph node was observed to be independent of the state of health, although upon inflammation the composition of migrating ILC subgroups as well as their activation profile subsequently changed. This indicates that the migration and activation profile is indeed influenced by infection; however, the number may be limited by intercellular dynamics. The ability of migratory ILC3s to express IFN-γ alone as well as in combination with GM-CSF suggests that they may directly contribute to the defense against *Salmonella* Typhimurium. Indeed, IFN-γ production is key in *S.* Typhimurium infection.[6] Here, ILC3-derived IFN-γ has been shown to regulate goblet cell formation and inflammatory response upon *Salmonella* infection.[7] In addition, ILC3-derived granulocyte–macrophage colony-stimulating factor GM-CSF is important to recruit inflammatory monocytes, trigger dendritic cells and promote acute intestinal inflammation.[8] Kästele *et al.* now in detail investigated the activation and cytokine profile of migratory ILC3s, linking their capacity to dislocate to the LN as well as their characteristic cytokine profile to fight off intestinal pathogens. Importantly, previous work[9,10] has shown that ILCs, including ILC2s and ILC3s, are located in the interfollicular regions of lymph nodes. Kästele *et al.* confirmed this observation and additionally demonstrated that ILCs migrate via the lymph to reach this area. CCR7 and CD62L have been previously implicated in the migration of ILC progenitors as well as trafficking of ILC1s to the lymph node.[5,11] Importantly, migration of Lymphoid Tissue inducer (LTI)-like ILC3s, a distinct subpopulation of ILC3s, was shown to be CCR7 dependent upon infection with the parasite *Heligmosomoides polygyrus*.[9] Upregulation of CCR7 transcript expression was also observed by unbiased RNA-Seq analysis comparing tissue-resident ILCs in the lamina propria with migratory lymph ILCs, further highlighting the potential role of this chemokine receptor in ILC migration via the lymph. It is evident now that ILCs utilize the lymphatic system to traffic to other organs. However, the precise functional role of ILCs in the lymph node remains elusive. During *Citrobacter rodentium* infection, ILC3s have been shown to play a key role by triggering T-follicular helper responses and immunoglobulin A production.[10] Based on their specific location, ILCs could influence T-cell responses as well as the development and recruitment of myeloid cells in *Salmonella* infection by production of IFN-γ or GM-CSF, respectively. Moreover, splenic IFN-γ has been recently reported to trigger MHC-II expression by LTi-like ILC3s and thereby T-cell activation.[12] T-bet$^{+}$ ILC3-released IFN-γ may thereby impact LTi-like ILC3 responses in the intestinal lymph node upon *Salmonella* infection. Interestingly, ILC3s are also present in other lymph nodes including the lung-draining mediastinal lymph nodes,[9] although ILC2s represent the dominant helper ILC population at this site, highlighting their potential to direct adaptive immune responses.

In this study, Kästele *et al.* clearly demonstrate that all investigated ILC populations are able to migrate from the intestinal tissue to the lymph node at steady state as well as under

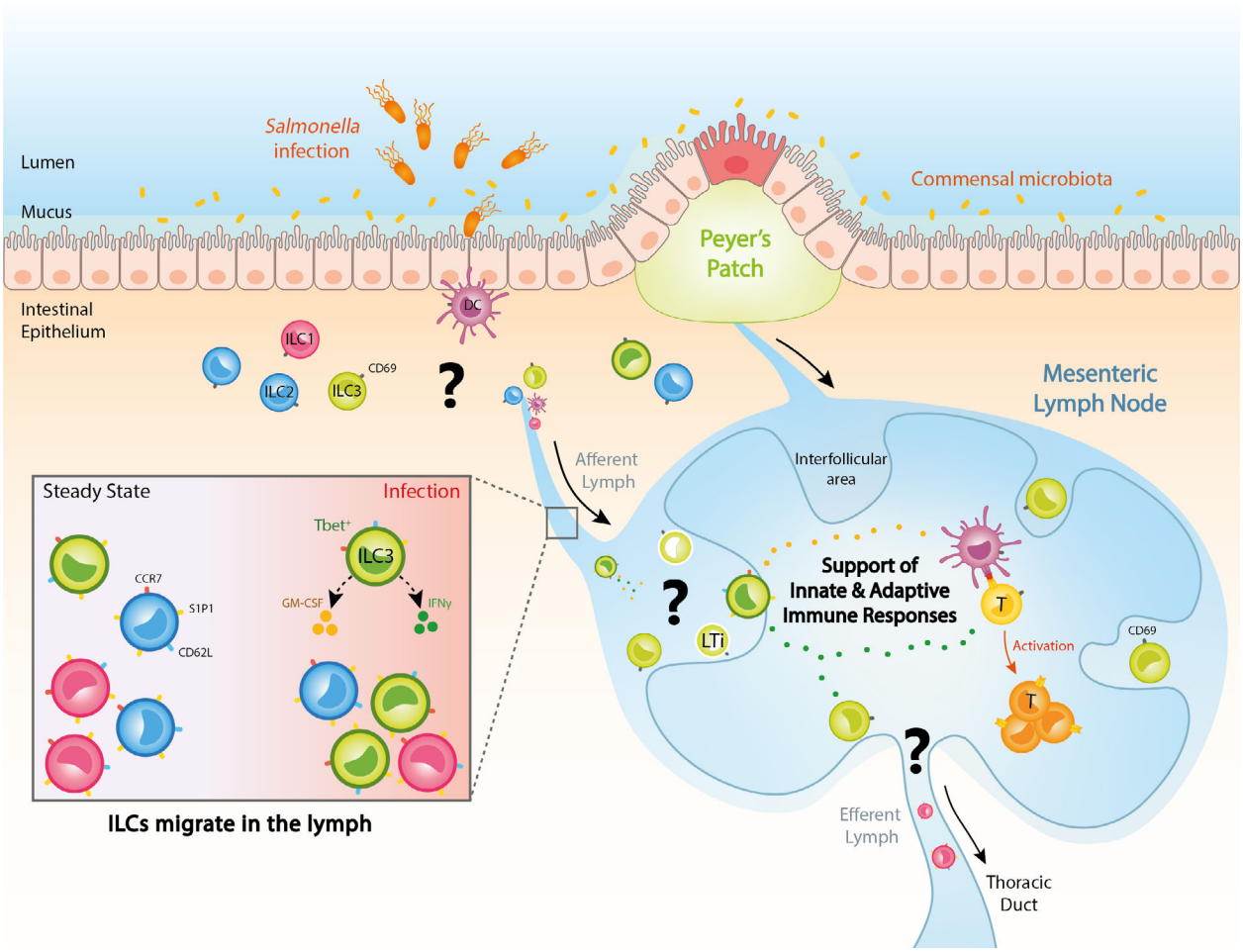


**Figure 1.** Migratory ILC3s travel in the lymph and trigger immune responses upon infection. The scheme depicts the findings by Kästele *et al*. All ILC groups are able to migrate via the lymph under homeostasis. Upon acute *Salmonella* infection, increased numbers of T-bet$^{+}$ ILC3s traffic in the lymph and are present in the mesenteric lymph node expressing IFNγ alone or in combination with GM-CSF. The work raises several key questions for future studies, which are highlighted in the figure by question marks: Which mechanisms trigger ILC3 migration? Do ILC3s collaborate with each other upon infection? How is ILC egress regulated? T-bet$^{+}$ ILC3s are depicted in dark green and LTi-like ILC3s in light green, ILC1s in red and ILC2s in blue. LTi, Lymphoid Tissue inducer; GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN, interferon; ILC, innate lymphoid cell.

inflammatory conditions. However, not all ILC subgroups traffic to the same extent and the exact underlying mechanisms that trigger migration of ILC3s specifically upon *Salmonella* infection and retention in, but also egress of ILCs from the lymph node remain unclear. Interestingly, Kästele *et al*. identified an IFN signature on migrating ILC3s upon infection. IFN may be an important candidate, which could influence restraining of ILC2s and affect the migration of ILC3s in a direct or indirect manner. However, to what extent IFN affects the migration of ILCs still needs to be elucidated. The acquisition of antigen by ILC3s together with additional (maturation) signals may be important in *Salmonella* infection. Interestingly, ILC migration and positioning in the lymph node has been shown to be regulated by the receptor GPR183 sensing cholesterol metabolites such as oxysterol.[10] Whether this is also the case for ILC3s upon *Salmonella* infection is not known. Furthermore, whether changes in nutrient or microbiota composition affect ILC3 migration and positioning in the lymph node upon *Salmonella* infection, and how their retention in the lymph node may regulate adaptive immune responses require further investigation. Overall, the ability of ILCs to migrate to the LN, their expression of cytokines and their positioning are of great interest. Active regulation of these processes may be a target of

scientific and clinical interest to counteract adverse immunopathologies of the intestine.

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