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INSIGHTS

IgM's exit route

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In this issue of *JEM*, Thierry et al. (https://doi.org/10.1084/jem.20180344) demonstrate that, once secreted by freshly activated plasmablasts, IgM leaves the lymph node via the microarchitecture of the fibroblastic reticular cell conduit. This work demonstrates how the very peculiar stromal compartment of lymphatic organs optimizes the systemic distribution of immune effectors.

The release of IgM is the first line of an antibody response and precedes the generation of high affinity IgG in germinal centers. Once secreted by freshly activated plasmablasts, IgM is released into the efferent lymph of reactive lymph nodes as early as 3 d after immunization. As pentameric IgM has an enormous size of 1,000 kD, its diffusibility is low, and one might wonder how it can pass through the densely lymphocyte-packed environment of a lymph node parenchyma in order to reach its exit. In this issue of *JEM*, Thierry et al. show that, in order to reach the blood stream, IgM molecules take a specific micro-anatomical route via lymph node conduits.

Insights from Anne Reversat and Michael Sixt.

The conduit system, the interstitial compartment of secondary lymphatic organs, is the site where extracellular matrix (ECM) molecules (collagens, glycans, etc.) constitute the architecture through which solute extracellular signaling molecules or plasma components move. However, the interstitium of lymphatic organs is substantially different from other mesenchymal tissues, as it is extremely compacted, to an extent that it does not surround its producing cells. The lymphatic fibroblasts, called fibroblastic reticular cells (FRCs), rather enwrap the ECM, which is organized in a 3D meshwork of thin strands with a diameter <1 µm. The functional unit of the FRC and its ECM has been termed FRC conduit because it acts like a microvascular network transecting the lymph node parenchyma. Conduits communicate with afferent lymph in the subcapsular sinus as well as the lumen of blood vessels, and tracer studies showed that conduits not only represent the preferred route of solutes but also act as a molecular sieve. They selectively channel solutes <70 kD in size from the afferent lymph into the blood vessel lumen. Larger substances do not have access to the conduit system and use the lymphatic sinuses to bypass the lymph node parenchyma. They travel via the efferent lymph and the thoracic duct back into the blood. Hence, the lymph node is a two-level filter for interstitial fluid: large molecules pass through the sinus into the efferent lymph. Small solutes pass via the conduit system directly into the blood (Malhotra et al., 2013).

Thierry et al. (2018) started off by immunostaining lymph nodes of freshly immunized mice when they are at the peak of an IgM response. They found that IgM localizes in a reticular pattern, and by costaining with ECM components and reporters for FRCs combined with electron microscopy, they found that IgM localizes in the lumen of conduits. Wondering how it got there, the authors considered two possibilities: either IgM is already in the systemic circulation and enters the lymph node reversely via the blood or the lymph, or it is locally produced in reactive lymph nodes and accesses conduits from within the parenchyma—meaning that IgM is on its way out of the node. A decisive hint arguing against the systemic option came from the fact that IgM was only found in conduits of the lymph node draining the site of inoculation, but not in others. When the authors injected IgM containing serum into Ig-deficient mice via different routes (blood and lymph), the assumption was confirmed, and IgM did not reach the conduit compartment via the lymph (subcutaneous injection) or via the blood (intravenous injection). The only maneuver that led to the reticular localization pattern was injecting IgM directly into the lymph node parenchyma, and the same was true for injecting other high molecular weight tracer molecules. These data were in line with previous tracer studies showing that large mol-

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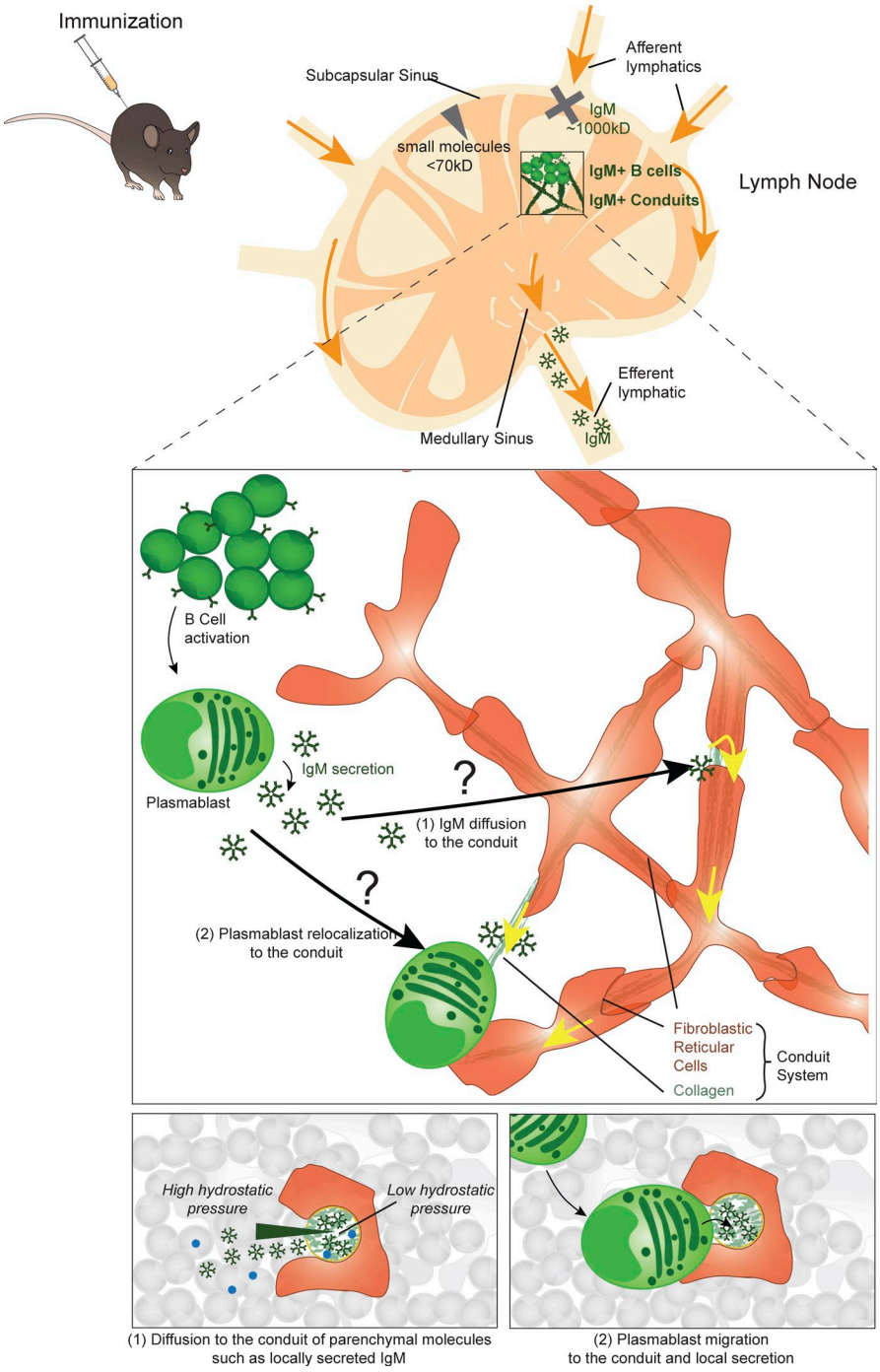

Upper panel: Upon immunization or infection, IgM locates to the draining lymph nodes conduit system. The ∼1,000-kD pentameric antibody is produced locally by plasmablasts during the early phase of infection (i.e., 4 d). The conduit system, composed of FRCs surrounding a network of ECM molecules such as collagens, allows the fast transport of secreted IgM toward the medullary sinus and the high endothelial venules. IgM transported by the afferent lymphatics cannot access the lymph node parenchyma due to the subcapsular sinus acting as a molecular sieve; instead, IgM arriving with the afferent lymph are channeled via the subcapsular sinus around the parenchyma of the lymph node and enter the blood circulation via the thoracic duct. Lower panel: Two possible paths of IgM into conduit lumen. (1) A hydrostatic pressure gradient between lymph node parenchyma and conduit drives fluid and its solutes into the lumen of the conduit. (2) IgM-secreting plasmablasts can migrate, actively couple to FRCs, and directly secrete their antibodies into the conduit system.

ecules do not have access to conduits via the afferent lymphatic route and that there is generally no flux of solutes (not even small ones) from the blood into the lymph node conduits (Gretz et al., 2000). This finding, that upon parenchymal injection IgM enters the conduits, is noteworthy for two reasons. (1) The size-exclusion phenomenon of the conduit system was often compared with a gel filtration-like function of the ECM that sieves out larger components (Gretz et al., 1997). However, a recent paper located the site of size exclusion to the conduit entry sites in the subcapsular sinus, where anatomical structures morphologically and molecularly similar to endothelial fenestrations regulate entry (Rantakari et al., 2015). Thierry et al. (2018) find that large molecules also locate to conduits when directly injected into the parenchyma, thus confirming that the sieve is not the conduit itself but must be at its interface with the subcapsular sinus. (2) Previous studies argued that the conduit system resembles a rather closed "micro-vascular" compartment. In such a scenario only some cells, mainly dendritic cells and macrophages, which are embedded into the FRC layer or stick protrusions into the conduit lumen, have access to the filtrate (Catron et al., 2004). Based on the findings by Thierry et al. (2018), it seems likely that within the parenchyma, the conduit system is not hermetic but openly communicates with the interstitial space between the lymphocytes. Nevertheless, the drainage route of IgM argued that the preferred site of solute transport is the conduit system. Hence, it seems likely that the FRC conduit rather acts like a river delta or an open drainage system than a closed pipe system: it freely communicates with the interstitial space between the lymphocytes but acts as a collector to drain fluid and solutes.

After applying IgM externally, which might perturb the potentially delicate architecture and physiology of the lymph node, the authors also devised two sophisticated genetic approaches to demonstrate that endogenous IgM also enters conduits during immunologically relevant responses. They created two mouse models where IgM is produced either in response to antigenic vaccination or to viral infection. Due to genetic mismatches, freshly produced antigen-specific IgM could be selectively detected in situ. Both models confirmed the initial finding: once produced by the plasmablast, IgM is not only located on the surface of the producing cells but also within the conduit system. This adds compelling evidence that for IgM, the conduit system is the preferred drainage route out of the lymph node.

The work of Thierry et al. (2018) allocates a very plausible function to an anatomical structure that is still poorly understood. Ini-

tial work on the conduit system led to the assumption that it might primarily serve as an input system, where peripherally produced cytokines, chemokines, and antigens that arrive with the afferent lymph get channeled to resident dendritic cells, B cells, and also into the lumen of high endothelial venules. The findings of Thierry et al. (2018) suggest that conduits also have an output function by allowing substances to leave the lymph node. This adds to a previous study, which showed that upon peripheral bacterial infection, a fragment of the ECM protein Cochlin is cleaved from the lumen of conduits and released into the periphery, where it serves to amplify cytokine responses (Py, 2013).

Mechanistically, many open questions remain. How does IgM travel from the plasmablast into the conduit lumen? Thierry et al. (2018) contemplate two possibilities. (1) The migratory plasmablast may actively establish physical contact with the conduit and directly secrete IgM into its lumen. (2) The plasmablast secretes into the parenchyma, and IgM follows a fluid current along a hydrostatic pressure gradient that drains into the conduit. From the conduit, IgM might then directly enter the blood circulation (most likely via drainage into high endothelial venules) or be discharged into the medullary sinus, from where it reaches the blood circulation via the efferent lymph and the thoracic duct. To better understand these important processes, it will be essential to test basic physiological parameters of the homeostatic and the inflamed lymph node. What are the forces driving fluid and its solutes through the conduit system? In peripheral tissues, fluid exchange between vasculature and interstitium is driven by Starling forces—the difference between osmotic suction and hydrostatic pressure across the vessel wall (Levick and Michel, 2010). Is the same true in lymphatic organs, where the interstitium is organized in such a peculiar way? Or is there another force driving flux within the conduits? Interestingly, the conduits of the spleen and the thymus, organs that lack the additional fluid supply by afferent lymphatics, are filled "retrogradely" via the blood, probably meaning that the blood vessels of these organs are in a state of constant fluid secretion, as opposed to the fluid-resorbing state of the lymph node blood circulation. Could lymph nodes also switch to this mode, e.g., when venous pressure increases, the osmotic state of the lymph node parenchyma changes, or when the muscular tone of blood vessels or conduits is tuned? None of these parameters have been studied, but they might have profound implications for the orchestration and regulation of adaptive immune responses.

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