cGAMP-dependent processes beyond antiviral immunity.

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T Cells: Bridge-and-Channel Commute to the White Pulp

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In contrast to lymph nodes, the lymphoid regions of the spleen—the white pulp—are located deep within the organ, yielding the trafficking paths of T cells in the white pulp largely invisible. In an intravital microscopy tour de force reported in this issue of *Immunity*, Chauveau et al. show that T cells perform unidirectional, perivascular migration through the enigmatic marginal zone bridging channels.

Most of our knowledge on T cell trafficking in secondary lymphoid organs comes from studies in lymph nodes. Naive T cells of the blood circulation continuously move through lymph nodes by crossing through a specialized set of postcapillary venules, the high-endothelial venules (HEVs). T cells interact with HEVs in a well-defined sequence of molecular events, involving the presence of chemokines and adhesion receptors, before they transmigrate through the endothelial layer and enter the T cell cortex of the lymph node. After scanning the T cell parenchyma for antigens presented by antigen-presenting cells, T cells leave the lymph node by entering efferent lymphatics, allowing their return to the systemic blood circulation. In contrast to lymph nodes, which are plugged into the lymphatic system and filter interstitial fluid, the spleen is the immunological filter of the blood circulation. Due to its direct location in the circulatory system, the spleen is unique among all secondary lymphoid organs with a special splenic architecture that is grossly divided into the lymphocyte-rich white pulp (WP) and the erythrocyte-rich red pulp (RP). A region termed the marginal zone (MZ) forms the border between these two compartments in rodents. In the WP, T cells ensheath central arterioles that supply the spleen with arterial blood, while B cells organize as follicles at the outer parts. The MZ surrounds the B cell follicles and harbors special subsets of B cells, macrophages, dendritic cells, and stromal cells (Mebius and Kraal, 2005). As a major difference to lymph nodes, spleens are missing HEVs, and newly arriving T cells from the blood are released with the arterial blood supply. Almost 50 years ago, it was shown that intravenously injected ink particles primarily label the MZ but that at some sites, WP channels penetrate the MZ envelope and directly enter the RP. Similarly, injection of radiolabeled

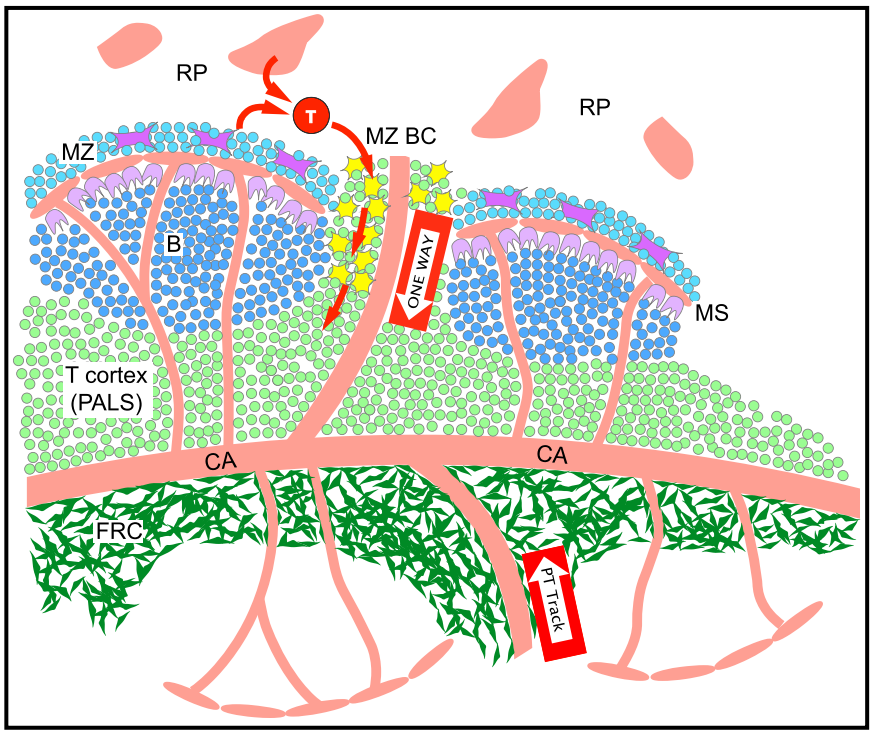

Figure 1. Homeostatic Trafficking of Naive T Cells into the Splenic White Pulp

Blood-circulating naive T cells enter the spleen preferentially from vessels in the red pulp and the marginal sinus (Tadayon et al., 2019). Chaveau et al. (2020) demonstrate that T cells are collected from their initial release sites and follow the outer layer of blood vessels, which serve as guidance structure to lead T cells via the marginal zone bridging channels into the T cell cortex. The blood vessel is coated with a unique subset of reticular cells that provide a migration scaffold for T cells during their passage of the bridging channel. This pathway is one directional for naive T cells and exclusively used for entry into the white pulp, but not for egress. Color labeling of immune cells is as follows: T cells (light green), follicular B cells (dark blue), MZ B cells (light blue), marginal metallophilic macrophages (light purple), marginal zone macrophages (dark purple), DICR2$^+$ CD4$^+$ dendritic cells (yellow), fibroblastic reticular cells (dark green). Not depicted in the image are dendritic cell subsets in MZ and T cell cortex and stromal cells in B cell follicles, MZ and RP. B, B cell follicle; CA, central arteriole; FRC, fibroblastic reticular cells; MS, marginal sinus; MZ, marginal zone; MZ BC, marginal zone bridging channel; PALS, periarteriolar lymphoid sheath; PT track, perivascular T cell track, RP, red pulp; T, T cell.

lymphocytes revealed that channels of lymphocytes breach the MZ at sites that were called "marginal zone bridging channels" (MZ BCs) (Mitchell, 1973). MZ BCs are identified by a gap in the ring of metallophilic macrophages (Lewis et al., 2019) and an enrichment of a specific CD4$^+$ subpopulation of dendritic cells, which are retained at this site through oxysterol ligands that are produced by the local stroma cells (Lu et al., 2017). How MZ BCs contribute to the trafficking of T cells has been highly debated. Early studies favored a model where MZ BCs serve as an exit route out of the WP via which T cells could directly access the RP venous system and return to the circulation (Brelińska et al., 1984; Mitchell, 1973). In contrast, sequential static imaging studies suggested that after release from the circulation into the marginal sinus, T cells use MZ BCs to enter the WP (Bajénoff et al., 2008). These findings have led to a view that MZ BCs are a pathway for bidirectional T cell trafficking, but the inaccessibility of the spleen to intravital imaging did not allow direct validation of the models. Now, through real-time observation of T cell trafficking through the spleen, Chauveau et al. (2020) demonstrate that, once released from the blood flow, T cells enter a perivascular compartment and back trace the vessel, which leads them through the BC and into the T zone.

In contrast to the small-sized lymph nodes, the splenic WP is buried underneath a thick capsule and a layer of RP with its highly light absorbing and light scattering red blood cells (Herz et al., 2012). These features make intravital imaging of the spleen challenging. Arnon and colleagues (Chauveau et al., 2020) leveraged expertise in splenic WP imaging in living anesthetized mice (Arnon et al., 2013). To visualize B cell follicles, T cell areas, stromal compartments, and freshly transferred T cells, the authors devised a whole battery of new genetic models. Combining mice bearing reporter alleles for the stromal compartment and mixed bone marrow chimerism of mice bearing reported alleles marking B and T lymphocytes, the authors were able to achieve combined life visualization of the B and T cell compartment and MZ. They first focused on T cell behavior in the MZ and RP and found that, while T cells vigorously migrated in the T zone, T cells in MZ and RP were largely stationary and rounded. However, upon closer inspection of the different anatomical compartments, they found that a subpopulation of T cells was actively motile and that this behavior was confined to the MZ BCs. The T cells migrating in these areas organized in relatively straight tracks, and importantly, the trafficking direction was largely oriented from the RP toward the WP. Characterization of the compartment that fostered directional migration revealed that T cells always surrounded a blood vessel and that this perivascular compartment, the perivascular T cell track (PT-track), was surrounded by a specific subset of lymphatic stromal cells. These stromal cells resembled in many (but not all) aspects the typical T zone fibroblastic reticular cells and expressed the T cell attracting chemokine CCL21. Thus, once released from the blood circulation, T cells trace back the path of the blood vessel and enter the WP via the MZ BCs (Figure 1).

To functionally characterize the migratory behavior, Chauveau et al. (2020) first interfered with adhesion receptors of the integrin family using blocking antibodies. Although simultaneous blockade of VLA-4 and LFA-1 slowed cells, they were still able to make it into the WP and also kept their route along the PT-tracks. This indicated that, similar to migration within lymph nodes, integrins contribute to the transmission of traction forces but are not essential for locomotion and localization. Deletion of CCR7, the receptor for the T cell attracting chemokines, had a more substantial effect, and although CCR7-deficient T cells still associated

with PT-tracks, they lost their directional preference and moved both away and toward the WP. Interestingly, while half of the CCR7-deficient cells still reached the WP via the BCs, CCR7 was absolutely essential for final entry into the WP. These findings suggested that CCR7 ligands likely determine the direction of movement toward and also invasion into the WP. At the same time, other factors must exist that define movement along the PT-tracks, and these are likely G protein-coupled receptor (GPCR) ligands, as blockade of G alpha i signaling via Pertussis toxin completely stalled migration of the T cells.

These findings firmly establish T cell trafficking routes in the spleen in homeostasis. Do these routes change during an immune challenge? To address this question, the authors tested how acute inflammation affects T cell migration along PT-tracks. Acute inflammation causes a shutdown of lymph nodes and spleen, where both entry and exit of lymphocytes are drastically reduced. Systemic injection of lipopolysaccharide led to a substantial block of T cell entry via PT-tracks into the WP and had similar effects on the T cells as the application of Pertussis toxin. This suggests that systemic inflammation not only reduces the expression of CCR7 ligands but might also affect the yet-to-be identified GPCR signal that triggers PT-track association of the T cells.

In summary, the study of Chauveau et al. (2020) provides a view on T cell dynamics within a yet-enigmatic anatomical compartment of the spleen. This sets the stage for many unexplored and exciting questions. How are other immune cells guided through the MZ BCs during spleen homeostasis and inflammation? Is it possible that CCR7-expressing dendritic cells and B cells follow the same guidance cues during their travel in MZ BCs? How does their positioning and migration kinetics influence the trafficking of T cells on PT-tracks? It will be exciting to study if human spleens also have a BC equivalent. As advanced imaging techniques have not yet been extensively applied to the human spleen, it remains unknown if the perifollicular zone, forming the border between WP and RP in humans, is analogous on the anatomical level to the MZ in rodents (Lewis et al., 2019). From the study of Chauveau et al. (2020), it is now clear that T cells use the MZ BC as entry port into the WP and not as exit route. But where in the spleen do naive T cells then reenter the systemic circulation—through venous sinusoids in the RP or the controversially discussed efferent lymphatics? Since road planning in the spleen has designed a one-way street toward "downtown" T cell cortex, it will be interesting to identify the exit roads back to the outskirts.

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