SUPPLEMENTAL MATERIAL

Hickman et al., http://www.jem.org/cgi/content/full/jem.20102545/DC1

Video 1. Separation of macrophages and DCs after dextran injection. 20-min MPM movies of maximum intensity projections of draining inguinal lymph nodes (DLNs) 6 hpi with a nonfluorescent VV. Approximately 40 μm depth below the capsule is shown (many of the DCs that appear to be in the MRR are actually above it (note that the two colors do not co-localize)). Two different magnifications are shown. DCs=green Dextran+ macrophages= red. Collagen (blue) visualized using second harmonic generation (SHG). Scale bars are shown in micrometers. Time is shown in minutes.

Video 2. Intravital visualization of macrophages and DCs after dextran injection. Rotation of a 3D blended image (not maximal intensity projection as in other videos) showing distinct DC areas (green) outside the MRR (red) of the LN 10 hpi with a nonfluorescent VV. OT-I cells (magenta) are largely obscured in the blended view. Collagen (blue) visualized using second harmonic generation (SHG).

Video 3. OT-I cells interact with DCs outside of the MRR after VV infection. 10⁷ blue cell tracker–labeled OT-I cells (light blue) were transferred into CD11c-eYFP mice, which were infected 12 h later with nonfluorescent VV (right). Mice were given rhodamine dextran (red) labeling the MRR 45 min before multiphoton microscopic imaging. Maximal intensity projection of the top 30 μm (in depth) just beneath the SCS of the LN is shown. Uninfected LN (left), VV-infected LN 6 hpi without cognate antigen (middle), or VV-infected LN 6 hpi with cognate Ag (SIINFEKL; right). Collagen fibers were imaged using second harmonic generation (SHG, dark blue). Time is shown in minutes.

Video 4. OT-I cells interact with VV-infected DCs and non DCs in the ILN. 10⁷ blue cell tracker-labeled OT-I cells (light blue) were transferred into CD11c-eYFP mice 12 h before infection with VV-NP-S-mCherry (red, nuclear expression). LNs were imaged from 10–12 hpi interactions with each APC type (infected DC, “uninfected” DC, and infected non DC) are shown in higher magnification. Time is shown in minutes.

Video 5. T cells interact with DCs outside of the LN MRR. Higher magnification MPM images (maximum intensity projections) of T cell/DC interactions. 10⁷ blue cell tracker-labeled OT-I cells (light blue) were transferred into CD11c-eYFP mice which were infected 12 h later with nonfluorescent VV (right). Mice were given rhodamine dextran (red) labeling the MRR 45 min before MPM imaging. Maximal intensity projection of the top 30 μm (in depth) just underneath the SCS of the LN is shown. Uninfected LN (left), VV-infected LN 6 hpi with cognate Ag (SIINFEKL, nonfluorescent; right). Collagen fibers were imaged using second harmonic generation (SHG, dark blue). Time is shown in minutes.

Video 6. Most of the infected cells in a VV-infected LN are macrophages. Control mice (C57BL/6; left) or CD11c-DTR-eGFP mice treated with DTx (right) were infected for 6 h with VV-NP-S-eGFP (green, nuclear). 30 min before imaging, mice were given rhodamine dextran (red). A white dotted line delineates the borders of the MRR. Arrowheads point to some of the many infected macrophages that have red cytoplasmic staining (dextran in endocytic vesicles) and green nuclear staining (virus). Note that many infected cells are found outside of the MRR in control animals (left, indicated by arrowheads), and the absence of infected cells outside the MRR in DTx-treated mice. Time is shown in minutes.
Video 7. **CD8+ T cells move into the MRR in DC-ablated, VV-infected lymph nodes.** CD11c-DTR-eGFP mice were treated with Dtx for 12 h before infection with nonfluorescent VV-SIINFEKL. −12 h before Dtx-treatment, mice received 1.0 × 10⁷ CMPTX-labeled OT-I cells (red). 30 min before imaging, mice were given tetramethylrhodamine dextran s.c. to label macrophages in the inguinal LNs. LNs were imaged 6–10 hpi. A white dashed line delineates the MRR. Time is shown in minutes.

Video 8. **OT-I cells form tight, long-lasting contacts with infected cells in DC-ablated lymph nodes.** Right panel) WT mice were given 10⁶ CMTPX-labeled OT-I cells (red) ~24 h before infection with VV-NP-S-eGFP (green, nuclear). LNs were imaged between 6 and 8 hpi. Circles highlight some of the contacts between OT-I cells and VV-infected cells that lasted the course of the intravital imaging period (30 min). White dots follow the path of one OT-I cell that failed to form tight contacts in an untreated mouse during the observation window. (left) Same as above but in Dtx-treated CD11c-DTR-eGFP mice. Time is shown in minutes.

Video 9. **OT-I cells move into the MRR when infection occurs in the presence of chemokine-neutralizing Abs.** 10⁷ blue cell tracker–labeled OT-I cells (light blue) were transferred into CD11c-eYFP mice 12 h before s.c. infection with VV-ovalbumin (nonfluorescent). At the same time as infection, mice were given chemokine neutralizing Abs against CCL3, CCL4, and CCL5 i.v. At 5.25 hpi, mice were given rhodamine-dextran (red) to identify macrophages. Nodes were imaged 6–8 hpi. The boundary of the MRR is marked with white dashed lines. Collagen fibers were imaged using second harmonic generation (SHG) and are shown in dark blue. Approximately 30 μm (in depth) of the LN is shown just underneath the SCS. Time is shown in minutes.

Video 10. **OT-I cells rapidly scan APCs in the presence of exogenous rCCL3.** 10⁷ red cell tracker–labeled OT-I cells (red) were transferred into untreated (WT) mice 12 h before s.c. infection with VV-SIINFEKL (nonfluorescent). 2 h 15 min after infection, mice were given FITC dextran (green) with saline (left) or with 1 μg rCCL3 (right). LNs were MPM imaged at 3 hpi. A maximum intensity projection of ~40 μm of the LN is shown. T cell tracks over time are indicated by white "dragon tails" (which show the last 10 min of an individual cell’s track). Time is shown in minutes.

Video 11. **CCR5KO OT-I cells are located both outside and within the MRR after VV infection.** 10⁷ red cell tracker–labeled OT-I cells (red) were transferred into WT mice 12 h before s.c. infection with VV-NP-S-eGFP (green). 6 hpi, MPM images were acquired from the inguinal LN. A maximum intensity projection of ~65 μm of the LN is shown. The MRR was visualized by the intrinsic autofluorescence of macrophages (with detector voltages increased for sensitivity), and is outlined with a white dashed line. Collagen fibers were imaged using second harmonic generation (SHG) and are shown in dark blue. Note the many CCR5KO OT-I cells located in a blood vessel just next to the time indicator in the image. Time is shown in minutes. Scale bars are shown in micrometers.