

Movie S1: Phase-1 interactions of CD4⁺ T cells with LPS or Papain Treated DC.

Related to Figure 1.

Polyclonal B10.A CD4⁺ T cells and antigen-specific 5CC7 CD4⁺ T cells interacting with LPS- or papain-treated, 0.1μM pPCC pulsed CD11c⁺ DC. The LPS- or papain-treated peptide-pulsed DCs (100μM CMF2HC, blue) were injected into the footpad of a recipient CD45.1⁺ B10.A mouse. Eighteen hrs later, control polyclonal CD4⁺ T cells (1.25μM CMFDA, green) and antigen-specific 5CC7 CD4⁺ T cells (1.25μM CMTPX, red) were injected i.v. Images were collected immediately afterwards from 0-2.0 hrs. following CD4⁺ T cell transfer in the popliteal LN of an anesthetized mouse using 2P-IVM. (x, y, z = 213μm, 213μm, 84μm; merge of z-stack), time-lapse over 2hr.

Movie S2: Phase-2 and Phase-3 interactions of CD4⁺ T cells with LPS- or p-apain treated DC. Related to Figure 2.

Polyclonal B10.A CD4⁺ T cells and antigen-specific 5CC7 CD4⁺ T cells interacting with LPS- or papain-treated, 0.1μM pPCC pulsed CD11c⁺ DC. The papain-treated peptide-pulsed DCs (100μM CMF2HC, blue) were injected into the footpad of a recipient CD45.1⁺ B10.A mouse. Eighteen hrs later, control polyclonal CD4⁺ T cells (1.25μM CMFDA, green) and antigen-specific 5CC7 CD4⁺ T cells (1.25μM CMTPX, red) were injected i.v. followed 2h later by 100mg Mel-14 antibody i.v. Images were collected at either 12 hours or 22 hours post-transfer for 1hr in the popliteal LN of an anesthetized mouse using 2P-IVM. (x, y, z = 283μm, 283μm, 97μm; merge of z-stack), time-lapse over 1hr.

Movie S3: LPS- vs. papain-treated DC competitive CD4⁺ T cell interaction assay. Related to Figure 3.

Antigen-specific 5CC7 CD4⁺ T cells (red) interacting with LPS treated, 0.1μM pPCC pulsed CD11c⁺ DCs (green) vs. papain treated, 0.1μM pPCC pulsed CD11c⁺ DCs (blue). LPS-treated peptide-pulsed DCs (100μM CMF2HC, blue) and papain-treated peptide-pulsed DCs (2.5μM CMFDA, green) were co-injected into the footpad of a recipient CD45.1⁺ B10.A mouse. Eighteen hrs. later, antigen-specific 5CC7 CD4⁺ T cells (1.25μM CMTPX, red) were injected i.v. Images were collected immediately after from 0-2.0hr

following CD4⁺ T cell transfer in the popliteal LN of an anesthetized mouse using 2P-IVM. (x, y, z = 283μm, 283μm, 72μm; merge of z-stack), time-lapse over 2hr.

Movie S4: Proximal Ca²⁺ flux during in vivo activation. Related to Figure 4.

CD11c⁺ splenic DC were isolated and incubated in 0.1μM pPCC at 37°C for 4hr with either LPS or papain or with CpG or SEA. DC were adoptively transferred into the rear foot pads of naïve CD45.1+ B10.A animals. Eighteen hrs. post transfer 5CC7 CD4⁺ T cells labeled with CMTPX and Fluor-4 were adoptively transferred by i.v. injection and imaged immediately afterwards for 2hr in the popliteal LN of an anesthetized mouse using 2P-IVM. (x, y, z = 213μm, 213μm, 52μm; merge of z-stack), time-lapse over 2hr 0 min 23s (10 frames/s).

Movie S5: Whole LN reconstruction of LPS- vs. papain-treated DC competitive CD4⁺ T cell interaction assay. Related to Figure 4.

Antigen-specific 5CC7 CD4⁺ T cells (red) interacting with LPS-treated, 0.1μM pPCC pulsed CD11c⁺ DCs (green) vs. papain-treated, 0.1μM pPCC pulsed CD11c⁺ DCs (teal), second harmonic signal from collagen (blue). LPS-treated peptide-pulsed DCs (100μM CMF2HC, teal) and papain-treated peptide-pulsed DCs (2.5μM CMFDA, green) were co-injected into the footpad of a recipient CD45.1+ B10.A mouse. Eighteen hrs. later, antigen-specific 5CC7 CD4⁺ T cells (1.25μM CMTPX, red) were injected i.v. Two hours following CD4⁺ T cell transfer LN were harvested, fixed immediately, serially sectioned into 100μm sections and imaged by 2P-IVM. Serial sections were then compiled using Imaris imageworks to reconstruct the whole LN. (x, y, z = 1416μm, 1416μm, 384μm)