SUPPLEMENTAL MATERIAL

Kunder et al., http://www.jem.org/cgi/content/full/jem.20090805/DC1

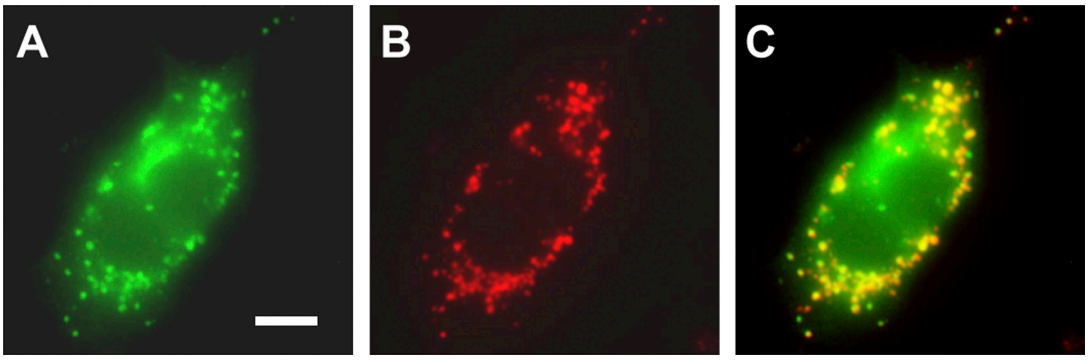


Figure S1. TNF-GFP fusion protein colocalizes with granule markers in RBL-2H3 cells. (A) TNF-GFP expression. (B) Serotonin labeling. (C) Colocalization. These images are representative of observations made in two independent experiments. Bar, 5 µm.

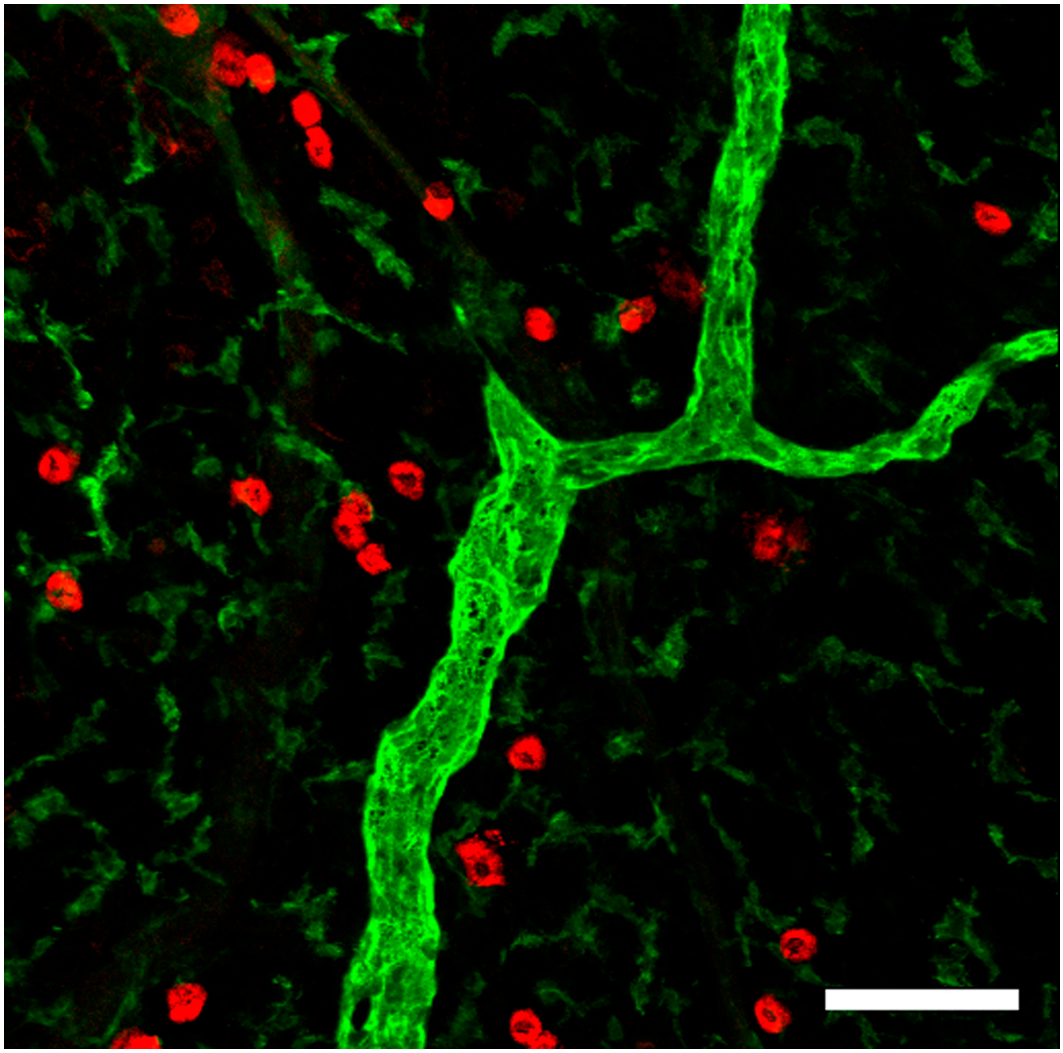


Figure S2. Intact MCs in untreated rat mesentery. Green, LYVE-1; red, heparin. The image is representative of observations made on two independent experiments. Bar, 125 µm.

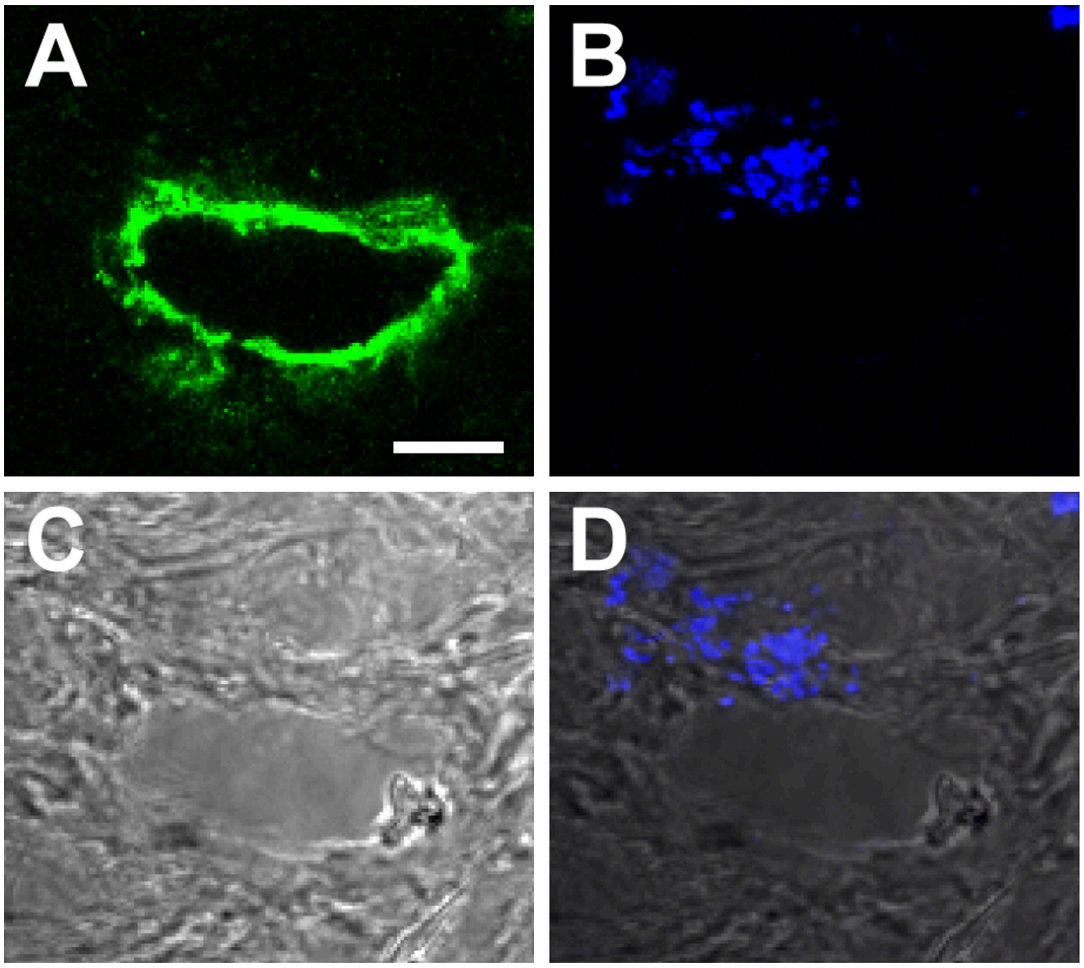


Figure S3. Intralymphatic MC-derived particle is not inside a migratory phagocyte. (A) Green, LYVE-1. (B) Blue, heparin. (C) Differential contrast interference micrograph of local morphology. (D) DIC image and heparin signal superimposed. Bar, 8 µm.

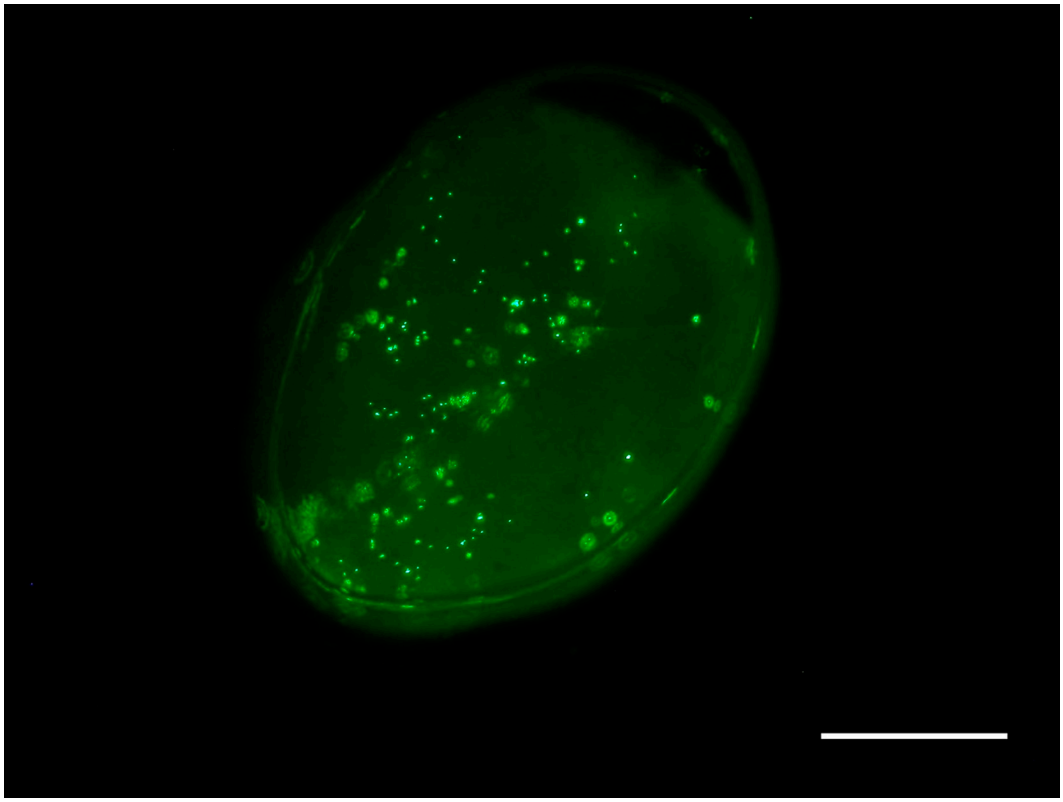


Figure S4. Particles the size of MC-derived particles can enter lymphatics and rapidly reach the DLN under physiological conditions. Epifluorescence micrograph of whole-mounted mouse popliteal LN 30 min after the introduction of fluorescent microspheres into an experimental laceration. The result shown is representative of more than three independent experiments. Bar, 250 µm.

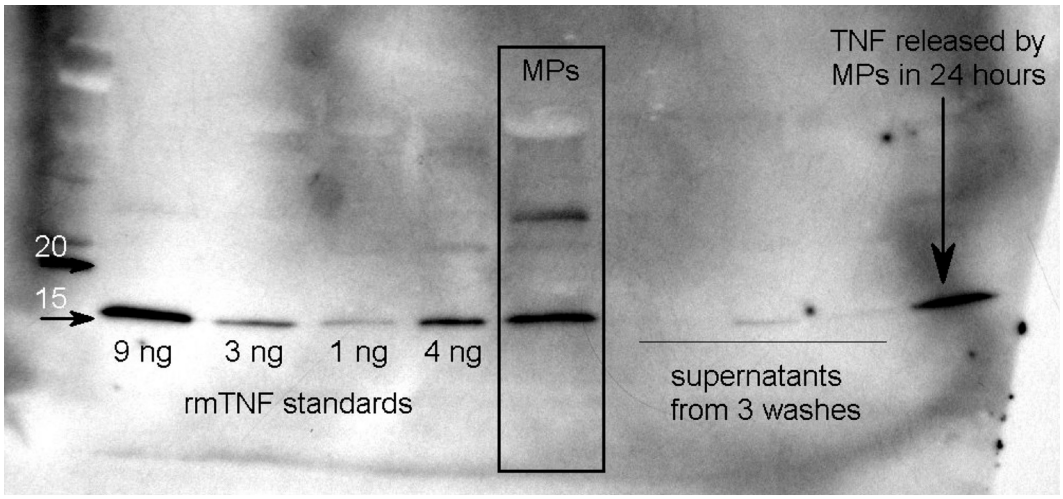
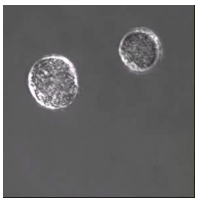
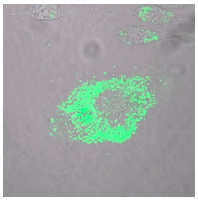


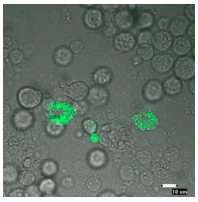
Figure S5. Recombinant TNF is gradually released from heparin-based microparticles. Immunoblot showing the disposition of recombinant mouse TNF (rmTNF) during encapsulation. After the initial precipitation of the particles, they were washed three times with the same volume PBS. The particles were then allowed to stand for 24 h at room temperature, after which they were sedimented a final time. The sediment (MP-associated TNF) is shown in the boxed lane. The supernatant (TNF released from MPs over 24 h) is shown in the right lane. The five left lanes show a molecular size standard and quantitation standards of known amounts of rmTNF. The data shown are representative of more than three independent experiments.



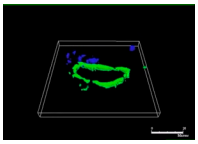
Video 1. DIC video microscopy of compound 48/80–activated rat peritoneal MCs releasing granule particles. This observation is representative of more than three independent experiments.



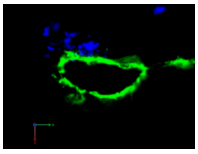
Video 2. Green fluorescent/DIC overlay video of ionomycin–activated TNF-GFP–expressing RBL-2H3 cells. This observation is typical of more than three independent observations made on two separate batches of virally infected cells.



Video 3. Green fluorescent/DIC overlay video of ionomycin–activated TNF-GFP–expressing 3T3 fibroblast–cocultured BMMCs. This observation is typical of more than three independent observations made on two separate batches of virally infected cells.



Video 4. Three-dimensional reconstruction of laser-scanning confocal data showing MC-derived particles inside a dermal lymphatic vessel of compound 48/80–injected mouse rear footpad. This experiment is detailed in Fig. 3 G.



Video 5. Isosurface reconstruction of the same region shown in Video 4.