The Journal of Experimental Medicine

SUPPLEMENTAL MATERIAL

Wuest et al., http://www.jem.org/cgi/content/full/jem.20091385/DC1

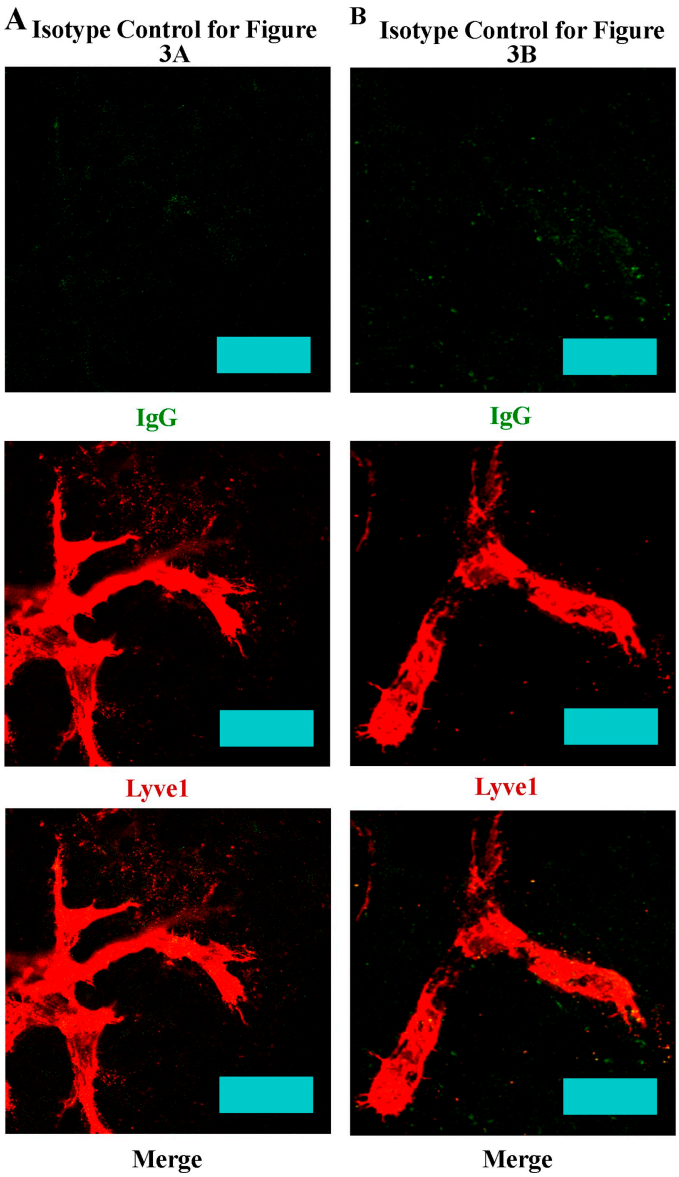

Figure S1. Isotypic controls for Fig. 3 (A and B). Images showing isotypic controls (goat IgG) for VEGFR-2 and VEGFR-3 staining. Images are representative of two experiments, $n = 6$ corneas, using a 400× objective to acquire these images. Bars, 100 µm.

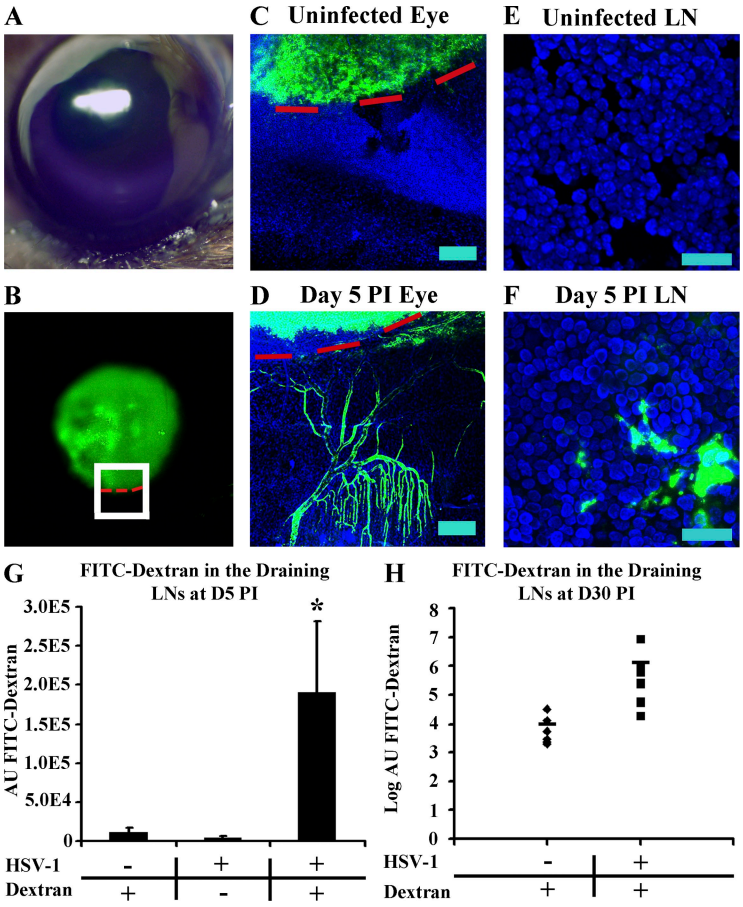

Figure S2. HSV-1–induced lymphatic vessels transport soluble antigen. The capability of HSV-1–associated corneal lymphatics to transport antigen was tested by dye transport assay. Animals were infected with HSV-1 and, at day 5 PI, corneas were injected with 1 µl of FITC-labeled 2,000-kD dextran intrastromally. (A) 40× white light image of injected eye of uninfected animal. (B) 40× green fluorescent image to show dye injection site with a diagram showing the approximate point of the microscope image frame in C and D. (C and D) Uninfected eyes (C) and eyes harvested from animals at day 5 PI (D) were harvested 1 h after FITC-Dextran injection and imaged for the transport of FITC-Dextran (green) from the cornea. FITC-Dextran was observed in lymphatic vessels draining the cornea of infected but not uninfected animals. Nuclei are counterstained with DAPI (blue). Bars, 500 µm. Images are representative of two experiments. $n = 6$. (E and F) At 24 h after injection, FITC-Dextran draining from the cornea was infrequently observed in the draining lymph nodes of uninfected mice (E), whereas dextran was abundant in the draining lymph nodes of mice at day 5 PI (F). Representative images are of lymph node sections showing FITC-dextran dye (green) with nuclei stained with DAPI (blue). Bars, 20 µm. Images are representative of two experiments. $n = 6$. (G) LN cells were analyzed by flow cytometry for staining with FITC-Dextran. LN cells were harvested at 24 h after intrastromal dye injection in either uninfected animals or animals at day 4 PI with HSV-1. FITC-Dextran levels are expressed in arbitrary units (AU), defined as the multiple of total FITC-Dextran$^+$ events in the draining lymph nodes times their mean fluorescence intensity. FITC-Dextran arbitrary units were significantly elevated in HSV-1–infected animals at day 5 PI. Data are from three experiments, with a total of $n = 11$ for uninfected and HSV-1–infected animals that were injected with FITC-Dextran and of $n = 8$ for HSV-1–infected animals not injected with FITC-Dextran. *, $P < 0.05$ comparing the indicated group to the other two groups. Error bars represent the SEM based on the results of each cornea sample summarized for three experiments. (H) We did observe a trend for elevated FITC-Dextran$^+$ arbitrary units in latently infected animals. However, this trend was not statistically significant despite the mean FITC-Dextran$^+$ arbitrary units being >2 logs higher (1.36×10^6 AU $\pm$ 9.8×10^5) than that observed after injection into uninfected animals (9.6×10^3 AU $\pm$ 4×10^3). Horizontal bars represent the mean. Data are from two experiments. $n = 6$.