Supplementary Material

Methods

SNARF-1 Labeling and Adoptive Transfer of Cells

As an alternative to CFSE labeling of monocytes, we also conducted adoptive transfer studies where we labeled isolated peripheral blood monocytes with SNARF-1 carboxylic acid, acetate, succinimidyl ester (carboxy SNARF-1). Peripheral blood mononuclear cells were isolated and purified from donor day 8 BDR and Sham mice as described in the 'Methods' sections in the main text. Purified monocytes were loaded with carboxy SNARF-1 (Molecular Probes) at a concentration of 10uM/10^6cells for 30 minutes at 37°C (Anthony ML et al., J Biol Chem 1999). Approximately 60,000 SNARF-1 labeled monocytes were then injected per mouse in 200uL of PBS intravenously by retro-orbital injection into respective recipient day 8 BDR or Sham mice. At day 10 post-surgery, brains were removed and embedded in paraffin. For immunohistochemical staining for Von Willebrand Factor (vWF), the same protocol as outlined in the 'Methods' section in the main text was carried out. For detection of primary rabbit anti-vWF antibody (Abcam), sections were incubated with a secondary Alexa Flour 488 goat anti-rabbit IgG antibody (Molecular Probes) for 1 hour at room temperature. Sections were mounted in glycerol and viewed using a confocal microscope (Fluoview, Version 1.6; Olympus America Inc., Melville, NY).

Results

Monocytes are Recruited into the Brains of day 10 Recipient BDR mice

Donor purified Sham CFSE labeled monocytes were not found in the brains of recipient day 10 Sham mice which had been infused with CFSE labeled monocytes isolated from the peripheral blood of the donor Sham mice (Supplemental Fig. 1A). In contrast to Sham mice, in brain sections from day 10 recipient BDR mice CFSE labeled monocytes were readily identified in the regions of the brain adjacent to cerebral blood vessels and within the brain parenchyma (Supplemental Fig. 1B). SNARF-1 labeling of isolated and purified peripheral blood monocytes (red fluorescence) was performed to confirm our results obtained with CFSE labeled monocytes in day 10 BDR mice. Supplemental Fig. 1C demonstrates both a SNARF-1 labeled monocyte present within a blood vessel (open arrow) in addition to a second monocyte located outside the blood vessel (closed arrow) and within the brain parenchyma of a day 10 BDR mouse. Cerebral endothelium stained for vWF (ie. green fluorescence) clearly identifies a SNARF-1 labeled monocyte (ie. red fluorescence) to be present within the brain parenchyma (Supplemental Fig. 1D).

Figure Legends

Figure 1: CFSE and SNARF-1 labeled monocytes are recruited into the brains of day 10 BDR mice. Panel A demonstrates the absence of CFSE labeled monocytes in the brains of day 10 Sham mice (blood vessels are indicated by arrowheads). Panel B demonstrates a CFSE labeled monocyte present within the cerebral parenchyma (closed arrow) and a CFSE labeled monocyte that appears to be adherent to the cerebral vascular endothelium (open arrow) in a brain section from a day 10 BDR mouse. Panel A and B are

representative of the periventricular regions in brain sections from n=3 day 10 Sham (Panel A) and BDR (Panel B) mice. Scale bar = 10μm. Panel C indicates a SNARF-1 labeled monocyte present in the cerebral parenchyma (closed arrow) and a SNARF-1 labeled monocyte that appears to be adherent to the cerebral vascular endothelium (open arrow) in a brain section from a day 10 BDR mouse. Panel D demonstrates a SNARF-1 labeled monocyte within the cerebral parenchyma in an area remote from the cerebral blood vessel as identified by staining the endothelium for vWF. Panels C and D are representative of blood vessels in the periventricular region of n=3 day 10 recipient BDR mice. Scale bar = 5μm.

CFSE: Carboxyfluorescein diacetate succinimidyl ester, BDR: Bile Duct Resection; vWF: Von Willebrand Factor