CXCR2\(^+\) Neutrophils play an essential role in cuprizone-induced demyelination:

Relevance to multiple sclerosis

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Figure 1. Toluidine blue staining in the corpus callosum after 4 wks of cuprizone feeding

(a) The corpus callosum of Cxcr2<sup>+/−</sup> mice, containing abundant macrophages with cytoplasmic lipid inclusions and sparse myelinated axons. (b) The corpus callosum of Cxcr2<sup>−/−</sup> mice with numerous myelinated axons and few macrophages. Arrowheads indicate foamy macrophages. Scale bar: 4μM.
Figure 2. EM studies of corpus callosum showing nuclear morphology consistent with apoptotic cells in the corpus callosum of Cxcr2^{+/+} after 4 wks of cuprizone feeding. Arrowheads indicate nuclei with condensed chromatin consistent with apoptosis. Scale bar: 5μM.
Figure 3. Quantification of G-ratios of myelinated axons in the corpus callosum of Cxcr2+/+ and Cxcr2−/− mice after 4 wks of cuprizone feeding.
Figure 4. PDGFRα⁺ OPCs in the cortex adjacent to corpus callosum across the time course of cuprizone feeding in either Cxcr2⁺⁺⁺ or Cxcr2⁻⁻⁻ mice. (a) Immunohistochemical staining of PDGFRα antibodies at 3 wks or 6 wks in cuprizone-fed mice. (b) Quantification of PDGFRα⁺ cells in cortex. Scale bar: (a), 50 μm;
Figure 5. Flow cytometric analysis of CXCR2 on peripheral leukocytes. Peripheral blood cells were stained with polyclonal rabbit anti-CXCR2 antibodies from Santa Cruz (sc-683) (a) or T. Lane (UCI) (b), followed by Alexa fluor® 488 goat anti-rabbit IgG. In Cxcr2+/+ mice (a, b, red line) but not Cxcr2−/− mice (a, b, green line), antibodies from T. Lane (b, red line), but not sc-683 stained neutrophils (in Gr1 gate) (a, red line). These data are representative of three experiments.
Figure 6. Microglial cells do not express CXCR2. CD45<sup>dim</sup> microglial cells lacked CXCR2 immunoreactivity either at baseline (upper panel) or after cuprizone feeding for 3 wks (middle panel), as defined by polyclonal anti-CXCR2 abs (T. Lane, UCI). CXCR2 was stained in parallel on peripheral blood Gr1<sup>+</sup> cells as the positive control (lower panel).
Figure 7. CXCR2 expression by neutrophils in Cxcr2+− or Cxcr2+/* mice and normalized populations in Cxcr2+−→Cxcr2+− bone marrow chimeras. Blood cells from mice of indicated genotype were analyzed by flow cytometry. In the myeloid gate, cells from Cxcr2+/* mice and Cxcr2+−→Cxcr2+− and Cxcr2+−→Cxcr2+/* bone marrow chimeras were uniformly Gr1hi/CXCR2+ neutrophils. Cells from Cxcr2−/* mice in the expanded myeloid population were Gr1hi/CXCR2− neutrophils.
Figure 8. Successful Cxcr2−/−→Cxcr2+/+ chimerization is verified by flow cytometry.

Blood cells from mice of indicated genotype were analyzed by flow cytometry. In the myeloid gate, cells from Cxcr2+/+ mice and Cxcr2+/+→Cxcr2+/+ bone marrow chimeras were uniformly Gr1hi/CXCR2+ neutrophils (upper panel; lower panel, left). Cells from Cxcr2−/−→Cxcr2+/+ mice in the expanded myeloid population were Gr1hi/CXCR2− neutrophils (lower panel, right). These data represent 4 mice.
Limited apoptosis in neutrophil-depleted mice at 3 weeks of cuprizone feeding

Mice were injected with Gr1 antibodies (supernatant from cultures of hybridoma RB6-8C5, 200µg/mouse) or comparable mass of rat IgG every other day for 1 week beginning two days before feeding with cuprizone. Mice were sacrificed at 3 weeks of cuprizone feeding. (a) TUNEL staining showed apoptotic cells were significantly elevated in rat IgG injected mice, but not neutrophils depleted mice. DAPI counterstaining showed the nuclei of TUNEL positive cells. (b) Quantification of TUNEL positive cells in corpus callosum. (c) Flow cytometry showed complete depletion of neutrophils 2 days after Gr1Ab injection. **, P<0.01. Scale bar: (a), 25µm.
**Figure 10.** Demyelination caused by LPC injection of the spinal cord dorsal columns occurs in the absence of CXCR2 signaling. LPC injections to Cxcr2\(^{+/+}\) or Cxcr2\(^{−/−}\) mice caused an equivalent demyelinating lesion after 1, 2 and 3 days. Scale bar: 250 \(\mu\)m.