

# Selective Expression and Cellular Localization of Pro-Inflammatory Chemokine Ligand/Receptor Pairs in the Sciatic Nerves of a Severe Murine Experimental Autoimmune Neuritis Model of Guillain-Barré Syndrome

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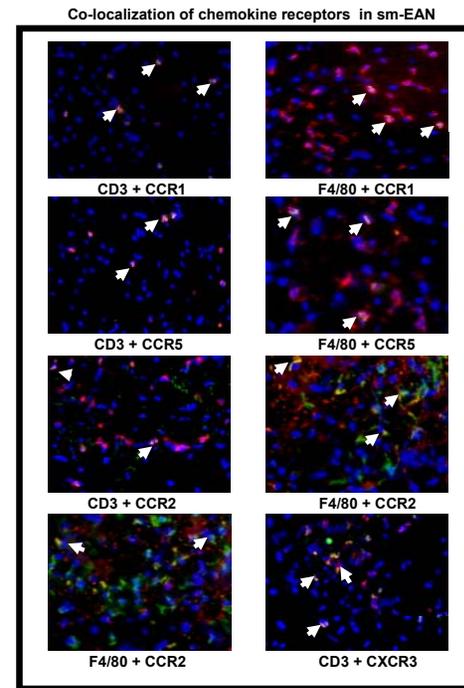
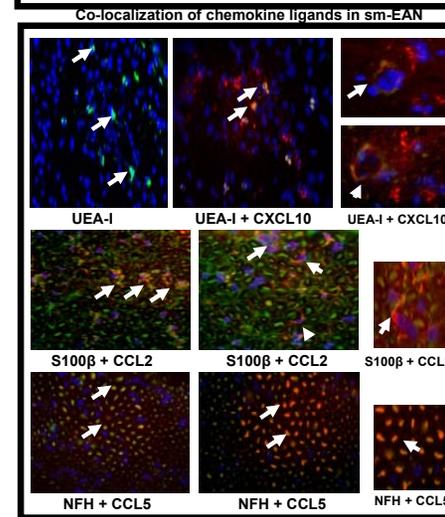
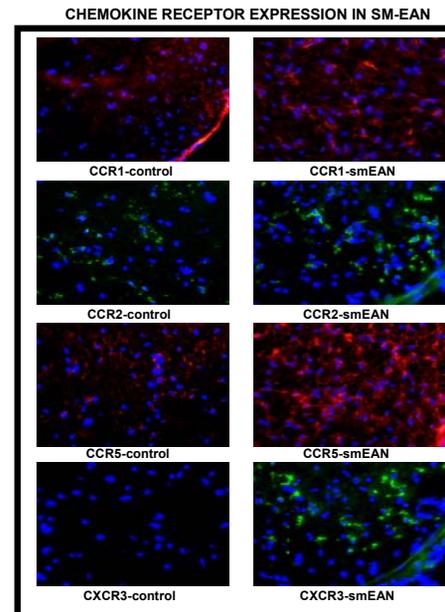
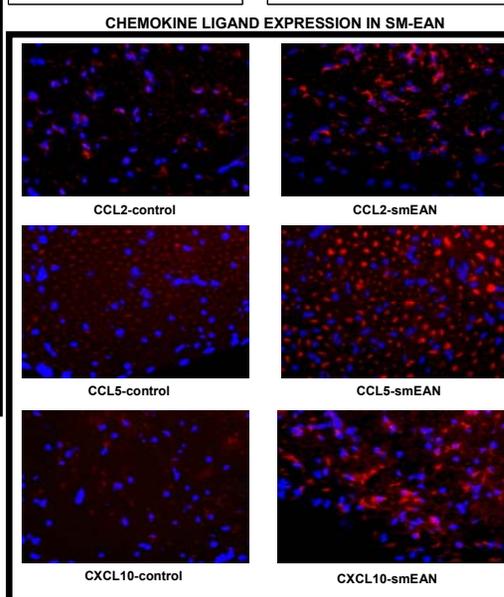
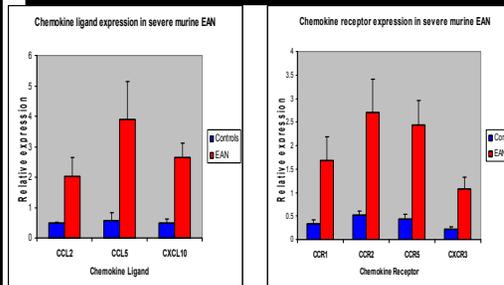
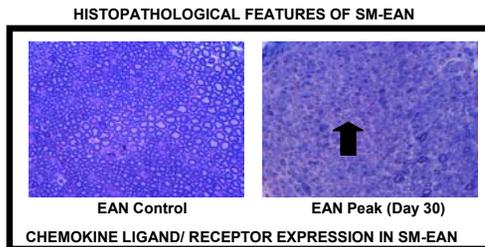
## ABSTRACT

**Aims/Objectives:** The aim of this study is to determine if specific pro-inflammatory chemokine ligand/receptor pairs are expressed in severe murine experimental autoimmune neuritis (sm-EAN), an animal model that recapitulates key clinical, electrophysiological and pathologic features of Guillain-Barré Syndrome. Their cellular expression would aid elucidate the sequence of pathogenic events relevant to peripheral nerve inflammation. Chemokine receptors, being G-protein coupled, are potentially amenable to targeted blockade.

**Methods:** Sm-EAN was induced in 8-12 week old female SJL/J mice using bovine peripheral nerve myelin emulsified in complete Freund adjuvant with Pertussis toxin and recombinant mouse IL-12 acting as co-adjuvants, with appropriate controls. Mice were assessed for neuromuscular weakness and weighed daily. Electrophysiological studies of the right dorsal caudal tail nerve and bilateral sciatic nerves were performed at peak severity (Days 28-32). Sciatic nerves were then harvested from deeply anesthetized mice. Chemokine ligand/receptor expression was determined using semi-quantitative reverse transcriptase polymerase chain reaction on homogenates and indirect fluorescent immunohistochemistry of acetone-fixed frozen sections.

**Results:** There were statistically significant increases in chemokine ligand and receptor expression in sm-EAN nerve homogenates compared to controls (fold-increase in brackets): CCL5 (6.78)/ CCR1 (4.96), CCR5 (5.53); CXCL10 (5.31)/ CXCR3 (5.04); CCL2 (4.13)/ CCR2 (5.23). CCL5 localized to axons, with CCR1 and CCR5 expression on Schwann cells, F4/80+ macrophages and rare CD3+ T-cells. CCL2 was expressed on Schwann cells with CCR2 expressed on F4/80+ macrophages and CD3+ T-cells. CXCL10 was expressed on endoneurial endothelial cells and within the endoneurial connective tissue, with CXCR3 expressed on CD3+ T-lymphocytes.

**Conclusions:** The expression and cellular localization of the above chemokine ligand/receptor pairs suggests that CCL2 expressed by Schwann cells and CXCL10 expressed by endoneurial endothelial cells may play a key role in initiating F4/80+ macrophage and CD3+ T-cell mediated demyelination in sm-EAN. CCL5-CCR1/CCR5 interactions may be involved during the process of myelin phagocytosis and axonal degeneration. These molecules are potential targets for therapeutic intervention in peripheral nerve inflammation. The constitutive expression of these chemokine ligand/receptor pairs in control mice suggest roles during normal immunosurveillance and axonal support/repair processes.



Severe murine experimental autoimmune neuritis is a reliable, and reproducible animal model of Guillain-Barré Syndrome. There is increased expression of specific pro-inflammatory chemokines and their receptors at peak severity, associated with maximal cellular infiltration and demyelination in the sciatic nerves.

**CCL2** (also known as MCP-1) co-localized with Schwann cells (S100β+) suggesting that these cells secrete this chemokine to induce the chemotaxis of CCR2+ macrophages and T-cells.

**CXCL10** (also known as IP-10) co-localized with endoneurial microvessels (UEA-I+) suggesting that the endothelial cells secrete this chemokine to facilitate CXCR3+ T-cell entry into the nerve. There was also interstitial expression of CXCL10 close to microvessels suggesting abluminal secretion.

**CCL5** (also known as RANTES) co-localized with axons (NFH+) suggesting that axons secrete this chemokine to attract CCR1+ or CCR5+ macrophages and T-cells. CCL5 expression was increased in axons close to infiltrating mononuclear leukocytes and absent in areas with axonal loss, implying that this chemokine may be involved in the process of axonal degeneration.

CCR1/CCR5 inconsistently co-localized with Schwann cells implying a possible inducible phagocytic role during inflammation.

