INTRODUCTION

Alterations in blood-brain barrier (BBB) permeability accompany neuroinflammatory disorders such as multiple sclerosis (MS). It nonetheless remains unclear whether such permeability changes are a “cause” or “effect” of the neuroinflammatory process, and the mediators responsible await clarification. Despite this, the chemokine CCL2 has emerged as a critical mediator in MS and its animal model, experimental autoimmune encephalomyelitis (EAE). Specifically, CCL2 is linked to leukocyte-extravasation at the sites of BBB-breakdown in the central nervous system (CNS), implying an intricate functional correlation between CCL2 expression, paracellular leukocyte influx into CNS parenchyma, and BBB-leakage. To begin resolving CCL2’s role in neuroinflammatory disease, we recently created the first cell-conditional chemokine knockout-mice, separately eliminating CCL2 gene from two cell types considered crucial in neuroinflammation: astrocytes (Astro-KO) or endothelial cells (Endo-KO).

AIMS

AIM 1: To qualitatively characterize the heightened BBB-permeability and leukocyte-influx in CNS during various stages of EAE progression in wild-type (WT) animals, and correlating these observations with CCL2 expression in various segments of the spinal cord and brain.

AIM 2: To employ Astro-KO and Endo-KO mice to determine the relative contributions of astrocyte- and endothelial cell-derived CCL2, respectively, to the histopathological changes in BBB-permeability and parenchymal leukocyte invasion that accompany different stages of EAE.

EXPERIMENTAL DESIGN

Immunization with MOG\textsubscript{35-55}/CFA

Before an intracerebral (i.c.) antigen injection, 300µg MOG\textsubscript{35-55} in CFA was injected subcutaneously with 500 ng of pertussis toxin (i.p. at d0 & d2).

METHODS

- EAE induction was carried out by active-immunization with myelin oligodendrocyte glycoprotein (MOG) peptide-CFA.
- Epifluorescence and confocal microscopy was employed to identify leukocytes, TJs, basement membranes (BMs), leaked IgG and CCL2 in the WT, Astro-KO & Endo-KO mice.
- Three-dimensional (3-D) projections of Z-stacked confocal images were surface rendered by IMARIS, uniquely allowing qualitative assessment of holistic pathological changes around the entire surface of CNS microvessels during EAE progression (e.g., expression/localization of tight-junction (TJ) proteins, IgG leakage, step-wise penetration of leukocytes through endothelium and successive endothelial and parenchymal BMs, and sites of CCL2 expression).
- ELISA was performed to detail the time-course of changes in CCL2 expression in the lumbar, thoracic and cervical spinal cord, as well as brain, with ascending progression of EAE.

RESULTS

CCL2 expression in spinal cord with EAE progression

BBB permeability (endogenous serum IgG Leakage) with EAE progression

CONCLUSIONS

Our study unveiled a close association between increased CCL2 production by reactive astrocytes or endothelial cells (major CCL2 sources during EAE) and an elevated permeability at the BBB during EAE. Temporal changes in TJ breakdown and a concomitant increase in parenchymal invasion of leukocytes accompanied this BBB damage. Cell-specific knockout of CCL2 alleviated the neuroinflammation observed in WT EAE mice with EAE progression. These results highlight CCL2 as a potential therapeutic target for neuroinflammatory diseases.

REFERENCES


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