CCR5 Deficiency Exacerbates Oxidative Stress is Associated with Impairs Senescence Marker Protein-30 Generation in Bile Duct Ligation Mice

Fang-Yu Chen1, Hen-Hong Chang1,2, Tzung-Yan Lee1

1Graduate Institute of Traditional Chinese Medicine, Chang Gung University; Taoyuan, Taiwan
2Center for Traditional Chinese Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan

Abstract

Background and Aim: In the case of liver immunology, the recent studies have indicated that CCR5 deficiency exacerbated the severity of hepatic inflammation and injury in some T cell-mediated liver diseases. The primary purpose of this study was to investigate the role of CCR5 deficiency mice during bile duct ligation-induced liver injury. Second end points were to determine the relationship between CCR5 deficiency and oxidative stress within the liver.

Methods: C57BL/6 wild-type (WT) or CCR5-deficinet (CCR5-/-) mice were received bile duct ligation. Three days after surgical, blood were collected for CD8+ and regulatory T cells calculated by flow cytometry. The antioxidant capacity (ACL, lipid-soluble antioxidants) was analyzed by Photo-chemiluminometer. Hepatic samples for histopathology and oxidative stress parameters by Western blot.

Results: H&E staining of liver sections showing confluent hepatocellular necrosis >30% could be seen in the liver in CCR5-/- mice following BDL, whereas liver from WT mice exhibited limited foci of hepatocellular necrosis not >10% after BDL. In the absence of CCR5, the percentage of CD8+ and CD4+CD25+ regulatory T cells was significantly decreased. Notably, WT but not CCR5-/- mice have an increasing T cell expression in response to BDL stress. Immuno-blotting shown hepatic platelets-derived growth factor receptor (PGDFR), endothelial growth factor receptor (VEGFR), VCAM-1, and ICAM-1 proteins were more producing in CCR5-/- mice related to WT mice following BDL stress. These various proteins may interact and regulate stellate cell proliferation by promoting alfa-smooth muscle actin and procollagen I expression. The plasma ACL concentration was significantly higher in the CCR5-/- mice related to WT mice following BDL stress (p<0.05). The severe lipid peroxidation in CCR5-/- mice is associated with increased hepatic superoxide dismutase enzyme activities (p<0.05), and MnSOD protein levels more production than that observed in WT mice after BDL challenge. In addition, down-regulation in the expression of senescence marker protein-30 might decrease resistance to hepatic oxidative stress in CCR5-/- mice.

Conclusions: CCR5 deficiency exacerbates BDL–mediated hepatic oxidative stress, and leads to increased levels of superoxide anion production and a more pronounced liver inflammation, suggesting that CCR5 expression can modulate severity of immunomediated liver injury.

Materials and Methods

- Animal
  - C57BL/6 wild-type (WT) or CCR5-deficinet (CCR5-/-) mice were received bile duct ligation for 3 days.
- Histopathology and Immunohistochemistry
- Western blotting
- Antioxidant capacity of liposoluble substance and superoxide dismutase analysis
- Flow cytometry analysis
- Statistical analysis

Results

- Representative photomicrographs show a pathologic granulocyte neutrophile from C57BL/6, CCR5(-/-), CCR5(-/-)+BDL and C57BL/6+BDL mice. At the time of sacrifice (3 days after bile duct ligation), the liver was removed, then sectioned and staining with granulocyte neutrophile kit. Central vein area (A, B, C, D), or portal triad area (E, F, G, H).
- Various protein markers expression were deter-mined by immunoblotting technique in the liver tissues from C57BL/6, CCR5(-/-), CCR5(-/-)+BDL and C57BL/6+BDL mice. Representative results of more than three individual experiments are shown.
- Total liver homogenate lysates were collected 3 days from various experimental condition of C57BL/6 and CCR5(-/-) mice exposure to bile duct ligation challenge. β-actin was used as an internal control.

Conclusions

CCR5 deficiency exacerbates BDL–mediated hepatic oxidative stress, and leads to increased levels of superoxide anion production and a more pronounced liver inflammation, suggesting that CCR5 expression can modulate severity of immunomediated liver injury.