The Importance of NFkB Proteins in Dextran Sodium Sulphate Colitis

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Introduction

The inflammatory bowel disease ulcerative colitis (UC) affects up to 24.5/100,000 people worldwide. Clinical symptoms include diarrhea and rectal bleeding. UC is characterised histologically by Th2 like inflammation in the mucosa of the colon (Figure 1) and epithelial cell disruption.

Dextran Sodium Sulphate (DSS) is used to model UC in mice. It disrupts the mucosal barrier and causes an adverse immune response to commensal bacteria in the distal colon, causing inflammation with a Th2 cytokine profile.

The NFkB family of transcription factors regulate inflammatory mediators via the classical and alternative signalling pathways. NFkB subunits NFkB1, c-Rel and RelA are involved in the classical pathway, whereas NFkB2 and RelB regulate the alternative pathway.

We have recently shown that NFkB2 null mice are protected from DSS colitis. However the cell population responsible for this phenotype remains to be determined.

Methods

Induction of DSS colitis:
- Male C57BL/6, NFkB2 null, c-Rel null, NFkB1 null mice aged 10-12 weeks.
- 2% DSS in drinking water from day 0-5 and drinking water from days 5-11 of study.
- Control mice given drinking water throughout study.
- N=3 mice culled on day 5, 8 and 11 of study Colons taken for standard histology.
- Mouse weights taken daily throughout studies.

Bone Marrow reconstitution:
- Mice are subjected to full body irradiation- two doses of 550rad (5.5Gy) 3 hours apart.
- Animals then tail vein injected with bone marrow from donor mice and left for 6 weeks to recover.
- DSS administered to induce colitis.

Cryopreservation assay method:
- Mice from DSS study with day 8 end point. 10x circumferences counted per mouse.
- Crypts in a circumference counted, and 10 widths per circumference counted.
- Mean average surviving crypt per group adjusted using following formula to adjust for crypt width.

Results 1. Dextran sulphate sodium induces clinical signs of colitis in C57BL/6 (wild-type) mice.

Results 2. Colitis is ameliorated following depletion of alternative pathway member NFkB2.

Results 3. NFkB1 null and c-Rel null mice are susceptible to DSS induced colitis.

Results 4. Epithelial cell specific deletion of NFkB2 reduces colitis severity.

Conclusions

NFkB2 null mice are protected from colitis, but c-rel and NFkB1 null mice are sensitive to DSS colitis. The effect of NFkB subunit knockouts on DSS colitis indicates that the classical and alternative pathways may have different roles in colitis induction, and that the alternative pathway (to which NFkB2 belongs) may have a significant role in colitis progression. Using bone marrow reconstitution we have shown that the genotype of the bone marrow derived cells contributes less to DSS colitis induction than the genotype of the epithelium.

Further work: To determine the cellular downstream mechanisms by which NFkB2 signalling contributes to colitis pathogenesis.

References