BLOCKADE OF EPHB-EPHRINB SIGNALLING PROTECTS AGAINST MURINE TNBS-INDUCED COLITIS

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Background and aim

Crohn's disease (CD) is a complex multifactorial disorder whose etiology, still only partially elucidated, entails the disruption of intestinal homeostasis and defects in immune responses (1). Eph (Erythropoietin-producing hepatocellular carcinoma) receptor tyrosine kinases and their ephrin ligands are a complex of proteins, characterized by bidirectional signalling, with critical roles in embryonic growth and in cancer development and progression (2). Recently, their involvement is emerging also in the regulation of inflammatory conditions. In particular, the well-documented intervention of Eph-ephrin system in cell adhesion-based responses and gut epithelial homeostasis (3, 4) and the up-regulation of Eph-ephrin expression in mucosal lesions of CD patients (5) prompted us to explore the role of Eph-ephrin system in the pathogenesis of CD. Concurrently, our research group synthesized UniPR1331, an original potent and selective Eph-ephrin antagonist, ideal tool to investigate the relevance of Eph-ephrin system in pathological conditions (6). Accordingly, the effects produced by activation of forward (ephrin-B1-Fc) or reverse (EphB1-Fc) signalling or by blockade of both signals (monomeric EphB4 and UniPR1331) were studied in murine TNBS-induced colitis.

Methods

Colitis was induced in C57BL/6 mice by enema administration of 5mg/mouse 2,4,6-Trinitrobenzene sulfonic acid (TNBS) in 50% ethanol. Normal mice (N) received only saline solution. Subcutaneous (s.c. - recombinant proteins) or oral (p.o. – UniPR1331) pharmacological treatments started 8 hours after induction of colitis and were applied daily till euthanasia, 3 days later; control mice (C) received only saline solution. Disease Activity Index (DAI), colonic macroscopic score (MS), colon length and thickness and colon and lung myeloperoxidase (MPO) activity, index of leukocyte recruitment, were determined. Splenic and mesenteric lymph nodes (MLN) CD3+, CD3+CD4+ and CD3+CD8+ lymphocytes were counted by flow cytometry. IL-1□, TNF□ and IL-10 levels were measured in colonic tissues excised from N, C and UniPR1331-treated mice. All experiments were performed according to the guidelines for the Care and Use of Animals (DL26/2014).

Results

EphB1-Fc 30microg/kg, equimolar EphB4 20microg/kg and UniPR1331 25 mg/kg b.i.d. dramatically reduced DAI ($P<0.001$) and MS ($P<0.05$), minimized colon shortening ($P<0.01$) and thickening ($P<0.05$) and curtailed local ($P<0.05$) and systemic ($P<0.01$) neutrophil infiltration by about 80% compared to C. Treatment with equimolar ephrinB1-Fc (17microg/kg) lowered only lung MPO levels ($P<0.05$), while equimolar Fc had no effect on colitis parameters. As regards splenic T cells, UniPR1331 was able to prevent the reduction of CD3+CD4+ and CD3+CD8+ lymphocytes number

induced by colitis, while none of the treatments affected MLN cells decrease. Finally, UniPR1331 was able to attenuate also the TNBS-induced increase of colon IL-1β levels.

Conclusions

These results demonstrate that endogenous EphB-ephrinB system promotes the local inflammatory responses in TNBS-induced colitis, mainly through forward signalling: pharmacological agents selectively disrupting EphB-ephrinB transmission, like our newly synthesised, low molecular weight Eph-ephrin antagonist UniPR1331, may therefore represent a novel strategy for the treatment of inflammatory conditions like CD.

References

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