Disruption of Eph-ephrin B signaling pathway mitigates inflammation in a murine model of Crohn's disease

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BACKGROUND AND AIM

Eph receptors are the largest family of tyrosine kinases receptors, divided in A and B classes on the basis of their structural features and affinity for cell-bound ephrin ligands. Eph-ephrin interaction generates a bidirectional signalling affecting both the receptor bearing (forward signaling) and the ligand bearing cells (reverse signaling) (1).

Besides its critical role in tumor development and progression (2), the Eph-ephrin system is also involved in inflammatory and immune responses: in particular, B-type Eph-ephrins regulate endothelial cells activation, leukocytes adhesion and migration as well as T cell maturation and activation, taking part also in gut epithelial tissue remodelling (3, 4). Interestingly, B-type Ephs and ephrins mRNA are overexpressed in the gut epithelium of patients affected by Crohn's Disease (CD) (5), a chronic inflammatory intestinal disorder where defects of the gut barrier associated to an aberrant immune response to luminal antigens are pivotal players (6).

Our aim is to investigate the effects produced by the interference with B-type Eph-ephrins signaling on the local and systemic inflammatory responses induced in a murine model of CD by administering different dosages of the recombinant monomeric EphB4 receptor.

METHODS

Colitis was induced in female C57BL/6 mice, 8-12 weeks old, by enema (i.r.) administration of 5 mg/mouse of 2,4,6-Trinitrobenzene sulfonic acid (TNBS) in 50% ethanol. Subcutaneous pharmacological treatments started 8 hours after induction of colitis and were applied daily, until the sacrifice, 3 days later. Animals were randomly divided in different experimental groups:

SHAM: i.r. administration of 50 µL saline (0.9% NaCl) + saline (10 mL/kg/die)

TNBS: TNBS + saline (10 mL/kg/die)

EphB4: TNBS + EphB4 (5; 10; 20 µg/kg/die)

We determined clinical outcome as Disease Activity Index (DAI) (7), colonic macroscopic damage score (MS) (8), colonic length and thickness, colon and lung myeloperoxidase (MPO) activity, index of granulocyte infiltrate. All experiments were performed according to the guidelines for the Care and Use of Animals (DL26/2014).

RESULTS AND CONCLUSIONS

With respect to **SHAM**, **TNBS** mice showed markedly higher DAI ($p<0.001$) and MS ($p<0.01$), a pronounced shortening and thickening of the colon ($p<0.01$) and strongly increased colonic and lung MPO activity ($p<0.01$). **EphB4** dose-dependently reduced DAI: a marked improvement of clinical outcome was observed at 20 µg/kg ($p<0.001$). Colonic shortening and thickening, macroscopic damage and granulocyte infiltrate were moderately counteracted in a dose-dependent way, while a mild reduction of lung MPO was observed with a bell-shaped dose-response curve.

These preliminary findings show that bidirectional signaling triggered by B-type Eph-ephrins interaction is involved in the inflammatory response induced in this model of colitis, suggesting that pharmacological agents selectively perturbing this signaling pathway might represent a novel strategy for the treatment of inflammatory conditions such as CD.

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