P0794 EFFECTS OF HUMAN AMNION-DERIVED MESENCHYMAL STEM CELL TRANSPLANTATION AND CONDITIONED MEDIUM ENEMA IN RATS WITH TRINITROBENZENE SULFONIC ACID-INDUCED COLITIS

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Introduction: Mesenchymal stem cells (MSCs) have been reported to be a valuable cell source in regenerative medicine, and bone marrow represents a major source of MSCs. Recently, it has been shown that MSC can be easily isolated from human amnion, which is generally discarded after delivery, and a large amount of cells can be obtained. We have previously reported that intravenous administration of human amnion-derived MSCs (hAMSCs) provided significant improvement in rats with colitis induced by dextran sulfate sodium or g-irradiation. In addition, conditioned medium (CM) obtained from MSCs contains a variety of humoral factors to improve damaged tissues. In this study, we investigated the effects of hAMSCs and CM in rats with 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis.
Aims & Methods: hAMSCs were isolated and expanded by digestion with collagenase, followed by culturing in uncoated plastic dishes. CM was collected by culturing subconfluent hAMSCs with serum-free MEMa for 48 hrs. CM gel was prepared by mixing CM with 2% carboxymethyl cellulose. On day 0, 200 μl of TNBS (15 mg/rat) in 30% ethanol was intrarectally administered to the ten-week-old male Sprague-Dawley (SD) rats. One-million hAMSCs were intravenously administered 3 hrs after TNBS treatment, and rats were sacrificed on day 7 for histological examination and quantitative PCR. In another experiment, 400 μl of CM gel was intrarectally administered 3 hrs after TNBS treatment, and day 1 and day 2.
Results: hAMSC transplantation and CM gel enema significantly improved the endoscopical score, and tended to improve the histological score. Quantitative PCR demonstrated that the expression levels of TNF-α, CXCL1 and CCL2 tended to be decreased by hAMSC transplantation and CM gel enema. Infiltrations of CD68-positive macrophages and myeloperoxidase-positive neutrophils were significantly decreased by hAMSC transplantation and CM gel enema.
Conclusion: Transplantation of hAMSCs and CM gel enema provided significant improvement in rats with colitis induced by TNBS. hAMSCs or CM from hAMSCs may be new therapeutic strategies inflammatory bowel disease.
Disclosure of Interest: All authors have declared no conflicts of interest.

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P0795 TERTIARY LYMPHOID ORGANS IN GUT MUCOSA OF NEWLY DIAGNOSED, UNTREATED INFLAMMATORY BOWEL DISEASE PATIENTS

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Introduction: There is recent evidence of increased naïve and central memory T lymphocytes (T_N and T_{CM}) mucosal infiltration of the inflamed gut in Inflammatory Bowel Disease (IBD), while T_N and T_{CM} cells were thought to migrate exclusively to secondary lymphoid organs (SLOs). Ectopic formation of tertiary lymphoid organs (TLOs) containing peripheral lymph node adressin $(PNAd)^+$ high endothelial venules (HEVs) might explain the homing of these lymphocytes to the gut, as PNAd is the ligand of L-selectin which is expressed on T_N and T_{CM} lymphocytes.
Aims & Methods: The aim of this study was to investigate the presence of PNAd expressing HEVs and TLOs in the inflamed intestinal mucosa of newly diagnosed, untreated, IBD patients in relation to the presence of T_N and T_{CM} lymphocytes. Thirty-nine newly diagnosed, untreated IBD patients and eight healthy controls were prospectively included. Intestinal biopsy samples were analysed by immunohistochemistry for blood vessels (CD31) and PNAd expression (MECA-79), the density of MECA-79^+ vessels was calculated and the presence of lymphoid follicles was assessed. Different lymphocytes subsets in the tissue samples were identified by flowcytometric immunophenotyping, including T_N ($CD45RA^+CD27^+$), T_{CM} ($CD45RA^-CD27^+$) and effector memory T cells ($CD45RA^-CD27^-$).
Results: A statistically significant higher number of extra-follicular $PNAd^+$ vessels were found in the inflamed colon of patients with ulcerative colitis (median density of 3.05 $PNAd^+$ vessels/mm^2 (IQR 0–6.39)) and ileum of patients with Crohn's disease (median density of 1.40 $PNAd^+$ vessels/mm^2 (IQR 0–4.34)) compared with healthy controls (median density of colon: 0 $PNAD^+$ vessels/mm^2 (IQR 0–0, $p = 0.033$) and ileum: 0 $PNAd^+$ vessels/mm^2 (IQR 0–0.50, $p = 0.033$)). The heterogeneity of extra-follicular $PNAd^+$ vessels in IBD patients allowed classification in two different groups: HEV^{high} and HEV^{low}. A high density of $PNAd^+$ HEV-like vessels was associated with increased numbers of T_N and T_{CM} in the inflamed gut mucosa (median 87% (IQR 82–93%) of total T cell population), compared with the inflamed mucosa of patients from the HEV^{low} group (58% (IQR 38–81%) $p = 0.003$). The number of colonic follicles was higher in HEV^{high} patients (median 0.54/mm^2 (IQR 0.28–0.84)) when compared with HEV^{low} patients (median 0.25/mm^2 (IQR 0.08–0.45) $p = 0.031$) and controls (0.31/mm^2 (IQR 0.23–0.45) $p = 0.043$).
Conclusion: For the first time, evidence has been delivered of extra-follicular HEV-like vessels and TLOs, strongly associated with T_N and T_{CM} cell mucosal infiltration, in a subgroup of newly diagnosed IBD patients. Different T cell migration phenotypes based on TLO formation in the early phase of IBD might allow risk-stratification of patients and enable more effective, individualized treatment.
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P0796 TOLL-LIKE RECEPTOR 9 MODIFIES INTESTINAL SEROTONERGIC SYSTEM

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Introduction: Toll-like receptor 9 (TLR9) is expressed in intestinal epithelial cells, which recognize microbiota developing different responses [1]. Several studies have shown that TLR9 seems to be involved in Inflammatory Bowel Diseases (IBD) due to an inappropriate defensive response against microorganisms [2]. Moreover, intestinal serotonergic system is also altered in IBD, where extracellular serotonin (5–HT) levels are increased [3]. 5-HT bioavailability is mainly regulated by the serotonin transporter (SERT), expressed in enterocytes [4].
Aims & Methods: The aim of the present study was to analyse whether TLR9 activation affects SERT expression and activity, and expression of other elements from the serotonergic system (TPH1, TPH2 and 5-HT receptors). Human enterocyte-like Caco-2 cells, and ileum and colon from $TLR9^{-/-}$ mice and Dextran Sulphate Sodium (DSS) mouse colitis model were used as experimental models. mRNA expression was determined by RT-qPCR, and protein expression by western blot.
Results: TLR9 activation in Caco-2 cells decreased SERT mRNA and protein expression. TLR9 activation also reduced SERT activity by different intracellular pathways, depending on activation period. Indeed, TLR9 long-time activation altered 5-HT uptake through ERK pathway, whereas short-time activation modified SERT by p38/MAPK pathway. Moreover, 5-HT addition to culture media increased TLR9 protein expression in the brush-border membrane of Caco-2 cells. In $TLR9^{-/-}$ mice were observed different expression patterns. SERT was not modified in ileum, but increased in colon. TPH1 was increased in ileum, and TPH2, in colon. Regarding 5-HT receptors, 5HT2A, 5-HT2B, and 5-HT3 were increased in ileum; however, 5-HT1A, 5-HT2A and 5-HT4 were increased in colon. In both ileum and colon of DSS mice, TLR9 and 5-HT7 mRNA expression were increased, whereas SERT expression was diminished.
Conclusion: Our results suggest that TLR9 modulates intestinal serotonergic system. Indeed, TLR9 activation decreases SERT function and expression by different pathways. In part, this is corroborated by the decreased expression of SERT in colon of TLR9 knockout mice and altered 5-HT receptors expression. Finally, SERT and TLR9 are inversely disturbed in DSS mice colitis model.
Disclosure of Interest: All authors have declared no conflicts of interest.

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