Sa1129

WATER AVOIDANCE STRESS DECREASES INNATE LYMPHOID CELL 3 FLOWING THROUGH MESENTERIC LYMPHATIC VESSELS

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Introduction: Innate lymphoid cells (ILC) play a role in the maintenance of intestinal homeostasis.ILC mainly consist of ILC1, ILC2 and ILC3. Especially, ILC3, the most abundant subset in the intestine, are reported to be rapid source of protective cytokines following initial exposure to a variety of pathogens and we reported ILC3 were significantly increased in mesenteric lymphatic vessels (MLV) in intestinal inflammation of rat supposedly for protective role (AGA 2019). Psychological stress has been reported to be a deteriorating factor for functional gastrointestinal dysfunctions (FGIDs). But, there is no study investigating relationship between psychological stress and ILC. We hypothesized that alteration of ILC, especially ILC3, might be induced in early phase of psychological stress. In this study, we aimed to investigate the impact of psychological stress exposure on ILC in rat. **Materials and Method:** Wistar male rats (5 weeks) received mesenteric lymphadenectomy (MLNx). Six weeks later, the rats were exposed to water avoidance stress (WAS) for 1 hour during successive 3 days to induce psychological stress. On the next day of the last WAS exposure, thoracic duct (TD) was cannulated for collecting lymphatic fluid. Collected lymphocytes were analyzed by flow cytometry to investigate the ILC components. ILC were defined as IL-7Rα^+ Lin (B220, CD3, CD5 and CD11c)$^-$ cells, and then separated into ILC1, ILC2 and ILC3 by the expression of T-bet, GATA-3 and ROR γt, respectively. **Results:** First, we confirmed that percentage of ILC among all lymphocytes in MLNx rats was about 1.0 %, which was significantly higher than ILC of TD in normal rats (about 0.4 %), showing that more ILC exist in MLV (lymphatic vessels before MLN) than TD (lymphatic vessels after MLN). ILC of MLNx rat are composed of 3.0 % in ILC1, 33.8 % in ILC2 and 24.4% in ILC3, respectively. Then, we focused on ILC in MLNx rats with or without WAS exposure. In sham rats, there was no significant difference in ILC components compared with MLNx rats. In rats with WAS exposure, there was no significant difference in ILC1 (1.59 ± 1.59 %) and ILC2 (49.3 ± 17.14 %), but, surprisingly, ILC3 was significantly decreased to 10.97 ± 0.74 %. **Conclusions:** In this study, we clarified ILC3 components were significantly decreased by WAS exposure. Although WAS is usually performed for 10 days according to original reports, we performed WAS only for 3 days based on the assumption that ILC were involved in early phase of psychological stress. Since it was reported ILC3-derived IL-22 is crucial in preventing dissemination of commensal bacteria, decreased ILC3 by psychological stress might contribute to pathophysiology of FGIDs. We now hypothesize that aryl hydrocarbon receptor (Ahr), expressed on ILC3 and essential for the IL-22 production, might be a therapeutic target of FGIDs and will investigate in the future.

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THE EFFECT OF PHOENIXIN-14 ON SEPSIS-INDUCED HEPATIC INJURY AND IMPAIRED INTESTINAL CONTRACTILITY

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Background: Liver injury is critical for sepsis-induced mortality and multiple organ dysfunction, which includes gastrointestinal dysfunction due to impaired contractility of the gastrointestinal smooth muscle. Phoenixin (PNX), a recently identified neuropeptide, is expressed in the gut, the brain, and the vagal nuclei. PNX is postulated to have a variety of functions in several organ systems, but they are not fully unraveled yet. The present study was aimed to clarify the effects of PNX on impaired ileal contractility and oxidative injury of the liver due to sepsis. Secondly, the role of vagal fibers in the putative effects of PNX were elucidated. **Materials and Methods**: Under ketamine anesthesia, male Sprague-Dawley rats (310-390 g) underwent sham-surgery (n=8) or cecal ligation and puncture to induce sepsis. Septic rats were treated intraperitoneally with 3 consecutive doses of PNX-14 (50 µg/kg; n=8) or saline (n=7). In some rats, four weeks prior to sepsis induction, vagal denervation (VD; n= 8) was made by applying capsaicin on cervical vagal trunks, and rats were treated with PNX-14. All rats were then euthanized at 16th hour of surgery. Ileum and liver samples were obtained to evaluate lipid peroxidation (LP) and glutathione levels. From other sets of sham-operated, sepsis and VD-sepsis (n=24) rats that have not received PNX-14 treatment, ileal rings were mounted in organ baths to study isometric contractions elicited by carbachol (10^{-10}-10^{-4} M) in the absence or presence of PNX-14 (1 nM; 20 min). Data were analyzed using Student's t-test and ANOVA, and expressed as mean ± SEM. **Results**: Sepsis resulted in elevated LP in the ileum and liver with a concomitant increase in hepatic glutathione ($p<0.05$). PNX-14 had no significant effect on sepsis-induced oxidative injury of the intestines. However, PNX-14 inhibited LP in the liver ($p<0.05$), which was abolished by vagal denervation. Maximum contractions (E_{max}) of ileal strips from septic (49.8 ± 9.3 %) and VD-septic rats (33.1 ± 3.0 %) were significantly decreased as compared to sham-operated ones (98.1 ± 7.4 %; $p<0.05$). PNX-14 depressed E_{max} in sham-operated rats (53.3 ± 3.7 %; $p<0.05$), and this inhibition was greater in septic (34.4 ± 2.8 %; $p<0.05$) and VD-septic rats (21.9 ± 1.81%; $p<0.05$). **Conclusion**: Sepsis resulted in impaired ileal contractility, which was exaggerated upon vagal denervation. When applied *in vitro,* PNX-14 also inhibited ileal contractility and this inhibitory effect was further enhanced by sepsis and vagal denervation. However, despite its exaggerative effect on sepsis-induced impairment in smooth muscle contractility, PNX-14 reduced sepsis-induced hepatic injury that appears to involve intact vagal innervation. Further studies will help to identify the pathophysiological role and therapeutic potential of phoenixin in hepatic and intestinal failure in association with sepsis.

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EXOSOMES FROM HUMAN MESENCHYMAL STEM CELLS PREVENT DEXTRAN SULFATE SODIUM (DSS)-INDUCED ULCERATIVE COLITIS IN MICE

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Background: Mammalian cells continuously secrete 30 - 120 nm diameter extracellular lipid membrane vesicles known as exosomes (EXOs), which contain genetic material (mRNA, miRNA and lncRNA), proteins and lipids. Although the therapeutic roles of mesenchymal stem cells (MSCs) with regenerative and immunosuppressive properties have been extensively investigated, the potential therapeutic value of EXOs in ulcerative colitis (UC) is unknown. **Aim**: To investigate whether EXOs from human MSCs attenuate DSS-induced UC in mice. **Methods**: EXOs isolated from human MSCs grown in serum-free conditioned medium were characterized using transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA). UC was induced in mice (C57BL/6; 21 days old) by giving 3% DSS in drinking water *ad libitum* for 7 days and divided into 4 groups that were *i.p* administered 0, 10^5, 10^7 and 10^9 EXOs/day for 7 days. Normal animals given water were administered 10^9 EXOs/day for 7 days. Fecal blood was detected using a Hemoccult kit. Histopathology and goblet cells were evaluated by H&E and alcian blue staining, respectively. RT-qPCR and western blot analyzes were performed using standard protocols. **Results**: Homogeneous population (~100 ± 20 nm by NTA) of donut shaped (by TEM) EXOs were enriched with TSG101, CD9, CD63 and HSP70 (western/RT-qPCR analyses). Stool blood was detected starting day-4 until day-7 in DSS-animals with 0 EXOs. Stool blood levels decreased with increasing EXO concentrations with no trace of blood in DSS-animals administered 10^9 EXOs. Colon lengths were shortened by ~30% in DSS-animals with 0 EXOs (normal vs DSS: 9.3 ± 0.6 vs 6.0 ± 1.1 cm), while colon lengths were not significantly altered in DSS-animals administered 10^9 EXOs. Distorted crypts, crypt abscess, increased immune cell infiltration, decreased goblet cells, and increased proinflammatory cytokines were present in distal, but not in proximal colon of DSS-animals with 0 EXOs. The severity of DSS-induced histopathological changes and proinflammatory cytokine levels both decreased progressively with respect to increasing EXO concentrations, with no significant evidence of disease in DSS-animals administered 10^9 EXOs. **Conclusion**: EXOs from human MSCs prevent DSS-induced UC in mouse distal colon. **Speculations**: We speculate that treatment with EXOs may be a safe and viable option to treat patients with acute UC, and may help promote recovery in patients with more chronic disease.

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CHARACTERIZATION OF IL10 GENE-DEFICIENT MOUSE INTESTINAL STEM CELLS PROLIFERATION BASED ON ORGANOIDS CULTURE

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Background The intestinal epithelium has the fastest self-renewing rate in mammal. Stability and accuracy of intestinal epithelial cells (IECs) self-renew is the foundation of intestinal mucosal regeneration and repair. Crypt where the intestinal stem cells (ISCs) locate is the physiologic headstream of IECs. As we know that IL-10 gene-deficient mouse fails to develop Crohn's-like colitis, such as inflammation and mucosal injury, under germ free conditions. While, it could spontaneously develop colitis under specific pathogen free (SPF) conditions. It is commonly believed that mucosal injury in IL10 gene-deficient mouse occurs due to the IL10 gene-deficient associated dysregulated immune response to normal enteric microbiota. Moreover, IL10 gene-deficient mouse has been shown to have increased intestinal permeability without inflammation at 2-week old under SPF condition. Therefore, it is necessary to explore whether the deletion of IL10 gene can lead to the imbalance of crypt ISCs niche and the epithelial dysfunction in early life or not. We adopt the intestinal organoids to investigate the ISCs proliferation and epithelial structure in vitro to avoid the influence from immune cells and inflammatory/ anti-inflammatory cytokines. **Method** 2-4 weeks Wile type and IL10-/- mice were used for intestinal crypts isolation and organoid culture. Procedures were established according to previously published methods (H. Clevers, et al.Gastroenterology 2011). The organoid proliferation was evaluated by the change of Organoids' cross sectional area which was measured, calculated, recorded by the Operetta High-Content Imaging System and Harmony software 4.8. Immunofluorescence image were captured by Leica confocal micro- scope. **Result** There was no difference in intestinal inflammatory cell infiltration and tissue damage between WT and IL10-/- at 2 weeks of age (Figure 1). The intestinal organoids growth rate showed no difference. The cross sectional area of organoids generating from WT mice and IL10-/-mice at 96 hours were 10934.39±1808.09 µm^2/well and 10763.70±2792.08 µm^2/well, respectively (Figure 2,3). The organoids derived from WT and IL10-/- mice both were stained positively for Ki67 (Figure 4). The single cell layer of organoids labeled by E-cadherin were intact in both organoids generating from WT and IL10-/-mice (Figure 4). **Conclusion** Above results suggest that, in IL10-/- mouse, the proliferation and organization of ISCs remained normal before the presence of intestinal inflammation.