Conclusions: We show clear differences in the distribution of circulating cell subsets in inflammatory conditions compared with healthy controls, including distinct differences in the immunological signatures of patients with PsA, AS, Ps, and CD.

P029
Modulation of intestinal epithelial permeability by plasma from patients with Crohn’s disease in a 3D cell culture model
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Background: Intestinal epithelial barrier is affected by multiple factors, such as tumour necrosis factor (TNF)-α. Plasma concentration of TNF-α is markedly higher in Crohn’s disease (CD) patients than healthy controls and correlates positively with disease activity. However, studies comparing effect of plasma from active and inactive CD patients on intestinal barrier function are currently lacking. Therefore, this study aimed to determine the impact of plasma from active, inactive CD patients and healthy controls on paracellular permeability and tight junction expression using a three-dimensional (3D) Caco-2 culture model, and to investigate the role of MAPK as a potential signalling mechanism.

Methods: Plasma samples were collected from patients (NL31636.068.10) with active CD (SES-CD ≥3, N = 7), inactive CD (SES-CD ≥2, N = 7) and healthy controls (HC, N = 7) using sodium heparin tubes. 3D Caco-2 spheroids were grown in MatrigelTM and treated basolaterally with plasma (37.5% v/v) or TNF-α (25 pg/ml) for 24 h, with or without pre-incubation of TNF-blocker adalimumab (10 or 20 µg/ml) or JNK inhibitor SP600125 (100 µmol/l). Epithelial permeability of the Caco-2 spheroids was assessed by the flux of fluorescein isothiocyanate-labelled dextran 4 kDa (FITC-D4) from the basal to the luminal compartment using confocal microscopy. Gene expression and localisation of ZO-1 and Occludin were analysed by qRT-PCR and immunofluorescent staining respectively. Phosphorylation of the MAPK isoforms p38, ERK1/2 and JNK were analysed using cell-based ELISA.

Results: Compared with HC, plasma from active and inactive CD patients showed a significant increase of luminal/basal FITC-D4 ratio (0.012 ± 0.002, 0.194 ± 0.024, 0.052 ± 0.005 respectively, p < 0.01). The effect was less pronounced for inactive vs. active CD patients (p < 0.001). Compared with HC, active CD plasma decreased ZO-1 and Occludin expression on both mRNA (1.000 ± 0.073 vs. 0.203 ± 0.027, p < 0.05; 1.00 ± 0.206 vs. 0.205 ± 0.081, p < 0.05 respectively) and protein levels, and led to an increased phosphorylation of MAPK JNK isoform (1.000 ± 0.071 vs. 1.896 ± 0.039, p < 0.001). Pre-incubation with adalimumab or a JNK inhibitor both ameliorated the TJ disruption and barrier dysfunction induced by plasma from CD patients (p < 0.05).

Conclusions: Compared with HC, plasma from CD patients is able to induce epithelial barrier disruption, at least in part by TNF-α induced TJ modulation. This is most pronounced in active disease and may contribute to a vicious circle affecting disease outcome. Our data also demonstrate an involvement of MAPK signalling pathway, in particular the JNK isoform, in CD patient plasma-induced barrier dysfunction.

P030
Intravital observation system of the colon by two photon excitation microscopy
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Background: Cell dynamics throughout the body is an essential event for immune cells to work properly against pathogens or other targets. When intestines are inflamed, many immune cells including lymphocytes migrate from blood vessels to lamina propria. On this process, immune cells adhere to endothelium, change the morphology and migrate to interstitial tissue. Cell adhesion and shape change is controlled by a group of adhesion molecules, integrins and their ligands. Recently, many anti-adhesion molecule antibodies are on market or under development for therapeutic use of inflammatory bowel disease. To investigate this immunological event, we have developed intravital observation system using two photon excitation microscopy.

Methods: Fluorescent YFP or EGFP transgenic mouse is anaesthetised and a small incision is made at the lower abdomen. A part of large intestine is pulled out and clipped. The organ is filled with soft agar and attached to the custom-built suction device to immobile. Blood vessel is coloured by intravenous administrating of Evansblue. The wave length of two photon excitation laser is set to 840 nm. Colitis is induced by oral administration of 1.5 to 3.0% dextran sulfate sodium for seven days. Mice for this study are treated under the guideline for proper use of experimental animals of the institute.

Results: Immune cell movement in small vessels and in lamina propria can be observed in the colon of living mouse. The maximum observation time was 3 h. The change of cell shape was also seen clearly. Under the inflamed condition, more immune cells are recruited to the region.

Conclusions: Intravital observation system of the colon by two photon excitation microscopy is a powerful tool to study immune cell dynamics in physiological condition. Real-time cell movement in intestines of living mouse can be observed and analysed. This system could be applied to evaluate the effect of therapeutic reagent candidates for inflammatory bowel disease including adhesion inhibitory reagents, e.g. anti-α4 β7 integrin antibody.

P031
Effects of tobacco alkaloids on DSS-induced colitis mouse model
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Background: IBD is the collective term for chronic immune-mediated diseases of unknown, multifactorial aetiology with complex interactions between genetic and environmental factors, comprising two main disease manifestations, Ulcerative colitis (UC) and Crohn’s disease (CD). Decades of epidemiological and clinical based evidence from robust case controlled studies clearly point to an inverse association between smoking and the onset and development of ulcerative colitis (UC). Contrarily, cigarette smoke is associated with a higher incidence
risk and increased severity of Crohn’s disease progression. However, the biological mechanisms responsible of the underlined smoking effects on UC progression remain largely elusive. Several studies have demonstrated the anti-inflammatory action of cholinergic agonists, such as the main tobacco alkaloid nicotine. The contrasting results observed in clinical studies addressing the role of nicotine in UC opened new questions on the molecular mechanisms at the base of the intrinsic nicotinic anti-inflammatory activity and the possible involvement of other tobacco alkaloids in the observed reduced ulcerative colitis disease risk, progression and relapse rate in smokers. In the present study we aimed to investigate the potential preventive anti-inflammatory activity of a tobacco alkaloid (Alkaloid #1), structurally similar to nicotine, in a murine model of intestinal inflammation.

Methods: UC-like symptoms were induced in C57BL/6 male mice (n = 14) by 3.5% DSS administration for 7 days. Mice were then treated with nicotine and Alkaloid #1 at 2 different concentrations in drinking water for a total of 21 days (14 days pre-DSS + 7 days during DSS). During the treatment animals were evaluated for progression and severity of UC symptoms.

Results: Daily observations (body weight, intestinal bleeding, hematocrit, etc.) and cytokine analysis revealed a protective alkaloid-related effect. Although no differences observed on the histological analysis of colons, alkaloid-treated mice showed a reduced body weight loss, intestinal bleeding and hematocrit. Further molecular analysis are ongoing to investigate the molecular mechanisms implicated in the disease progression and amelioration.

Conclusions: Further molecular analysis are ongoing to investigate the molecular mechanisms implicated in the disease progression and amelioration.

P032 has been withdrawn.

P033

The efficacy of tonsil-derived mesenchymal stem cells conditioned medium in chronic colitis model


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Background: Tonsil-derived mesenchymal stem cells (TMSCs) obtained from tonsillectomy have many advantages such as a short doubling time, high differentiation capacity, and immune modulatory activity. In previous studies, intraperitoneal administration of TMSC in DSS-induced acute and chronic colitis animal models have shown results of improvement in disease activity index and down regulation of histological grading and pro-inflammatory cytokine expression levels. However, the TMSCs were not observed to migrate to the inflamed intestine in vivo. These results are presumed to be due to the paracrine effect of TMSCs. In this study, we tried to verify the therapeutic effect of TMSCs conditioned medium (TMSCs-CM) in the mouse model of DSS-induced chronic colitis.

Methods: In vitro, immunosuppressive effects of TMSCs-CM were confirmed by splenocyte immunosuppression assay. C57BL/6 mice splenocytes were stimulated with mitogen such as LPS or PMA/Ionomycin, and then cultured in TMSCs-CM or co-cultured with TMSC. After 24 h, proliferation of splenocytes were measured using the cck-8 kit. In vivo, eight-week-old C57BL/6 mice were randomly assigned into four groups: normal, colitis, TMSC, and TMSC-CM groups. Chronic Colitis was induced by oral administration of 1.5% dextran sulfate sodium (DSS) for 5 days followed by 5 days of drinking water continuously for three cycles. TMSC (1x106/500 µl) and TMSC-CM (500 µl) were administered via intraperitoneal injection four times and 12 times. The severity of the colitis was assessed by measuring the disease activity index (DAI), colon length, histologic grading, and cytokine levels.

Results: The splenocyte stimulated by LPS showed decreased proliferation when co-cultured with TMSC (3.18 ± 0.07 vs. 2.03 ± 0.12, mean ± standard error mean, control group vs. p = 0.0008, ANOVA), and cultured in TMSC-CM (3.18 ± 0.07 vs. 1.81 ± 0.06, p < 0.0001). Proliferation was significantly reduced by the number of TMSC (p = 0.0035) and TMSC-CM concentration (p = 0.0028). In a chronic colitis animal model injected TMSC [X4] or TMSC-CM [X12], reduction of DAI (3.25 ± 0.25 vs. 1.50 ± 0.28 vs. 1.50 ± 0.28, control vs. TMSC vs. TMSC-CM group, p = 0.0038), increased of weight gain (1.65 ± 2.88 vs. 5.08 ± 3.31 vs. 6.00 ± 0.52, p = 0.4562, 0.1888 (respectively), and recovery of colon length (61.4 ± 2.82 vs. 70.0 ± 2.74 vs. 72.60 ± 2.68, p = 0.0602, p = 0.0205 respectively.) was observed at day 30.

Conclusions: In the DSS induced chronic colitis animal model, the administration of TMSC as well as TMSC-CM showed almost the same effect on improvement of inflammation. Therefore, we suggest the use TMSC-CM for IBD treatment utilising paracrine factors of TMSC without any cell transplantation.

P034

Validation of a novel xenograft mouse model for intestinal fistulas


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Background: Fistulas are a frequent, relapsing complication in Crohn’s disease (CD), affecting up to 50% of patients. Surgical resection is regularly required, as medical treatment outcome with conventional drugs is often insufficient. Previously, we demonstrated that epithelial-to-mesenchymal transition (EMT) plays a critical role for fistula development in CD patients. We found particular cytokines and their receptors upregulated along the tracts, supporting fistula development via the stimulation of EMT. Despite some progress in understanding the pathogenesis, there is still an urgent need for more effective medical treatments for CD fistulas. Due to a lack of a reliable in vivo model, new drug developments are complicated. Here, we validated a promising new human gut xenograft (XGR) mouse model of intestinal fistulas, clearly resembling the human situation.

Methods: 12–18 weeks (w) old human foetal small intestine was transplanted subcutaneously onto the backs of SCID mice. After 12–16w, ~15% of the mature xenografts spontaneously developed enterocutaneous fistulas. Using systemic LPS treatment followed by mild skin irritation adjacent to the transplant, we established...