TNBS application). Two groups: Platform + Infliximab (1 mg/ml), s.c. Infliximab (5 mg/kg). Clinical and histological evaluations were done in both studies (ponderal evolution, bacterial translocation to liver, colon weight as a marker of oedema and inflammation, inflammatory cell infiltrate and intestinal architecture). ADAs levels were determined in the chronic model.

Results: On the acute model, treatment with our drug-eluting platform significantly improved clinical evaluations (ponderal evolution), macroscopic (colon weight), and histological tissue evaluations. On the chronic model, both drug-eluting platform and subcutaneous administration showed a similar fashion in resolving the disease, but the formation of ADA's was significantly diminished with our drug-eluting platform (0.9 vs. 1.97 µg/ml-c, p = 0.0025) at day of euthanasia (Day 28).

Conclusions: Endoscopic placement of drug-eluting platforms opens a new possibility for therapeutic endoscopy. We have been able to reduce the formation of ADA's when a biological therapy is used. This could be of great importance for the future management of patients with IBD and other pathologies where mAb's are used.

P003

C86/CD16 macrophages may act as a source of WNT2b in intestinal tissue from B3 Crohn's disease patients

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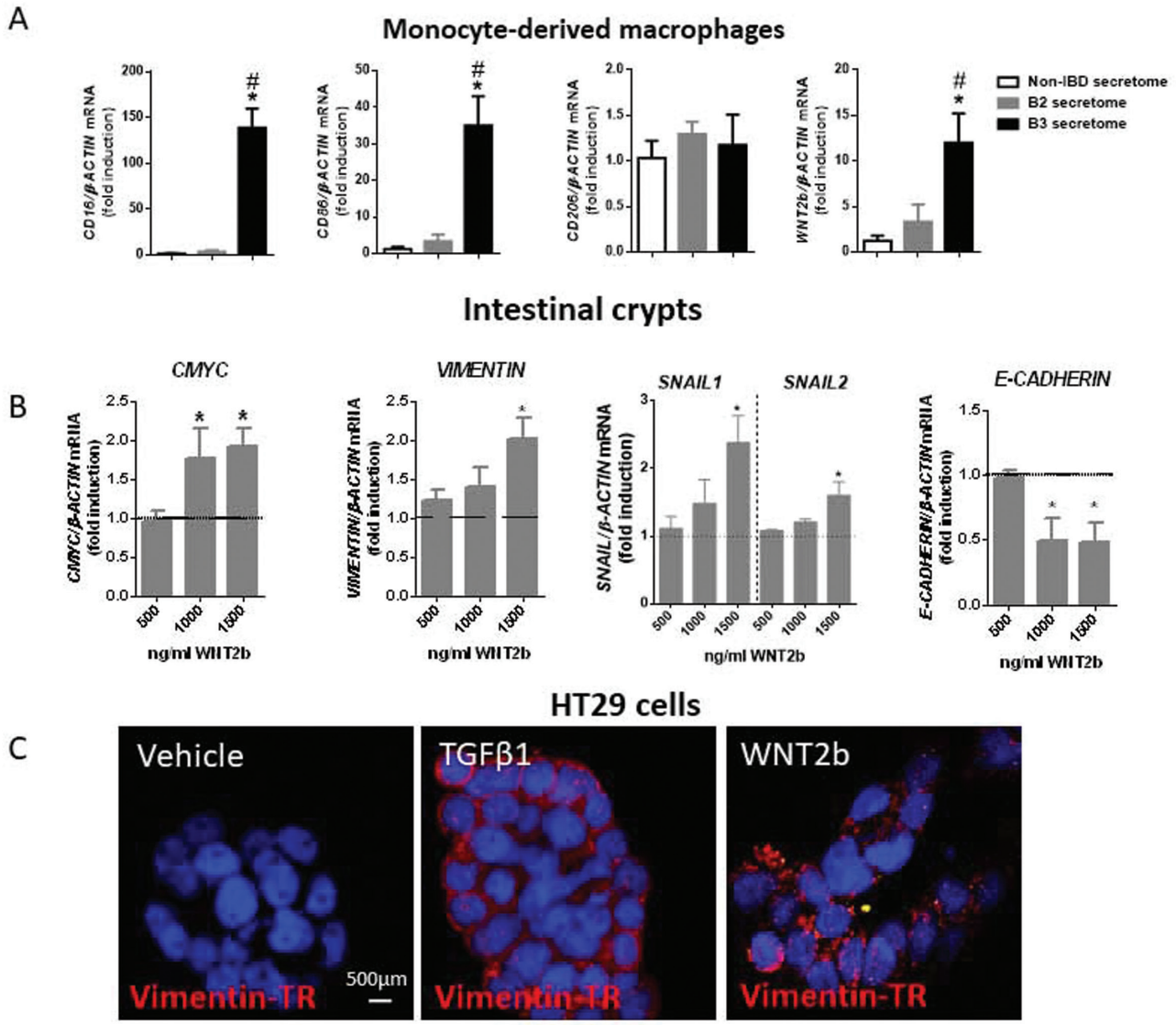

Abstract P002 – WNT2b induces EMT. (A and B) Relative mRNA expression vs. β-ACTIN and represented as fold induction vs. vehicle-treated. (C) Images showing VIMENTIN and nuclear staining in HT29 cells.

Background: Macrophages contribute to fibrosis through the release of different mediators and the pattern of secretion may vary according to their phenotype. The expression of WNT ligands has been related with the macrophage phenotype and strong evidence identifies the WNT signalling pathway as an emerging modulator of fibrosis.
Methods: The aim of the present study was to analyse the pattern of expression of macrophages and the expression of WNT ligands in surgical resections from Crohn's disease (CD, n = 43) patients which were categorised according to Montreal classification (B2 or B3; unaffected mucosa of patients with colorectal cancer was used as control). mRNA was isolated from intestinal samples and the expression of macrophage markers and WNT2b was analysed by RT-PCR. The number of macrophages positive for the different markers (CD206, CD86, CD16, and WNT2b) was determined by flow cytometry. PBMCS were isolated from healthy donors and treated during 5 days with secretomes, from control, B2 or B3 surgical resections; the mRNA expression of macrophage markers and WNT2b was determined by RT-PCR. Intestinal crypts were isolated from control samples and were incubated for 24 h with WNT2b and the expression of EMT genes was analysed by RT-PCR. HT29 were treated for 7 days with WNT2b or TGFβ1 and immunofluorescence was performed. Results are expressed as mean ± SEM ($n \geq 5$). Statistical analysis was performed by ANOVA + Newman–Keuls test. $^{*}p < 0.05$ significant differences vs. Non-IBD group or vehicle, $\#p < 0.05$ vs. B2-CD group.
Results: The expression of WNT2b was significantly higher in intestinal samples from B3 CD patients (2.3 ± 0.4) than in controls (1.1 ± 0.1) or B2 patients (0.7 ± 0.1). The number of CD16 or CD86-positive macrophages was significantly higher in intestinal tissue from B3 CD patients (69.7 ± 24.4% and 88.8 ± 18.4%, respectively) than in that from B2 CD patients (36.12 ± 5.8% and 30.58 ± 10.9%, respectively). A high percentage of CD16 positive macrophages in intestinal tissue from B3 CD patients were also positive for WNT2b (24.7 ± 8.8%). The mRNA expression of CD16, CD86, and WNT2b was significantly higher in PBMCS treated with B3-secretomes than in those treated with B2- or control secretomes (A). Exogenous administration of WNT2b to intestinal crypts induced the mRNA expression of EMT genes (B). WNT2b and TGFβ1-induced VIMENTIN expression in HT29 cells (C).
Conclusions: A macrophage phenotype expressing CD86/CD16 may act as a source of WNT2b in intestinal tissue from CD patients with a penetrating (B3) behaviour. WNT2b induces EMT in intestinal crypts and HT29 cells.

P004

IL-22 affects barrier function and cell polarity by MAPK/PI3 kinase signal transduction

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Background: Polarity in intestinal epithelial cells (IECs) is crucial to the barrier function. IL-22 is a cytokine that has been related to directly affect the integrity of the epithelial layer. IL-22 receptor/signalling complex is found mainly in epithelial cells membranes. The activated complex leads to the activation of various cellular signalling pathways including STAT-3, MAPK and PI3K/AKT. The effect of IL-22 on epithelial cells concerning cell polarity and barrier defect is not clearly understood. Therefore, this study aimed to understand the mechanism underlying the development of dyspolar epithelia and barrier defect caused by IL-22.
Methods: To investigate the role of IL-22, we exposed various intestinal epithelial cell lines (Caco-2, T84 and HT29/B6) with IL-22. Single IECs implanted in Matrigel were grown to 3-dimensional cysts +/– IL-22 and analysed by confocal microscopy. The integrity of the barrier was monitored by measurements of transepithelial resistance (TER). Calcium switch experiments (Ussing chamber) was used to evaluate tight junction (TJ) assembly. To evaluate cell motility wound healing and invasion assays were performed. Intracellular localisation of immunostained proteins related to TJ (JAM and ZO-1) was investigated using confocal microscopy. Activated signal transduction pathways were identified in phosphoblots and inhibitors of STAT-3, MAPK/ERK, and PI3K pathways were applied to uncover the signal transduction of barrier and polarity effects.
Results: IL-22 treatment reduced TER, altered distribution of TJ proteins and caused multi-lumen cysts, suggesting disturbed cell polarity and secondary to that disturbance of barrier function of IECs. In addition, invasion and migration were increased after IL-22 treatment. It was, furthermore, observed that IL-22 treatment induced STAT-3, ERK, and AKT phosphorylation, which were associated with the observed IL-22 effects. Interestingly, only blocking of PI3K/AKT and MAPK pathways rescued barrier effects of IL-22 exposure, while STAT-3 primarily caused effects on cell viability.
Conclusions: IL-22 treatment alters cell polarity and has an effect in barrier function in IECs. Altogether, our data suggest that this effect is associated with the activation of PI3-kinase and ERK-pathways rather than STAT-3 pathways

P005

Persistent transcriptional reprogramming in the choroid plexus during chronic colitis: towards understanding persistent fatigue in patients with quiescent inflammatory bowel disease?

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Background: Neurobehavioural comorbidities such as depression, anxiety, and fatigue are correlated with disease activity in patients with inflammatory bowel diseases (IBD). The persistence of fatigue during disease remission, however, remains a clinical challenge, and lacks any scientific basis. In the present study, we described behavioural changes in mice with extinguished chronic colitis, and mapped the transcriptional profiles at the blood–cerebrospinal fluid barrier, constituted by the choroid plexus.
Methods: Chronic gut inflammation was induced in C57BL/6J mice by repeated administration of dextran sodium sulphate (DSS). Following a recovery period of 3 weeks, mice were subjected to behavioural tests, and the choroid plexus tissue was analysed by RNA sequencing.