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Circulating inflammatory protein and cellular profiles at time of diagnosis classify inflammatory bowel disease patients according to their underlying immune response and clinical disease course

M. Heredia*[1], M. Charrout[2], R. Klomberg[3], M. Aardoom[3], M. Jongsma[3], P. Kemos[4], D. van Haaften[1], B. Tuk[1], L. van Berkel[1], B. Bley Folly[1], A. Mahfouz[2], M. Reinders[2], F. Ruemmele[5], N. Croft[4], J. Escher[3], L. de Ridder[3], J. Samsom[1]

[1]Erasmus University Medical Center-Sophia Children's Hospital, Laboratory of Pediatrics- division Gastroenterology and Nutrition, Rotterdam, The Netherlands, [2]Delft University of Technology, Delft Bioinformatics Lab, Delft, The Netherlands, [3]Erasmus University Medical Center-Sophia Children's Hospital, Pediatric Gastroenterology, Rotterdam, The Netherlands, [4]Blizard Institute-Queen Mary University of London, Centre for Immunobiology, London, United Kingdom, [5]Necker-Enfants Malades University Hospital- Institut Imagine- AP-HP- Université de Paris, Pediatric Gastroenterology, Paris, France

Background: Chronicity of inflammatory bowel disease (IBD) is driven by reactivation of inflammatory memory CD4$^+$ T helper (Th) cells which activate an inflammatory cascade involving innate immune cells and structural intestinal tissue cells. Because of disease heterogeneity, novel treatment strategies tailored to more precisely target the patient's individual immune defect are required to prevent disease reactivation. We hypothesize that analysis of changes in circulating inflammatory protein abundance combined with phenotyping of circulating Th cells allow to dissect underlying immune pathogenesis and we aim to stratify pediatric IBD patients accordingly.
Methods: We performed plasma analysis of 92 inflammatory proteins in a cohort of pediatric IBD patients (CD: n=62; UC/IBD-U: n=20), patients with suspicion of IBD but negative diagnosis (n=13) and age-matched healthy controls (HC: n=30). Peripheral blood was obtained at diagnosis and after induction treatment (t=10–14 weeks). Plasma protein concentrations were assessed with Olink Proximity Extension Assay technology® and Th cells were analyzed with flow cytometry. Samples were clustered using hierarchical clustering with Ward linkage. Differential protein abundance was assessed with t-tests at t=0 and a mixed effect model after treatment.
Results: Thirty-six plasma proteins discriminated pediatric IBD patients from HC. CD and UC/IBD-U patients shared increased abundance of 17 proteins amongst which interleukin-6 and oncostatin-M. Increased abundance of the Th1 cytokine interferon-γ was strictly associated with CD while Th17-associated interleukin-17A was significantly more abundant in UC/IBD-U. Hierarchical clustering of plasma protein profiles discriminated 2 clusters of UC patients with different clinical disease activity and disease extent. In CD, three patient clusters were identified. CD#1 patients had lower clinical disease activity, lower C-reactive protein and higher blood albumin concentrations. Clusters CD#2 and CD#3 had comparable clinical parameters. CD#3 patients had higher abundance of 14 proteins associated with neutrophil function and interferon-γ signaling while CD#2 patients had a marked increase in frequencies of activated (HLA-DR$^+$) memory Th cells. The three CD clusters responded differently to therapy with CD#1 patients exhibiting only a few changes, CD#2 patients showing intermediate modulation and CD#3 patients exhibiting more modulated proteins and greater fold changes.
Conclusion: Combined plasma immune protein and circulating Th cell profiling discriminates subgroups of pediatric IBD patients during active disease which differ in their response to therapy.
Abbreviations: CD: Crohn's disease; UC: ulcerative colitis; IBD-U: IBD-unclassified.

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Pathologic involvement of the farnesoid X receptor/fibroblast growth factor 19 axis to the Crohn's disease early postsurgical recurrence.

R. Suau*[1], A. Garcia-Jaraquemada[2], V. Lorén[1], P. Torres[2], E. Jou[2], M. Llaves[2], A. Aterido[3], T. Julià[3], Y. Zabana[4], J.J. Lozano[5], E. Domènech[6], M. Mañosa[6], J. Manyé[1]

[1]CIBER-"Germans Trias i Pujol" Research Institute, IBD Research Group, Badalona, Spain, [2]"Germans Trias i Pujol" Research Institute, IBD Research Group, Badalona, Spain, [3]Vall d'Hebron Research Institute, Rheumatology Research Group, Barcelona, Spain, [4]Hospital Mutua de Terrassa, Gastroenterology Department, Terrassa, Spain, [5]CIBER in Liver and Gastroenterology, Bioinformatics Unit, Barcelona, Spain, [6]"Germans Trias i Pujol" University Hospital CIBER, Gastroenterology Department, Badalona, Spain

Background: Crohn's disease (CD) is a chronic inflammatory bowel disease that normally requires bowel surgical resection due to intra-abdominal inflammatory complications such as fistulae and stenosis. However, 90% of patients that underwent surgery and did not receive prophylaxis, suffer from postsurgical endoscopic recurrence within one year.
Methods: The objective of this study is to understand the *de novo* postsurgical lesion formation in order to stablish a potential mechanism of action and its derived predictive signals to devise preventive strategies. To achieve it, transcriptome analyses of the inflamed and macroscopically unaffected zone of the ileocecal resection from 20 patients from a hospital cohort and 10 controls were performed. Patients were classified for early postsurgical recurrence by means of Rutgeerts index (i0<i1<i2<i3<i4) and transcriptome was analysed using different comparatives and clustering strategies. In order to replicate the results from the transcriptome analyses, the statistically significant genes and rationally related genes have been tested by qPCR in a corroboration cohort formed by 24 different patients.
Results: The transcriptome results showed there were no differences neither between i0, i1 and i2a (anastomotic lesions) nor when comparing i2b (non-anastomotic lesions) vs ≥i3. However, when comparing i0+i1+i2a vs. i2b+i3+i4 there were differently expressed genes highlighting ADIPOQ in the inflamed zone and the FXR/FGF19 axis in the unaffected zone. When replicating the results in the corroborative cohort, we identified FXR and other xenobiotic nuclear receptors as the genes differentially expressed in i2a vs i2b, i3 and/or i4, mainly in the macroscopically unaffected zone. Specifically, the differentially expressed genes were: MOGAT2, PXR, FXR and AHR (i2a vs i4), PPARα and FXR (i2a vs i3), TMIGD1, PPARα, FXR and AHR (i2a vs i3+i4) and FXR (i2a vs i2b+i3+i4).
Conclusion: Nuclear receptor FXR is activated in the ileum by bile acids action and it is key in the lipid metabolism regulation and in the bile acids production by means of FGF19. Moreover, ileal FXR, as well as other xenobiotic nuclear receptors, actively participates in the enhancement of the intestinal barrier function and in the innate immunity regulation. In conclusion, the results obtained in

this study suggest a link between bowel xenobiotic metabolism and the *de novo* lesion formation in previously unaffected areas in the postsurgical recurrence in CD.

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SZN-1326, a Wnt agonist, improved epithelial healing and ameliorated colitis in a chronic DSS model, in stark contrast to anti-TNF and anti-IL-12/23p40 antibodies

L. Xie*[1], P. Handa[1], C. Tun[1], A. Liu[1], C. Lu[2], H. Baribault[1], M. Newman[3], L. Le[3], D. Shah[2], S. Deshmukh[2], D. Mutha[1], T. Le[1], R. Fletcher[2], H. Chen[4], J. Ye[4], J. Tibbitts[3], U. Srinivasan[3], Y. Li[4], G. Vanhove[3], W.C. Yeh[1]

[1]Surrozen. INC, In vivo pharmacology, South San Francisco, United States, [2]Surrozen. INC, Discovery biology, South San Francisco, United States, [3]Surrozen. INC, Pre-Clinical, South San Francisco, United States, [4]Surrozen. INC, Protein science, South San Francisco, United States

Background: All currently approved treatments for Inflammatory bowel disease (IBD) and the majority of drugs in the pipeline are anti-inflammatory agents. Clinical remission rates have reached a plateau, and there is an unmet need for agents that directly repair and regenerate the intestinal epithelial barrier as mucosal healing has been associated with improved clinical outcomes. Wnt/β-catenin signaling promotes intestinal stem cell renewal and epithelial regeneration. We have engineered a Wnt activator, SZN-1326, which increased Wnt target gene expression in the colon of mice treated with Dextran Sulfate Sodium (DSS), an animal model of IBD and demonstrated efficacy in the acute and chronic forms of this model. The goal of this study was to compare the efficacy of SZN-1326 to the anti-inflammatory agents anti-TNFα or anti-IL-12/23p40 antibodies in a chronic DSS model.

Methods: 7- to 8-week old female C57BL6/J mice were administered 3% DSS for three 7-day cycles separated by 7-days off, followed by a 3-day 1% DSS wash-out period. SZN-1326 was injected intraperitoneally (I.P.) at 1, 3, or 10 mg/kg for 2, 4 or 6 doses. Anti-TNFa (MP6-XT22, Biolegend) was injected I.P. at 5 or 25 mg/kg for 4 or 7 doses, and the mice were euthanized on day 38. A separate study examined the efficacy of SZN-1326 relative to anti-IL-12/23p40 (C17.8, Bioxcell) in the same model. Anti-IL12/23p40 antibody at 3 or 10 mg/kg for 4 or 8 doses was given on days 23 or 7 respectively.

Results: During each cycle of DSS treatment, animals exhibited a marked increase in disease activity index (DAI), characterized by body weight loss, diarrhea and bleeding. SZN-1326 at various dosing regimen (1 mg/kg for 4 doses to 10 mg/kg for 2, 4 or 6 doses) significantly improved body weights and decreased DAI compared to isotype control, while anti-TNFa or anti-IL12/23p40 had no efficacy on body weight restoration or DAI. The antibodies could be detected in the serum in accordance with their doses. Treatment with SZN-1326, but not with anti-TNFα significantly decreased colon inflammation, mucosal erosion and overall histopathological score. Further, SZN-1326 treatment significantly reduced serum levels of IL-6 and lipocalin-2, while anti-TNFa decreased only lipocalin-2 levels and anti-IL12/23p40 reduced serum IL-6 level.

Conclusion: In a chronic mouse IBD model, SZN-1326 at various dosing regimens stimulated intestinal epithelial regeneration, induced mucosal healing and restored the epithelial barrier resulting in reduced inflammation, improved body weight and reduced disease activity. In contrast, treatment with anti-TNFα or anti-IL-12/23p40 antibodies led to reduction only in certain serum inflammatory cytokines but had no efficacy on DAI or colon histology in this model.

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Development of a treatment for short bowel syndrome using small intestinal organoids

S. Sugimoto*[1], E. Kobayashi[2], M. Fujii[3], Y. Ohta[3], K. Ishikawa[1], K. Nanki[1], Y. Hakamata[4], T. Kanai[5], T. Sato[1]

[1]Keio University School of Medicine, Department of Organoid Medicine- Department of Gastroenterology, Tokyo, Japan, [2]Keio University School of Medicine, Department of Organ Fabrication, Tokyo, Japan, [3]Keio University School of Medicine, Department of Organoid Medicine, Tokyo, Japan, [4]Nippon Veterinary and Life Science University, School of Veterinary Nursing and Technology, Tokyo, Japan, [5]Keio University School of Medicine, Department of Gastroenterology, Tokyo, Japan

Background: Massive small intestinal resection leads to short bowel syndrome (SBS), which is a severe malabsorption disorder. Crohn's disease is one of the most frequent cause of surgical removal of the small intestine in adults. Severe SBS patients need to receive permanent parenteral nutrition, which can cause serious complications. Intestinal transplantation currently remains the only curative option for such patients but has not widespread due to its high mortality/rejection rates. Here, we propose a concept to generate a rejection-free small intestinalized colon (SIC) by replacing the native colonic epithelium with small intestinal organoids.

Methods: Human normal intestinal organoids and rat intestinal organoids derived from luciferase-expressing LEW transgenic rats were established and cultured as previously described (Fujii et al. *Cell Stem Cell* 2018). Human colon or ileum organoids were xenotransplanted onto the EDTA-injured colon of immunodeficient mice via transanal infusion as previously described (Sugimoto et al. *Cell Stem Cell* 2018). In LEW rats, a 4-cm segment of the ascending and proximal transverse colon was dissected with the preservation of the vasculature. After EDTA-based removal of the colon epithelium, rat colon or ileum organoids were transplanted in a blinded manner. Following organoid transplantation, the colon segment was fixed to the abdominal walls as stoma outlets for a week. Afterwards, organoid-transplanted colon segment was trimmed and interposed between the jejunum beginning and the ileocolic valve following total jejunoileum resection. Overall survival and detailed histological analyses were performed.

Results: Xenotransplanted human ileum organoids reconstituted nascent villus structures reminiscent of the ileum epithelium in mouse colon. Furthermore, ileum xenografts exhibited a formation of Lyve-1$^+$ lacteal-like structure equipped with the absorption-related machinery, but not colon xenografts. In rats, engrafted ileum organoids initially formed crypt-like structures in the colostomy and, after interposition, they developed mature villus structures. The villus formation was small intestine-specific and flow-dependent. The SIC gained small intestinal function along with the remodelling of the underlying lymphovascular networks. Ileum organoid-transplanted rats exhibited milder body weight loss and significantly higher survival rate compared to colon organoid-transplanted rats.

Conclusion: The SIC with villus structure, intact vasculature and innervation, and the lacteal, had absorptive and peristalsis functions.