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Close Monitoring of CRP and Fecal Calprotectin is Able to Predict Clinical Relapse in Patients With Crohn's Disease in Remission After Infliximab Withdrawal. a Sub-Analysis of the Stori Study

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Introduction: In Crohn's disease (CD), predicting clinical relapse in patients in clinical remission by using non-invasive biomarkers could allow early therapeutic intervention. Serum C-reactive protein (CRP) and fecal calprotectin (calpro) have great potential in this regard. Aim: To assess the value of monitoring CRP and calpro levels to predict a relapse in patients with CD in clinical remission after infliximab (IFX) discontinuation. Materials and Methods: Patients with luminal CD treated for at least one year with scheduled IFX combined with an immunosuppressant (IS) and in stable remission without steroids for at least 6 months were prospectively recruited in the STORI study (1). IFX was discontinued at baseline and IS treatment was kept at a stable dose over the study period. CRP and calpro were measured every 2 months until 18 months of follow-up or until clinical relapse. CRP and calpro levels were compared between relapsers and non-relapsers at each time point using a linear mixed model. The optimal threshold of each biomarker to predict clinical relapse was determined using ROC curve analysis. Results: 113 patients were included and analyzed. Among them, 51 presented a relapse after a median follow-up of 10 months. Overall 475 CRP and 454 calpro measurements were performed in relapsers and non-relapsers with a median of 4 measurements/patient for each marker. Median [IQR] CRP at inclusion was 2 mg/l [0.9;4.9] and median calpro was 51 µg/g [30;224]. The evolution of CRP and calpro levels was significantly different between relapsers and non-relapsers ($p<0.0001$ and $p<0.001$). In non-relapsers, a slight but significant increase in CRP and calpro levels was observed throughout the follow-up ($p=0.0018$ and $p=0.0016$ respectively) with a median value at maximal follow-up of 3.7 mg/l for CRP and 66.9 µg/g for calpro. In relapsers, after a slight and progressive increase, a sudden and more pronounced increase in CRP and calpro levels was observed during the 4 months preceding clinical relapse ($p<0.0001$ and $p=0.0004$ respectively) with a median value before the relapse of 8 mg/l for CRP and 534 µg/g for calpro. Using ROC curve, the best compromise between sensitivity and specificity to predict relapse was 6.1 mg/l for CRP (sensitivity 71%, specificity 66%) and 305 µg/g for calpro (sensitivity 70%, specificity 74%). Conclusion: After discontinuation of IFX in patients with CD in clinical remission, a sudden increase in CRP and calpro levels predicts the occurrence of a relapse during the next 4 months. Further studies are needed to evaluate the therapeutic implications of these findings. 1. E Louis, GETAID et al. Gastroenterology 2011, Sept 22 epub.

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PIANO: A 1000 Patient Prospective Registry of Pregnancy Outcomes in Women With IBD Exposed to Immunomodulators and Biologic Therapy

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Introduction: Women with IBD and their physicians have concerns regarding the safety of biologic and immunosuppressant medication use during pregnancy. Data regarding the safety of these medications are sparse due to limited sample size at any one center and lack of uniform data collection methods. We created a prospective cohort of pregnant women at 30 US IBD centers to determine whether the complication rates are higher among women with IBD and their offspring who are exposed to azathioprine (AZA), 6-MP, or anti-TNF agents during pregnancy compared to women with IBD who do not take these medications. Methods: Pregnant women with IBD were prospectively enrolled and contacted every trimester, at the birth of their baby, and at 4, 9, and 12 months of age. Newborn complications for the first year of life and the mothers' medications, disease activity and complications of pregnancy were recorded. Patients were divided into four groups according to exposure between conception and delivery: Unexposed (no thiopurines or anti-TNF agents); Group A (6MP/AZA); Group B (infliximab, adalimumab, certolizumab) and Group AB (both thiopurines and anti-TNF). Results: 1052 women have been enrolled in the study to date, of whom 797 have completed their pregnancy (Unexposed=337; Group A=265; Group B=102, Group AB=59). There were 33 (4.1%) spontaneous abortions (SAB) and 37 infants with congenital anomalies (CA) (4.6%). The use of thiopurines and anti-TNF agents were not associated with an increase in "any complication", SAB, CA, preterm birth, intrauterine growth retardation, caesarean section, or NICU stays even when adjusted for disease type or disease activity. The majority (72%) of newborns were breastfed. Breastfeeding was not associated with an increase or decrease in infection risk among drug exposures and within each drug category. There was a significant increase in infant infections at 12 months of age in the combination therapy group relative to the unexposed group (Group AB, RR 1.50 (1.08-2.09)). Infant height, weight and developmental milestones, adjusted for disease activity, were similar among infants in all groups at 4, 9 and 12 months of age. Conclusions: Among infants born to women with IBD, the use of biologics and immunosuppressants was not associated with an increase in congenital anomalies, abnormal newborn growth and development or other complications compared to infants of mothers not exposed to these medications. The increase in infections from 9 to 12 months of age among infants exposed to a combination of immunomodulators and biologics during pregnancy merits further investigation. As drug should no longer be detectable in infants at 9 to 12 months, this finding may suggest dysfunctional immune development. Infants will continue to be followed until 4 years of age to determine whether an increase in infections persists.

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Microbiota Support Increased Production of IL-22 by Lamina Propria Innate Lymphoid Cells in Patients With IBD

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Interleukin (IL)-22 is an IL-10 family member that acts on epithelial cells to promote healing. Although initially characterized as a cytokine made by T cells (predominantly Th17 cells), more recent data has revealed the contribution of non-T cells, including lymphoid tissue inducer cells and innate lymphoid cells (ILCs), in IL-22 production. These ILCs plays an important role in mouse models of colitis, but their role in inflammatory bowel disease (IBD) and regulation by intestinal microbiota remain less clear. Here, we characterize Th17 cytokine production by lamina propria mononuclear cells (LPMCs) from IBD patients and non-IBD controls. Our results reveal an increase in IL-22 in IBD patients, particularly in non-T cells. The surface markers of these IL-22 producing cells are consistent with an ILC and include c-Kit, CD56, and CD127. These cells additionally express retinoic acid-orphan receptor (ROR)γt, a nuclear hormone receptor central to the Th17 transcriptional program. ILC profiling from three diverted patients suggest that exposure to bacteria associated with the fecal stream is required for induction of IL-22. In order to test the sufficiency of human microbiota to induce IL-22 by ILCs, we employed a mouse model of acute colitis induced by dextran sodium sulfate (DSS). ILC production of IL-22 requires mucosal ulceration (initiated by DSS), commensals, and RORγt. By gavaging gnotobiotic mice with PBS, human feces, or mouse feces, we show that human microbiota are sufficient to induce ILC production of IL-22 in DSS-induced colitis. Microbial induced IL-22 production by ILCs requires MyD88-dependent signaling. Moreover, IL-23 is required for IL-22 production by ILCs in both mouse and human colitis. While analysis of mucosal-associated bacteria in a subset of the patients did not reveal notable changes at the phyla level, deeper characterization is needed to examine the possible contribution of particular microbial species. These results offer the first characterization of intestinal ILCs producing IL-22 in IBD, show a correlation of ILC activity with exposure to bacteria in the fecal stream, establish the sufficiency of human microbiota for ILC activation in mouse models of colitis, and reveal the dependence of microbial induced induction of IL-22 on both MyD88-signaling and IL-23. The production and regulation of IL-22 by intestinal ILCs may have important implications for diagnostic and therapeutic developments in the clinical management of IBD.

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NFIL3 Deficient Mice Develop Severe Innate Immune Mediated Spontaneous Colitis

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Background & Aims: Regulation of inflammatory responses against the enteric microbiota is essential for the maintenance of intestinal homeostasis. We identified NFIL3 (nuclear factor, IL-3 regulated, also known as E4BP4) as a transcriptional repressor of IL-12 p40 in macrophages (Kobayashi T et al. J Immunol 2011). Expression of NFIL3 is induced by the microbiota in wild type (WT) mice, but is impaired in colitis-prone *Il10*$^{-/-}$ mice. Moreover, decreased intestinal NFIL3 expression was found in patients with Crohn's disease and ulcerative colitis. In this study, we characterize the occurrence of severe spontaneous colitis in *Nfil3*$^{-/-}$ mice. **Results:** Approximately 20% of *Nfil3*$^{-/-}$ mice developed rectal prolapse by 20 weeks and 50% by 36 weeks of age. On necropsy, *Nfil3*$^{-/-}$ mice demonstrated significantly thickened and foreshortened colons with lack of formed fecal pellets. Serum IL-12 p40 levels correlated with histological severity of colitis and colonic expression of inflammatory cytokines (IL-12 p40, TNF-α, IL-6, IFN-γ, and IL-17). Histologically, significant elongation of colonic crypts and transmural inflammation were observed. CD3^{+} and F4/80^{+} cells were notably increased in inflamed colons of *Nfil3*$^{-/-}$ mice. Both IFN-γ and IL-17 producing lamina propria CD4^{+} T cells were increased in *Nfil3*$^{-/-}$ mice, whereas the number of CD4^{+} FoxP3^{+} T cells was similar to WT mice. Since NFIL3 is induced by IL-10 in macrophages, we generated mice deficient in both NFIL3 and IL-10 (NIDKO) to examine whether NFIL3 is responsible for IL-10-mediated immune regulation. NIDKO mice developed more severe colitis with 50% incidence of rectal prolapse at five weeks and 100% penetrance of severe colitis by eight weeks, associated with higher levels of IL-12 p40 in serum and colonic tissue compared to *Nfil3*$^{-/-}$ and *Il10*$^{-/-}$ mice. NIDKO bone-marrow derived macrophages (BMDMs) showed considerably higher LPS-induced IL-12 p40 compared with either NFIL3 or IL-10 single knockout mice. Exogenous IL-10 inhibited IL-12 p40 to the same extent in NIDKO and *Il10*$^{-/-}$ BMDMs, suggesting that IL-10 and NFIL3 independently regulate IL-12 p40 in BMDMs and *In Vivo*. We next generated *Nfil3*/*Rag2* double knockout (NRDKO) mice. NRDKO mice adoptively transferred with WT unfractionated CD4^{+} T cells developed colitis in three weeks whereas *Rag2*$^{-/-}$ recipients did not. This result indicates that WT CD4^{+} T cells are sufficient to induce colitis in *Nfil3*$^{-/-}$-recipients due to defects in non-lymphocyte populations. **Conclusion:** *Nfil3*$^{-/-}$ mice develop severe spontaneous colitis. Our study implicates NFIL3 as an IL-10-independent regulator of macrophage-derived IL-12 p40. NFIL3 expression in non-lymphocyte population is crucial for the maintenance of intestinal homeostasis.

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Immunochip-Based Analysis of a Large IBD Case-Control Cohort Identifies 50 Novel Loci, Refining Definitions of Disease Pathways

Judy H. Cho on behalf of the IIBDGC

INTRODUCTION: A seminal finding of the genome-wide association study (GWAS) era is the identification of a marked overlap of loci between immune-mediated diseases. The Immunochip Consortium designed a customized chip with ≈200.000 single nucleotide polymorphisms focused on: 1) fine-mapping 190 known loci for 9 different immune-mediated diseases and; 2) replication of top GWAS signals not previously followed up, including 2000 markers from CD and UC GWAS. METHODS: A meta-analysis was performed combining HapMap3 imputed CD and UC GWAS data, with a larger case-control cohort genotyped on the Immunochip. Altogether, 19,416 CD, 17,016 UC and 36,602 controls,