Background: ulcerative colitis (UC) is a complex disease with poorly understood pathogenesis. In recent years, enormous genome-wide association studies have identified 242 single-nucleotide polymorphisms (SNPs) which cause UC susceptibility. However, their exact functions and effects remain unknown. To help discover novel pathogenic pathways in UC, we developed network biology approaches to study these SNPs in the context of their signalling and regulatory landscapes.
Methods: We used immunochip profiles of 941 UC patients and focussed on UC-associated SNPs which altered miRNA target sites or transcription factor (TF) binding sites. We identified the SNP affected proteins, and mapped them to a comprehensively curated signalling database, OmniPath (http://omnipathdb.org/), to uncover their known interactions. We run a simulation using an approach called random walks to link the effect of the SNP affected proteins to TFs. We calculated how many signals reached each TF from the SNP affected proteins in each patient. Afterwards, we connected the TFs to their target genes, using a manually curated TF-target gene dataset we developed in-house (TFlink) and the Gene Transcription Regulation Database. Following a randomised control, we kept those genes that were significantly affected in more than 50% in the analysed patients.
Results: We found 24 genes with putative links to UC. The 24 genes linked the immune-related kinase LYN and STAT4 to the immune-based pathogenesis of UC. UC SNPs affected CSKA1, CSKA2, and PKCA kinases. These kinases regulate major parts of cellular signalling networks, indicating their key role in pathogenic rewiring. Furthermore, we identified TFs involved in myofibroblast development including MYOD1 and MEF2A and MEF2D. We also identified EPCAM and ACTN4A which are involved in the focal adhesion complex, which is regulated indirectly by LYN. The involvement of these genes suggests a defected wound healing mechanism in the colon as a key player in UC pathogenesis.
Conclusions: Our findings suggest that the SNPs in UC can affect, via their signalling interactions, a wide variety of cellular functions with known pathogenic relevance. The functions of the affected genes indicate the focal adhesion complex and the myofibroblast development to be involved in UC pathogenesis. The described effects suggest novel pathogenetic pathways involved in UC which may be used to illuminate potential novel therapeutic intervention points.

P012

IL22 expression in intestinal immune cells is not augmented by AHR activation in health or Crohn's disease

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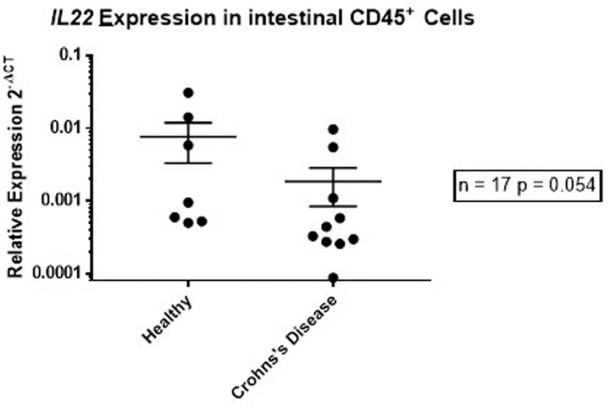
Background: IL-22 produced by mucosal immune cells plays an important role in maintenance of the intestinal barrier; production is increased in response to intestinal injury. The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that responds to specific dietary and bacterial ligands. In mice, activation of AHR is critical for the expression of *IL22*. In diverse models of colitis, genetic deletion of AHR or low AHR ligand availability leads to reduced IL-22 activity and increased disease severity. Although enhancing IL-22 release by activating AHR is an attractive therapeutic approach it is unclear if this would reduce inflammation in the human IBD. In this study, we determine activation of AHR *in vivo* using quantitative measurement of *CYP1A1* expression, which closely correlates with AHR activation, and examine the impact of AHR blockade or activation on *IL22* expression in health and Crohn's disease.
Methods: CD45+ cells isolated from endoscopic biopsies using antibody labelling and immunomagnetic sorting, were cultured with AHR ligand (FICZ 10 nM) or antagonist (CH-223191 100 μM). Whole biopsies were also immediately homogenised in RLT buffer and expression of *AHR, CYP1A1*, and *IL22* determined by qRT-PCR.
Results: Whole biopsies and CD45+ intestinal immune cells expressed both *AHR* and *CYP1A1 ex vivo* suggesting the presence of a functional AHR signalling pathway; *AHR and CYP1A1* expression was higher in CD. *IL22* expression was also detectable *ex vivo* but did not correlate with *CYP1A1* expression and was lower in CD.



AHR signalling was significantly inhibited by antagonist but was minimally enhanced by agonist. However, *IL22* expression *in vitro* by CD45+ cells was not significantly affected by either AHR antagonist or agonist. Baseline AHR activation or response to agonist did not correlate with *IL22* expression in response to agonist. However, the degree to which *CYPY1A1* expression was inhibited by antagonist, a potential surrogate for *in situ* activation, did correlate with baseline *IL22* expression in the same tissue suggesting a more complex relationship.
Conclusions: In humans the AHR pathway is activated *in vivo* in both health and Crohn's disease. Resting *IL22* expression is lower in CD compared with health. However, the expression of *IL22* in intestinal immune cells was not augmented in vitro by AHR ligand in either health or disease, perhaps because the pathway is already near maximally activated. This suggests the relationship between AHR and IL22 is complex and simply supplementing AHR ligand intake may not be helpful in IBD.

P013

Novel immunomodulatory role of food bioactive peptide lunasin in the healthy human intestinal mucosa

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Background: The gastrointestinal mucosa represents the main interface between dietary components and the organism. Lunasin is a 43-amino acid peptide naturally present in soybean protein with a variety of biological functions demonstrated by in vitro assays, cell cultures and animal models. Nevertheless, its physiological relevance in human primary intestinal cells remains elusive.
Methods: Peptide was obtained by chemical synthesis. Human colonic biopsies were obtained from healthy controls and conditioned with peptide lunasin (5, 50, and 200 μM), both in the presence and absence of pro-inflammatory lipopolysaccharide (LPS, 100 ng/ml). Peptide integrity during overnight culture was monitored by liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). After culture, the relative gene expression of colonic biopsies as well as the intestinal cytokine milieu in culture supernatants were characterised.
Results: HPLC-MS/MS analysis showed that lunasin maintained its stability during biopsy culture up to 90%. Lunasin was bioactive in the human mucosa inducing IL-1β, TNF-α, IL-17A, CCL2, and PGE2/COX-2 gene expression, typically in a dose-dependent manner. Moreover, lunasin also enhanced mucosal expression of tolerogenic cytokines IL-10 and TGFβ and down-regulated the expression of iNOS and subunit p65 from NF-κB. LPS induced a pro-inflammatory immune response which was, however, partially abrogated in the presence of lunasin as it down-regulated pro-inflammatory IL-17A and IFN-γ, and enhanced mucosal gene expression of regulatory IL-10 and TGFβ. Moreover, results were further validated at the protein level as IL-1β, TNF-α, and IL-10 secretion were enhanced while IL-6, CCL2, and IFN-γ production were abrogated by lunasin. Indeed, the latter cytokine was also neutralised in the presence of LPS.
Conclusions: Food-derived peptide lunasin is biologically active in the human intestinal mucosa determined by changes on the global cytokine milieu both at the messenger and protein levels. Lunasin displayed its anti-inflammatory effect by abrogating the production of pro-inflammatory cytokines even in the presence of LPS, and expanding the production of tolerogenic IL-10 and TGFβ. This peptide might represent, therefore, a novel agent as functional compound for the prevention of immune and inflammatory-mediated intestinal disorders.

P014

Disruption of epithelial barrier function by coeliac peripheral blood mononuclear cells

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Background: Immune cells are present in the small intestine mucosa in normal and inflammatory conditions. Once activated, these cells cause direct effect in the barrier function of epithelial cells in inflammatory bowel diseases (IBD). It is known that the epithelial barrier function is altered in coeliac disease (CD), common disease affecting the small intestine. In CD patients, the immune cells in the small bowel mucosa are activated after contact with antigen-presenting cells exposing gliadin-derived peptides, which leads to an inflammatory cascade causing villous atrophy and disruption of the epithelial barrier. Nonetheless, the mechanisms underlying the disrupted barrier function in CD is not clearly understood. This study aimed to verify the effect of immune cells derived from coeliac patients on the barrier function of intestinal epithelial cells
Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from the blood sample of heathy donors (n = 3), CD patients on gluten-free diet (CD GFD; n = 2) and active CD patient (n = 2). CacoBBe cells were co-cultered with PBMCs and CD14+ cells (monocytes). To verify the role of active gliadin stimulation, the intestinal cells were treated with or without IL15/Tglia. In addition, to exclude direct toxic effect of gliadin on the epithelium, control CacoBBe cells were treated with IL-15/Tglia alone. The integrity of the barrier in the monolayer cells was monitored by measuring transepithelial resistance (TER). The localisation of proteins with role in epithelial barrier function (CD71, occludin, claudin-2 and ZO-1) was investigated using confocal microscopy after immunostaining
Results: A more pronounced decrease in TER was observed in intestinal epithelial cells after co-culture with coeliac PBMCs and CD14+ cells (active CD or CD GFD patients) comparing with healthy donors. However, no difference in TER was observed comparing active CD and CD GFD. As found in completely untreated cells, in cells treated with IL-15/Tglia alone, the TER did not decrease. Exposure of intestinal epithelial cells to coeliac PBMCs resulted in a decreased expression of occludin, while no effect was observed in claudin-2 localisation and expression. In addition, it was observed an abnormal structure in ZO-1 after co-cultered epithelial cells with coeliac PBMCs (CD GFD and active CD). Confocal microscopy revealed an altered localisation of CD71 after treatment with coeliac PBMCs and CD14+ cells, with evidence of a diffuse intracellular localisation when compared with untreated cells.
Conclusions: Coeliac PBMCs have an effect on epithelial barrier function of intestinal epithelial cells. This is associated with an altered expression pattern of key proteins for tight junction assembly.

P015

PNAd+ and MAdCAM+ high endothelial venules correlate with disease activity in ulcerative colitis

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Background: Tertiary lymphoid organs (TLOs) comprising peripheral node addressin positive (PNAd+) and/or MAdCAM+ high endothelial venules (HEVs) have been found to play an important role in local immunological dysregulation in chronic immune-mediated disorders and malignancies. Their presence have a predictive value for disease course and response to therapy. Identification of these HEVs in the early phase of ulcerative colitis (UC) might help stratify patients to enable personalised medicine. We aimed to investigate the presence of these HEVs at UC diagnosis and their development during follow-up. Furthermore, we studied their association with disease activity and response to therapy.