Forty-six genes showed a significant association (≥1 SNP with \( p_{\text{corr}} < 0.05 \)) with IBD, of which 8 belonged to the differentially expressed genes (MUC1, MUC4, TFF1, CLDN8, DSG3, MAGI1, TCF4, MEP1A). Interestingly, SNPs in DSG3, MAGI1, and TFF1 did not only confer risk to IBD, but also were eQTLs for their expression in inflamed colon and ileum respectively.

**Conclusions:** Our data show a dysregulated expression of several barrier genes in active IBD patients, while only in inactive ileal CD patients few barrier genes remained dysregulated, suggesting a primary barrier defect in these patients. The expression data, as well as genetic data and eQTL analysis pointed to DSG3, MAGI1, and TFF1 as possibly important and functional candidate genes for IBD pathogenesis.

**P066**

**Crohn’s disease-associated adherent-invasive Escherichia coli induce secretion of exosomes with pro-inflammatory activity by intestinal epithelial cells**

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**Background:** Crohn’s disease (CD) is a chronic inflammatory bowel disease of which the etiology involves environmental, genetic and microbial factors. Our group and others have shown a high prevalence of the invasive Escherichia coli strains, designated adherent-invasive E. coli (AIEC), in the intestinal mucosa of CD patients. Exosomes are small endosomal-derived vesicles involved in cell-to-cell communication and have been implicated in various diseases including cancer and infectious disorders. It has been reported that mammalian cells infected with pathogens can release exosomes containing microbial compounds. Here, we investigated the capacity of CD-associated AIEC bacteria to induce secretion of exosomes by intestinal epithelial cells and to determine the inflammatory characteristics of the released exosomes.

**Methods:** Human intestinal epithelial T84 cells were infected with the AIEC reference strain LF82. Exosomes were purified using the ExoQuick exosome precipitation reagent. Exosomes released by LF82-infected T84 cells were tested for their ability to promote pro-inflammatory responses in naïve macrophagic cells. Identification of exosomal proteins was performed by mass spectrometry.

**Results:** Electron microscopy and immunogold-labeling analyses for CD63, an exosomal marker, showed that differentiated T84 cells infected with AIEC LF82 secreted an increased amount of exosomes compared to uninfected cells. This was confirmed by increased levels of CD63 as assessed by Western blot. Stimulation of human macrophages with exosomes secreted by LF82-infected T84 cells, but not by uninfected cells, significantly induced production of the pro-inflammatory cytokines TNF-alpha and IL-6, and this was not due to the presence of lipopolysaccharide, known to induce a pro-inflammatory response. Mass spectrometry analysis revealed that exosomes released by T84 cells upon LF82 infection carried microbial antigens such as the outer membrane protein C, known to be involved in AIEC adhesion and invasion.

**Conclusions:** Our study shows that in response to CD-associated AIEC infection, intestinal epithelial cells release exosomes that can trigger pro-inflammatory responses in naïve macrophagic recipient cells.

**P067**

**Binding of infliximab (IFX) to human serum exosomes.**

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**Background:** The loss of response to infliximab has been associated with the presence of antidrug antibodies (ATI). However, other protein complexes in blood could bind this drug blocking its effect. Several studies have suggested that the exosomes (30-100 nm) present in plasma could bind biological drugs decreasing their bioavailability.

**Aims:** To analyze the molecules that bind IFX. To assess the interaction of IFX with exosomes. To know the composition of exosomes by proteomic methods.

**Methods:** Human sera from healthy volunteers (n=3) were analyzed. The samples were incubated with IFX-alexa 488 and the mixture was injected onto SE-HPLC column. Exosomes were isolated from human serum by Size Exclusion-HPLC using a Yarra3000 column from Phenomenex, USA. The void volume from the chromatographic profile was submitted to ultracentrifugation (160,000g at 4°C for 90 min). The pellet was analyzed by western blot and using a Nano LC ESI-MSMS shotgun proteomics approach. In the last method was used an Eksigent 1D- nano HPLC coupled via a nanospray source to a 5600TripleTOF QTOF mass spectrometer (ABSciex, Framingham, MA, USA).

**Results:** We observed IFX-alexa 488 bound to protein complexes that eluted in the void volume, indicating a diameter (greater than 30 nm) consistent with the size of exosomes. After ultracentrifugation of this last fraction, the pellet was characterized by western blot against CD63 (a classical marker of exosomes). Another part of the sample was used to identify by proteomic Methods, other molecules that co-eluted with IFX-alexa 488. Interestingly, we observed new proteins in the exosomes, as polymeric immunoglobulin receptor (PIGR), fibulin-1, utrophin, complement factor H, dermcidin, immunoglobulin J chain, among others. As predictable, we also identified proteins previously described in exosomes such as keratins, Ig alpha-2 chain C region, Ig gamma-4 chain C region, or thrombospondin-1. At present, we have immunoprecipitated the protein complexes from exosomes using IFX aiming to define news targets of this therapeutic antibody.

**Conclusions:** Our study demonstrated that IFX was able to bind to human serum exosomes, which were characterized by immunological and proteomic Methods. That should be taken into account when measuring antibodies to IFX as they could be wrongly considered ATI. The study of the structure and the functions of these exosomes could be important to evaluate the bioavailability and efficacy of IFX in patients with inflammatory bowel disease.

**P068**

**PSC - IBD is associated with different microbiota composition as compared to UC**

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