lower in both CC and LC, whereas IL10 was found increased in CL but not in CC.

Conclusions: We have detected remarkable differences in cellular immune response between CC and LC, suggesting that they do not share the same pathophysiological mechanisms. The decrease of apoptosis may play a role in the increased cellularity observed in both CC and LC.

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PO47 Involvement of PSGL1 and its ligands P-, E- and L-selectins in inflammatory bowel disease (IBD)

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Background: PSGL1 and L-Selectin on leukocytes and P- and E-Selectins on endothelial cells are responsible for the initial steps of leukocyte extravasation to the inflamed tissue. Our group has shown that PSGL1/P-Selectin interaction triggers tolerance signals in human monocyte-derived dendritic cells and that PSGL1 acts as a tolerogenic receptor, essential to maintain the colonic lamina propria homeostasis in mice. Our aims were: (1) To quantify soluble PSGL1 and its ligands P-, E- and L-selectins in patients with ulcerative colitis (UC) and Crohn’s disease (CD), in order to find a characteristic profile of these molecules in inactive and active IBD. (2) To study the expression of PSGL1 in the colonic mucosa of patients with inactive and active UC and CD.

Methods: PSGL1 and P-, E- and L-Selectins levels were measured by ELISA in serum samples from patients with IBD and healthy volunteers. PSGL1 tissue expression was studied by immunohistochemistry in colon biopsies from patients with IBD and patients without immune-mediated diseases as controls. Clinical IBD activity was assessed by the Mayo score for ulcerative colitis (UC), and by the Harvey–Bradshaw index for Crohn’s disease (CD). Tissue IBD activity was determined by histological criteria.

Results: 48 serum samples were obtained from 16 controls, 22 patients with CD (11 active and 11 inactive) and 10 patients with UC (5 active and 5 inactive). Biopsies were taken from 5 controls, 22 patients with UC (8 inactive and 14 with histological activity) and 7 CD (3 inactive and 4 active). Our preliminary results show that patients with UC have lower serum concentration of PSGL1 and higher levels of P-Selectin than controls, regardless of IBD activity, while L-Selectin is increased in the active disease. Patients with CD have lower concentration of PSGL1 but levels of P-, E- and L-Selectins are not different from controls. Gating tissue expression, PSGL1 is present in the membrane of colonic mucosa leukocytes from controls and patients with inactive IBD. Membrane pattern disappears as IBD histological activity increases.

Conclusions: Decreased PSGL1 and increased P-Selectin serum levels are associated with UC, regardless of the disease activity, while high levels of L-Selectin are indicative of active UC. CD is associated with lower concentration of PSGL1, without changes in Selectins levels. Membrane PSGL1 expression in colonic mucosa leukocytes is lost as IBD histological activity increases.

PO48 In vitro pancreatitis by azathioprine but not 6-mercaptopurine


Background: Among drugs often used in the treatment of inflammatory bowel disease (IBD), thiopurines and 5-aminosalicylic acid (5-ASA) can cause pancreatitis. The underlying mechanism remains largely unclear, but may include an immune mediated drug reaction. Knowing that azathioprine (AZA) can stimulate pancreas secretion, we postulate that, like in the cerulein model of acute pancreatitis, this might cause acinar cell damage and consequently to autodigestion of the pancreas by its proteases. To bolster this hypothesis, we evaluated in vitro cytotoxic effects of thiopurines and 5-ASA, tested as single drugs and as combination treatment, on three pancreatic cell lines.

Methods: The human pancreatic cell lines PANC-1 (epithelial carcinoma), AsPC-1 and Capan-1 (both adenocarcinoma) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (PANC-1) or RPMI medium (ASPC-1 and Capan-1). After seeding in 96-wells plates (1.48×10^5 cells/cm²), cells were incubated with the single drugs AZA, 6-mercaptopurine (6-MP), tioguano (TG) or 5-ASA in the concentration range of 3.9-4000 μM. Every 24 hours culture medium and drugs were refreshed. After 24, 48 or 72 hours cytotoxicity was established by performing the water-soluble tetrazolium salt-1 (WST-1) assay. Cell survival curves were obtained and half maximal inhibitory concentrations (IC₅₀) were calculated. Next, combination experiments were conducted, with various concentrations AZA, 6-MP or TG (3.9-4000 μM) in combination with a fixed non toxic concentration of 5-ASA (200 μM). Three independent experiments were conducted in triplicate.

Results: IC₅₀ values of AZA were between 300 and 530 μM after 72 hours of incubation in the three cell lines. Incubation with TG resulted in IC₅₀ values, ranging from 360 to 2508 μM, depending on the cell line used. Incubations with 6-MP or 5-ASA did not result in decreased cell survival. Combinations of thiopurines with 5-ASA resulted in increased toxicity of AZA and TG in AsPC-1 cells, and decreased toxicity of AZA in Capan-1 cells. TMPT genotyping did not reveal polymorphisms associated with decreased TPMT activity in the three cell lines.

Conclusions: AZA, and to a much lesser extent TG, but not 6-MP and 5-ASA, exerted a cytotoxic effect on the tested pancreatic cell lines in the present study. The difference in toxicity between AZA and 6-MP could be explained by differences in their metabolism. A large comparative study between AZA and 6-MP would be necessary to verify if there is also an in vivo difference.

PO49 Investigation into the binding affinity of certolizumab pegol to FcRn and functional consequences for FcRn-mediated transcytosis: comparison to infliximab, adalimumab and etanercept

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Background: Certolizumab pegol (CZP) is an anti-TNF that lacks the monoclonal antibody Fc portion. In contrast, anti-TNF antibodies infliximab (IFX) and adalimumab (ADA) and receptor fusion protein etanercept (ETA) all possess an IgG1 Fc. It has been reported that lower levels of CZP, compared to ADA/IFX, are transferred from treated mothers to the neonate. This transfer differential may be due to the one-way active transport of antibodies across the placenta thought to be mediated by the neonatal Fc receptor (FcRn). The objective
was to quantify binding of CZP, IFX, ADA and ETA to FcRn and to measure FcRn-mediated transcytosis of these agents.

Methods: A Biacore® assay was used to determine the binding of CZP, ADA and IFX to human FcRn. Anti-TNFs were passed over an FcRn-coated chip for 5 min at a range of concentrations from 21–670 nM to determine the on-binding rate; a buffer at pH 6.0 was used to allow optimum binding. The off-rate was followed for a further 5 min by running buffer alone over the chip. MDCK II cells transfected with human FcRn were used to measure FcRn-mediated transcytosis using a pH 5.9 buffer on the apical side and pH 7.2 on the basolateral side. The anti-TNFs and the control antibody (P146), which possessed an Fc modified to prevent binding to FcRn, were biotinylated to allow visualization. The amount of each anti-TNF transcytosed across the cell layer over 4 hrs was measured by MSD assay.

Results: IFX (132 nM) and ADA (225 nM) had relatively high binding affinity to FcRn while the binding affinity of ETA to FcRn was approximately 5 to 10-fold lower (1500 nM). In contrast, CZP did not bind to the FcRn with any measurable affinity. The mean levels of transcytosis seen with IFX and ADA were 24.8 ± 0.06 ng/mL and 159.5 ± 0.05 ng/mL, respectively (n = 3). Transcytosis of ETA (81.3 ± 0.0 ng/mL) was lower than that of ADA and IFX. In contrast, the level of CZP transcytosis (3.2 ± 0.0 ng/mL) was significantly lower than that observed with the other anti-TNFs tested. The control antibody P146 also showed lower transcytosis (5.9 ± 0.0 ng/mL). Since neither the control antibody nor CZP bind to FcRn, the levels detected are probably due to low level nonspecific leakage across the cell layer.

Conclusions: CZP does not have an Fc and thus did not bind FcRn. Moreover, no FcRn-mediated CZP transcytosis was detected. In contrast, ADA and IFX had relatively high binding affinity to FcRn and were actively transcytosed. ETA showed lower binding affinity to FcRn and subsequent transcytosis, compared to IFX/ADA, but FcRn-mediated ETA transport could still be measured. These results explain previously observed active transport of anti-TNFs across the placenta seen in patients treated with IFX and ADA, whereas only low levels were observed with CZP.

P050
Intrapertioneally injected mesenchymal stromal cells home significantly more often to the intestines in colitis mice compared to healthy controls

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Background: Mesenchymal stromal cells (MSCs) are multipotent cells with immunomodulatory and tissue regenerative properties. Therefore, they may be useful in the treatment of inflammatory bowel disease (IBD). Whether or not MSCs need to home to and engraft at the site of inflammation to exert their beneficial effect in colitis needs to be elucidated. We compared the homing and engraftment capacity of MSCs and prestimulated MSCs in experimental colitis.

Methods: MSCs were isolated from bone marrow of wild type BALB/c mice and transduced with a firefly-luciferase and GFP construct. The week before infusion, some MSCs were prestimulated with interferon-gamma to give iMSCs. TNBS-colitis was induced in BALB/c mice and $2 \times 10^6$ transduced (i)MSCs were injected intraperitoneally. (i)MSCs were visualized in vivo by bioluminescence imaging (BLI). At sacrifice, 3 days after injection of the cells, inguinal lymph nodes (ILNs), mesenteric lymph nodes (MLNs), spleen, small intestine (SI) and colon were also imaged by BLI. Organs were imbedded in paraffin for immunohistochemistry.

Results: A 5.5 fold higher amount of injected MSCs and 4 fold higher amount of injected iMSCs was traceable by BLI in the abdomen of mice with colitis compared to control mice without colitis 2 days after infusion of the cells ($p < 0.0001$ and $p = 0.0029$). A similar difference was observed a day later ($p = 0.0005$ (MSCs); $p = 0.0048$ (iMSCs)). BLI at sacrifice 3 days after (i)MSC injection showed the same trend in colon, SI and MLN. Nineteen days after infusion of the (i)MSCs BLI-signal was completely disappeared in the mice that were not sacrificed at day 3. Colons and SI stained for GFP showed spherical (i)MSC formations situated in the fat surrounding the serosal site of the intestines. In these spheres macrophage were found close to (i)MSCs. No (regulatory) lymphocytes were observed inside the spheres, whereas in the surrounding area some CD3 and FoxP3 positivity was found. Furthermore, collagen deposition and some proliferation was observed in these (i)MSC-spheres.

Conclusions: The amount of traceable injected (i)MSCs in the abdomen was significantly higher in mice with colitis compared to healthy controls. (i)MSCs were more likely to cluster around the intestines when colitis was present. No difference in homing or engraftment was observed between MSCs and iMSCs.

P051
Interleukin 22 and interleukin 23R gene expression in Crohn’s disease: preliminary results

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Background: A causal role was recently attributed to inflammation in many diseases, the inflammatory mediators like cells and cytokines, which are involved in inflammatory disease including Crohn’s disease (CD), have both tissue-protective and inflammatory effects in the gut.

Aim: To investigate the expression level of the Th17 related cytokines: IL22 and the IL23 receptor. these levels were compared between inflamed and non inflamed colonic tissue from biopsies in the same patients.

Methods: The molecular analysis of these genes was performed on 20 Tunisian patients with known Crohn’s disease who underwent a colonoscopy between March 2012 to September 2012 in our department. Biopsies from inflamed and non-infamed tissue of each patient were collected during colonoscopy after legal consent. The mRNA expression level of each gene was determine by RT-PCR. IL22 and IL23R mRNA expression was normalized to beta-actin expression in the respective DNA preparation.

Results: Twenty patients were colliged. Median age was 45 years, disease location was colonic in ratio of 85% with a moderate flare in most cases. Evaluation of mRNA expression levels in colonic and control samples revealed that IL22 and IL23R were significantly over expressed in inflamed tissue versus non-infamed ($p = 0.000087$ for IL23R and $p = 0.000033$ for IL22).

Conclusions: This is the first study demonstrating that tissue inflammation in CD patients is clearly associated with increased expression levels of Th17 related cytokines: IL22 and IL23R.

P052
Interleukin 10 (IL10) expression and distribution pattern in healthy and inflamed bowel. Relationship with steroid response in Crohn’s disease (CD)


Background: Interleukin-10 (IL-10) is expressed by many cell types of the innate and adaptive immune system. Its effect