European
Crohn's and Colitis
Organisation

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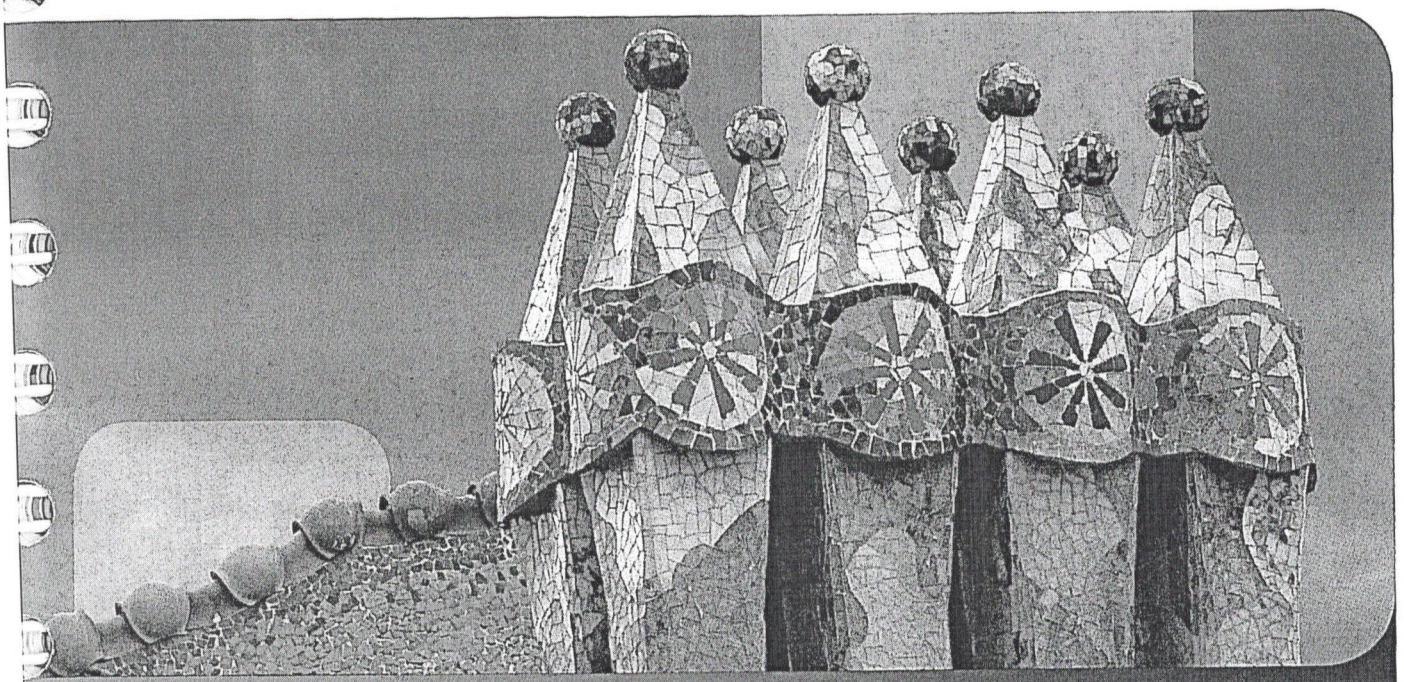
Inflammatory Bowel Diseases

Final Programme



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are recently recognized genes that encode cytoplasmic proteins from the same family that play a role in the apoptotic signaling cascade and activation of Nuclear Factor kB.

Aim: To determine the expression of some members of the CARD family (CARD10, CARD11 and CARD14) in patients with UC and controls without inflammation.

Methods: A total of 183 individuals divided into 3 groups (63 patients with active UC, 60 patients with UC in remission and 60 controls without histologic inflammation). In all cases the diagnosis was confirmed by histopathology. Total ribonucleic acid (RNA) was extracted from intestinal tissue, subsequently complementary deoxyribonucleic acid (cDNA) was obtained by polymerase chain reaction (PCR). For each gene, relative quantification was made by real-time PCR. The statistical analysis was performed using SPSS version 17.0, using Kruskall Wallis non-parametric test, Spearman's correlation, Fisher's exact test and Odds Ratio (OR) in order to determine the strength of association. A P value < 0.05 was considered as significant.

Results: The CARD14 gene expression was significantly higher in the group with active UC compared to controls (P=0.008) and statistical trend of significance in the group of UC in remission (P=0.07). The low expression of CARD14 gene was associated with a more benign clinical course of UC, characterized by initial activity followed by long-term remission longer than 5 years (P=0.01, OR= 0.07, CI 95%: 0.007-0.70). No statistically significant differences were found in the CARD10 and CARD11 gene expression.

Conclusions: The CARD family might be involved in UC pathophysiology. The CARD14 gene expression was increased in patients with active UC and the low expression of CARD14 gene was associated with a benign clinical course characterized by long-term remission.

P040
The biosimilars of infliximab are equally well quantified in a clinically validated infliximab assay

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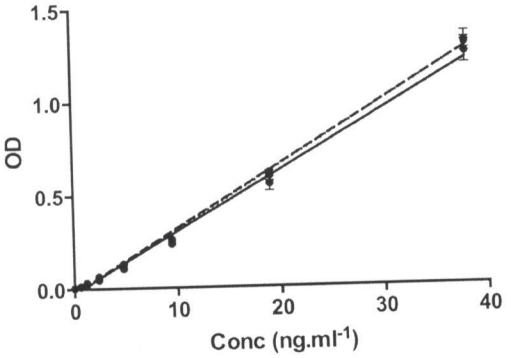
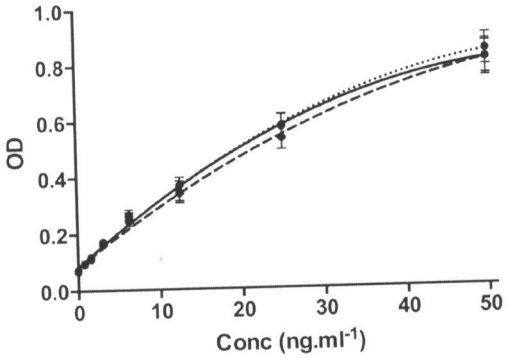
Background: A panel of 55 monoclonal antibodies towards the anti-tumour necrosis factor (anti-TNF) biological Remicade® (infliximab, IFX) was previously generated. The highly specific monoclonal antibody, MA-IFX6B7, was selected to quantify Remicade® in a clinically validated in house-developed infliximab ELISA, which was recently converted into CE-marked kits (distributed by ApDia and R-Biopharm). Recently, two biosimilars of Remicade®, Remsima® and Inflectra® were launched. We evaluated the 'cross-reactivity' of the MA-IFX panel towards these biosimilars and we evaluated the quantification of biosimilars in the infliximab ELISA.



"**Figure 1** Dose-response curve of MA-IFX6B7 for reaction with Remicade® (solid line), Remsima® (dashed line) and Inflectra® (dotted line) (p >.5). Data are presented as mean ± SE (n = 24)."



"**Figure 2**: Dose-response curve of Remicade® (solid line), Remsima® (dashed line) and Inflectra® (dotted line) in the infliximab ELISA. Data are presented as mean ± SE (n = 6). The fitted curves show no significant differences (p >.4)."

Methods: First, the reactivity of the MA-IFX panel was tested using an ELISA in which either Remicade®, Remsima® or Inflectra® was coated on microtiter plates. Different concentrations (ranging from 0.8 ng/ml to 50.0 ng/ml) of each MA-IFX were applied and binding was detected using horseradish peroxidase (HRP)-conjugated rabbit-anti-mouse IgG. Secondly, calibration curves of Remicade®, Remsima® and Inflectra® (ranging from 0.6 ng/ml to 38.0 ng/ml) were applied in the in-house developed infliximab ELISA using TNF for capture and HRP-conjugated MA-IFX6B7 for detection. Statistical analyses were performed using mixed model analysis in SAS version 9.2. Thirdly, different concentrations of Remicade®, Remsima® and Inflectra® (ranging from 0.5 µg/ml to 12.0 µg/ml) were spiked in phosphate-buffered saline containing 1% bovine serum albumin. Samples were diluted and the recovery was calculated versus the Remicade® calibration curve.

Results: Screening each of the 55 MA-IFX revealed similar reactivities towards Remicade®, Remsima® and Inflectra®. An identical reactivity of MA-IFX6B7 towards all biologicals was statistically proven (Figure 1). No significant differences were observed between the calibration curves of the three anti-TNF biologicals in the infliximab ELISA (p >.4) (Figure 2). Spiked samples revealed recoveries of 92 ± 5%, 95 ± 6% and 95 ± 5% for Remicade®, Remsima® and Inflectra®, respectively (n=3).

Conclusions: We have demonstrated that MA-IFX6B7 raised against Remicade® exhibits an identical reactivity towards Remsima® and Inflectra®. We analytically validated that the infliximab assay, using MA-IFX6B7, is well suited for quantification of Remsima® and Inflectra®.

P041
Mesenchymal stem cells protect from acute dextran sulphate sodium-induced colitis by attenuating function of antigen presenting cells

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Background: Acute dextran sulphate sodium (DSS)-induced colitis is a well-established murine model of colitis, because of the high degree of uniformity, reproducibility and similarities to acute human colitis. DSS induces mucosal injury and inflammation which is accompanied with migration of inflammatory cells in the colon. Because of their immunomodulatory characteristics, mesenchymal stem cells (MSC) are considered as promising therapeutic agents for the therapy of immune mediated diseases. Recently published studies suggested therapeutic potential of MSC for the treatment of colitis, but the mechanisms remain unknown. The main aim of this study was to evaluate possible cellular targets of MSC in the pathology of acute colitis.

Methods: DSS (3%, molecular weight 40kDa) was dissolved in water and given to C57Bl/6 mice in place of normal drinking water (ad libitum) for 7 days. Mouse bone marrow-derived MSC (2 x 106 cells) were intravenously injected daily. Disease Activity Index (DAI: weight loss, stool consistency, visible blood in feces), was used to assess the clinical signs of colitis. The histology score of colitis was calculated as the sum of "infiltration" and "damage of epithelium" sub-scores for each mouse. The cellular make up of colon and phenotype of colon-infiltrated immune cells was determined by flow cytometry.

Results: DAI and histology score were significantly attenuated in DSS+MSC-treated mice (n=16) compared to DSS-only treated animals (n=16). This was associated with the reduced infiltration of innate immune cells in the colon, particularly antigen presenting cells (CD11c+CD11b+ inflammatory dendritic cells and F4/80+CD11b+macrophages). In addition, intravenous injection of MSC attenuate expression of major histocompatibility complex II and co-stimulatory molecules on antigen presenting cells. The percentage of protective CD3+NK1.1+ NKT cells and F480+CD206+ alternatively activated macrophages was higher in colons of DSS+MSC-treated mice compared to DSS-only treated animals. Injection of MSC did not affect infiltration and phenotype of CD45+Ly6G+CD11c- neutrophils, CD45+SinglecF+CD11c- eosinophils and FcERI+CD117+mast cells.

Conclusions: MSC attenuate function of inflammatory antigen presenting cells in colon and protect from acute DSS-induced colitis

P042
Response to corticosteroids in Ulcerative Colitis may be related to modulation of mTOR signaling pathway genes by microRNAs

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Background: Up to 40% of patients with active Ulcerative Colitis (UC) do not have an adequate response to corticosteroids (CS). Mechanisms of resistance to CS in UC are not well understood. MicroRNA (miR) are small non-coding RNA fragments that modulate messenger RNA (RNAm) at a post-transcriptional level, playing a critical role in many biological processes. Little is known about the influence of miR in the response to CS in UC. Objective: To compare the transcriptomic profile (miR and RNAm) in rectal mucosa of patients with active UC responding and non-responding to CS.

Methods: Rectal biopsies were obtained from UC patients before and after three days of CS treatment for a moderate-to-severe flares. Patients were grouped according to clinical response (non-responder = moderate or severe activity according to Montreal's classification or need of rescue therapy at day 7; responder = mild activity or remission without rescue therapy at day 7). miR were identified by means of a sequencing method (Tru Seq Small RNA kit from Illumina) and RNAm were study by microarrays method (HumanHT-12 kit from Illumina) on those rectal biopsies with high integrity. After the comparison between groups those miR and RNAm with a fold change greater than 1.5 and adjusted p-value less than 0.05 were further studied. Potential targets of selected miR were checked in "Target Human Scan database" (www.targetscan.org), and their impact on biological activity was searched in "GeneCodis database" (http://genecodis.cnb.csic.es).

Results: 8 responders and 7 non-responders tissue samples reached an integrity that allowed miR sequencing and microarrays study. Comparison between responders and non-responders before CS showed a differential miR expression of miR-1246, miR-1291, miR-5701 and miR-625-3p. Comparison between responders before and after treatment showed differential expression of miR-183-5p, miR-3607-3p, miR-1246, miR-5701 and miR-625-3p. And comparison between non-reponders before and after treatment showed differential expression of miR-4770, miR-449, miR-145-3p, miR-1246 and miR-1291. The only gene with differential expression after microarrays study was DDIT4, which was down-regulated in responders before CS in comparison with responders after 3 days of treatment. A further in silico study reveals that DDIT4 is a potential target of three of the differential expressed miR (miR-183-5p, miR-625-3p , miR-3607-3p) and also that this gene is linked to the mTOR pathway (and indirectly with autophagy).

Conclusions: There is a different miR profile in rectal mucosa of patients with active UC responding and non-reponding to CS. Our findings suggest that regulation of mTOR and autofagy pathways by miR might be involved in the response to CS in active UC.

P043
Immunohistochemical expression of angio and lymphangiogenic factors in colonic mucosa of patients with inflammatory bowel disease.

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Background: To evaluate the possible differences between inflammatory bowel disease (IBD) and non-IBD, and between ulcerative colitis (UC) and Crohn's disease (CD), in the expression of VEGFA, -C, -D, VEGFR1, -R2, -R3, PlGF, Ang1, Ang2 and Tie2 in colonic mucosa

Methods: Biopsies from patients with and without IBD that underwent to a colonoscopy by medical criteria were prospectively included and their mucosal samples studied by immunohistochemistry. VEGFA, -C, -D, -R1, -R2, -R3 and PlGF expression Results were graded as follows: (++) over 50% of the tissue cells were stained, (+) below 50%, and (-) completely negative. Ang1, Ang2 and Tie2 were assessed as the average density of five hot spots at a magnification of x40. Endoscopic activity was assessed by endoscopic Mayo