RESULTS: A significant differential expression of genes is found comparing Treg from HDC to aCD (69 genes, 39 up-, 30 down-regulated) and HDC to ICD (52 genes, 25 up-, 27 down-regulated). In both groups, genes were up-regulated more than 2.5fold. Transcripts for CCR4 were among the strongest expressed, achieving an up-regulation of 5.76 (HDC vs aCD) and 6.4fold (HDC vs ICD), respectively. FACS analysis showed that Treg express the highest level of CCR4 in peripheral T cells. In addition, migration studies showed that Treg migrate preferentially towards TARC - the ligand for CCR4. No significant differences were found in the percentage of CCR4+ Treg comparing HDC to ICD (57.9 ± 11.3 to 54.4 ± 12.7). It could be detected, but comparison of normalized mean fluo- rescence intensity (MFI CCR4+/MFI CCR4−) showed a significant decrease in CD (15.8 ± 3.7 vs 10.4 ± 3.4; p=0.019). Immunohistochemistry showed a significant reduction of CCR4-expressing Treg in CD compared to DIV (44.4 ± 3.9 vs 29.0 ± 8.0; p=0.019).

Conclusions: CCR4 is a possible target gene for Treg pathobiology in CD. Since TARC is abundantly expressed in diseased mucosa, the decreased expression of CCR4 points to an impaired mucosal Treg migration. Therefore, the migration capacity of Treg in CD is currently under investigation.

P252 EPITHELIAL-SPECIFIC BLOCKAGE OF THE MYD88-DEPENDENT TLR SIGNALING PATHWAY CONTRIBUTES TO SPONTANEOUS INTESTINAL INFLAMMATION
J. Gong1, N. Li1, W. Zhu2, X. Gao2, J. Li1. 1Research Institute of General Surgery, Jilinling Hospital, Nanjing, China; 2Model Animal Research Center, Nanjing University, Nanjing, China

To elucidate the potential role of epithelium-intrinsic myd88-dependent Toll-like receptor (TLR) in the pathogenesis of inflammatory bowel disease (IBD), a transgenic mouse model in which the flag-epitope tagged dominant-negative Myd88 (dnMyd88) driven by a 12.4kb villin promoter was generated. Expression analysis revealed that the transgene was specifically expressed in the epithelium of small intestine and colon. At 6 weeks, the transgenic mice demonstrated elongament of the small intestine, increased crypt depth and width, and significantly increased proliferation compartment (Brdu positive cells/crypt, 20.6 vs 6.2, P<0.01). By the age of 48 weeks, the transgenic mice showed increased intestinal cell infiltration, crypt abscess, crypt branching, and increased inflammatory cytokine levels. Alcian-Blue staining revealed that the number of goblet cells were significantly decreased (A-B positive cells/crypt, 6.11± 14.44, P<0.01), but real-time quantitative PCR did not detect any change in MUC2 mRNA expression transgenic in mice. TUNEL assay also revealed increased apoptosis in transgenic mice. Further more, we examined the possible mechanism of intestinal inflammation. Real-time quantitative PCR revealed that expression of des-dfensin 3 and Claudin-1/4,7 levels did not change significantly in transgenic mice. However, the Paneth-cell-derived α-des-dfensins (also named cryptdins) were significantly decreased (general cryptdins, relative mRNA levels, 1.0 ± 0.33, P<0.05).

Our study identified that myd88-dependent TLR signaling in the intestinal epithelium was a critical regulator of epithelial defense and intestinal immune-mucostasis, and the results might provide important implications for understanding the possible mechanisms controlling the pathogenesis of human IBD, such as Crohn’s disease.

P253 NEW SEROLOGICAL MARKERS FOR INFLAMMATORY BOWEL DISEASE ARE ASSOCIATED WITH EARLIER AGE AT ONSET, COMPLICATED DISEASE BEHAVIOR, RISK FOR SURGERY, AND NOD2/CARD15 GENE MUTATION IN A HUNGARIAN IBD COHORT
M. Papi1, I. Altorjay1, N. Dotan1, K. Palatsa1, I. Fóldi1, J. Tumpek1, J. Sipka1, M. Udvardy1, L. Lakatos1, A. Kovacs4, T. Málnás1, T. Tulassay6, P. Mehlter1, T. Szamos1, P.L. Lakatos1. 1University of Debrecen, Debrecen, Hungary; 2Glycominds Ltd, Lod, Israel; 3Csolnoky F. County Hospital, Veszprem, Hungary; 4Peterfi Hospital, Budapest, Hungary; 5University of Szeged, Szeged, Hungary; 6Semenweis University, Budapest, Hungary

Background: Antibodies to Saccharomyces cerevisiae(ASC) and porin protein-C of Ershcherichia coli(anti-OmpC) are associated with disease phenotype and may be of diagnostic importance in inflammatory bowel diseases(IBD). Our aim was to determine whether a panel of new antibodies against bacterial proteins and carbohydrates could help differentiate between various forms of IBD, and whether they were associated with particu- lar clinical manifestations in a Hungarian cohort of IBD patients.

Methods: 652 well-characterized, unrelated, consecutive IBD patients (CD: 357, m/f: 262/295, duration: 8.1±11.3 years; UC: 95, m/f: 44/51, duration: 8.9±9.8 years), 100 healthy and 48 non-IBD gastrointestinal controls were in- vestigated. Sera were assayed for anti-Omp and antibodies against a mannepitope of Saccharomyces cerevisiae(ASC), laminiaribioside(ALCA), chito- bioside(ACCA), and mannobioside(AMCA). TLR4 and NOD2/CARD15 variants were tested by PCR-RFLP. Detailed clinical phenotypes were determined by reviewing the patients’ medical charts.

Results: CD patients had at least one of the investig- ated antibodies. Among glycans antibodies, ASCA or the combination of gASCA/atypical pANCA were most accurate for differentiating between CD and ulcerative colitis(UC). ASCA and gASCA assays performed similarly. Increasing amount of CCR4+ level of antibody responses toward gASCA, ALCA, ACCA, AMCA and Omp were associated with more complicated disease behaviour (p<0.0001) and need for surgery in CD (p=0.023). A serology dosage ef- fect was also observed. gASCA and AMCA antibodies were associated with NOD2/CARD15, in addition to a gene dosage effect. No serotype-phenotype associations were found in UC.

Conclusions: Antibody response to this new panel of serological markers was associated with complicated disease phenotype, NOD2/CARD15 genotype, and a need for surgery in this Eastern European IBD cohort.

P254 GALECTIN-2 AND GALECTIN-4 MODULATE INTESTINAL EPITHELIAL CELL FUNCTION AND INTESTINAL WOUND HEALING
D. Pack1, B. Wiedenmann1, A. Dignass1, A. Sturm1. 1Charite, Berlin, Germany; 2Markus-Krankenhaus, Frankfurt, Germany

Aim: Inflammatory bowel diseases are characterized by various degrees of mucosal surface damage resulting in subsequent impairment of intestinal barrier function. Galectins are increasingly recognized as novel regulators of inflammation and autoimmunity. However, their ability to modulate intestinal epithelial cell function, and thus wound healing, remains still unclear. Thus, we aimed to explore the effect of galectin (Gal)-2 and Gal-4, both predominantly expressed within the gastrointestinal tract, on epithelial cell function and wound healing and to compare its effects with Gal-1, which is widely expressed outside the gastrointestinal tract.

Materials and Methods: Cell binding was determined with biotinylated Gal-2 and Gal-4, stained with streptavidin-APC and analysed by flow cytometry. Binding sites were detected with coated magnetic beads followed by SDS- PAGE electrophoresis. Cell migration was determined by a well-established wound healing assay with confluent monolayers of Caco-2. For cell cycle analysis, cells were stained with cyclin B1-FITC and propidium iodide, to quantify apoptosis stained with annexin-V, all followed by flow cytometric analysis.

Results: Gal-2 and Gal-4 bound to epithelial cells at the E-cadherin/β-catenin complex. Gal-2 and Gal-4 both significantly enhanced intestinal epithelial cell migration in vitro by 84 ± 81 respectively 150 ± 10% through a TGF-β independent pathway as assessed by blocking studies with neutralizing TGF-β antibodies. In contrast, Gal-1 decreased epithelial cell migration. This inhibitory effect was abrogated by neutralizing TGF-β antibodies. By performing cell cycle analysis, we demonstrated that Gal-2 and Gal-4 both increased cyclin B1 expression and consequently cell cycle progression, while Gal-1 inhibited the cell cycle. Determining the influence of Gal-2 and Gal-4 on epithelial cell apoptosis, we detected no induction of apoptosis whereas Gal-1 significantly induced epithelial cells apoptosis.

Conclusion: Gal-2 and Gal-4, in contrast to Gal-1, both significantly enhanced intestinal epithelial cell restitution and cell cycle progression in vitro, both essential for rapid resealing of the epithelial surface barrier. We thus provide for the first time evidence, that these galectins differentially modulate intestinal epithelial cell function and may also play a significant role in wound-healing processes. Our data suggest a beneficial effect of Gal-2 and Gal-4 in diseases which are characterized by epithelial barrier disruption like IBD, colonic disease or chemotherapy or radiation induced epithelial surface injury.

P255 AUTO-REGULATION OF HUMAN TCF-4 BY WNT-β-CATENIN-TCF-4 SIGNALING
G. Wang1, O. Burk1, E.F. Stange1, J. Wehkamp1. 1University of Tübingen/Dr. Margarete Fischer-Bosch - Institute of Clinical Pharmacology, Tübingen, Germany; 2Department of Internal Medicine II/Robert Bosch Hospital, Stuttgart, Germany; 3University of Tübingen/Dr. Margarete Fischer-Bosch- Institute of Clinical Pharmacology and Department of Internal Medicine II/Robert Bosch Hospital, Stuttgart, Germany

Aim: Tcf-4 is a major transcription factor in the Wnt/β-catenin pathway. Wnt signaling plays a critical role in stem cell maintenance and Paneth cell differ- entiation in intestinal crypts. An over-activation of this pathway is thought to be important in intestinal carcinogenesis. We recently reported a reduced expression of Tcf-4 in ileal Crohn’s disease (CD) leading to reduced expres-