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Efficiency of evaluating the expression of Toll-like receptors 2, 4, 6 and proteins proteomic profiling as integral indicators for predicting the risk of early relapse of ulcerative colitis

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Background: Success was made in the study of TLR in congenital and adaptive immunity, which determined a new look at immune processes at ulcerative colitis (UC). Modern achievements of proteomic methods of analysis research allow to define molecular characteristics of the inflammation in colon mucosa of patients with UC.
Methods: The study included 86 patients with UC, an average age of 39.0 ± 1.4 years. Groups: 1–15 (17.4%) patients with distal form of UC, 2–42 (48%) left-sided form, 3–29 (33.7%) patients with total UC. The expression of TLR on peripheral blood monocytes was determined in the immunofluorescence test. Two-colour analysis was performed on a flow-through laser cytofluorimeter (Cytomics FC500, Beckman Coulte). The percentage of monocytes (CD14 + cells) carrying TLR2, TLR4, and TLR6 on their surface was assessed. The separation of proteins of colon mucosa was based on technologies of IEF, SDS–PAGE, 2DPAGE, by standard sets (MB-HIC C8 Kit, MB-IMAC Cu, MB-Wax Kit, Bruker, USA). The getting of mass spectrogram was determined by matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry (MALDI-TOF-MS/MS, Ultraflex II, Bruker, USA). Statistical analysis was performed using the software Statistica 10.0 (Statsoft).
Results: The direct average relationship was established between the number of monocytes expressing CD14 + CD282 +, CD14 + CD284 +, CD14 + CD286 + and the area of inflammation ($r = 0.49$, $r = 0.55$, $r = 0.42$, $p < 0.05$). The nonlinear regression equation was used. Calculation example: the risk of recurrence UC = exp (–26.1 + (0.4) × TLR 2)/(1 + exp (–26.1 + (0.4) × TLR 2)), $\chi 2 = 130, 59$, $p < 0.0001$. Thus, when the number of monocytes expressing TLR2 is not more than 60%, the risk of recurrence of the UC is not more than 11%, with values above 70%, the probability of recurrence exceeds 80%. We identified potentially new molecular markers of the early relapse of ulcerative colitis: SMAD2 activates the transcription of TFG1β and leads to development of fibrosis in colon submucosa in patients with UC; significant decrease of the expression of PPARγ promotes the activation of STAT and AP-1 signalling pathways that promotes the increase of the synthesis of IL-2, 6, 8, 12, TNFα, the activity of immune and inflammation processes in colon mucosa; the reduction of expression of β-defensin-1 in cells of colon mucosa is accompanied with increased expression of CCR6 that promotes the formation of inflammatory infiltrates in colonic submucosa in UC.
Conclusion: Expression of TLR 2, 4, 6 in blood monocytes (the risk of recurrence UC ratio) and new protein molecular markers of colon mucosa (SMAD2, PPARγ, and apoC-III) can be used as a tool for prediction of early relapse UC.

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C86/CD16 macrophages accumulate in the mucosa of B3 patients and could mediate EMT in Crohn's disease

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Background: Macrophages contribute to fibrosis through the release of different mediators and the pattern of secretion may vary according to their phenotype.
Methods: The aim of the present study is to analyse the pattern of expression of macrophages, of EMT-related genes and cytokines in surgical resections from Crohn's disease (CD, $n = 43$) patients which were categorised according to Montreal classification (B2 or B3); unaffected mucosa of patients with ileocecal cancer was used as control ($n = 20$). mRNA was isolated from intestinal samples and the expression of macrophage, EMT markers and cytokines were analysed by RT-PCR. PBMCS were isolated from healthy donors and treated during 5 days with secretomes, from control, B2 or B3 surgical resections; the mRNA expression of macrophage markers was determined by RT-PCR. U937 cells were differentiated to macrophages and then treated with IFNγ (20 ng/ml) for 4 days, the mRNA expression of macrophages markers were determined by RT-PCR. Results are expressed as mean ± SEM ($n \geq 5$). Statistical analysis was performed by ANOVA + Newman–Keuls or *t*-test. Correlations between data were analysed using Pearson's correlation coefficient ($^*p < 0.05$).
Results: The expression of CD16 and CD86 was significantly higher in intestinal samples from B3 CD patients (7.2 ± 1.1 and 7.7 ± 1.3, respectively) than in controls (1.4 ± 0.2 and 2.5 ± 0.4, respectively) or B2 CD patients (4.8 ± 0.9 and 4.5 ± 0.6, respectively). The mRNA expression of CD16 and CD86 were significantly higher in PBMCS treated with B3-secretomes than in those treated with B2- or control secretomes. The expression of CD16 and CD86 significantly correlated with FSP1 ($r = 0.74$, $p = 0.002^*$ and $r = 0.66$, $p = 0.003^*$, respectively), VIMENTIN ($r = 0.60$, $p = 0.02^*$ and $r = 0.82$, $p = 0.001^*$, respectively), SNAIL1 ($r = 0.61$, $p < 0.01^*$ $r = 0.52$, $p = 0.04^*$, respectively), IL4 ($r = 0.63$, $p = 0.01^*$ and $r = 0.60$, $p = 0.02^*$, respectively) and IFNγ ($r = 0.56$, $p = 0.001^*$ and $r = 0.58$, $p = 0.01^*$, respectively) in intestinal tissue from the fistulising CD group. U937 cells treated with IFNγ increased significantly the mRNA expression of CD16 (1.94 ± 0.24* vs. vehicle) and CD86 (1.60 ± 0.17* vs. vehicle).
Conclusion: A macrophage phenotype expressing CD86/CD16 may act as a source of EMT mediators in intestinal tissue from CD patients with a penetrating (B3) behaviour. IFNγ could be responsible for the increase in the number of CD86/CD16 macrophages in the B3 behaviour.

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Per- and polyfluoroalkyl substances (PFAS) are significantly increased in patients with late-onset of ulcerative colitis

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Background: Environmental factors have been implicated in the pathogenesis of inflammatory bowel disease (IBD), particularly late onset disease. Per- and polyfluoroalkyl substances (PFAS) are man-made chemicals with a long biological half-life that have been extensively used since the 1950s and have been proposed to interfere with the bile acid synthesis. Therefore, to investigate if late onset IBD correlates with higher PFAS levels, we measured serum levels of PFAS and bile acids in patients diagnosed with IBD later in life.

Methods: Serum samples were collected from patients diagnosed with ulcerative colitis (n = 20) and Crohn's disease (n = 20) at the age of ≥55 years. Blood donors (n = 20) were used as healthy controls and were matched by gender and age. The levels of PFAS and bile acids were assessed by ultra performance liquid chromatography coupled to a triple quadrupole mass spectrometer.

Results: The total amount of PFAS was significantly higher in patients with ulcerative colitis compared with healthy controls (p = 0.021) or patients with Crohn's disease (p = 0.015). No difference was found in total PFAS levels between Crohn's disease patients and healthy controls (p = 0.841). Seven out of 30 bile acids correlated to the total PFAS level.

Conclusion: Our results demonstrate that PFAS levels are increased in patients with late-onset of ulcerative colitis compared with Crohn's disease patients and healthy controls. This finding indicates that PFAS might represent an environmental risk factor for ulcerative colitis. However, additional studies assessing the functional consequences of increased PFAS in late-onset ulcerative colitis are required to confirm this hypothesis.

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Colonic mucosal kinase activity, cytokine and chemokine profiles in inflammatory bowel disease

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Background: With the approval of tofacitinib, an oral Janus Kinase (JAK) inhibitor, modulation of kinase activity has been added to the therapeutic armamentarium of inflammatory bowel disease (IBD). Despite its established efficacy, at least a third of patients will not respond to this or other therapeutic options such as anti-tumour necrosis factor (TNF), anti-interleukin (IL)23/IL12 compounds or vedolizumab. A better understanding of the inflammatory profile

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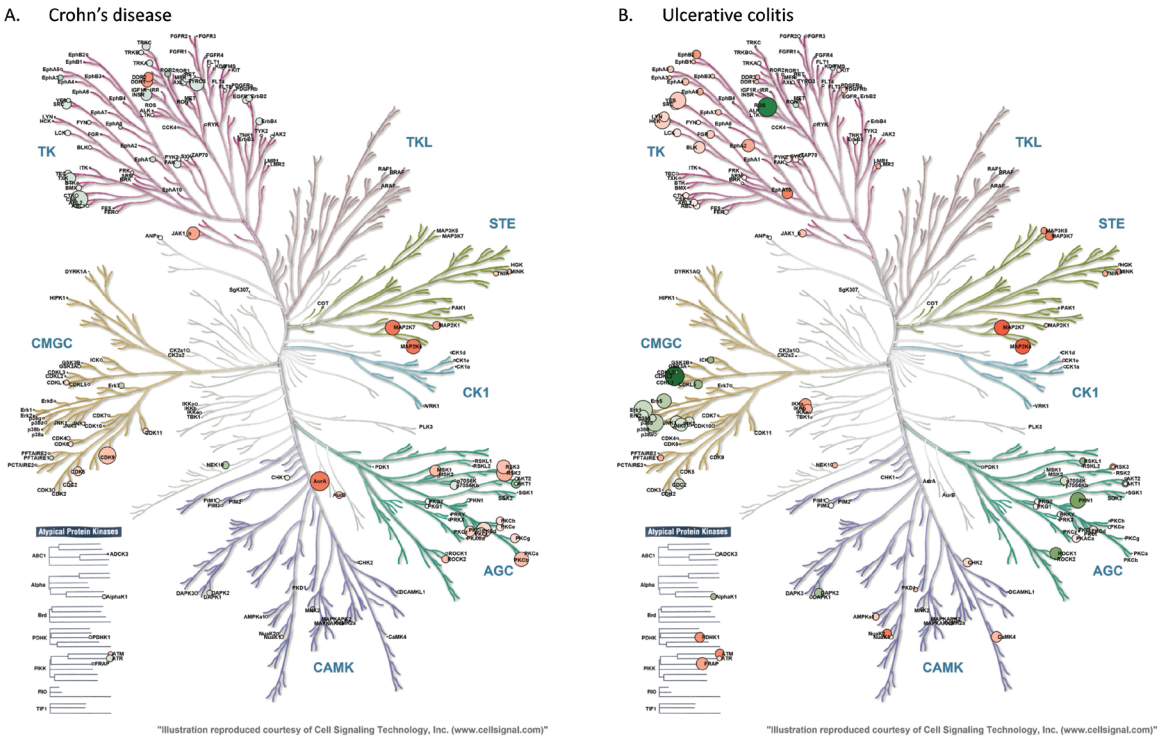

Figure 1. Kinase activity comparing inflamed and non-inflamed mucosa of IBD-patients.
Kinase phylogenetic trees are plotted depicting predicted kinase activity employing Pamgene technology. Red circles depict more kinase activity in inflamed tissue, while green depicts more activity in non-inflamed tissue. The brightness of the colour reflects the certainty of the effect, and the size of the circle reflects the size of the effect.