recruitment and that AT-1001 could delay progression of enteropathy in patients with gluten sensitivity.

T1270

Gliadin Induced Increase in Intestinal Epithelial Permeability Is Independent of MEK

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Background: A characteristic of coeliac disease during ingestion of gluten is increased intestinal permeability, dysregulation of junctional proteins located at the apical lateral membrane and phosphorylation of the extracellular signal-regulated kinase proteins ERK1 and ERK2 (ERK1/2). The aim of this study was to test whether gliadin induced phosphorylation of ERK1/2 proteins contributes to increased permeability. Methods: We challenged established confluent T84 monolayers grown on Transwells with pepsin and trypsin digested gliadin (1mg/ml; PT-gliadin) for 4 hours. Parallel T84 cells were pre-incubated with the MEK inhibitor (PD98509; 50 μM) for 1 hour before gliadin challenge. Permeability was analysed by measuring transepithelial resistance (TER) and influx of 4 kDa FITC-dextran. Tight junction protein expression and ERK1/2 phosphorylation were detected by Western blot analysis and intracellular relocation of tight junction proteins was detected by confocal microscopy. Results: Gliadin increased ionic permeability of T84 monolayers rapidly. A 96% decrease in TER occurred within 30 minutes of PT-gliadin challenge (172 ± 6 Ohms/cm2) when compared to T84 monolayers treated with BSA (4,535 ± 33 Ohms/cm2). A statistically significant increase in the influx of 4 kDa FITC dextran was first detected at 60 minutes ($p<0.015$) in monolayers treated with PT-gliadin (1.824 ± 0.052 μg/cm2) compared to monolayers treated with BSA (0.073 ± 0.014 μg/cm2). Increased permeability coincided with intracellular re-distribution of claudin -1, -3, -4 and -5 proteins from the lateral membrane to the cytoplasm but not occludin or ZO-1. Western blot analysis revealed that the overall level of expression of claudin-1, -3, -4, -5 and occludin did not change. PT-gliadin induced an increase in the phosphorylation of ERK1/2 proteins (44 kDa and 42 kDa) within 10 minutes correlating with an increase in permeability. The MEK inhibitor prevented PT-gliadin-induced ERK1/2 phosphorylation but did not prevent an increase in permeability. Conclusion: Our findings show that PT-gliadin induced increase in permeability in T84 intestinal cells is MEK independent.

T1271

Enhanced Levels of Interleukin -1beta in Stools from Children with Inflammatory Bowel Disease, But No Local Production in Children with Coeliac Disease

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Background: Interleukin-1beta (IL-1beta) is a proinflammatory cytokine that induces fever and acute phase protein synthesis. In active inflammatory bowel disease (IBD) large amounts of IL-1beta is produced by mucosal macrophages and increased amounts of IL-1 beta has been found in the rectal mucosal fluid. IL-18 is a pro-inflammatory cytokine that has a crucial role in maintaining the T helper cell type 1 (Th1) response. IL-18 is, together with other Th1 cytokines, expressed in the small intestinal mucosa of patients with coeliac disease (CoD). Aim: The aim of this study was to compare the levels of IL-1beta and IL-18 in children with CoD, IBD and other gastrointestinal diseases. Methods: IL-1beta were measured in stool samples and IL-18 in serum samples from 172 children with gastrointestinal complaints (median age 12 years). Included were 17 untreated children with CoD; 35 CoD children on a gluten-free diet; 40 children with IBD; 40 children with food hypersensitivity, and 40 children with other diseases. Results: Enhanced levels of IL-1beta were found in stool samples from 20 children and 16 of them had an active IBD (median 13 ng/g). Only one celiac child had slightly increased IL-1beta levels (1.8 ng/g) and the median level for the 17 untreated celiac children were 0.37 ng/g. The untreated CoD children had the highest median level of IL-18 (259 pg/ml). However, of the 17 children with increased IL-18 levels, 4 had CoD, 6 food hypersensitivity and 6 had other gastrointestinal complaints. Notably, only one IBD patient had increased levels of IL-18, a child with Crohns disease, who in addition was the only child with elevated concentrations of both IL-1beta (152 ng/g) and IL-18 (663 pg/ml). Conclusion: The production of intestinal IL-1beta and peripheral IL-18 did not coincide and the results indicate that separate inflammatory mechanisms are involved in the disease development of CoD and IBD. The different cytokine expression can also elucidate why IBD patients often suffer from fever, while patients with CoD or food hypersensitivity seldom are affected.

T1272

Role of NKT Cell-Driven IFN-γ in Postoperative Adhesion Formation

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Background: Adhesion formation is a common and often severe complication of abdominal surgery. However, the mechanism of adhesion formation is still poorly understood. The aim of this study is to establish new experimental mouse model of surgical adhesion formation and to examine immunological mechanisms underlying organ adhesions. Method: Anesthetized mice were underwent an operation for cauterization of cecum by coagulate mode of surgical forceps for a second. Each mouse was sacrificed 7days later and evaluated degree of adhesion formation following standard scoring system (Score from 1 to 5), which has been widely used in this field. Cecums were prepared for measurement of mRNA expression of cytokines by real time PCR. Result: 1.All animals survived and developed severe adhesion formation (Score 5; very thick adhesion). Histological analysis of cecum exhibited severe inflammation and fibrosis. 2.To evaluate the role of CD4+T cells for surgical adhesion formation, mice were depleted of CD4+T cells by treatment with anti-CD4 antibody. These CD4+T-depleted mice formed mild or no adhesion formation (Score 0-1). 3.The jα18 KO mice lacking NKT cells also formed mild or no adhesion formation (Score 0-1). 4.To determine which cytokines mediate this surgical adhesion formation, we isolated cecums and examined mRNA expression. In general, cytokine mRNAs were undetectable in cecum, however, IFN-γ mRNA expression increased and peaked at 3 h after operation. 5.IFN-γ KO mice formed mild or no adhesion formation (Score 0-1). However, IFN-γ KO mice given IFN-γ formed severe adhesion formation (Score 5). Furthermore, Wild-type mice given anti IFN-γ antibody formed mild adhesion formation (Score 2). 6.To evaluate the significance of IFN-γ from NKT cells, we transferred NKT cells from wild-type or IFN-γ KO mice to jα18 KO mouse. Only jα18 KO mouse given wild-type NKT cells formed severe adhesion formation (Score 5). 7.Since development of Th1 cells requires IL-12, we compared the degree of adhesion formation between STAT4 KO and STAT6 KO mice. Both mice formed severe adhesion formation (Score 5), excluding contribution of Th1 or Th2 cells to development of adhesion. In contrast, STAT1 KO mouse formed mild or no adhesion formation, substantiating further that IFN-γ is a causative factor for this surgical adhesion formation. Conclusion: These studies demonstrated the immunological mechanism underlying surgical adhesion formation. Contribution of IFN-γ from NKT cells to adhesion development sheds a new insight in understanding the mechanism of adhesion formation and provides target cells or molecules for regulation of organ adhesion.

T1273

CD30 Antigen Expression Is Involved in Celiac Disease

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Background/aims: CD30 antigen is expressed after activation of normal B and T lymphoid cells. Different T cell activating stimuli induce CD30 expression on CD45RO+ precursors. The putative role of CD30 in celiac disease (Cel) is poorly known. Our aims are to determine CD30 expression after nonspecific activation of peripheral blood lymphocytes (PBL) and to examine CD30 expression in duodenal T cells from biopsies ex-vivo challenged with gliadin. Materials/methods: PBL from 10 healthy controls (Co) and 10 active adult Cel patients were incubated with 1μg/ml of anti-CD3 for 5 days. Triple immunofluorescence (anti-CD45RO PE or -CD25 PE, -CD3 PerCP and -CD30 FITC) and flow cytometry analysis were performed at baseline and days 3 and 5 after incubation. CD30 antigen was immunohistochemically evaluated in paraffin sections of duodenal biopsies from 6 Cel patients and 6 Co. Triple immunofluorescence and flow cytometry analysis was performed on isolated intraepithelial and lamina propria lymphocytes (IEL and LPL). Biopsy specimens from 3 Co and 3 Cel were similarly evaluated after 3-hour incubation of biopsies with 100 μg/ml of crude gliadin. Results: Meanwhile CD30 is not expressed on resting PBL, a peak is shown after 3-day incubation with anti-CD3 (Cel vs Co, p=ns). Compared with Co samples, CD30 expression persists increased by day 5 in Cel (1.2±1.0 vs.13.3±3.0, $p<0.05$). CD30 frequency is increased on CD3+CD45RO+ and CD3+CD25+ subsets (10.5±2.0% vs. 0.3±0.1%, p=0.0238 and 11.1±1.9% vs. 0.6±0.2%, p=0.0025, Cel vs Co, respectively). While a similar low frequency of CD30+ cells was found at baseline in isolated IEL from Cel and Co (p=ns), the expression was increased in Cel LPL (vs. Co: 12.4±5.1% and 7.9±3.3%, p=0.0367). Incubation with gliadin up-regulates CD30 expression in a subpopulation of LPL from both, Co and Cel. Conclusions: Cel patients show a persistent expression of CD30 on a subset of memory, activated cells that might be capable of signal transduction and differential immunoregulatory activity in peripheral compartment. Similarly, the higher frequency of CD3+CD30+ LPLs observed points to the presence of a differential activated subset of duodenal T cells probably involved in CD pathogenesis within the intestinal mucosa. After challenging of biopsies with gliadin, CD30 triggering might provide costimulatory signals for activation/proliferation of LPLs in both patients and controls. However, a functionally differential response remains to be demonstrated.

T1274

Dominant Mucosa-Associated Microbiota in Celiac Children At Diagnosis and After GFD

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Background Celiac disease (CD) is an immune-mediated enteropathy, characterized by small bowel chronic inflammation. Its pathogenesis is multifactorial: exposure to toxic prolamins and appropriate HLA-DQ haplotype are necessary but not sufficient for contracting CD. Althoug modification of gut microbiota seems to be involved in the pathogenesis of other chronic inflammatory bowel diseases (IBD), its possible role in CD has never been investigated. Aims To identify by a molecular approach the microbiota colonizing the upper small bowel of children with CD at diagnosis and after 8 mounths of gluten free diet (GFD). Methods Mucosal-associated bacteria from duodenal biopsies of 10 celiac children aged 5-15 years ,(at diagnosis and after 8 mounths of GFD), and of 8 healthy controls were investigated. Total DNA was extracted, and 16S ribosomal DNA was amplified by PCR. Amplification products were separated by temporal temperature gradient gel electrophoresis (TTGE) a powerful technique for comparing the biodiversity of the dominant microbiota in different biological samples. The profiles obtained were analyzed using GelQuest software(Sequentix) providing a dendogram based on the agglomeration method of UPGMA. Result The dendogram obtained reveals two clusters sharing high degrees of similarity. The first cluster includes all active disease patients, the second one those in GFD. TTGE profiles of controls clustered with celiac children in GFD. Interestingly dominant microbiota in active disease differed very much from remission and controls. Conclusion This is the first pediatric report investigating the duodenal mucosa-associated microbiota in celiac children at diagnosis and after GFD. The main result of the present study, although limited by the sample size, hjghlighted TTGE profiles clusters with clinical status of CD. These preliminary data showed that dominant duodenal microbiota in active disease seems to differ very much from that in remission. These findings suggest a pathogenetic role in CD. Further studies will sequence different DNA fragments, specific for each profile, to identify dominant microbial species characterizing intestinal microbiota in active and in remission disease.