P1699 ASSOCIATION OF CX3CR1 EXPRESSION IN THE PERIPHERAL BLOOD WITH MUCOSAL INFLAMMATION IN IBD: A NATURAL KILLER STORY?

Uniken Venema W.T.C.[1], Visser F.[1], Hagting M.[1], Voskuil M.[2], Jansen B.H.[1], Weersma R.K.[2], Festen E.A.M.(.[3]
[1]University Medical Center Groningen, Gastroenterology, Groningen, Netherlands, [2]University Medical Center Groningen, Gastroenterology and Hepatology, Groningen, Netherlands, [3]University Medical Center Groningen, Groningen, Netherlands

Contact E-Mail Address: w.t.c.uniken.venema@umcg.nl
Introduction: CX3CR1 is a chemokine receptor expressed on various (immune) cell types, and involved in adhesion on and migration of leukocytes through epithelial and endothelial cells (1,2). The proportion of CX3CR1+ cells within the blood CD4 effector T cell population, has been found to be correlated with clinical disease activity in IBD (3). Moreover, blocking CX3CR1 has been shown to reduce colitis in mice. This renders CX3CR1 a promising drug target for IBD and a potential biomarker for disease activity. We assessed expression of CX3CR1 on a variety of cell types in the blood of IBD patients and healthy controls to find potential target cells for CX3CR1-blockade therapy in humans, and questioned whether expression of CX3CR1 is associated with histologically proven disease activity in the gastrointestinal tract in patients with IBD.
Aims & Methods: Peripheral blood from patients with Crohn's disease (CD, n=11), ulcerative colitis (UC, n=11) and healthy controls (HC, n=11) was collected prior to endoscopy. Whole blood was isolated using washing and lysis of red blood cells, stained with a panel containing various antibodies (CD3, CD8, CD19, CD16, CD14, CD15, CX3CR1, CD56) and subsequently analyzed using an LSR II FACS analyzer. Disease activity was assessed through evaluation of biopsies of macroscopically inflamed areas by a pathologist. Using flow cytometry, the proportion of CX3CR1-positive cells as well as the level of CX3CR1 expression in a variety of cell types was evaluated. Differences between CD, UC and HC, as well as between patients with and patients without disease activity in the gastrointestinal tract were assessed.
Results: We noted CX3CR1 expression on blood (NK) T cells, NK cells, monocytes and granulocytes, but not on B cells. Neutrophils constitute the largest proportion of CX3CR1+ cells in the blood (median 3.7% [IQR 3.3]), whereas within the subpopulations, non-classical monocytes (median 98% [IQR 2.6]) and NK cells (median 90.3% [IQR 8.7]) had the highest proportion of CX3CR1 positive cells. In blood derived from patients with histologically active UC, intermediate monocytes showed a significantly higher proportion of CX3CR1+ cells (p=0.036), whereas CD4+ NK T cells showed upregulation of CX3CR1 on the cell surface (p=0.013), compared to patients with UC in remission. In CD, the proportion of CX3CR1+ CD8+ NK T cells was higher in patients with inflammation versus patients without inflammation (p=0.05).
Conclusion: CX3CR1 is expressed on a wide variety of peripheral blood cells. Although the proportion of CX3CR1+ cells within the CD4 T cell population has previously been reported to be associated with *clinical disease activity* in IBD, we do not find an association between the proportion of CX3CR1+ CD4 T cells and *histologically proven disease activity* in IBD. We do find an association between inflammation and CX3CR1+ CD8+ NK T cells in CD and CX3CR1+ intermediate monocytes in UC, which highlights the role of these cells in IBD. These results provide a baseline for follow-up studies on the role of CX3CR1 in IBD.
References: 1. Imai T, Hieshima K, Haskell C, et al. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. Cell 1997;91:521-30. 2. Nishimura M, Umehara H, Nakayama T, et al. Dual functions of fractalkine/CX3C ligand 1 in trafficking of perforin+/granzyme B+ cytotoxic effector lymphocytes that are defined by CX3CR1 expression. J Immunol 2002;168:6173-80. 3. Kobayashi T, Okamoto S, Iwakami Y, et al. Exclusive increase of CX3CR1+CD28-CD4+ T cells in inflammatory bowel disease and their recruitment as intraepithelial lymphocytes. Inflamm Bowel Dis 2007;13:837-46.
Disclosure: Nothing to disclose

P1700 NO DIFFERENCE IN THE DISTRIBUTION OF PATHOGENIC FACTORS FOUND IN *ESCHERICHIA COLI* ISOLATES FROM PATIENTS WITH CROHN'S DISEASE AND HEALTHY INDIVIDUALS

Siniagina M., Markelova M., Laikov A., Boulygina E., Khusnutdinova D., Kharchenko A., Fedorova M., Romanova V., Grigoryeva T.
Kazan Federal University, Kazan, Russian Federation

Contact E-Mail Address: marias25@mail.ru
Introduction: Dysbiosis of the gut microbiome in Crohn's disease (CD) patients is associated with reduced bacterial diversity and higher relative abundance of Proteobacteria phylum, in particular *Escherichia coli*. In recent decades, *E. coli* is thought to be implicated in CD pathogenesis and considered as one of the possible causes of the disease occurrence and progression.
Aims & Methods: The aim of the study was to characterize *E. coli* strains obtained from diagnosed CD patients and individuals (controls), and to evaluate their contribution to the pathogenesis of CD.
E. coli strains were isolated from stool samples of 14 patients and 18 controls. Fecal suspensions were inoculated on selective Endo medium and colonies were identified by MALDI Biotyper and serological test. Based on quadruplex PCR identification according to Clermont *et al.* (2013), the colonies were assigned to phylogenetic groups. Ninety seven selected isolates were sequenced on MiSeq platform. Reads were assembled using SPAdes v.3.11.1 followed by assemblies' annotation using Prokka v.1.9. Serotypes were assigned using SerotypeFinder-2.0 tool. Using MLT ENTEROtest 24N kit, the strains were tested for the production of H_2S and the ability to utilize various sugars, polyhydric alcohols, glycosides, and amino acids.
Results: Out of 97 sequenced isolates, 33 duplicates were revealed using the comparative genome analysis, i.e. isolates sequenced more than once due to varying colony phenotypes. Sixty four strains had unique genomes - 27 isolates from CD patients, and 37 isolates from the control group.
The phylogenetic group distribution did not differ significantly between CD patients and controls. However, *E. coli* strains belonged to phylogroups E/clade 1 and F were identified only in healthy donors. Clinical significance of strains belonging to these groups is not well understood.
The pathogenicity and virulence of *E. coli* strains were explored by searching for 61 previously reported genes of adhesion and invasion system in their genomes. We found no statistically significant difference in these genes distribution when comparing isolates from both CD and control groups. So, there was no evidence for the association between CD status and the presence of pathogenic or virulent genes in *E. coli* strains. According to the classification of *E. coli* serotypes, among isolated strains 3 enteroaggregative types (EAEC: O17/O44:H18, O15:H18, O126:H27), 3 enteropathogenic types (EPEC: O128ac:H12, O88:H25, O154:H9), and 1 enteroinvasive type (O112ac:H16) were found. The distribution of these types did not differ significantly between CD and control groups, either, though strains of these serological subgroups prevailed in fecal samples from individuals with CD and constituted a smaller proportion in healthy ones. Prevalence of these strains in CD patients may be associated with the development of the disease.
Strains from studied cohorts showed no significant difference in ability to catalyze sugars, polyhydric alcohols, glycosides and amino acids. It is of interest to note, 3 different strains from one CD patient showed ability to produce H_2S. It is supposed that hydrogen sulfide involves in IBD development by disrupting the integrity of the intestinal mucosa.
Conclusion: The whole genome analysis of *E. coli* strains in CD patients and healthy controls did not reveal any connection between *E. coli* virulence and pathogenicity and disease status. It may be assumed that the development of the disease is associated with altered interaction between the bacteria with certain serotypes and human immune system and their balance in community.
References: Clermont, O., Christenson, J. K., Denamur, E., Gordon, D. M. (2013). The Clermont Escherichia coli phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environmental microbiology reports, 5(1), 58-65.
Disclosure: This work was funded by Russian Foundation for Basic Research (RFBR) according to the research project №17-00-00433