Patients with Inflammatory Bowel Disease show IgG immune responses towards disease-associated small intestinal bacteria

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Published in:
Journal of Crohn's and Colitis

DOI:
10.1093/ecco-jcc/jjab232.205

Publication date:
2022

bleeding in UC. Fibrin layers contained ample amounts of neutrophils co-aggregated with neutrophil extracellular traps (NETs) with detectable activity of peptidyl-arginine deiminases (PAD). Transcriptome analyses showed significantly elevated *PAD4* expression in active UC. In experimentally inflicted wounds, we found that neutrophils underwent NET formation in a PAD4-dependent manner hours after formation of primary blood clots, and remodelled clots to immunothrombi containing citrullinated histones, even in the absence of microbiota. PAD4-deficient mice experienced an exacerbated course of DSS-induced colitis with markedly increased rectal bleeding (96 % vs 10 %) as compared to controls. PAD4-deficient mice failed to remodel blood clots on mucosal wounds eliciting impaired healing. Thus, NET-associated immunothrombi are protective in acute colitis, while insufficient immunothrombosis is associated with rectal bleeding.

Conclusion: Our findings uncover that neutrophils induce secondary immunothrombosis by PAD4-dependent mechanisms. Insufficient immunothrombosis may favor rectal bleeding in UC.

P076

Patients with Inflammatory Bowel Disease show IgG immune responses towards disease-associated small intestinal bacteria

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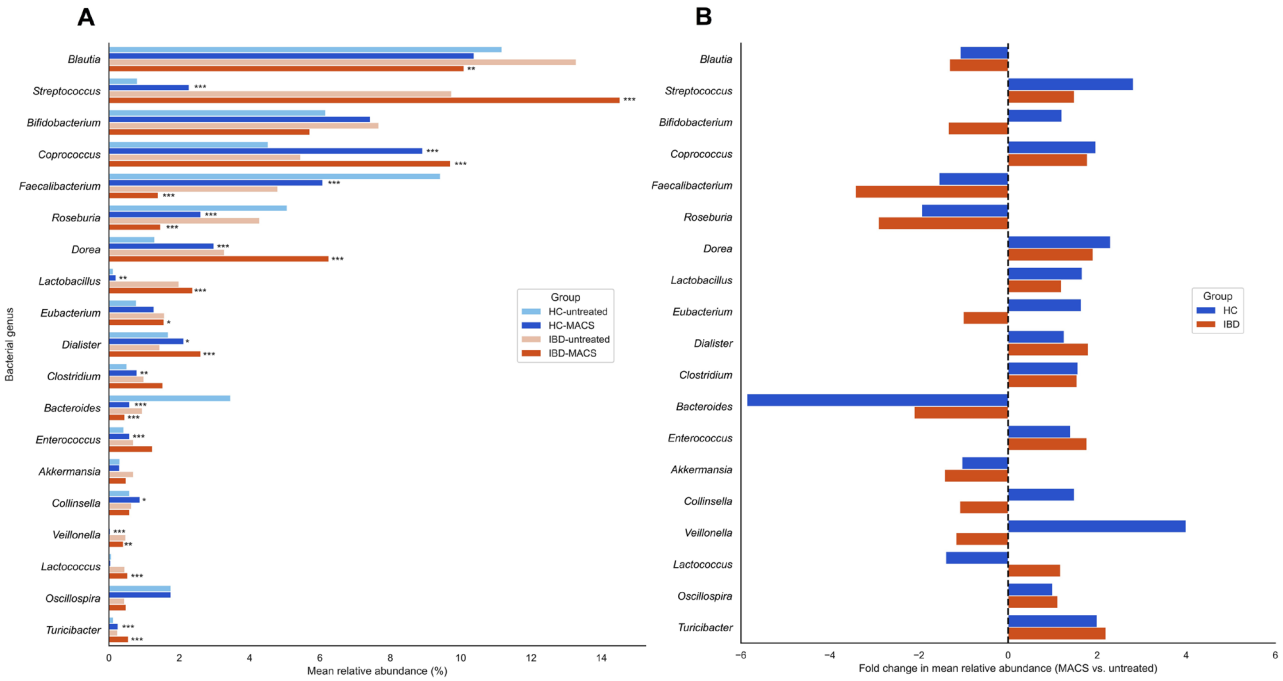
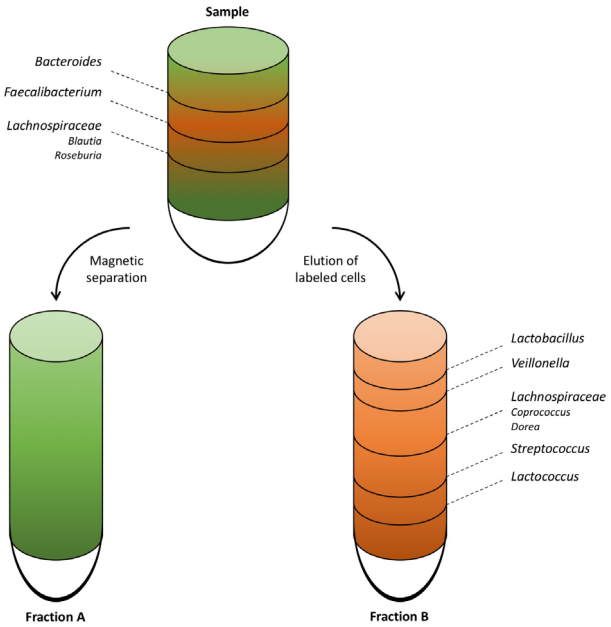
Background: Inflammatory bowel disease (IBD) is characterized by a disturbed gut microbiota composition. Patients with IBD have elevated levels of mucosal and serum levels of IgG-antibodies directed against bacterial antigens, including flagellins. In this study, we aimed to determine to which faecal bacteria the humoral immune response is directed to in patients with IBD.

Methods: Faecal and serum samples were collected from patients with IBD (n=55) and age- and sex-matched healthy controls (n=55). Faecal samples were incubated with autologous serum and IgG-coated fractions were isolated by magnetic-activated cell sorting (MACS) and the coating efficiency was assessed by flow cytometry. Bacterial composition of both untreated and IgG-sorted fecal samples was determined by 16S rRNA-gene Illumina sequencing.

Results: Serum IgG responses were primarily directed to typical small intestinal bacterial genera, including *Streptococcus*, *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Veillonella* and *Enterobacteriaceae*, as well as against specific *Lachnospiraceae* bacteria, including *Coprococcus* and *Dorea* (all P<0.001) (Figures), and to the species *Ruminococcus gnavus* (P<0.05). In contrast, serological IgG responses against typical commensal, anaerobic and colonic microbial species were rather low, e.g. to the *Lachnospiraceae* members *Roseburia* and *Blautia*, to *Faecalibacterium* as well as to *Bacteroides*. IgG-sorted faecal samples were characterized by significantly lower microbial diversity. Patients with IBD showed more IgG-coating of *Streptococcus*, *Lactobacillus* and *Lactococcus* bacteria compared with healthy controls (all P<0.05). No differences in IgG-coated bacterial fractions were observed between CD and UC, between active or non-active disease, nor between different disease locations in CD.





Conclusion: The IgG immune response is specifically targeted at typical small intestinal bacterial genera, whereas responses against commensal, rather colonic-type microbiota are lower in patients with IBD. These findings may be indicative of a strong immunological exposure to small intestinal bacteria in concordance with relative immune tolerance against commensal bacteria.

P077

Different granulocyte subsets are involved in the pathogenesis in Crohn's disease fistula

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Background: Early in inflammation granulocytes migrate from the circulation into inflamed areas and rapidly accumulate. They form an important role in first defense as well as the orchestration of downstream immune responses. In the circulation, several different subsets of granulocytes have been described, for example immature, intermediate and mature granulocytes. Surprisingly, despite their abundance and prominent function, this cell type is rarely studied from a tissue perspective, most likely due to their short lifespan ex vivo.
One example of gastrointestinal disease with an extremely high number of granulocytes is the occurrence of perianal fistula which contain a considerable amount of granulation tissue. As curettage of this tissue is part of the treatment, the fistula tract is good source to study tissue derived granulocyte subsets. The aim of this study was to characterize granulocyte subsets in the fistula tract.
Methods: Curettage material of perianal fistula tracts was obtained during surgical intervention from patients with CD (n=15) and cryptoglandular fistulas (n=5). From 2 CD patients venous blood was taken and a ficoll step was performed. Single-cell suspensions were stained with a 35-antibody panel, including CD66, CD14, CD33, CD16 and CD11b. Samples were analyzed using mass cytometry (CyTOF).
Results: Granulocytes, characterized as CD45+CD66a+ cells, were the main cellular component of the fistula tract (64%±24) compared to other immune cells. This was more pronounced in cryptoglandular fistula compared to CD fistula (81% vs. 67%; p=0.019). Within these cells we could differentiate between 4 subsets, mature granulocytes ($CD33^{+}CD11b^{+}CD16^{+}$), intermediate ($CD33^{int}CD11b^{+}CD16^{int}$), immature granulocytes ($CD33^{int}CD11b^{-}CD16^{int}$) and eosinophils ($CD33^{-}CD11b^{-}CD16^{-}$). Immature granulocytes were the most abundant in de fistula tract (31%±15), closely followed by the mature (30%±23) and intermediate (23±9). No significant difference was seen in the percentages of granulocyte subsets between CD and cryptoglandular fistula.
Conclusion: Here, we show the presence of different subtypes of granulocytes in fistula derived granulation tissue. We show a heterogeneous group of cells ranging from immature neutrophils to mature neutrophils. Due to their high number in active ulcerating tissue, further evaluation of granulocyte subsets and function could be an interesting target for medical treatment options.

P078

Vedolizumab Treatment of ex-vivo Human Ulcerative Colitis (UC) Explants Results in Altered Inflammatory Protein Secretion Profiles

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Background: Patient-derived inflammatory bowel disease (IBD) explants have potential for biomarker and therapy discovery. Vedolizumab (VDZ) is a monoclonal antibody targeting a4b47 integrin whose mechanism of action is the reduction of inflammatory immune cell trafficking to the intestinal tract. The association between VDZ exposure and treatment response is unclear and appears insufficiently explained by serum levels. For this reason it is hypothesised that VDZ may also have effects at the tissue level.
Methods: We aimed to evaluate the effect of VDZ on inflammatory protein secretion profiles in *ex-vivo* human ulcerative colitis ex-plants (UC ex-plants).
Patients with UC, undergoing endoscopy, were prospectively recruited. Endoscopic biopsies were collected and UC ex-plants generated as per previously described methods. UC ex-plants were then co-cultured for 24 hours with an IgG control vehicle or VDZ. After 24 hours tissue conditioned media (TCM) from UC ex-plants was collected. TCM secreted protein profiles were quantified using 54 V-plex ELISA (Meso Scale Diagnostics, USA). Secreted cytokine profile were compared between IgG vehicle (control) and VDZ treated ex-plants. P values < 0.05 were considered significant in analyses.
Results: Thirteen patients with UC were included; age (mean, [range]) 45.8 years [30–78], 54% male; disease duration (mean, [range]) 8 [1–24] years; 62% of patients were anti-TNF naïve. Baseline total Mayo score (median [range]) was 6 [0–9]; endoscopic Mayo score (median [range]) was 2 [0–3]. Comparing VDZ with control treatment, 5 of 54 ex-plant secreted proteins differed significantly. GMCSF, IL-16, IL-22, IL-23 and sVCAM-1 secretion were significantly decreased comparing VDZ and control treatments, p < 0.03 for all comparisons.
Conclusion: The predominant mechanism of action of VDZ is the blocking of gut homing pro-inflammatory immune cell trafficking from the peripheral circulation to the intestinal tract. These data demonstrated that VDZ reduces pro-inflammatory protein secretions from UC ex-plants suggesting an additional local effect of this therapy. Further evaluation is required to determine whether a component of VDZ therapeutic effect occurs at the tissue level.