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Background: One of the most important consequences of chronically active inflammatory bowel disease (IBD) is the development of colorectal cancer (CRC) in a form called colitis-associated CRC (CAC) that significantly shows more aggressive features compared with sporadic CRC. It has been demonstrated that innate immune response plays a key role in inflammation-associated tumourigenesis, but little is known about the role of mucosal-associated invariant T (MAIT), innate lymphoid cells (ILC), and gamma-delta T-cells in sporadic and IBD-associated CRC. The aim of our study is to understand the contribution of several different components of innate immunity to inflammation-associated CRC.

Methods: We analysed 40 samples: IBD at onset (n = 10), IBD after therapy (n = 7), CRC (n = 16), CAC (n = 2), and healthy donors (n = 5). To identify specific lymphocyte subsets, immune cells were isolated from fresh intestinal biopsies, digested, and stained with fluorochrome conjugated monoclonal antibodies against intracellular and surface markers.

Results: The frequency of type-1 innate lymphoid cells (ILC1) was higher in IBD (mean 20%), compared with CAC and sporadic CRC, and these infiltrating ILC1 produced high amounts of TNF-alpha but not IFN-gamma. IBD patients at onset also had higher frequencies of infiltrating Vdelta1 T-cells compared with CRC, whereas Vdelta 2 T-cells had an opposite pattern. Vdelta 1 T-cells produced IL-17 in sporadic CRC and IFN-gamma in IBD, whereas Vdelta 2 T-cells produced TNF-alpha and IFN-gamma in IBD and CRC, but IL-17 only in treated IBD patients. Interestingly, MAIT cells were present in CRC compared with IBD, whereas a very poor percentage was observed in healthy colon from the same patients.

Conclusions: Our results, albeit preliminary, suggest an appreciable and unexpected role for distinct subsets of innate lymphoid cells and MAIT cells in CAC, and provide a tool to evaluate the contribution of these subsets to the development of chronic inflammation-associated cancer.

P076
The role for T-cells in the pathogenesis of Crohn's disease–associated fistulae

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Background: Fistulae represent a frequent complication in Crohn's disease (CD), and surgical resection is often required. We have previously demonstrated that epithelial-to-mesenchymal transition (EMT) plays a critical role for fistula development. The T-cell cytokines tumour necrosis factor (TNF), interleukin (IL)-13, interferon gamma (IFN γ), IL-17A, and IL-22 are highly expressed in transitional cells along fistula tracts in CD patients. Similar to transforming growth factor beta (TGF β), TNF is able to induce EMT and the expression of molecules being associated with cell invasiveness and migration. IL-13 has no effect on epithelial cell morphology, but it induces expression of genes being associated with invasive cell growth, such as SLUG transcription factor. Here, we analysed the implication of the T-cell-derived cytokines IFN γ, IL-17A, and IL-22 in the event of EMT. Moreover, we investigated if there are differences in the composition of lymphocytes in the blood of CD patients suffering from fistulae, compared with patients without fistulae or healthy controls.

Methods: Three-dimensional intestinal epithelial cell (IEC) constructs (spheroids) were stimulated with IFN γ, IL-17A, and IL-22 to investigate the effects on EMT development. Further, CD4+ and CD25+ T-cells were isolated from fistulising CD patients' blood or control blood samples and co-cultured together with HT29 cells. Afterwards, mRNA expression levels of EMT-associated genes were analysed.

Results: Treatment of the spheroids with IFN γ resulted in a loss of the well-defined globular shape after day 7. We observed a clear separation of IECs, whereas mRNA levels for EMT-related transcription factors like SNAIL-1 were not up regulated. IL-17A and IL-22 had no effect on cell morphology, suggesting that they do not induce EMT in our cell model. On a molecular level, both cytokines had no effect on the mRNA expression of EMT-associated genes, but prevented the TGF β -induced up regulation of, for example, ETS-1. The sorting of T-cells isolated from blood of CD patients with fistulae revealed an elevated expression level of CD4+ and CD25+ T-cells compared with healthy controls.

Conclusions: Our data demonstrate that T-cells may play a crucial role in the pathogenesis of CD-associated fistulae. Th1 cell–derived IFN γ may be involved in the event of EMT in IECs; however, Th17 cell–derived cytokines IL-17A and IL-22 are likely not implicated in EMT onset, and may prevent EMT-associated effects of TGF β. This observation supports the hypothesis that Th17 cell–derived cytokines exert a pivotal role for maintaining intestinal homeostasis. The different lymphocyte composition in CD patients' blood represents a further hint for the importance of these cells in fistulae formation.

P077
The efficacy of tyrosine kinase inhibitor dasatinib on colonic mucosal damage in murine model of colitis

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Background: Ulcerative colitis is an inflammatory condition limited to the colon in the gastrointestinal system. Currently the most potent medications used for medical treatment of ulcerative colitis produce no response in 20%–30% of cases, in spite of a high side-effect profile. There is a need for more efficient and reliable medications. Tyrosine kinase inhibitors have shown efficacy in some inflammatory diseases and in experimental colitis models. Even though dasatinib, a tyrosine kinase inhibitor, suppresses proinflammatory cytokines in colonic tissue, there are a few case reports of haemorrhagic colitis with use of the medication. There is no study investigating the effect of dasatinib on experimental colitis. We aimed to investigate the effect of dasatinib in a colitis model induced with acetic acid in our study.

Methods: In the study, 24 male Sprague-Dawley rats distributed into 4 groups of 6 rats each as control, dasatinib, colitis, and dasatinib+colitis groups randomly. For colitis induction, 4% acetic acid was used. Sacrificing of the rats was performed on the seventh day. Disease activity; morphologic and histological injury; superoxide dismutase, myeloperoxidase, and malondialdehyde activity; and TNFα and CD3 expression were assessed in colonic tissue.

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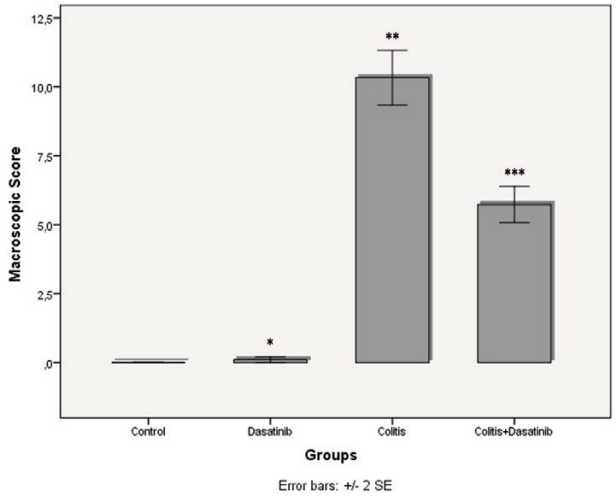

Figure 1. Macroscopic damage scoring.

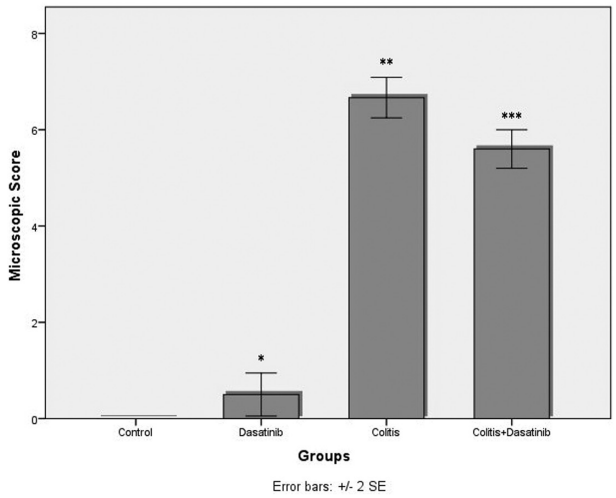

Figure 2. Microscopic damage scoring.

Results: Apart from malondialdehyde, significant difference in all parameters between the control and colitis groups was determined. Difference between the colitis and colitis+dasatinib groups was not significant only in weight loss and biochemical parameters. Though dasatinib does not fully resolve clinical and inflammatory changes related to colitis, there was significant regression.

Conclusions: A tyrosine kinase inhibitor, dasatinib, decreased the inflammation in a rodent model of colitis. It may provide this effect by the suppression of TNFα. Dasatinib may be one of the treatment options for ulcerative colitis; however, this finding must be supported with advanced clinical studies.

P078
Walnut extract ameliorates acute and chronic colitis in murine models of colitis

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Background: Walnut (Juglans regia) is known to have anti-cancer and immunomodulatory effects. However, little information is available on the effect of walnut extract on acute and chronic murine colitis.

Methods: In the acute colitis model, mice were given 4% dextran sulfate sodium (DSS) for 5 days. Walnut extract (ie, 5 mg/kg/day and 20 mg/kg/day) was dissolved in PBS and administered once daily by oral gavage, beginning 2 days before DSS administration. To evaluate the disease activity index (DAI), body weight, stool consistency, and the presence of bloody stool or blood around the anus were assessed daily by a researcher. Postmoterm, a quantitative evaluation for histology was performed. Immunohistochemical stainings for phospho-IκB kinase (IKK) and occludin were performed in mouse colon tissue. In the chronic colitis, interleukin (IL)-10-/- mice were administered with either the vehicle or walnut extract (20 mg/kg) by oral gavage daily for 2 weeks.

Results: Severe colitis was induced by DSS administration for 5 days. In contrast, administration of walnut extract significantly reduced the severity of DSS-induced murine colitis, as assessed by the disease activity index and colon length. In the histopathological analysis, histological grading showed that walnut extract significantly reduced overall colitis score in comparison to the scores of the PBS-treated controls. Immunohistochemical analysis showed that the DSS-induced phospho-IKK activation in intestinal epithelial cells was significantly decreased in walnut extract–treated mice. In addition, immunoreactivity for occludin was significantly inhibited by the treatments of walnut extract. Finally, walnut extract significantly reduced the severity of chronic colitis in IL-10-/- mice.

Conclusions: Walnut extract ameliorates acute and chronic colitis in mice, which suggest that walnut extract may have potential for the treatment of inflammatory bowel disease.

P079
Trehalose ameliorates colitis by increasing mucosal autophagy and M2 polarisation

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Background: Several polymorphisms in gene loci containing autophagy-related proteins such as ATG16L1, IRGM, and NOD2 have been associated with an increased risk of Crohn's disease, suggesting that a defective autophagy may have a role in its pathogenesis. Autophagy has been shown to mediate macrophage polarisation towards the anti-inflammatory phenotype M2a, and we have demonstrated that these macrophages mediate mucosal healing in a murine model of colitis. We analyse the effects of trehalose, an mTOR independent autophagy-inducer, in macrophage polarisation and mucosal regeneration in a murine model of colitis.

Methods: Balbc mice received trehalose (3%) in the drinking water during 3 weeks before an intrarectal injection of TNBS (3,5mg/20g) or its vehicle (40% EtOH) (day 0). Changes in body weight were determined daily (results are expressed as % vs the weight at day 0), and mice were sacrificed 4 days after TNBS administration. Mucosal histology was evaluated according to Wallace score (1–10). Colons were frozen, and the expression of M1 and M2 markers and pro-inflammatory cytokines was analysed by qPCR (results are expressed as fold induction). The mucosal autophagic flux was determined by

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