OP19
Genetic and functional evidence for a role for CYLD in Crohn’s disease: Results from a European consortium

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Background: Data from both humans and experimental animals underscore the critical role of intestinal bacteria in the establishment and maintenance of inflammatory bowel disease (IBD). Host defense to counteract bacterial colonization and maintaining mucosal integrity involves intestinal proteases and protease inhibitors.

Methods: We performed a genetic association study of all top-ranked protease (and inhibitor) genes in a previously published systematic review [1]. 185 haplotype tagging SNPs in 23 genes were genotyped in an exploratory dataset of 650 Crohn’s disease (CD) patients, and 542 healthy controls (HC). Validation was performed in 1670 CD and 1254 HC. Statistical analysis was done using SVS v7.5.2 (crude association analysis, additive genetic model), and plink v1.0.7 (meta-analysis of exploration and validation datasets, interaction analysis). A corrected p < 0.05 was considered statistically significant.

The T84 epithelial cell line was used for functional assessment of CYLD.

Results: 10 markers were found to be significantly associated with CD in the meta-analysis: 4 in USP40, 1 in APEH, 1 in USP3, and 4 in CYLD. The top signals were in CYLD, a cytoplasmic deubiquitinating enzyme located next to NOP2, on 16q12: rs12324931 (p = 1.64e-18), rs17314544 (p = 1.06e-9), rs7205423 (p = 1.89e-09), and rs1861762 (p = 1.07e-09). Upon infection of T84 cells with the adherent-invasive Escherichia coli (AIEC) strain LF82, the proteotypr strain of AIEC associated with ileal CD, decreased CYLD expression was observed, leading to an increased ability of LF82 AIEC to replicate within T84 cells (through CYLD siRNA transfection). Together with the AIEC LF82-induced CYLD decrease, we observed protease-dependent degradation of the NFκB inhibitor, IκB-α, in AIEC LF82 infected T84 cells, and an increased translocation of the NFκB p65 subunit into the nucleus.

Conclusions: Our data provide strong genetic and functional evidence for a role for CYLD in CD pathogenesis. We show that AIEC bacteria are able to take advantage of decreased CYLD to replicate within host epithelial cells, and that CYLD acts as a negative, NFκB-mediated regulator for E. coli colonization.

Reference(s)

OP20
TNF and MDP induce epithelial-to-mesenchymal transition in human intestinal cells: Implications for the pathogenesis of Crohn’s disease-associated fistulae and the use of anti-TNF antibodies

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Background: A common clinical complication of Crohn’s disease (CD) are fistulae. Current treatment options include anti-TNF-antibodies and antibiotics. We have previously shown that epithelial to mesenchymal transition (EMT) plays a crucial role in the pathogenesis of CD fistulae. Here, we investigated a possible role for TNF and the bacterial wall component, muramyl-dipeptide (MDP) in the pathogenesis of CD-associated fistulae.

Methods: We analyzed 7 intestinal fistulae from 7 CD patients by immunohistochemistry (IHC) for Ets-1 and dickkopf-homolog-1 (DKK-1). Primary human intestinal colonic lamina propria fibroblasts (CLPF) were isolated from CD patients with or without fistulising disease. mRNA levels were assessed by real-time PCR in CLPF or HT29 intestinal epithelial cells (IEC). Knock-down of Ets-1 was induced by siRNA.

Results: By IHC, we found strong staining of Ets-1 transcription factor and the Wnt-inhibitor, DKK-1, in TC covering fistula tracts. TNF induced mRNA expression of Ets-1 transcription factor in HT29-IEC (24 h, p < 0.01) and in CLPF from CD patients with fistulising (n = 4, p < 0.001; 24 h). This effect could be fully prevented by administration of an anti-TNF-antibody. TNF also induced mRNA levels of β6-integrin in HT29-IEC (72 h, p < 0.01). This effect was absent in Ets-1-deficient cells (24 h, p < 0.05) and could be effectively blocked by administration of anti-TNF antibodies (p < 0.01). Interestingly, TNF decreased DKK-1 mRNA levels in HT29 IEC (p < 0.05), but increased it in fistula CLPF. These effects were also sensitive to anti-TNF treatment. In HT29-IEC, TNF induced mRNA levels of TNF (p < 0.001) and TGF β (p < 0.001), but not of IL-13, SNAIL1 or SLUG by treatment for 24 h. In CLPF derived from CD patients, TNF-induced expression of β6-integrin (p < 0.05) and secretion of TGF β (p < 0.05) and IL-8 (p < 0.01) could be significantly blocked by anti-TNF treatment. Of note, the bacterial wall component, MDP, induced mRNA levels of TNF (p < 0.001) and DKK-1 (p < 0.05), TGF β (0.001), IL-13 (p < 0.001), SNAIL1 (p < 0.05) and β6-integrin (p < 0.001) in HT29-IEC and fistula CLPF by treatment for 24 h.

Conclusions: TNF and MDP induce the expression of genes associated with EMT and invasive cell growth in IEC and fistula CLPF, whereby TNF-induced effects can be effectively blocked by anti-TNF treatment. These findings indicate that TNF and bacterial components could synergize to induce EMT in IEC and, subsequently, cell invasion of EMT-cells, what finally leads to the development of fistulae during CD course.