Epithelial barrier and dendritic cell function in the intestinal mucosa
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Summary and discussion

Inflammatory bowel disease (IBD), including Crohn’s disease (CD) and ulcerative colitis (UC), are chronic inflammatory diseases of the intestine with an unknown aetiology. Although the pathogenesis of these diseases is not well understood, several components of the bacterial flora, the epithelial barrier, the immune system, the nervous system and mutations in genes that are a part of these components have been shown to play an essential role in the irregular nature of mucosal inflammation. In this thesis we have investigated different parts of the pathogenesis of IBD.

The first part of this thesis describes the role of apoptosis in IBD (Chapter 2) and evaluates a new TNF-α inhibitor in two different colitis models (Chapter 3). Evidence is increasing that a defect in apoptosis is involved in the pathogenesis of IBD. CD seems to be the cause of an intrinsic defect in the apoptotic pathway of (autoreactive) T cells, resulting in excessive T cell responses. In UC, however, an increased rate of apoptosis of epithelial cells is more likely involved in the pathogenesis. Several therapeutic approaches in IBD induce apoptosis of autoreactive T cells, such as sulphasalazine, azathioprine and infliximab. Infliximab is a common used drug in CD, which induces apoptosis of T cells via the transmembrane form of TNF-α. Since infliximab has many side-effects, companies are still developing new strategies to neutralise TNF-α. In the first part of this thesis, we describe the role of apoptosis in IBD (Chapter 2) and subsequently tested a new inhibitor of soluble TNF-α, based on the heavy chains of camel antibodies, the so-called nanobodies, in an acute and a chronic colitis mouse model (Chapter 3). Unfortunately, these nanobodies did not ameliorate colitis, suggesting that the transmembrane, and not the soluble form of TNF-α has to be targeted.

In the second part of this thesis we focused on the role of dendritic cells (DC). DCs are key players in the innate and adaptive immune response and are generally accepted to play a major role in the pathophysiology of CD. DCs located in the lamina propria of the intestine migrate to the mesenteric lymph nodes (MLNs)
where they present antigens to T cells. A number of different markers are described to define DC populations. We have investigated which DC populations are present in the colon and MLNs of CD patients by immunohistochemistry. It seems that there are three different subpopulations of myeloid DCs present in colonic mucosa and MLN of non-CD and CD patients (Chapter 4). These populations consist of the following: (1) immature DCs or macrophages that express DC-SIGN, (2) mature DCs that express S-100 or CD83, and (3) mature DCs that express BDCA3. BDCA1 and CD1a expressing DCs were virtually absent in the colon as well as in MLNs. Immature DCs and macrophages are mainly localised at antigen-capturing sites such as the mucosa and medullary cords, whereas mature DCs are present where antigen is presented, including the T cell areas in colonic lymph follicles and MLNs. All different DC markers give variable staining patterns so there is no marker for the DC. Since DC-SIGN+ DCs were increased in the mucosa of CD patients, we wanted to investigate whether polymorphisms in DC-SIGN are associated with IBD (Chapter 5). Moreover, previous studies showed that mutations in NOD2, which is a pattern recognition receptor similar to DC-SIGN, are associated with CD. Besides, DC-SIGN is located in a susceptibility locus, like three other C-type lectins: MGL, LLT1 and DCIR. Only polymorphisms in LLT1 seem to be associated with CD. This is interesting since LLT1 is a ligand for CD161 and as a complex it inhibits NK cell-mediated cytotoxicity and cytokine production. CD161 is a new surface marker for human IL-17 producing Th17 cells. The Th17 phenotype has recently been linked to CD by the fact that IL-22, IL-17 and IL-23 receptor levels are increased in CD.

The last section of this thesis describes how the enteric nervous system influences barrier function of the intestine (Chapter 6 and 8) and we investigated whether two new α7 nAchR agonists can ameliorate experimental colitis (Chapter 7). Besides the anti-inflammatory capacities of acetylcholine (Ach), evidence is accumulating that Ach also plays an important role in the homeostasis of the epithelial barrier integrity. Ach can signal via nicotinic or muscarinic acetylcholine receptors (nAchRs or mAchRs). We tested two selective α7 nAchR agonists
(ARR17779, (−)-spiro[1-azabicyclo[2.2.2] octane-3,5′-oxazolidin-2′-one and GSK1345038A) on disease severity in two mouse models of experimental colitis. Both α7 nAChR agonists worsened colitis or were ineffective in these colitis models, questioning the further development of α7 nAChR agonists as treatment for colitis.

In the last study we investigated the effect of Ach, nicotine and muscarine on the epithelial barrier function (Chapter 8). We showed that Ach and muscarine, but not nicotine, inhibit IL-8 production of Caco-2 cells induced by TNF-α and IL-1β, indicating that Ach mainly acts via the mAChRs. Interestingly, low concentrations of nicotine also inhibited IL-8 production induced by low concentrations of IL-1β. Nevertheless, only Ach and muscarine and not nicotine reduce IL-1β induced NF-κB activity of Caco-2 cells. Furthermore, we demonstrated that Ach reduces 4kDa dextran flux though Caco-2 monolayers and restores ZO-1 expression of Caco-2 cells after cytokine exposure. However, in healthy mice, Ach increases bacterial translocation. These data indicate that Ach protects epithelial cells from the detrimental effects of IL-1β and TNF-α on the integrity of the intestinal epithelial barrier via activation of mAChRs, but that under healthy conditions Ach increases epithelial permeability.

Since we showed in two studies that promising new therapies, a TNF-α inhibitor and specific α7 nAChR agonists, did not work in different mice models, it is important that future research will focus more at IBD pathology at molecular level. This means that we have to elucidate more about the role of soluble and transmembrane TNF-α in inflammation, because the different forms of TNF-α seem to have another function. In addition, it seems crucial to unravel the mechanisms controlling the production of TNF-α, in other words, what are the triggers for a cell to produce transmembrane TNF-α and which signals are responsible for the cleavage of transmembrane TNF-α into its soluble form, and perhaps most importantly, do these signals differ between IBD patients and healthy subjects. This knowledge will be of crucial importance to develop strategies to interfere with these
mechanisms and/or to develop new anti-TNF therapies that are more efficient and devoid side-effects.

Although our \( \alpha7 \) nAchR agonists did not ameliorate colitis in mice, these findings do not exclude that activation of other nAchRs may have therapeutic effects as the anti-inflammatory effect of vagal nerve stimulation may result from interaction with other nAchRs. Besides, not only nAchRs could be a potential target in the treatment of IBD and other intestinal diseases, but also mAchRs since activation of mAchRs decreases intestinal permeability and inhibits the NF-\( \kappa \)B pathway under inflammatory conditions. We do not know, however, which mAchR is involved in this processes, although the M3 muscarinic receptor may be a good candidate. Therefore, future research should focus on the type of mAchRs expressed by intestinal epithelial cells \textit{in situ} and \textit{in vitro} and whether activation of this receptor can ameliorate colitis in mice models.

Besides more research at molecular level, it is also necessary to do more research at cellular level. We demonstrated that there are several DC populations present in the mucosa of the colon and MLNs, but the exact role of these DC populations in the pathogenesis of IBD is not known yet. Especially, DC-SIGN\(^+\) and s-100\(^+\) DCs are interesting as they are increased in IBD patients. The challenge thus is to isolate these DC populations from the intestine and MLN for further in detail study. Probably, it is not possible to culture those cell populations, but RNA and protein isolation may give us some more information about the pathways, i.e. NOD2 and NALP3 signalling, that could be involved in the pathogenesis of IBD. Finally, more genetic research is necessary to make a better patient profile. Not all IBD patients have the same polymorphisms. Although the association of polymorphisms in LTT1 with CD is not very strong, it is important to investigate whether the combination of polymorphisms in LTT1 and other genes increases the risk to develop CD. Future studies will have to evaluate which combination indeed leads to an increased disease risk and should try to identify clusters of patients with different combinations of polymorphisms in for example NOD2, NALP3 and TLR4 or in
LLT1 and IL-23R. The final goal is to predict which treatment will be most effective in these patients and whether family members have an increased risk to develop IBD so that the disease could be prevented or the onset of the disease could be delayed.

In conclusion, our data demonstrate that many factors are involved in the pathogenesis of IBD and that all these components could be targets in the treatment of these diseases, although much more research is required to understand how all these processes interact.