Inducible nitric oxide synthase in intestinal inflammation
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Chapter 9

Summary
SUMMARY

Intestinal inflammation is almost invariably accompanied by intestinal dysfunction which constitutes a major clinical complication. Current therapy is often inadequate due to incomplete knowledge about the mechanisms causing intestinal dysfunction in intestinal inflammation. Nitric oxide (NO) may be a key component in this process. This thesis is focused on the expression, regulation and function of the enzyme inducible nitric oxide synthase (iNOS) in intestinal inflammation. In chapter 1 the function of the three NOS isozymes nNOS, eNOS and iNOS in the gastrointestinal tract are discussed. Small (nanomolar) amounts of Nitric Oxide (NO), produced by calcium-dependent nNOS, have a physiological role in peristalsis and sphincter function of the intestine. Decreased nNOS function can result in a-peristalsis and obstructive sphincters. NO produced by eNOS dilates mucosal blood vessels and prevents leucocyte aggregation and is thereby essential for the maintenance of mucosal blood flow. Absence of eNOS derived NO results in an increased susceptibility of the gastrointestinal tract to injury. Selective NO delivery by gene therapy or by NO donating compounds might offer new therapeutic options in motility disorders of the gut and the prevention of mucosal injury. The effects of large (micromolar) amounts of NO as produced by iNOS are less well understood. Large amounts of NO can increase gut permeability, induce apoptosis and stimulate intestinal secretion while NO can also kill bacteria, block apoptosis and reduce inflammation by inhibiting NF-κB activation. Therefore, inhibition of nitric oxide generation could have both beneficial and deleterious effects. In chapter 2 we identified intestinal epithelial cells as the main cellular source of inducible nitric oxide synthase (iNOS) in mucosal biopsies from patients with active inflammatory bowel disease (IBD). Since NO can react with superoxide anions (O2·−) yielding the toxic oxidizing agent peroxynitrite (ONOO−) we also studied peroxynitrite induced nitration of tyrosine residues (nitrotyrosine) in proteins. Superoxide anion producing cells were present in increased numbers in the lamina propria of IBD patients. Nitrotyrosine formation was not observed in epithelial cells but only in superoxide-anion producing inflammatory neutrophils and mononuclear cells. These findings indicate that tissue damage in IBD is probably more related to reactive oxygen species (ROS) producing inflammatory cells than to NO producing epithelial cells. In this study a low level of iNOS expression was also observed in tissue macrophages. Since tissue macrophages are derived from circulating monocytes, we studied iNOS expression in circulating monocytes and related this expression to disease activity and to markers of monocyte activation. This study is described in chapter 3. The expression of iNOS in circulating monocytes was increased in patients with active IBD compared to healthy controls. The patients who went into remission all had marked reduction of iNOS expression in circulating monocytes. Surface markers for monocyte activation CD63 and CD11b, were not elevated and HLA-DR expression was decreased in circulating monocytes from patients with active ulcerative colitis. This may suggest a suppressive function of NO
on systemic monocyte activation. In chapter 4 we used a model of endotoxemia and an intestinal epithelial cell line (DLD-1) to study the epithelial iNOS and heme-oxygenase-1 (HO-1) expression in response to inflammation and oxidative stress. Endotoxin, or lipopolysaccharide (LPS) injected in rats, induced iNOS, but not HO-1 in intestinal epithelial cells of the ileum and colon. The thiol-modifying agent diethylmaleate (DEM) strongly induced HO-1 both in epithelial cells and lamina propria inflammatory cells. Combined treatment with LPS and DEM decreased iNOS expression, but strongly induced HO-1 expression. Similarly, cytokine mix (CM) induced iNOS expression, but not HO-1 expression in cultured intestinal epithelial DLD-1 cells. DEM and the lipid peroxidation end product 4-HNE prevented iNOS induction in a NF-κB dependent manner, but increased the HO-1 expression in CM-exposed DLD-1 cells. Thus, we demonstrate an opposite regulation of iNOS and HO-1 in intestinal epithelial cells in response to cytokine exposure and DEM or 4-HNE-induced oxidative stress. These findings suggest that iNOS (NF-κB driven) and HO-1 (AP-1 driven) represent complementary survival mechanisms in intestinal epithelial cells. In chapter 5, we studied the kinetics of iNOS expression in relation to T cell infiltration and cytokine production in conventional-reared (CNV) and with one bacteria-associated severe combined immunodeficient (SCID) mice in the CDB45high CD4+ T cell transfer model of colitis. In CNV-SCID mice injected with CD45RBhigh CD4+ T-cells an early and focal, epithelial iNOS expression on the apical site of crypts was observed that preceded the infiltration of CD4+ cells and cytokine production. This epithelial iNOS expression extended to the entire epithelial surface along the entire crypt axis in the course of colitis. SCID mice mono-associated with the bacteria H. muridarum developed an accelerated colitis and showed high epithelial iNOS expression. CNV-SCID mice without T-cells and SCID mice mono-associated with Segment Filamentous Bacteria did not show any iNOS expression, SCID mice mono-associated with act A(-) mutant L. monocytogenes and O. anthropi showed some scattered epithelial iNOS staining on the apical site of a few crypts, but none of these mice developed colitis. These findings suggest that T-cells and bacteria-dependent epithelial iNOS expression is an early event in the CDB45high CD4+ T-cell transfer model of colitis. In order to determine whether epithelial iNOS induction is detrimental or beneficial and to establish the role of the two constitutive isoforms of NOS we investigated the development of TNBS-induced colitis in mice genetically deficient for inducible nitric oxide synthase (iNOS), constitutive endothelial (eNOS) and neuronal (nNOS) isoforms in chapter 6. Both eNOS and iNOS deficient mice showed a more severe colitis than wildtype and nNOS deficient mice. In wildtype mice eNOS localization in endothelial cells was unchanged by colitis, however, iNOS expression was dramatically increased in epithelial cells and lamina propria mononuclear cells. Fluorescent and electron microscopy identified bacteria translocating across the inflamed lamina propria of eNOS deficient mice, but not in wildtype, iNOS or nNOS deficient mice during colitis. The increased susceptibility of eNOS deficient mice was associated with a decrease in colonic goblet cell numbers.
and impaired mucin production. Intra-rectal delivery of the NO donor isosorbide dinitrate normalized mucin production and ameliorated subsequent colitis. These results suggest a protective role of both iNOS and eNOS during experimental colitis. The absence of eNOS caused an impaired intestinal defense against lumenal bacteria and a concomitant aggravation of colonic inflammation. Finally, in chapter 7, we discuss whether blockade of NF-κB activation and donation of nitric oxide could be new treatment options for inflammatory bowel disease. The transcription factor NF-κB is a key regulator of the expression of genes involved in immune and inflammatory responses in the gut. Various stimuli like oxidative stress, cytokines (IL-1, IL-6, TNF-α), bacteria and viruses can release NF-κB from its inactive cytoplasmic form to the nucleus. Drugs like corticosteroids, sulfasalazine, mesalazine and inhibitory cytokines (eg IL-10, IL-11) can prevent the activation of NF-κB. New, more potent and selective treatment strategies with anti-sense NF-κB-subunit p65, proteasome inhibitors which prevent degradation of IκB protein and viral IκBα expression vectors aim at the prevention of NF-κB activation in mucosal macrophages and T lymphocytes. However NF-κB regulated genes are also involved in survival responses of epithelial cells. For example inhibition of the NF-κB mediated induction of iNOS in epithelial cells could block important anti-apoptotic and anti-microbial survival mechanisms. Nitric Oxide (NO) may also serve in a negative feedback loop to antagonize prolonged activation of NF-κB, thereby limiting chronic inflammation. Therefore lumenal donation of nitric oxide could block NF-κB activation. Selective inhibition of NF-κB activation in inflammatory cells and donation of NO could be a treatment option in inflammatory bowel disease. Further studies with intestinal NO donation or very selective iNOS inhibitors are needed before this can be applied in clinical practice.