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DISSERTATION

Transgenic *fat-1* mice are protected from colitis

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Abbreviations/ Verwendete Abkürzungen

AA	Arachidonic acid
BSA	Bovine serum albumin
CD	Crohn's disease
COX-2	Cyclooxygenase 2
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DSS	Dextran sodium sulfate
EPA	Eicosapentaenoic acid
FITC	fluorescein isothiocyanate
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
IBD	Inflammatory bowel disease
IEC	Intestinal epithelial cell
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
iNOS	inducible nitric oxide synthase
IRAK	Il-1 receptor-associated kinase
LC	Liquid chromatography
LPMC	Lamina propria mononuclear cell
LPS	Lipopolysaccharide
LTB 4	Leukotriene B 4
MS	Mass spectrometry
N-3	Omega-3 PUFA
N-6	Omega-6 PUFA
NPD1	Neuroprotectin D1
NF-κB	Nuclear factor kappa B
PAMP	Pathogen-Associated Molecular Pattern
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PD1	Protectin D1
PGE 2	Prostaglandin E 2
PLA 2	Phospholipase A2
PMN	Polymorphonuclear neutrophil
PRR	Pattern Recognition Receptor
PUFA	polyunsaturated fatty acids
RvD3	Resolvin D3
RvE1	Resolvin E1
SEM	Standard error of the mean
TNFα	Tumour necrosis factor alpha
TFF 3	Trefoil factor 3
TLR	Toll-like receptor
TOLLIP	Toll-interacting protein
UC	Ulcerative colitis
UV	ultraviolet
WT	wild type
ZO-1	Zona occludens 1

1 Einleitung/ Introduction

1.1 Inflammatory bowel disease

1.1.1 Crohn's disease and ulcerative colitis

The epithelial surface of the gut is the largest mucosal surface in mammals and is particularly exposed to microbial attacks and trauma. The inflammatory bowel diseases (IBD), Crohn's disease and Ulcerative Colitis are characterized by idiopathic relapses and remitting chronic inflammation. While each entity has its characteristic hallmarks (**Table 1.1**), there is a significant number of IBD patients (8-13%) where no decisive diagnosis can be made, then classified as undetermined colitis. It is still in question whether CD and UC are fundamentally discrete diseases or merely phenotypes of a single underlying pathologic process (1-3).

	Crohn's disease	***Ulcerative colitis***
Clinical features	Mucous diarrhea Abdominal mass Perianal disease	Bloody diarrhea
Complications	Strictures and fistulae Cancer	Toxic megacolon Cancer
Endoscopic findings	Cobblestone appearance Skip lesions Any part of the GI tract may be affected	Pseudopolyps Continuous involvement Restricted to the rectum and colon
Histologic findings	Transmural inflammation Granulomas	Mucosal inflammation Crypt abscesses

Table 1.1 *Hallmarks of Crohn's disease and ulcerative colitis.*

1.1.2 Etiology and Epidemiology of IBD

While the pathoetiology of IBD is yet unknown, it is currently believed that multiple factors are involved in the pathogenesis of CD and UC. These involve most importantly genetics, immunoregulatory defects, luminal bacteria and environmental factors (4).

Genetic predisposition represents the strongest independent risk factor, as evidenced in familial aggregation and twin concordance studies. In first degree relatives, there is a 10-15 fold increase in IBD prevalence (5). CD and UC are polygenic diseases. Genome screenings in cohorts of patients with IBD have identified chromosomal susceptibility regions, named IBD1-9. Homozygous mutations in the gene CARD15 (on chromosome 16) that encodes for the protein NOD2 have been found in a large number of patients with CD and links to a defective interaction between the mucosal immune system and the enteric commensal bacteria. While no specific pathogen could be shown as a causative agent for IBD, development of CD and UC require bowel colonization and antibiotics can alleviate the course of the disease.

Crohns' disease and ulcerative colitis are diseases of the industrialized, developed world with the highest prevalence seen in North America and Europe. A 'westernized lifestyle' (pollution, diet, stress) has been associated with disease incidence and this is supported by the increased risk for developing IBD in second-generation Asians that migrated to developed countries and increasing incidence in countries that underwent significant socioeconomic changes over the past decades, such as Japan or Korea. In the United States, the prevalence of IBD is an estimated 1,000,000 individuals, with an annual incidence of 30.000 new cases. There is no difference between male and female incidence. Certain ethnic groups, e.g. the Ashkenazi Jews have an increased risk of developing IBD. Crohn's disease is the second most common chronic inflammatory disorder after rheumatoid arthritis. With a peak age of onset of 15 to 30 years, IBD for most of the patients is a lifelong condition requiring continous and intensive medical care. It follows that CD and UC have an important economic effect on the healthcare system and the society as a whole by direct (medical care, medications, procedures) and indirect (absence from work, decreased earnings etc.) costs (1-3).

1.1.3 Intestinal mucosal inflammation

With 200 to 300 m^2 in humans, the intestine represents the largest interface between the environment and the organism. There is an omnipresent exposition to orally ingested microorganisms (bacteria, virus, fungi). The intestinal immune system must distinguish selectively between the innocuous antigens of the physiological bacteria and those of pathogens that are potentially harmful to health. While it must provide an efficient immune response to the latter, distinct immunoregulatory mechanisms anticipate and prohibit a disproportionate inflammatory reaction to the resident intestinal flora.

Unspecific defense includes mechanical (epithelial barrier, secreted mucins) or functional (pH, bile acids, peristalsis, pancreatic enzymes, physiological flora) mechanisms. IBD is characterized by an abnormal, exacerbated and perpetuated mucosal immune response to otherwise innocuous stimuli. This immunopathogenesis involves luminal antigens, intestinal epithelial cells and cells of the innate and adaptive immune system.

The initial event of the acute inflammatory answer is characteristically represented by neutrophilic infiltration into the affected area and is part of the innate immune response. At the site of the injury, polymorphonuclear neutrophils (PMN) in their position as primary host-defenders release antimicrobial peptides and reactive oxygen intermediates intended to neutralize or kill invading pathogens. In ulcerative colitis, crypt abscesses represent a hallmark of the disease. PMN also produce a range of chemokines, proinflammatory cytokines, interleukines and eicosanoids and therefore initiate the activation of other leukocytes and the adaptive immune system. In IBD patients, inflammatory lesions of the intestine are replete with CD4+ T cells and distinct Th-responses have been characterized for Crohn's disease (Th1 response: Interferon gamma, IL-12) and ulcerative colitis (atypical Th2 response: IL-4,IL-5,IL-13) with their respective cytokine profile. Activated effector T-cells again trigger macrophages and monocytes to secrete TNFα, IL-1β and IL-6 (1-3).

Dysregulated proinflammatory mediator circuits perpetuate and amplify the inflammatory response, leading to a chronic process that results in pathognomic tissue destruction.

1.1.4 Current therapeutic approaches

Today, there is no specific causal treatment for human inflammatory bowel disease. Nonetheless, modulating the formation of proinflammatory mediators and/ or anti-inflammatory molecules at multiple stages of the inflammatory cascade is useful in the treatment of IBD and represents the principle of current therapeutic strategies (1-3). Current therapeutic agents include sulfasalazine, high-dose steroids (local enemas or systemic), immunomodulators (azathioprine, 6-mercaptopurine) and antibodies against TNFα. Surgery may be necessary in extensive disease and/or complications of IBD, e.g. toxic megacolon in UC or fistulas in CD.

Side effects of antiinflammatory/ immunoregulatory therapy in IBD are a major concern limiting the use of these therapeutic agents, especially since the disease remains a lifelong condition for most patients (except UC patients that can be healed by colectomy). High-dose steroid protocols are used in exacerbated disease, but serious complications in prolonged use include steroid-induced diabetes mellitus, osteoporosis, and opportunistic infections.

Introduction of anti-TNF therapy marked a great advance in the treatment of complicated (e.g. fistulizing, steroid-dependent) CD and UC (2, 6). Infliximab, a chimeric monoclonal antibody against TNFα features a complement binding human IgG_1 and a murine antigen-binding variable site that binds soluble TNFα. Again, serious side effects may result from the immune-compromising mechanism of action inherent to anti-TNF therapy. Most notably, infections, an elevated risk to develop lymphoma and other malignancies have been reported. Also, while highly effective for the treatment of Crohn's disease in the short term, repeated administration may induce the development of antibodies directed against the murine portion of the chimeric antibody (immunogenicity) of infliximab, leading to infusion reactions and loss of clinical effect.

Recently, a recombinant human IgG1 monoclonal antibody, Adalimumab (Humira), became available. Adalimumab exhibits high affinity and specificity to human soluble TNF and has been shown to be effective in CD patients that lost therapeutic response to prior infliximab treatment or became intolerant (7).

1.2 Experimental colitis

1.2.1 IBD animal models

Throughout the last years, IBD-related animal models have played a crucial role in the further pathoetiological understanding of CD and UC. For IBD research in vivo models remain an important tool to investigate for the pathogenic and protective factors in this complex disease. Four main different classes of experimental models are available to simulate the manifold aspects of human IBD in animals (**Table 1.2.1**). It is important to mention that none of these models can cover the complete spectrum of disease as seen in human IBD. However, each experimental approach offers designated benefits and limitations that must be considered in accordance with the strategic endpoints of the individual study (8-10).

	Species	**Pathology**	**Affected site**	**Pathogenesis**
Spontaneous *Cotton top tamarin*	Primate	Acute, chronic mucosal	Colon	T-cells Induction of cancer
Inducible *DSS*	Mice, rats	Acute, chronic mainly mucosal	Colon (left sided)	Toxic epithelial cell damage Mucosal immune system
TNBS	Mice, rats, rabbits	Acute, chronic transmural	Colon	T_H-1 mediated against bacterial antigens
Adoptive transfer *CD4+/SCID*	Mice	Acute, chronic transmural	Colon, Duodenum	T_H-1 mediated
Genetic *IL-10 Knock-out*	Mice	Acute, chronic transmural	Colon, Duodenum, Jejunum	T_H-1 cells in response to bacterial antigens
STAT4 transgenic	Mice	Acute, chronic transmural	Colon, Jejunum	T_H-1 cells in response to bacterial antigens
NEMO Knock-out	Mice	Acute, chronic transmural	Colon, Jejunum	Abrogation of NF-κB signalling

Table 1.2.1 *Animal models of Inflammatory bowel disease.* Adapted from Wirtz (9).

1.2.2 DSS induced colitis

Dextran sodium sulfate (DSS)-induced colitis is a well established experimental model of IBD used to study cytokine-triggered inflammation and injury in the colon (11, 12) as well as other mechanisms of colitis such as thrombin-triggered pathways of inflammation (13). It is furthermore particularly useful to examine mechanisms of epithelial regeneration and wound healing. Acute colitis can be induced by continuous oral administration of DSS polymers in the drinking water, while chronic inflammation develops after cyclical administration patterns over at least 3 weeks. The course of the disease essentially differs depending on the rodent strain (e.g. C57BL/6 vs. BALB/C mice), gender, age, concentration and molecular weight of DSS (14).

Histologically, DSS colitis is characterized by infiltration of inflammatory cells into the lamina propria, with lymphoid hyperplasia, focal basal crypt damage, and epithelial ulceration (8, 11-13). These pathological changes are thought to develop as a result of a barrier-destructive effect of DSS on the epithelium with increased apoptotic epithelial cells, subsequent phagocytosis of lamina propria cells, and production of cytokines (8, 11-13).

Although the relationship of murine DSS-induced colitis to the human disease remains to be established, this widely used IBD model has a number of advantages, including simplicity, immediate inflammation, high degree of uniformity of the lesions, high reproducibility and leukocyte infiltration (8).

In the present report we chose this model to examine the impact of an enhanced n-3 PUFA tissue status on the development of acute colitis in transgenic *fat-1* mice.

1.3 Lipid mediators in inflammation

1.3.1 From lipids to lipid mediators

Lipids have numerous functions in human physiology. They provide energy storage (triglycerides), are part of the cell membrane structure (phospholipids, cholesterin), participate in signal transduction (diacylglyceride) and hormonal circuits (steroids) and form precursors of pleiotropic mediators (eicosanoids, lipoxins, resolvins, protectins) .

Lipid mediators are biologically active lipid molecules that mediate host responses via their specific receptors and that form distinct, structurally diverse classes (as described below). An important resource for the generation of these messengers are membrane phospholipids (**Fig. 1.3.1**) such as phosphatidylinositol-4,5-biphosphate, phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine, that contain esterified essential fatty acid tails (e.g. n-3 and n-6 PUFA) at the sn2-position.

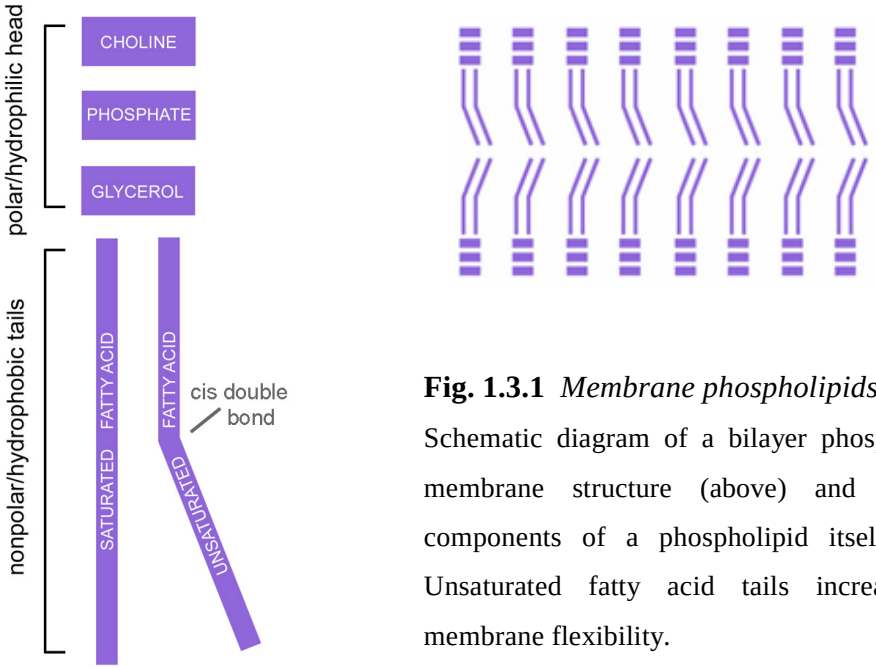

Fig. 1.3.1 *Membrane phospholipids*
Schematic diagram of a bilayer phospholipid membrane structure (above) and of the components of a phospholipid itself (left). Unsaturated fatty acid tails increase the membrane flexibility.

1.3.2 Omega-3 and omega-6 polyunsaturated fatty acids

In 1929 George and Mildred Burr discovered the importance of certain polyunsaturated fatty acids for the health of mammals. When they rigidly excluded fats from the diet of the experimental rats, they observed cessation of growth, inflammation and necrosis of the skin and tail, severe damage of internal organs and ultimately death (15).

Fatty acids are aliphatic, unbranched monocarbon acids that are classified according to the length of the acyl-chain, its functional groups, the amount of double bonds and the position of the first double bond, starting from the methyl (omega) end. Fatty acids without a double bond are called saturated fatty acids, with one double bond monounsaturated fatty acids and with more than one double bond polyunsaturated fatty acids (PUFA). Structurally, omega-3 (n-3) and omega-6 (n-6) PUFA feature a long hydrocarbon chain with 18 or more carbons that contains three to six double bonds. In n-6 PUFA, the first double bond is located at the sixth carbon-carbon bond from the terminal methyl end of the carbon chain. Similarly, in n-3 PUFA the first double bond is located at the third carbon-carbon bond. Physiologically, these double bonds are in cis-position.

The main n-3 PUFA are alpha-linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Their cardinal n-6 counterparts are linoleic acid (LA, 18:2n-6) and arachidonic acid (AA, 20:4n-6). N-3 and n-6 PUFA are essential fatty acids and are important for good health. The human body cannot synthesize these PUFA on its own, since mammals lack the enzymes to introduce double bonds at carbon atoms beyond C-9 in the fatty acid chain, and therefore requires their dietary intake. Hence, the actual composition of essential fatty acids in the body reflects to a large extent the nature of the diet. Importantly, mammals cannot convert n-6 to n-3 fatty acids and interconversion within n-3 PUFA itself through elongation, and desaturation of ALA is physiologically limited by inadequate enzyme capacity and the utilization of ALA in alternate metabolic pathways (16). The main alimentary resources for n-3 PUFA are canola oil for alpha linolenic acid (ALA) and cold water oily fish, such as salmon, herring, mackerel, anchovies and sardines, for EPA and DHA. The n-6 PUFA linoleic acid is found largely in vegetable oils and soy, while arachidonic acid, being a prominent part of animal cell membranes, is consumed mainly with meat and animal fats.

N-3 and n-6 fatty acids exert a wide range of actions and regulate physiological and pathological cellular processes by multiple mechanisms. They have effects on membrane receptors, transporters (e.g. ion channels) and enzymes, determine the physiochemical and functional properties of biological membranes (as described below), act as substrates for the production of signalling molecules or functioning mediators, regulate gene expression by modulating transcription factors (e.g. NF-κB) and finally, represent a source of energy production (17).

As mentioned above, n-3 and n-6 PUFA are incorporated mainly into membrane phospholipids (where they form a lipophil tail). As integral structural compounds, they do have a unique viscotropic impact on the physical properties of biological membranes, such as fluidity, thickness and deformability. Importantly, the number of double-bonds in highly unsaturated PUFA such as DHA (22:6n-3) is associated with an increased membrane flexibility and effectiveness of transmembrane protein activation. Therefore, a different acyl chain composition can have dramatic effects on cell function (16).

Mechanical triggers or chemical stimuli activate phospholipase A2 (phosphatidylcholine-2-acylhydrase, PLA2), an esterase that catalyzes the hydrolytic cleavage at the β-C atom of the fatty acid tail and leads to the release of the incorporated fatty acid. This is the rate-limiting step in the synthesis of prostaglandins and leukotrienes. Accordingly, the relative proportion of n-3 and n-6 fatty acids in membrane phospholipids reflects their availability as substrates for lipid mediator generation (18).

1.3.3 Eicosanoids

Eicosanoids, namely the prostaglandines, prostacyclines, leukotrienes and thromboxanes comprise different classes of biologically potent mediators with extensive effects on the cardiovascular and immune system such as regulation of inflammatory responses, nociception, renal function, hemodynamics and blood clotting (19). They are oxygenation derivates of polyunsaturated fatty acids, and are generated enzymatically through the cyclooxygenase (COX) and lipoxygenase (LOX) pathways. They are rapidly formed, have a short half life and act locally in an autocrine and paracrine fashion (autacoids). Both, n-3 and n-6 fatty acids are precursors of eicosanoid production and result in physiologically diverse series (**Fig. 1.3.3**).

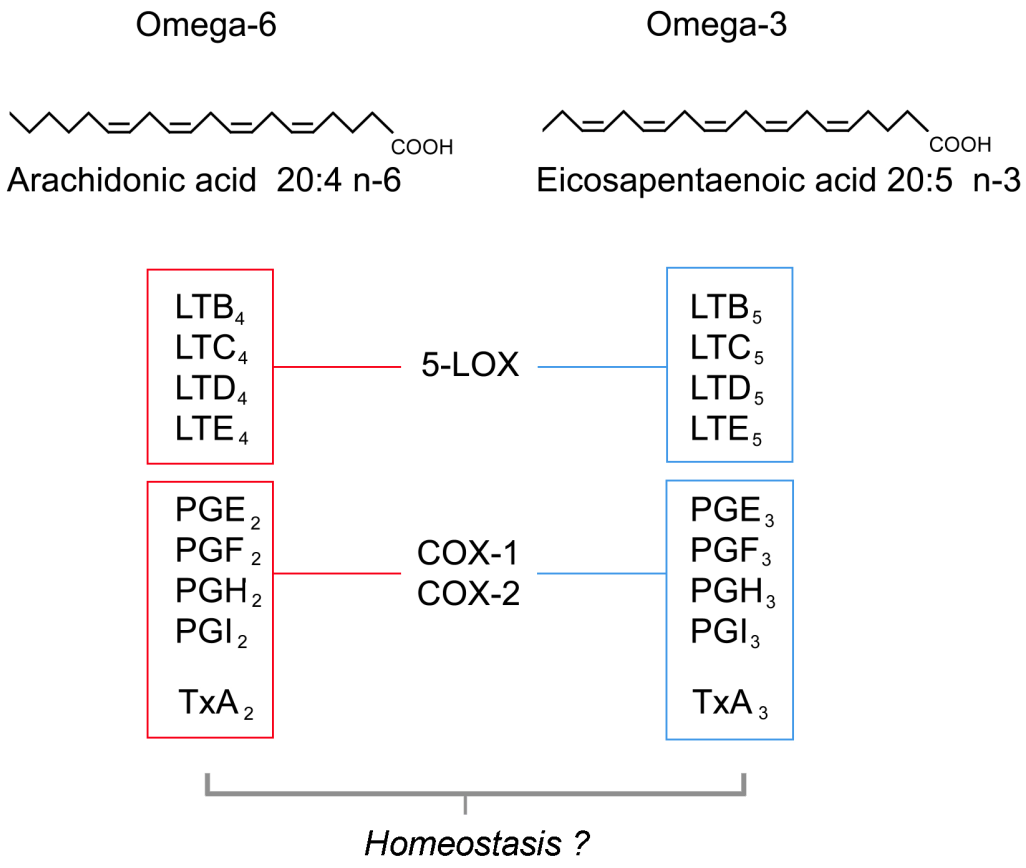

Fig. 1.3.3 *Schematic overview on Eicosanoid production from n-6 and n-3 PUFA .*
Adapted from Weylandt KH and Kang JX (20).

Eicosanoids derived from the n-6 PUFA arachidonic acid (AA), most prominently LTB_4 and PGE_2, are potent proinflammatory players that mediate the classic hallmarks of inflammation: fever, hyperalgesia, redness and swelling. These develop as a result of blood vessel dilatation with an increased blood flow, increased permeability across blood capillaries for macromolecules (e.g. complement factors, antibodies, cytokines) and increased translocation of leukocytes from the bloodstream into the encompassing local tissue. Thereby, these eicosanoids facilitate accumulation, adhesion and diapedesis of leukocytes and critically modulate the intensity and duration of inflammatory responses (**Table 1.3.3**).

Prostaglandin E_2	***Leukotriene B_4***
Induces fever	Enhances local blood flow
Increases vascular permeability	Increases vascular permeability
Increases vasodilatation	Chemotactic agent for leukocytes
Causes pain	Induces release of lysosomal enzymes
Enhances pain caused by other agents	Induces release of reactive oxygen species by granulocytes
Increases production of IL-6	Increases production of TNFα, IL-1β and IL-6

Table 1.3.3. *Proinflammatory properties of n-6 derived Eicosanoids.*
Adapted from Calder (21)

Baseline levels of PGE_2 are synthesized physiologically by the constitutively expressed COX-1 isoform, while the inducible COX-2 drives PGE_2 production during inflammation, particularly in cells of the immune system (19). Main effects of Aspirin and NSAIDs are thought to be based on the disruption of PGE_2-synthesis, highlighting the importance of eicosanoid manipulation as clinical therapeutic principle (20). Elevated PGE_2-production at inflammatory sites with local tissue defects, as evidenced in biopsies from patients with CD and UC (22), has been associated with anti-apoptotic properties and ulcer healing, while perpetuated PGE_2 activity promotes progression into chronic inflammation and is associated with a risk for cancerogenesis (23).

Leukotriene B_4 (LTB_4) is an AA-derived lipid mediator produced mainly by activated leucocytes. It is a potent chemoattractant and is critically involved in the recruitment of additional PMN, eosinophils and macrophages to the focus of injury. Furthermore LTB_4 activates the respiratory burst and triggers granule release from PMN. In concert with peptide mediators such as chemokines and cytokines it orchestrates leukocyte trafficking from the postcapillary lumen to the interstitial space and ultimately involves the adaptive immune system by attracting T-Cells (20, 21, 24).

The 3-series of prostaglandins (e.g. PGE_3) and 5-series of leukotrienes (e.g. LTB_5) are generated, respectively, via the COX and LOX pathways from the n-3 substrate eicosapentaenoic acid (EPA). They are devoid of biological actions or have much less intrinsic potency at the G protein-coupled receptors and have therefore been postulated as anti-inflammatory (25).

1.3.4 Novel omega-3 derived resolvins and protectins

Resolution of inflammation requires the elimination of granulocytes and reduction of the tissue mononuclear cell population (macrophages and lymphocytes) to normal numbers and phenotypes. This is achieved by an active and coordinated 'resolution program' that induces leukocyte apoptosis and clearance of dying cells by phagocytes. Newly identified resolvins (resolution-phase interaction products) and protectins, produced from n-3 PUFA, control key cellular events in the return to tissue homeostasis (catabasis). Importantly, in contrast to other products from n-3 PUFA described earlier, like the 3-series prostaglandins and the 5-series leukotrienes (which are structurally similar to the n-6 derived eicosanoids but less potent or devoid of biological actions), these novel lipid mediators possess highly potent anti-inflammatory and immunoregulatory properties and actively regulate the duration and extent of acute inflammatory states (26-28).

Resolvins of the E series are generated from EPA by an initial oxygenation via microbial P450-like enzymes to 18*R*-hydroxy-eicosapentaenoic acid (18*R*-HEPE). Subsequently, sequential conversions via the leukocyte 5-LOX lead to the bioactive 5,12,18*R*-trihydroxy-EPE (Resolvin E1, RvE1, **Fig. 1.3.4**). Interestingly, 18*R*-HEPE can also be generated (and has been primarily identified) by oxygenation via the acetylated COX-2 isoforms, e.g. of the vascular endothelium. This transcellular

process is enhanced by hypoxia in vivo. It is not known if the P450 pathway is the only mechanism to initialize resolvin synthesis in the absence of aspirin.

Resolvins of the D series, such as Resolvin D3 (RvD3), are synthesized from DHA by sequential action of the leukocyte 15-LOX (**Fig. 1.3.4**). While the 15-LOX leads to the generation of 17*S*-containing resolvins, metabolism via acetylated COX-2 results in a 17*R*-containing class (27).

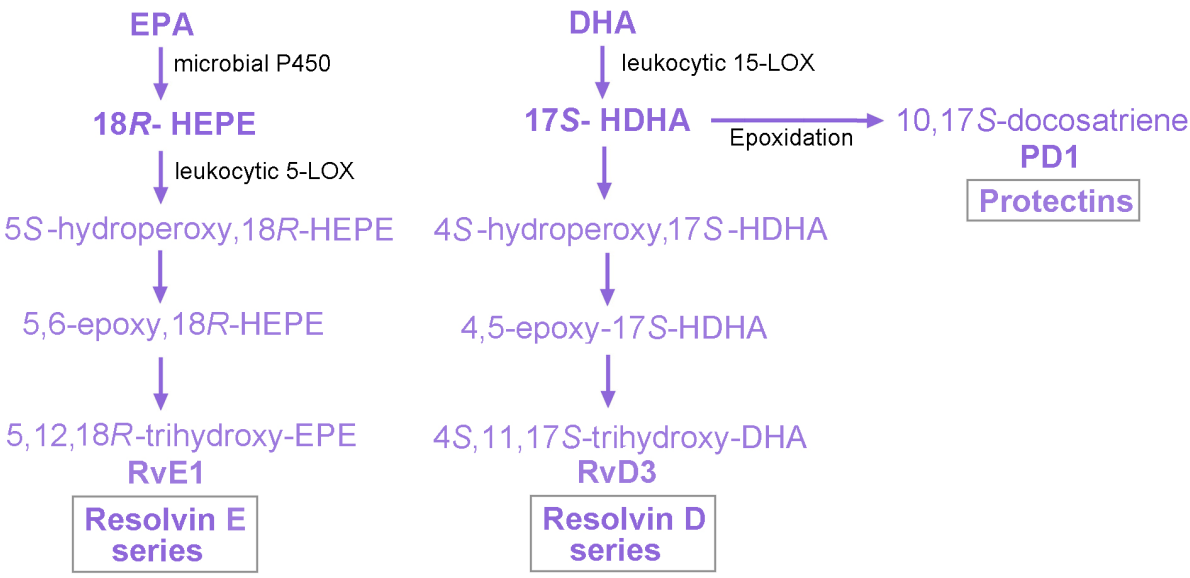

Fig. 1.3.4 *Pathways of resolvin and protectin synthesis.*

Both resolvins of the E and D series have inflammation-dampening effects in diverse models of inflammation (29) and have been shown to critically shorten resolution indices (time interval between maximum neutrophil infiltration and the drop to 50% of the maximum) in a model of murine peritonitis (30). In the same experiment, RvD3 gave ~50% inhibition and RvE1 gave ~75-80% inhibition when injected i.v. at 100 ng per mouse. This was compared to an inhibition of ~25% afforded by 100 ng indomethacin per mouse. A recently assigned specific G protein-coupled receptor, ChemR23, mediates the signal cascade of RvE1 to attenuate the pro-survival transcription factor NF-kappa B, therefore promoting apoptosis in the targeted cells. ChemR23 is expressed in a multitude of cell types, including leukocytes, dendritic cells and intestinal epithelial cells (29).

1.3.5 Omega-3 in inflammatory bowel diseases

Evidence suggesting that n-3 PUFA may play a role in IBD emerged from population-based studies. First, in an epidemiologic survey of Greenland Eskimos of the Upernavik district between 1950 and 1974, Kromann found a differing disease pattern in this piscivorous population compared to western countries. Besides the strikingly decreased prevalence of cardiovascular diseases in this people, chronic inflammatory diseases including IBD were significantly less common (31).

In a retrospective study, Shoda et al. analysed the changes in the Japanese diet in relation to Crohn's disease from 1966 to 1985. In the course of that period the traditionally prevailing fish diet shifted to a more western diet. Statistical evaluation resulted in the positive correlation between incidence of CD in the studied population and increased dietary contents of total fat, animal fat, n-6 PUFA and animal protein. There was a negative correlation with respect to dietary n-3 PUFA contents (32, 33). Fatty acid profiles from patients with IBD have been shown to have decreased total serum polyunsaturated fatty acids (34). Specific deficiencies in n-3 PUFA have been described in patients with CD (35).

In most westerners the amounts of tissue n-3 fatty acids are low, whereas the levels of n-6 fatty acids are high, with an n-6/n-3 ratio ranging from 10:1 to 20:1 (36). Clinical studies conducted to test the impact of n-3 PUFA supplements on clinical outcomes in IBD have reported mixed results. In a systematic review, MacLean et al. summarize that 7 out of 8 studies did not find an effect of n-3 PUFA supplementation on remission or relapse of disease (37). Nonetheless, the quality of the majority of these studies has been problematic and a methodical profiling of the n-6/n-3 lipid status in patients before and during the trials was missing. Taken together, these shortcomings render the data insufficient to draw conclusions about the effects of n-3 PUFA supplementation on clinical, endoscopic or histologic scores.

1.3.6 A transgenic approach

Marine vertebrates represent important sources of n-3 fatty acids in our diet. However, de novo generation of n-3 PUFA stems from the ability of single-cell phytoplankton and algae to convert linoleic acid (LA, n-6) to α-linolenic acid (α-LA, n-3), which enters the marine food chain and is further elongated and desaturated to the fish oil components EPA and DHA (**Fig. 1.3.6 A**).

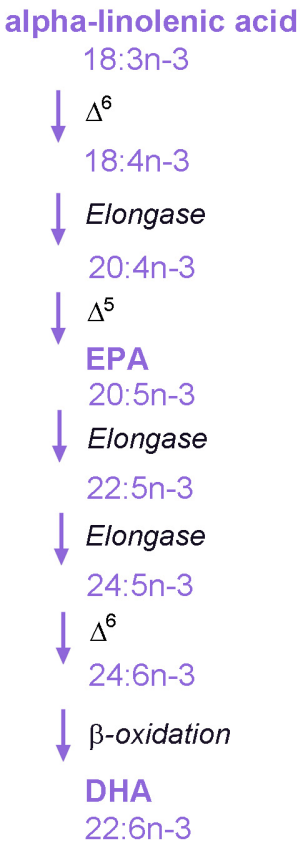

Fig. 1.3.6 A.

Metabolism of n-3 PUFA

Transgenic *fat-1* mice, engineered to express the *Caenorhabditis elegans fat-1* gene encoding an n-3 fatty acid desaturase, are capable of producing n-3 PUFA from n-6 PUFA (**Fig. 1.3.6 B**) and thereby have a low or balanced ratio of n-6/n-3 fatty acids in their tissues and organs without the need of dietary interventions.

In cells expressing the *fat-1* gene, all types of n-6 fatty acids can be converted to their respective n-3 fatty acids, namely 18:2n-6 (LA) to 18:3n-3 (α-LA), 20:2n-6 (eicosadienoic acid) to 20:3n-3 (eicosatrienoic acid), 20:3n-6 (dihomogammalinolenic acid) to 20:4n-3 (eicosatetraenoic acid), 20:4n-6 (AA) to 20:5n-3 (EPA), 22:4n-6 (docosatetraenoic acid) to 22:5n-3 (docosapentaenoic acid, DPA) and 22:5n-6 (DPA) to 22:6n-3 (DHA).

Hence, they do not only increase the n-3 PUFA status in all tissues, but simultaneously lower the amount of the corresponding n-6 PUFA (38). The genetic approach needs no incorporation of exogenous fatty acids into cells to alter the n-6/n-3 ratio and does not change the total amount of cellular fatty acids. Furthermore, in contrast to supplemental studies, n-6/n-3 PUFA status is balanced in *fat-1* mice since birth, allowing to address its effects on development, gene expression or physiological activity during the whole life cycle.

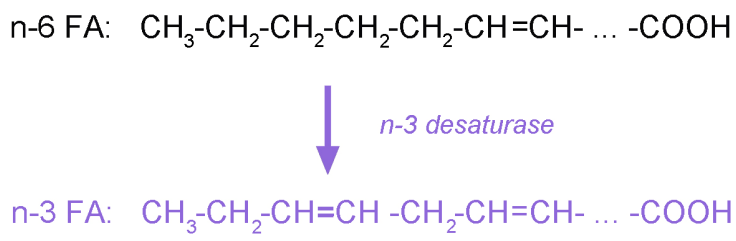

Fig. 1.3.6 B. *Conversion of n-6 fatty acids (FA) to n-3 fatty acids by n-3 desaturase.*
The n-3 desaturase catalyzes the introduction of a double bond into n-6 fatty acids at the n-3 position of their hydrocarbon chains to form n-3 fatty acids.

This model also allows carefully controlled studies to be performed in the absence of restricted diets, which can create confounding factors that limit studies of this nature. Therefore, transgenic mice offer unique opportunities to address the molecular events underlying the impact of n-3 fatty acids. In this study, transgenic *fat-1* mice are used for the first time to induce experimental DSS colitis and to investigate the association of n-3 fatty acids and proresolutionary action in intestinal inflammatory events .

1.4 Pattern recognition receptors and the regulation of NF-kappa B downstream

1.4.1 Toll-like receptors

The human gut is colonized by approximately 10^{14} microorganisms, comprising over 500 species. Bacteria are necessary for a normal maturation of the mucosal immune system and the induction of genes in intestinal epithelial cells (IECs) that are involved in electrolyte transport, nutrition and microbial protection (39). Toll-like receptors (TLR), comprising one class of pattern recognition receptors, play a key role in the perception of both commensal and pathogenic microbials and the initiation of innate immune responses of the intestinal mucosa .

To date, eleven distinct TLRs have been discovered, that are characterized by three common structural features: a divergent ligand binding ectodomain with leucine rich repeats, a transmembrane region and a highly homologous cytoplasmic Toll/interleukin 1 receptor (TIR) domain (40). They specifically recognize, and interact with, microbial macromolecules, the evolutionary conserved pathogen-associated molecular patterns (PAMPs). IECs constitutively express TLRs at their apical site, most prominently TLR2 and TLR4 (41). TLR2 recognizes bacterial lipopeptides and lipoteichoic acid, which are part of the cell walls of gram positive bacteria while the endotoxin of gram-negative microbes, lipopolysaccharide (LPS), is the major ligand for TLR4 activation (42).

1.4.2 Toll-interacting protein

Upon ligand recognition, the cytoplasmic TIR domain recruits the adapter protein MyD88 and serine/ threonine kinases of the IL-1R-associated kinase (IRAK-1). Autophosphorylation of IRAK-1 (by IRAK-4) further leads to interaction with another adapter protein, TNF receptor-associated factor 6 (TRAF6) and ultimately, induces translocation of NF-κB and gene transcription of distinct proinflammatory cytokine profiles.

Toll-interacting protein (Tollip) is an inhibitory regulator of the described MyD88 dependent TLR-pathway (**Fig. 1.4.2**). It acts downstream of TIR and MyD88 by binding to IRAK-1. Aggregated in a complex with Tollip, phosphorylation of IRAK-1 is prohibited and subsequently its kinase activity and signal transduction to TRAF6 are suppressed. Physiologically, in the colonized gut, Tollip is found to be highly expressed and contributes to the maintenance of intestinal hyporesponsiveness to the commensal bacteria (43-45).

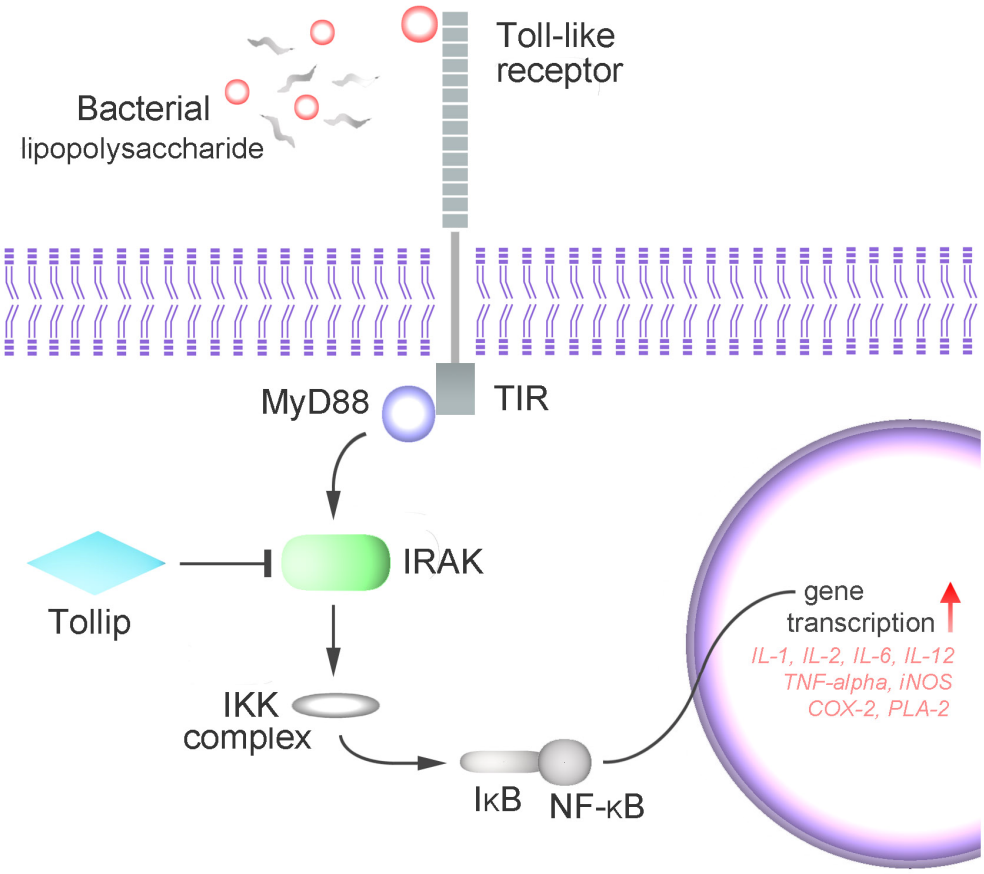

Fig. 1.4.2. *TLR downstream is inhibited by Toll interacting protein.*
A simplified schematic overview of TLR activation and the classic MyD88-dependent signal cascade leading to activation of NF-kappaB and nuclear gene transcription.

1.4.3 Nuclear factor-kappa B

Nuclear factor-kappa B (NF-κB) is an ubiquitous multi-subunit transcription factor of the Rel family that represents a key regulator of cell death, immune and inflammatory responses. It can be activated by bacterial products (e.g. LPS), proinflammatory cytokines (e.g. IL-1, IL-18) or viral components (dsRNA). It is often formed as heterodimers, e.g. p50/p65 with p65 (RelA) functioning as the transcription-activating subunit.

In unstimulated cells it remains inactive, sequestered in the cytoplasm with the inhibitory IκB (apart from B-Lymphocytes where it resides in the nucleus and is constitutively active). Several downstream cascades lead to a common final path by activating the IκB-kinase-Complex (IKK). IKK phosphorylates IκB and thereby initiates its ubiquitin-mediated proteasomal degradation. Once liberated from IκB, NF-κB translocates to the cell nucleus where it can bind to specific DNA sites (κB –motifs of ~10 base pairs) of over 200 genes, initiating *de novo* protein synthesis. Main target genes of NF-κB include IL-1, IL-2, IL-6, IL-12, TNF-alpha, COX-2, iNOS and phospholipase A2 (**Fig. 1.4.2**).

1.5 The role of proinflammatory cytokines and oxidative stress in IBD

Cytokines are small nonstructural proteins with molecular weights from 8 to 40,000 D that regulate host responses to infection, immune responses and inflammation. They can be produced by almost all nucleated cells and are classified by their biological activities. Differing from hormones, cytokines do not have a major role in homeostatic control mechanisms that are part of the intrinsic daily cycle. In fact, many cytokine genes are not expressed in healthy individuals unless induced by a certain cell stressor (trauma, infection, noxious events).

In general, one can distinguish between cytokines that promote proinflammatory genes (e.g. phospholipase A2, COX-2 and iNOS), such as IL-1 and TNF-alpha, and those that block or suppress these main triggers and therefore the intensity of the inflammatory cascade, such as IL-4 and IL-10. Both IL-1 and TNF-alpha are produced at local inflammatory sites and often act synergistically.

1.5.1 Tumor necrosis factor alpha

Tumor necrosis factor alpha (TNFα) plays a major pathogenic role in IBD and specific inhibition of this proinflammatory cytokine by its chimeric monoclonal antibody Infliximab or the recombinant human IgG_1 monoclonal antibody Adalimumab is a potent therapeutic strategy in exacerbated CD and UC. TNFα production is increased in plasma and intestinal biopsies in IBD patients and originates mainly from lamina propria mononuclear cells (46, 47).

The half-life of TNFα in human monocytes/macrophages, which represent its main source, is approximately 12 minutes. A broad spectrum of TNFα-mediated biological effects has been postulated to contribute to IBD pathophysiology. These include activation of monocytes/ macrophages and stimulation of proinflammatory mediator release, activation of the adaptive immune system, induction of fever, expression of adhesion molecules (E-Selectin, ICAM) on the vascular epithelium, alteration of the intestinal epithelial barrier and crypt hyperplasia. Moreover, TNFα autoinduces its own transcription via NF-κB activation (48).

1.5.2 Interleukin 1 beta

Interleukins comprise a diverse class of immunoregulatory signalling molecules that mediate communication between leukocytes and other immunocompetent cells. The IL-1 family consists of IL-1α, IL-1β and the receptor antagonist IL-RA. Both IL-1α and IL-1β result from a 31 kD precursor protein. The bioactive 17 kD peptide hormone IL-1β is a prototypal multifunctional cytokine that affects nearly all cell types and plays, in synergy with TNFα, a critical role in promoting inflammation and initiating host defense responses (e.g. upon lipopolysaccharide challenge). Among its pleiotropic actions is the stimulation of neutrophil granulocytes in the bone marrow, elevated release of adrenocorticotropic hormone (ACTH) and cortisone from the adrenal gland and an increase in IL-6, CD14 and COX-2 expression. It is secreted mainly by activated blood monocytes/ macrophages, B-lymphocytes, keratinocytes and fibroblasts (49).

There are two transmembrane IL-1 receptors (IL-1R) that are both members of the Toll/interleukin-1 receptor superfamily (TIR) and feature, in contrast to the leucine-rich regions of TLRs, three extracellular Ig domains (40). IL-1R type I, which is mainly expressed on T-lymphocytes and fibroblasts, initiates a downstream cascade leading to NF-κB activation, while IL-1R II does not transduce a signal and acts as a 'decoy' receptor. It has been proposed that IL-1 is involved in inducing early LPS endotoxin tolerance (50), since IL-1R and TLRs share the same downstream cascade beginning from the cytosolic TIR complex.

Individuals injected with IL-1 developed fever and pain, probably as a result of a COX-2 dependent increase in PGE_2 eicosanoid generation. These effects were ameliorated by the coadministration of COX inhibitors (51).

1.5.3 Inducible nitric oxide synthase

Nitric oxide (NO) is a free radical that is produced from the amino acid L-arginine by three distinct isoforms of the nitric oxide synthase enzyme. Small amounts of NO are synthesized under physiologic conditions by the constitutively expressed endothelial (eNOS) and neuronal (nNOS) nitric oxide synthase and act as an important signalling molecule, exerting multiple actions in the gut. These include regulation of intestinal motility, vascular permeability and blood flow (52).

In intestinal inflammation inducible nitric oxide synthase (iNOS) is upregulated and produces large amounts of NO, contributing critically to inflammation (53). Reaction of NO with superoxide (O_2^-) leads to the formation of the highly cytotoxic oxidant peroxynitrite anion (54, 55). The resulting oxidative stress is manifested in thiol oxidation, carbohydrate degradation, lipid oxidation and DNA cleavage. Another reaction pathway of NO with O_2 leads to autooxidation of NO and subsequently gives rise to the formation of cancerogenic nitrosamines (52).

1.6 The role of mucoprotective factors in IBD

1.6.1 Trefoil factor family

Mammalian intestinal trefoil factors comprise a class of three secretory peptides of the mucous epithelia that are mainly synthesized by intestinal mucin-producing cells and glands. The subtypes TFF1, TFF2 and TFF3 have distinct localisation profiles. TFF1 and TFF2 are predominantly secreted in the stomach and the duodenum while TFF3 is expressed primarily in the goblet cells of the small and large intestine (56).

They are secreted as integral constituents of the mucus to the apical site and have been termed ‘luminal surveillance peptides’ (57) since they can only bind to their respective receptors when the basolateral membrane is exposed, e.g. after local intestinal tissue damage. They then promote multiple protective actions at the local site of injury including formation of the mucus barrier, restitution of the mucosa, modulation of mucosal differentiation and modulation of the mucosal immune response (58).

Re-epithelialization of the mucosa is accomplished by rapid migration of neighbouring cells into the wounded area, subsequent formation of tight junctions and functional restoration of the intestinal epithelial barrier. This repair mechanism is essential to prevent contact of the luminal bacterial antigens with immunocompetent cells of the lamina propria. In chronic inflammatory conditions such as CD and UC, a characteristic glandular pattern known as the ulcer-associated cell lineage with an enhanced TFF synthesis can be found (59).

1.6.2 Zona occludens and the intestinal epithelial barrier

Zona occludens 1 is a 225 kD peripheral membrane-bound protein expressed on the cytoplasmic surface of intestinal epithelial cells, it contributes to intercellular tight junction. Within tight junction complexes it interacts with occludin, claudin and cingulin families and functions as the crucial intermediary link to the cytoskeleton (via F-actin at the C terminus, **Figure 1.6.2**) (60).

Epithelial tight junctions seal the intestinal mucosal surface, acting as a physiologically active barrier that prevents paracellular translocation of the luminal bacteria to the lamina propria and subsequent activation of immunocompetent cells residing there.

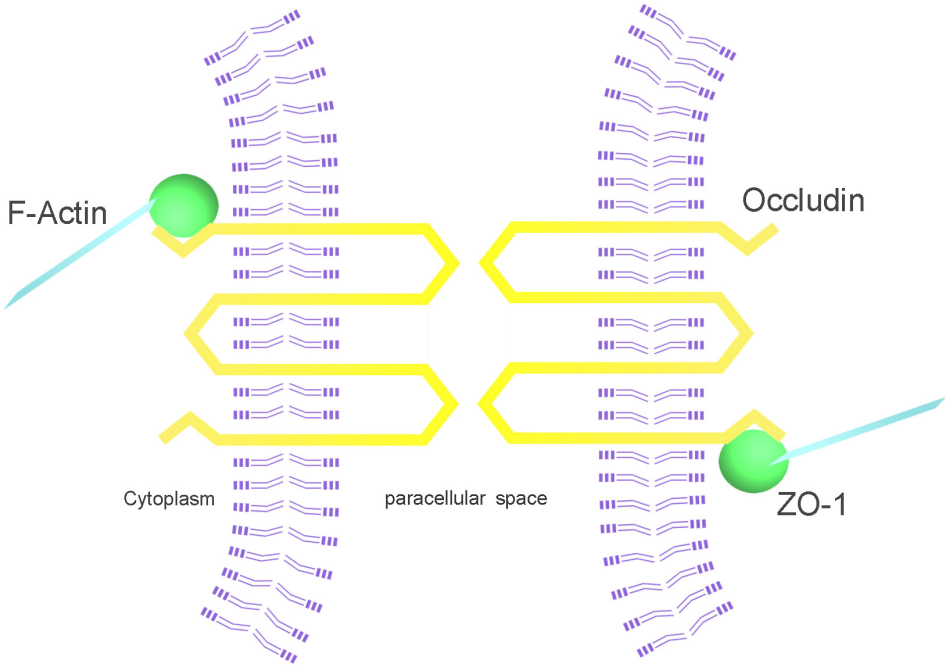

Fig. 1.6.2. *Zona occludens 1*
In tight junctions, ZO-1 links the transmembrane protein occludin to the cytoskeleton (F-Actin).

2 Fragestellung/ Research goals

2.1 Effect of an endogenously altered n-6/n-3 tissue status on the course of experimental colitis in the transgenic *fat-1* mouse.

The contribution of the n-3 fatty acid status itself to chronic disease progression such as in IBD and to the generation of local inflammatory mediators and mucoprotective factors remains of interest. The *fat-1* transgenic mouse offers a novel and unique approach to investigate the effects of an enhanced n-3 PUFA status in health and disease. It was the primary objective of this study to examine the extent of DSS induced colitis in the *fat-1* transgenic animal model described. Our hypothesis was that disease severity would be ameliorated in the experimental group by an altered n-6/n-3 PUFA status, based on different production of lipid mediators from the n-6 or n-3 precursor classes.

2.2 Lipid mediator systems in the transgenic *fat-1* mouse

Recently described lipid mediators deriving from n-3 PUFA have been associated with potent anti-inflammatory and proresolutionary actions. Yet, there has not been an investigation on the contribution of an altered n-6/n-3 PUFA tissue status on the generation of these mediators. Using the *fat-1* mouse, we analysed lipid mediator profiles of the eicosanoid family and the new resolvins and protectins during experimental colitis. Based on the recent work of Serhan et al., our hypothesis was that the n-3 derived resolvins and protectins would be endogenously produced in *fat-1* mice while none or less of these would be available in the wild-type mice with lower levels of the n-3 precursors. We also expected the levels of proinflammatory PGE_2 and LTB_4 to be lowered in the *fat-1* mice, based on the substrate competition between AA and EPA for enzymatic eicosanoid generation.

2.3 N-3-mediated effects on cytokines in inflammation

An eclectic body of cytokines and other biologically active factors has been implicated in the genesis, promotion and sustenance of inflammation. Gene expression analysis and detection of active protein levels were applied to elucidate potential molecular mechanisms by which an altered n-6/n-3 PUFA status might regulate immune responses and execute anti-inflammatory actions. We focused on the pathognomonic IBD parameters IL-1, TNFα, iNOS and NF-κB. We hypothesized that levels of NF-κB and, subsequently, of the proinflammatory cytokines will be lower in *fat-1* transgenic mice compared to their wild type littermates, based on the finding that activation of NF-κB can be directly inhibited by n-3 derived lipid mediators.

2.4 N-3-mediated effects on mucosal integrity in inflammation

Mucoprotective factors are actively employed in the structure, functional maintenance and restitution of the epithelial barrier. As such, they protect the host from erratic interaction with luminal antigens and potential pathogens. Upon local tissue damage, they promote healing mechanisms and prohibit an unbalanced immune reaction. We investigated three systemic levels of mucosal protection. The first, afforded by the apically secreted TFF3 represents a topical layer of a surveillance peptide that promotes restitutional actions after local epithelial injury. Second, ZO-1 as a main structural component of the tight junction complex, restricts intercellular transmigration and therefore impedes the contact of luminal microorganisms with immunocompetent cells residing in the lamina propria. Third, Tollip negatively regulates the downstream cascade of pattern recognition receptors such as TLR, and therefore mediates a controlled immunohomeostasis to the sensed luminal bacterial colonisation. We hypothesized that the enhanced n-3 tissue status might support these protective mechanisms. We focused on the question whether any differences seen in *fat-1* and WT mice may account for an active impact of the n-3 PUFA on these molecules themselves, or if they represent secondary events to an alleviated overall course of disease.

3 Methodik/ Materials and Methods

3.1 Mice

3.1.1 *Fat-1* transgenic mice

Transgenic *fat-1* mice were created as in ref. (38) and subsequently backcrossed (at least four times) onto a C57BL/6 background, a widely used inbred mouse strain that possesses a high degree of genetic and phenotypic uniformity. Generations of heterozygous *fat-1* mice and WT mice were then mated to obtain WT and transgenic mice from the same offspring.

In this study, all transgenic *fat-1* mice used were heterozygous. Fatty acid composition of the tail was analyzed by gas chromatography at 4 weeks of age (after the end of breast feeding) and the ratio between AA (n-6) and EPA (n-3) was determined for each individual to distinguish between WT and transgenic phenotypes. *Fat-1* mice typically had a AA/EPA ratio ≤ 1.

3.1.2 Animal housing

Animals were kept under specific pathogen-free conditions in standard cages and were maintained in an air-conditioned atmosphere with a controlled 12 hour light-dark cycle. WT as well as *fat-1* mice were fed the same special semi-purified diet (AIN-76A containing 10% safflower oil) high in n-6 and low in n-3 fatty acids until the desired age (9–10 weeks) and weight (19–21 g). Sterile drinking water was given ad libitum. Each cage housed two weight-matched female mice (one WT and one *fat-1* transgenic mouse).

All studies were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care.

3.2 Dextran sodium sulfate colitis

3.2.1 Induction of colitis and experimental setup

Four experimental groups were set up as in table 3.2.1 to investigate the impact of an endogenously altered n-3/n-6 PUFA status in DSS-colitis.

	Treatment	**Number of subjects**
Wild type colitis	5 days of 3% DSS, then 3 days of normal tap water	*n*=6
Fat-1 colitis	5 days of 3% DSS, then 3 days of normal tap water	*n*=6
Wild type control	Normal tap water for 8 days	*n*=3
Fat-1 control	Normal tap water for 8 days	*n*=3

Table 3.2.1. *Experimental groups in this study.*

Colitis was induced in both WT and transgenic mice by addition of 3% (wt/vol) dextran sodium sulfate (DSS molecular weight 35,000–40,000; ICN Biomedicals, Costa Mesa, CA, USA) to sterile drinking water. In pilot studies, this DSS concentration induced strong colitis, but a low mortality rate when given over 5 days.

On day 5, DSS supplementation was discontinued, and mice were killed by anesthesia with isoflurane (Abbott Laboratories, Abbott Park, Illinois, USA) on day 8 (3 days after cessation of DSS administration). The whole colon was excised from the anal verge to the ileocecal valve. Tissue was rinsed with PBS, and sections were prepared in a standardized way for further biochemical, histological and immunohistochemical investigation.

3.2.2 Disease activity assessment of macroscopic features

Clinical assessment of all DSS-treated animals for body weight, stool consistency, rectal bleeding, and general appearance was performed daily in a blinded manner. Mice were weighed twice at designated time points each day. Mice were killed on day 8. Colons were excised, and their length and thickening were documented.

3.2.3 Disease activity assessment of histological features

Histological examination was performed in a blinded manner and the degree of inflammation and epithelial damage on microscopic cross-sections of the colon was graded by an experienced pathologist. Colonic tissue samples for histological examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at full thickness and stained with hematoxylin and eosin.

The inflammation score is a combined score of (*i*) severity of inflammation (0 = no inflammation, 1 = mild, 2 = moderate, and 3 = severe) and (*ii*) thickness of inflammatory involvement (0 = no inflammation, 1 = mucosa, 2 = mucosa plus submucosa, and 3 = transmural); epithelial damage score consists of (*j*) character (0 = intact epithelium, 1 = disruption of architectural structure, 2 = erosion, and 3 = ulceration) and (*jj*) extent of lesions (0 = no lesions, 1 = punctuate, 2 = multifocal, and 3 = diffuse). For *i* and *jj*, half steps (e.g. *i:* 1.5 = mild to moderate inflammation) were used by the pathologist to further distinguish denomination of the histopathological status.

For each individual, values for *i* and *ii* were added and divided by two to obtain the score for inflammation. Scores for epithelial damage were calculated in the same manner (values for *j* and *jj* were added and divided by two for each mouse).

3.3 Immunohistochemistry

Immunohistochemistry allows to investigate the localization, distribution and expression profiles of bioactive proteins in biological tissues. Its principle is based on the binding of an antibody to its specific antigen. In immunofluorescence, visualization of the antigen-antibody complex is afforded by a fluorescent dye (e.g. FITC as in this study), and can be detected in a highly sensitive manner by confocal laser microscopy.

Colon tissue was fresh-frozen in Tissue-Tek® OCT medium (Ted Pella Inc., Redding, CA, USA), and sections were cut at 4 µm thickness. After air-drying, they were incubated with ZO-1 primary antibody (1:100 dilution; rabbit; Zymed) for 30 min at room temperature in a moist chamber, rinsed with PBS (Gibco), and incubated with an Alexa Fluor 488 FITC (fluorescein isothiocyanate)-conjugated secondary antibody (1:50 dilution; goat anti-rabbit IgG; Molecular Probes) in the same manner. Sections were mounted with Glycergel mounting medium (Dako, Cambridgeshire, UK) and evaluated with a LSM 5 Pascal confocal microscope (Carl Zeiss AG, Oberkochen, Germany).

3.4 Semiquantitative Real-Time PCR

3.4.1 Extraction of RNA

Tissue sections for RNA analysis were prepared immediately after removal of the colon, shock frozen in liquid nitrogen and stored at –80°C until further processing. Total RNA was isolated from whole colon tissue using TRIzol reagent (Invitrogen Life Technologies) following the manufacturer's instructions.

Briefly, whole colon tissue samples were added to 1 ml of TRIzol reagent and homogenized with Tissuemiser (Fisher Scientific, Hampton, NH, USA). Homogenates were then centrifuged at 12,000 x *g* and insoluble extracellular content was discarded. Supernatant containing RNA was transferred to a new tube. For phase separation, 0.2 ml chloroform was added to each sample, tubes were shaken thoroughly for 15 seconds and centrifuged for 15 min at 12,000 x *g*.

The upper aqueous phase was transferred to a fresh tube and 0.5 ml of isopropyl alcohol was added to precipitate RNA. After incubation for 10 minutes at room temperature and centrifugation at 12.000 x *g*, RNA precipitate appeared as a formed gel-like pellet attached to the tube. All liquid was evacuated and 1 ml of 75% ethanol was added to wash RNA.

After vortexing and centrifugation at 7.500 x *g* for 5 minutes, ethanol was removed and RNA precipitate was air-dried for 15 minutes. Then, RNAse-free water was used to dissolve RNA and samples were stored at –20 °C.

3.4.2 Determination of RNA concentration and quality

RNA concentrations and purity were determined spectrometrically with an Shimadzu UV160U spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA). First, 2 µl of sample RNA were diluted in 500 µl NaOH (= 250x diluted). Total RNA concentration was then calculated as follows:

$$\textit{RNA concentration } (\mu g/\mu l) = \frac{\textit{Absorbance at 260 nm } (OD_{260}) \textit{ x Dilution x 40}}{1000}$$

The ratio of readings at 260nm and at 280 nm (OD_{260}/OD_{280}) was calculated to give an estimate of RNA quality. Pure RNA has an OD_{260}/OD_{280} ratio of 1.9 to 2.1.

3.4.3 Generation of cDNA

Reverse transcription of mRNA was performed by using a Reverse Transcription System (Promega, Madison, WI, USA).

First, a reaction mastermix was prepared. For each sample, 4 µl 25mM $MgCl_2$, 2 µl reverse transcription buffer, 2 µl dNTP mixture, 0.5 µl recombinant RNAsin ribonuclease inhibitor, 1 µl random hexamer primers and 0.7 µl AMV reverse transcriptase were added into separate tubes and mixed thoroughly. Then, 1 µg of template RNA and nuclease free water (dH_2O) were incubated in a microcentrifuge tube at 70°C for 10 minutes.

Amount of nuclease free water was determined as follows:

Amount of dH_2O = 20 µl (total amount per tube) – 10.2 µl (mastermix) – template RNA (amount of sample containing 1 µg mRNA).

After incubation, template RNA/ dH_2O was added to the respective tube containing the mastermix and the reaction was incubated for 10 min at room temperature. Subsequently, reverse transcription was performed with a PTC-100 Programmable Thermal Controller (MJ Research Inc., Waltham, MA, USA). Cycler program was set at 42 °C for 45 min and 95 °C for 5 min.

DNA concentration was determined spectrometrically by adding 10 µl of reverse transcription product to 50µM NaOH (diluted in 500µl of sterile water). DNA concentration was calculated as follows:

$$\textit{DNA concentration (µg/ml)} = \frac{\textit{Absorbance at 260 nm } (OD_{260}) \textit{ x Dilution x 50}}{0.020}$$

3.4.4 Primers

Primer sequences were retrieved from Primer Bank of the Department of Molecular Biology, Harvard Medical School (http://pga.mgh.harvard.edu/primerbank/) and ordered from Invitrogen Corporation (Carlsbad, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene to normalize interindividual values (61).

Primer	Full name	Sequence	Product size
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	F: 5' ATGACATCAAGAAGGTGGTG R: 5' CATACCAGGAAATGAGCTTG	177
IL-1β	Interleukin 1 beta	F: 5' GCAACTGTTCCTGAACTCAACT R: 5' ATCTTTTGGGGTCCGTCAACT	89
TNFα	Tumor necrosis factor alpha	F: 5' CCCTCACACTCAGATCATCTTCT R: 5' GCTACGACGTGGGCTACAG	61
INOS	inducible Nitric oxide synthase	F: 5' GTTCTCAGCCCAACAATACAAGA R: 5' GTGGACGGGTCGATGTCAC	127
TFF-3	Intestinal Trefoil factor	F: 5' TTGCTGGGTCCTCTGGGATAG R: 5' TACACTGCTCCGATGTGACAG	117
ZO-1	Zona occludens 1	F: 5' GCCGCTAAGAGCACAGCAA R: 5' TCCCCACTCTGAAAATGAGGA	134
TOLLIP	Toll interacting protein	F: 5' CCTCAGCCCCGCTGTAATG R: 5' CCTCAGCCCCGCTGTAATG	115

Table 3.4.4. *Primers used in this study*

3.4.5 Performance of semiquantitative real-time PCR

Real-time PCR was carried out by using SYBR Green in a PRISM 9000 Light Cycler (Applied Biosystems, Foster City, CA, USA) following the manufacturer`s protocol.

A custom PCR mastermix was used containing (per sample) 2.5 µl Taq buffer, 0.25 µl Taq polymerase, 2.5 µl 25mM $MgCl_2$, 1 µl dNTP, 0.75 µl DMSO, 4 µl 50% Glycerol, 12.125 µl nuclease-free water, 0.75 µl ROX (control dye) and 0.125 µl SYBR Green® dye. Mastermix was prepared for each gene (adding 0.25 µl of forward and 0.25 µl of reverse primers x amount of tubes) and aliquoted into reaction tube strips. Ultimately, 0.5 µl of the template DNA was added directly to the respective tube, resulting in a total volume of 25 µl per tube.

To calculate semiquantitative mRNA expression, the delta CP method was used. First, CP values of the housekeeping gene were substracted from CP values of the target genes ($CP_{target\ gene}$ - $CP_{Housekeeping\ gene}$ = $\delta CP_{target\ gene}$). Then, δCP values of WT DSS, *fat-1* DSS and *fat-1* control mice (=experimental groups) were set into relation to WT control mice ($\delta CP_{experimental\ group}$ - mean $\delta CP_{WT\ control}$ = δδCP) for each target gene. Exponential δδCP values were then normalized with the following calculation:

n-fold expression (experimental group to WT control) = $2^{-\delta\delta CP}$.

Results are therefore expressed as a fold induction of the WT controls. All samples were processed in triplicate.

3.5 NF-κB Activation assay

To quantify the activated p65/RelA protein, TransAM NF-κB p65 Activation Assay (Active Motif, Carlsbad, CA, USA) was performed as follows.

3.5.1 Extraction of nuclear protein

Nuclear extracts from whole colon tissues were collected by using NE-PER (Pierce, Rockford, IL, USA) following the manufacturer's instructions. Briefly, tissue samples were cut into small pieces, transferred to glass vials and Dounce-homogenized in PBS buffer. After centrifugation at 500 x *g*, supernatant was removed and pellets were weighed to estimate the packed cell mass. A corresponding amount of CER I reagent was added and samples were vortexed at the highest speed for 15 seconds to fully resuspend the cell pellets. After incubation on ice for 10 minutes, 11µl of ice-cold CER II was added. Tubes were vortexed for 5 seconds and incubated for 1 min on ice. Again, tubes were vortexed for 5 seconds and then centrifuged at 16.000 x *g* for 5 minutes. Supernatants (cytoplasmic fraction) were immediately removed and discarded while the remaining pellet (which contains nuclei) was resuspended in 100 µl of ice-cold NER. Tubes containing the resuspended nuclear fraction were vortexed for 15 seconds and incubated for 10 minutes on ice. This was repeated 3 times for a total of 40 minutes. Then, they were centrifuged at 16.000 x *g* for 10 minutes and supernatants (containing the nuclear extracts) were immediately transferred to a clean pre-chilled tube. Samples were stored at –80°C until further usage.

3.5.2 Determination of protein concentrations

Protein concentrations in the nuclear extracts were determined by using a Coomassie Plus Assay (Pierce, Rockford, IL, USA). This method is based on the Bradford assay for protein determination (62). The Coomassie brilliant blue dye binds to the protein and subsequently the formed complex can be read spectrometrically at a proportional relationship between absorption at 595 nm and protein concentration. Absolute values were determined by employing a standard curve with bovine serum albumin (BSA).

3.5.3 Performance of TransAM NF-κB protein assay

Lysates (13 µg of total protein) were incubated at room temperature for 1 h in 96-well dishes containing immobilized oligonucleotides that comprise the NF-κB consensus DNA binding site (5'-GGGACTTTCC-3'). Consecutively, the primary antibody against p65 and the horseradish peroxidase-conjugated secondary antibody were incubated in the same manner, separated by washing steps. The reaction was developed for 5 min at room temperature, and its intensity was measured immediately at 450 nm by using a microplate reader (Victor 1420 Multilabel Counter, Wallac 1420 Workstation Software Version 3.00 Revision 2, Perkin Elmer, Wellesley, MA, USA).

3.6 Analysis of PUFA and lipid mediators

3.6.1 Gas chromatography

Fatty acid profiles were analyzed by using gas chromatography as described previously (63). Fresh colon tissues were grounded to powder under liquid nitrogen and subjected to extraction of total lipids and fatty acid methylation by heating at 100°C for 1 h in 14% boron trifluoride–methanol reagent. Fatty acid methyl esters were analyzed by gas chromatography using a fully automated Hewlett Packard 5890 system equipped with a flame-ionization detector. Peaks of resolved fatty acids were identified by comparison with fatty acid standards (Nu Chek Prep, Elysian, MN, USA), and area percentage for all resolved peaks was analyzed by using a Perkin Elmer M1 integrator.

3.6.2 Lipidomic analysis

Lipid mediators from n-3 and n-6 fatty acids were measured by using liquid-chromatography tandem mass-spectrometry methods (LC/MS/MS) on an LCQ quadrupole ion trap mass spectrometer (Finnigan MAT, San Jose, CA, USA) equipped with an electrospray atmospheric pressure ionization probe. Samples were extracted with 2 ml of cold methanol by C18 solid phase extraction, using d_4-LTB_4 (Cascade Biologics Inc.) as an internal standard. Compounds were then injected into the HPLC component, which consisted of a SpectraSYSTEM P4000 quaternary gradient pump (Thermo Separation Products, San Jose, CA, USA), a LUNA C18–2 (100 x 2mm x 5 µm) column (Phenomenex, Torrance, CA, USA), and a rapid spectra scanning SpectraSYSTEM UV2000 UV/VIS absorbance detector. This column was eluted isocratically with methanol/water/acetic acid (65:35:0,01; v/v/v) at 0,2 ml/min into the electrospray probe. The spray voltage was set to 5 kV and the heated capillary to 250°C. Resolvins and protectins were quantitated by selected ion monitoring for analyte molecular anions. Over a 2-second scan cycle, full-scan mass spectra (MS) were acquired by scanning between m/z 95-410 in the negative ion mode, followed by the acquisition of product ion mass spectra (MS/MS) of the most intense molecular anions for definitive compound identification.
For example, $[M-H]^-$ = m/z 291 and MS/MS 349 for Resolvin E1.

3.7 Statistical Analysis

Data analysis was performed with Prism 3.02 software (GraphPad). Comparison was made by using the Student *t* test. All values are presented as the mean ± SEM or as indicated. Statistical significance was set at $P = 0.05$.

4 Ergebnisbeschreibung/ Results

4.1 Fatty acid profiles of colon tissues

Both WT and *fat-1* transgenic littermates born to the same mother were maintained on a diet (10% safflower oil) high in n-6 and low in n-3 PUFA (n-6/n-3 =20). During this dietary regime, *fat-1* transgenic mice had significantly higher amounts of n-3 PUFA, such as EPA, DPA, and DHA, in all organs and tissues including the colon compared with WT mice (**Fig. 4.1** and **Table 4.1**).

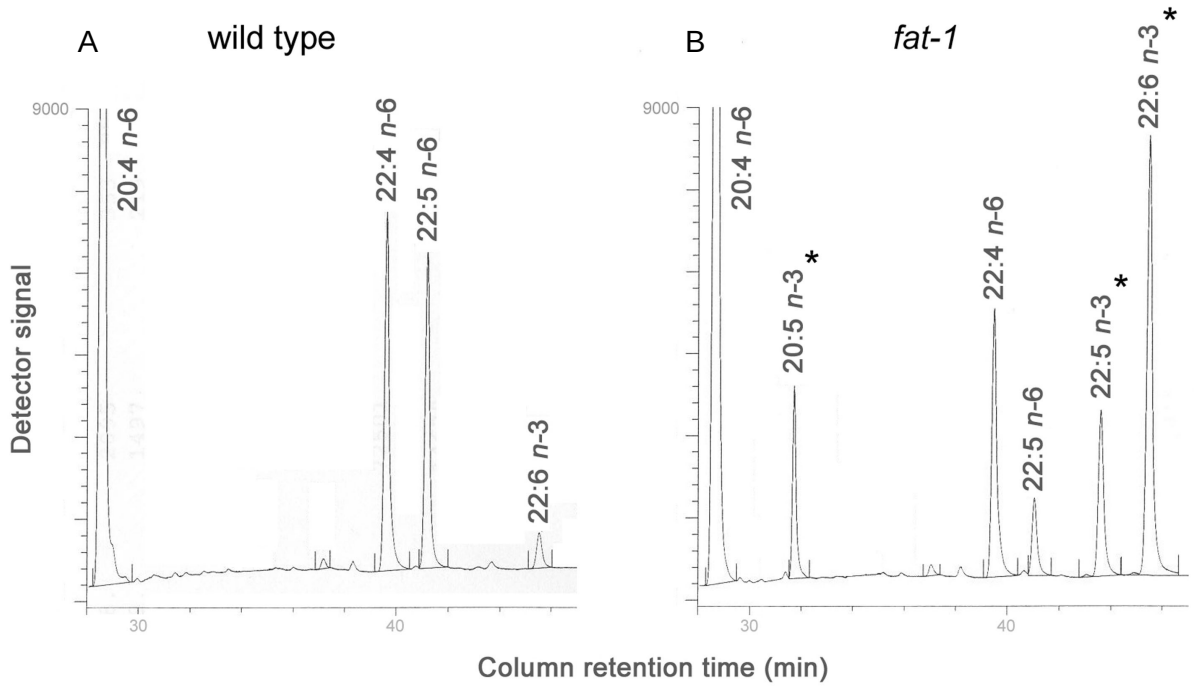

Fig. 4.1. *Differential fatty acid profiles in WT and fat-1 transgenic mice.*
Whereas high levels of n-6 fatty acids characterize WT samples (*A*), n-3 fatty acids are nearly absent. In contrast, an abundance of EPA (20:5 n-3), docosapentaenoic acid (22:5 n-3) and DHA (22:6 n-3) can be found in *fat-1* transgenic mice (*B*).
The n-3 PUFA are marked with an asterisk.

The ratio of the long-chain n-6 PUFA (20:4 n-6, 22:4 n-6, and 22:5 n-6) to the long-chain n-3 PUFA (20:5 n-3, 22:5 n-3, and 22:6 n-3) was 1.7 in *fat-1* transgenics and 30.1 in WT mice. Apparently, although both WT and *fat-1* eat the same diet, their body fatty acid profiles are distinct.

PUFA	WT (*n* = 3)	*fat-1* (*n* = 4)
n-6 (%)		
AA (20:4 n-6)	12.66 ± 2.94	12.47 ± 1.94
DTA (22:4 n-6)	3.02 ± 0.33	2.17 ± 0.38
DPA (22:5 n-6)	2.95 ± 1.13	0.57 ± 0.22
Total	18.62 ± 4.85	15.21 ± 2.22
n-3 (%)		
EPA (20:5 n-3)	0.06 ± 0.08	2.03 ± 0.47
DPA (22:5 n-3)	0.12 ± 0.15	2.45 ± 0.55
DHA (22:6 n-3)	0.43 ± 0.08	4.68 ± 0.36
Total	0.62 ± 0.15	9.86 ± 1.17
n-6/n-3 (of total fractions)	30.13	1.66

Table 4.1. *PUFA profiles of colons from WT and fat-1 mice.*

4.2 *Fat-1* transgenic mice are protected against DSS-induced colitis

Induction of colitis resulted in significant changes in body weight, stool consistence, appearance of fecal blood, and general status, typically associated with human and experimental DSS colitis.

4.2.1 Body weight change

Fat-1 mice showed significantly less body weight loss (Fig. 4.2.1) and a delayed progression of diarrhea but no apparent change in fecal bleeding. Interestingly, *fat-1* transgenic mice showed a recovery beginning from the second day after stop of DSS exposure whereas WT mice continued to lose weight progressively throughout the 3 days after cessation of DSS (**Fig. 4.2.1**).

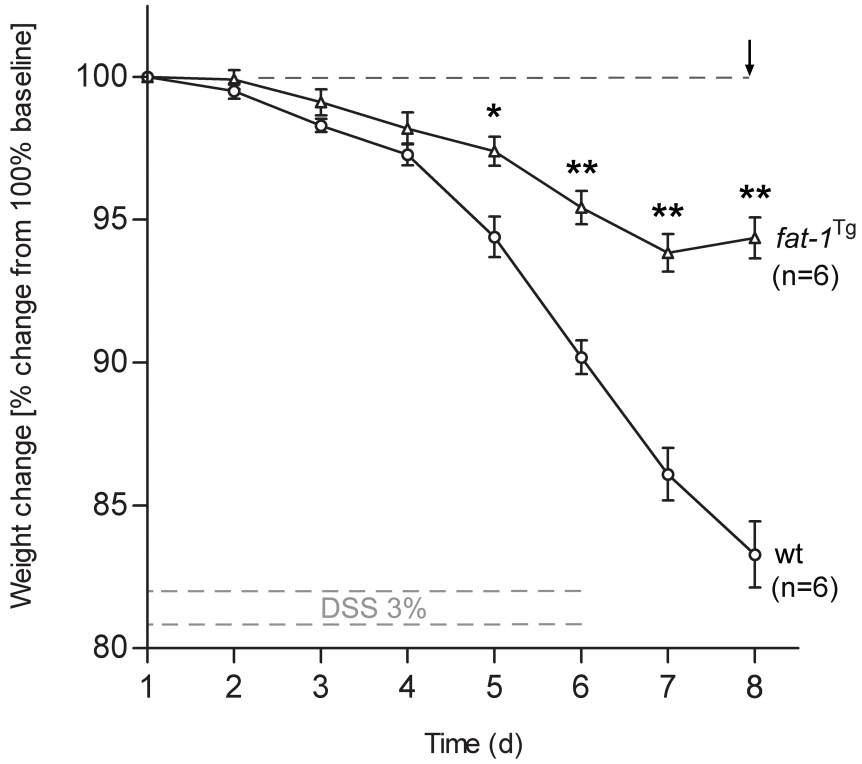

Fig. 4.2.1. *Body weight change during the course of colitis.*

Body weight change from 100% baseline over 7 days in *fat-1* mice and WT littermates (*n*=6 for each group), 5 days of DSS treatment and 2 days of normal drinking water.

*****, *P*=0.05 versus WT DSS; ******, *P*=0.01 versus WT DSS.

Mice were killed on day 8 (Arrow), and samples were taken for further analysis.

4.2.2 Macroscopic pathological properties

These clinical manifestations were reflected in the macroscopic pathological changes. Multiple adhesions, strictures, and a massive thickening of the colon were observed in WT mice but not in *fat-1* animals (**Fig. 4.2.2 A**).

Fig. 4.2.2 A. *Colon inflammation activity in WT and fat-1 transgenic mice.*
Macroscopic view of the distal colon in WT control mice (*Left*), DSS-treated WT nontransgenic littermates (*Center*), and *fat-1* transgenic mice (*Right*).

Furthermore, colon shortening amounted to 35% in WT mice but only 15% in *fat-1* transgenic mice when compared with that of untreated control mice (**Fig. 4.2.2 B**). The colon length is considered the parameter with the lowest variability in experimental DSS-induced colitis.

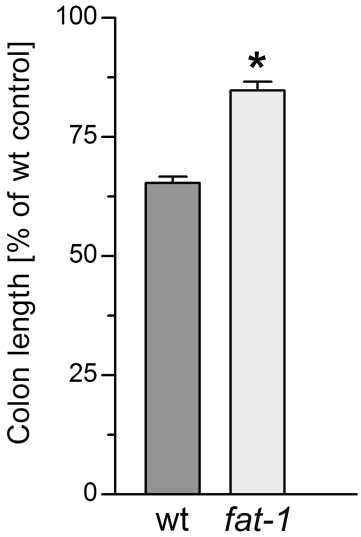

Fig 4.2.2 B. *Colon shortening.*
Colon shortening as a hallmark of DSS-induced colonic damage is reduced in *fat-1* mice.
n=6 for each group.

*, *P* =0.01 versus WT DSS

4.2.3 Histology

Microscopic assessment of the distal part of the colon revealed that severity and thickness of the inflammatory infiltrate as well as the extent of epithelial damage were significantly alleviated in *fat-1* mice (**Fig. 4.2.3 A**).

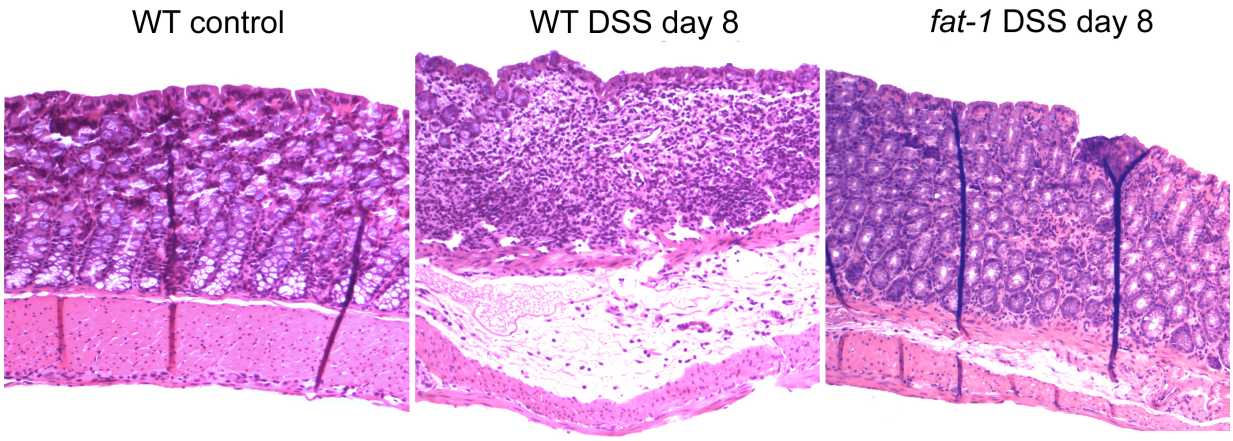

Fig. 4.2.3 A. *Histopathological changes during experimental colitis (10X).*
Microscopic hematoxylin and eosin staining of the distal colon in WT control mice (*Left*), DSS-treated WT nontransgenic littermates (*Center*), and *fat-1* transgenic mice (*Right*).

All hallmarks of colitis were reduced in *fat-1* mice except for minor punctate erosions and few ulcerations. In contrast, WT mice showed a massive fibrinous exudate on the luminal surface and marked epithelial infiltrate of leukocytes, as well as severe submucosal edema and diffuse ulcerations of the mucosa (**Fig. 4.2.3 A, B, C**).

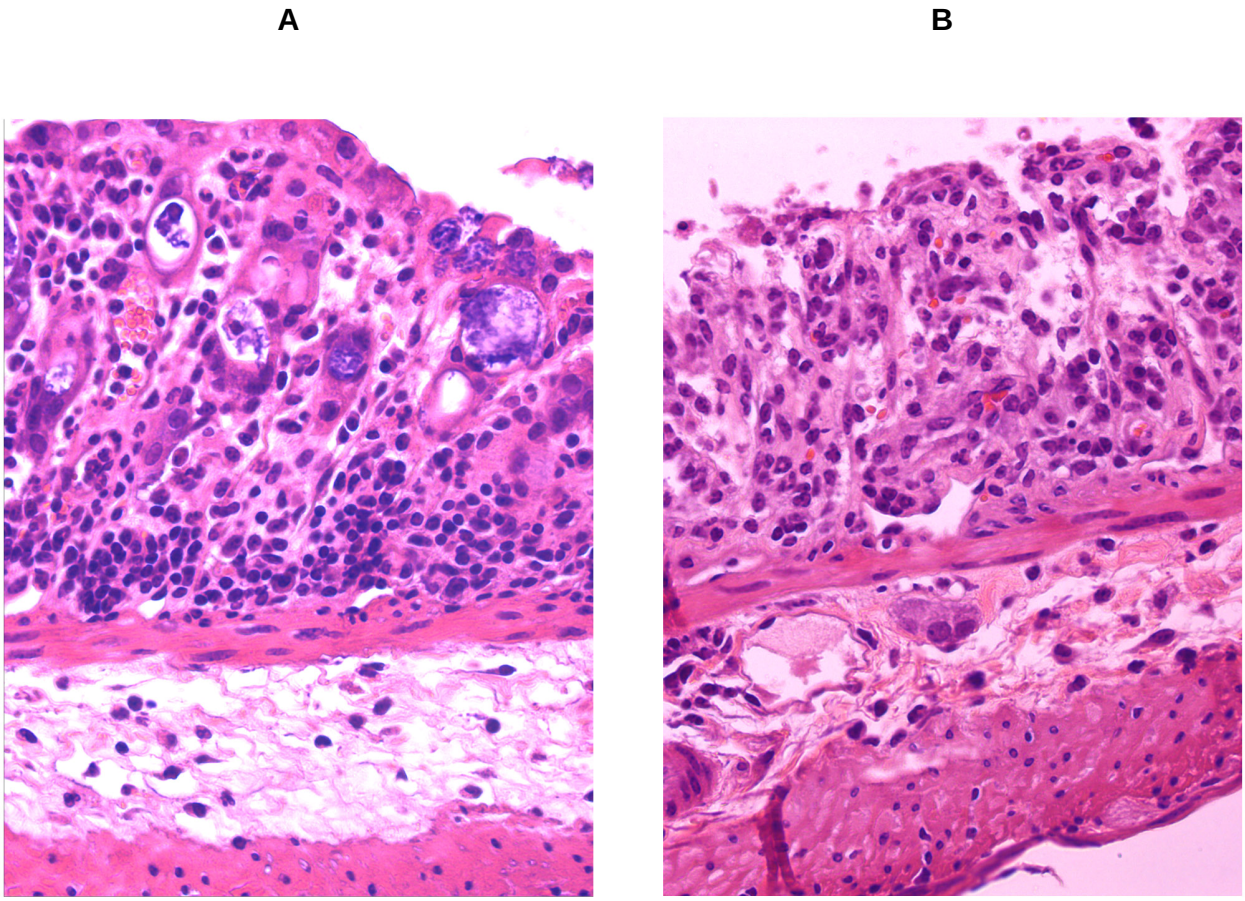

Fig. 4.2.3 B. *Histologic features of colons of WT mice (40X)*

Representative colonic cross-sections from WT mice at day 8 stained with Hematoxylin & Eosin show characteristic lesions after DSS treatment. Crypt architecture is disrupted with nearly absent goblet cells (left), or abraded in ulcerated areas (right). Massive edema of the lamina propria can be found and there is a thickly infiltrating mass of neutrophil granulocytes penetrating the intestinal wall.

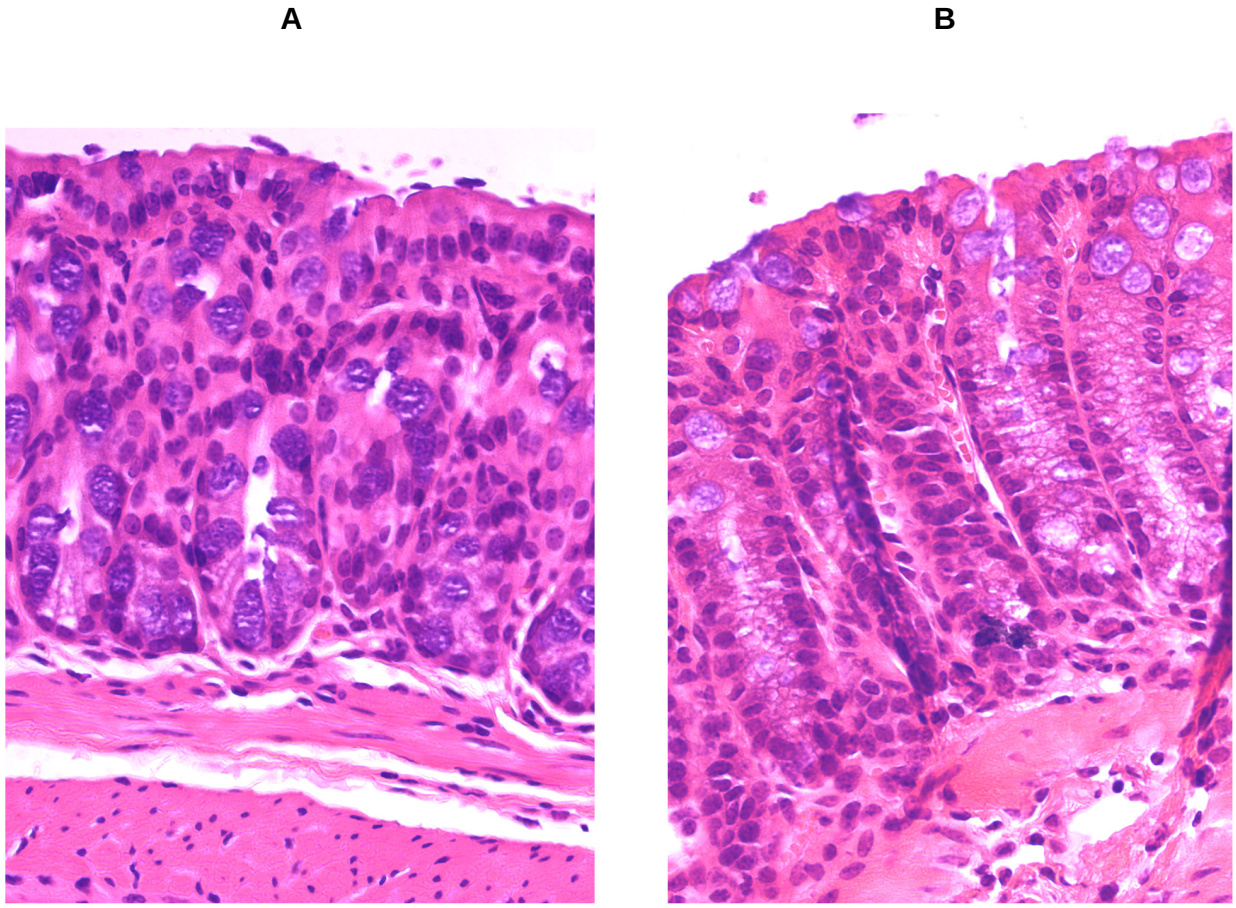

Fig. 4.2.3 C. *Histologic features of colons of fat-1 mice (40X)*

Representative cross-sections of colons from *fat-1* mice at day 8 stained with Hematoxylin & Eosin show an intact intestinal epithelial crypt architecture with abundance of mucin-producing cells. Little inflammatory exudate (edema of the lamina propria) is noticeable in the left image.

Histopathological lesions were evaluated in a blinded manner and systematically scored for severity of the inflammatory reaction and for the extent of tissue damage. Inflammatory score was 2.4 ± 0.415 in WT vs. 1.47 ± 0.371 in *fat-1* mice. Epithelial damage score was 2.83 ± 0.408 in WT and 1.58 ± 0.492 in *fat-1* mice (**Fig. 4.2.3 D**).

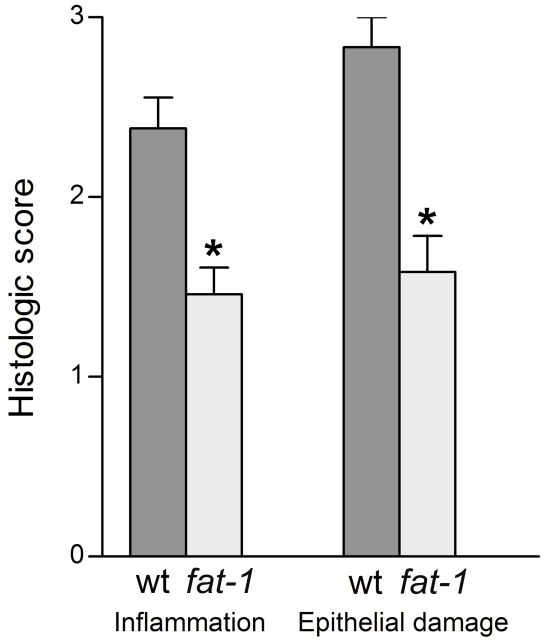

Fig. 4.2.3 D. *Histologic score.*
Histopathological scores for colonic inflammatory infiltration and epithelial damage in WT and *fat-1* mice at day 8. *n*=6 for each group.

*****, *P* =0.01 versus WT DSS.

These findings indicate that *fat-1* transgenic mice, rich in n-3 fatty acids, are protected from exacerbated inflammation after experimental DSS exposure.

4.3 Formation of n-3-derived anti-inflammatory mediators

Newly identified potent n-3 fatty acid-derived mediators such as the resolvins, including RvE1 and resolvin D3 (RvD3), and protectins, i.e., neuroprotectin D1 (NPD1)/protectin D1 (PD1), are anti-inflammatory (26-28, 64). We assessed both n-6 and n-3 PUFA-derived mediators from colons using liquid chromatography–UV–tandem MS profiling to determine whether the difference in DSS-induced colitis observed between WT and *fat-1* mice was associated with these pathways.

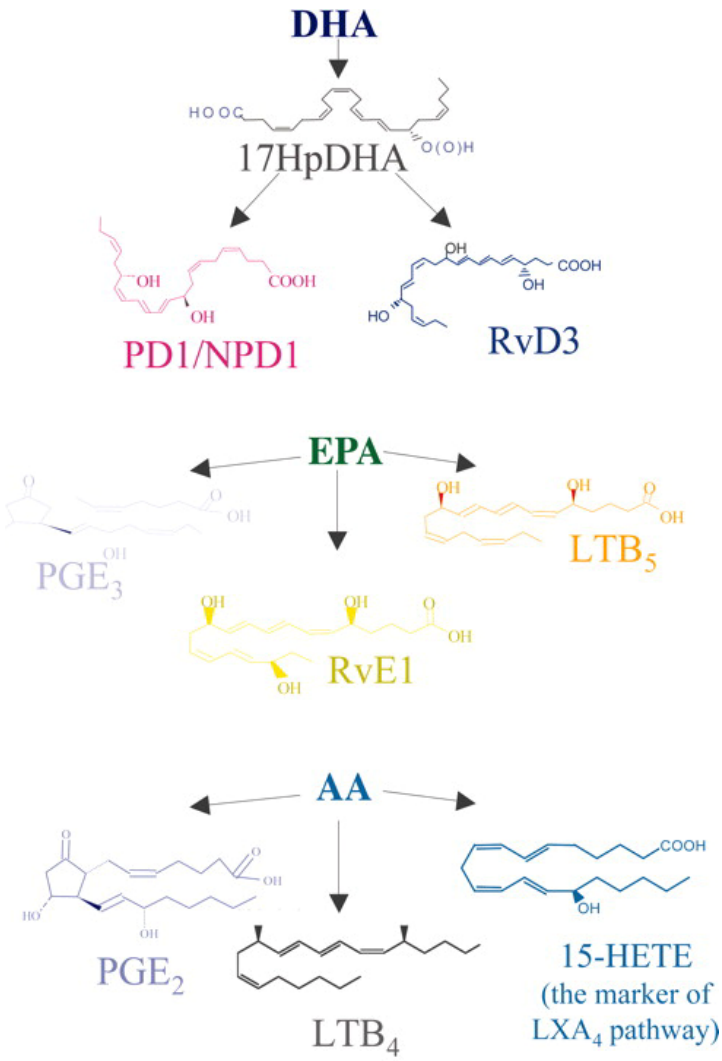

Fig. 4.3.1 *Lipid mediator genealogy.* Schematic overview of the genealogy of lipid mediators analysed by liquid chromatography-UV-tandem-mass–spectrography informatics.

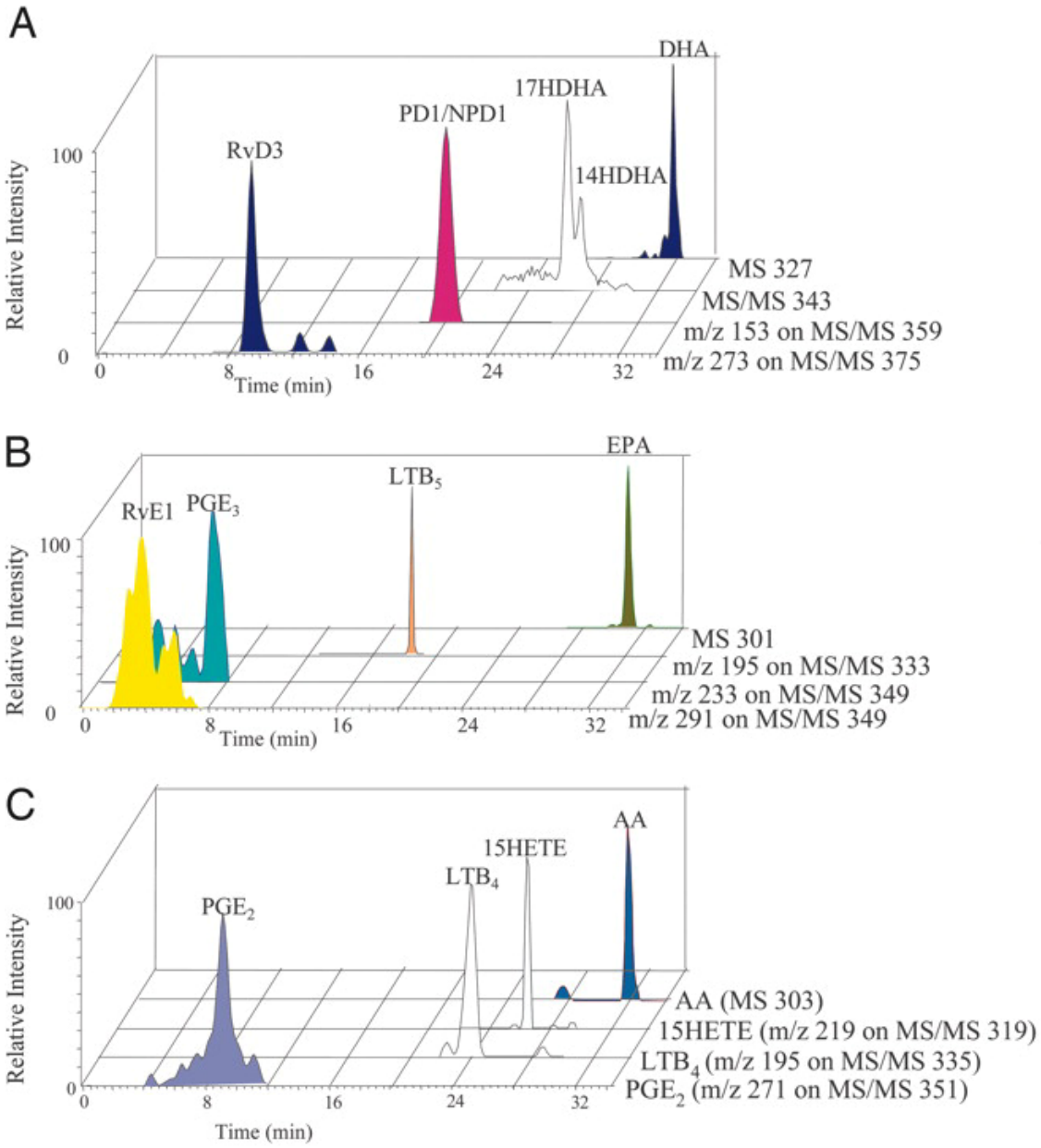

Fig. 4.3.2 ***LC–UV–tandem MS profiles of n-3 and n-6 PUFA-derived lipid mediators.***

(*A*) DHA-derived resolvins and protectins (main pathway products identified were RvD3 and PD1/NPD1).

(*B*) EPA-derived bioactive lipid mediators (identified mediators include RvE1, PGE_3, and LTB_5).

(*C*) Arachidonic acid-derived bioactive mediators [PGE_2, LTB_4, and 15-hydroxy-eicosatetraenoic acid (15-HETE) as precursor of the n-6 PUFA-derived lipoxin A4 (LXA4)].

The n-3-derived mediators, including RvE1, RvD3 and NPD1/PD1, were identified in physiologically active levels within colons of *fat-1* transgenics (**Fig. 4.3.3**). These mediators were not found in the WT colons. Also, both 17-hydroxy-DHA and 14-hydroxy-DHA as pathway markers of DHA utilization (22) were identified in *fat-1* mice. In addition to the resolvin lipid mediators, significant amounts of n-3 PUFA-derived prostaglandin E3 (PGE_3) and leukotriene B5 (LTB_5) were formed in *fat-1* mice. There were no significant differences in the levels of LTB_4, a potent chemoattractant, and the proinflammatory PGE_2. Similarly, there was no significant difference in the formation of 15-hydroxyeicosa-tetraenoic acid, the precursor for the n-6 PUFA derived anti-inflammatory lipoxin A4, between WT and *fat-1* mice.

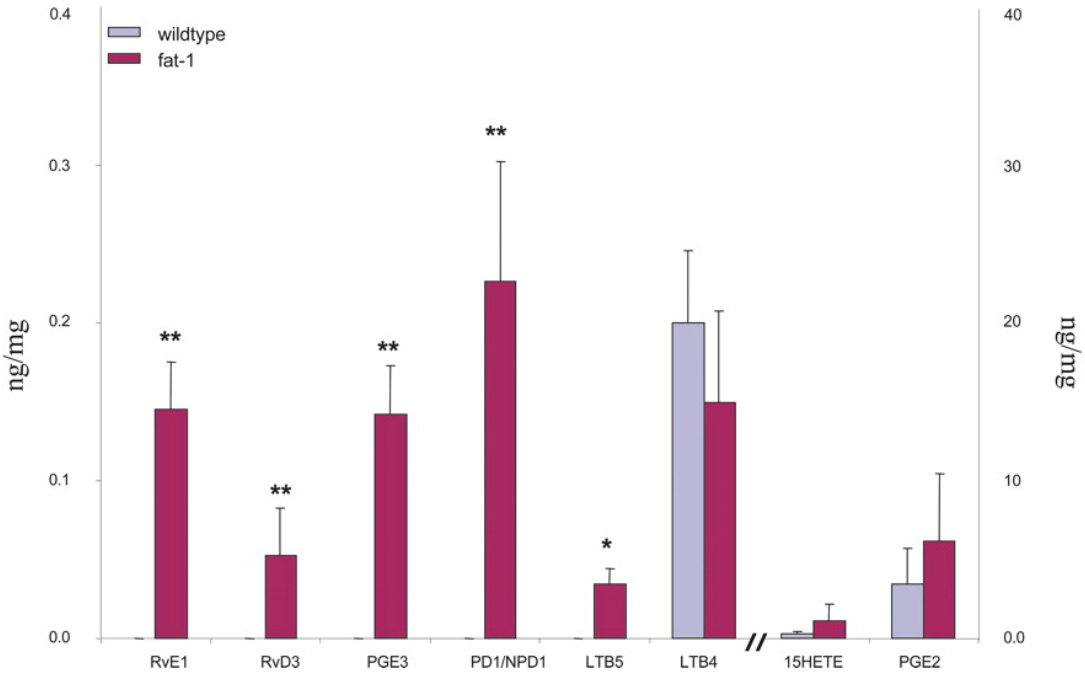

Fig. 4.3.3 *Lipidomic profiles of lipid mediators in inflamed colons.*

Presence of different lipid mediators in colon samples of *fat-1* transgenic mice (*n* =6) and WT animals (*n* =6) at day 8.

******, *P* = 0.01; *****, *P* = 0.05. Note the different scale for 15-HETE and PGE_2 (on the right).

4.4 Expression of Genes Involved in Inflammation and Colitis Pathogenesis

We next examined whether the protection from colitis observed in *fat-1* transgenics had an impact on inflammation-related gene expression.

4.4.1 Nuclear factor kappa B

Concordant with the protective action of the increased n-3 PUFA status in the *fat-1* group was a decrease in NF-κB protein activity at day 8 of experimental colitis (5.43 ± 0.64 fold induction in WT DSS vs. 2.97 ± 0.39 fold induction in *fat-1* DSS), as determined by activated p65 protein (**Fig. 4.4.1**). NF-κB is a key regulatory factor and promotes the transcription of multiple proinflammatory genes. It is noteworthy that *fat-1* control mice exhibited a significantly lower baseline activity of NF-κB (0.84 ± 0.03 fold induction) compared to WT controls without colonic inflammation.

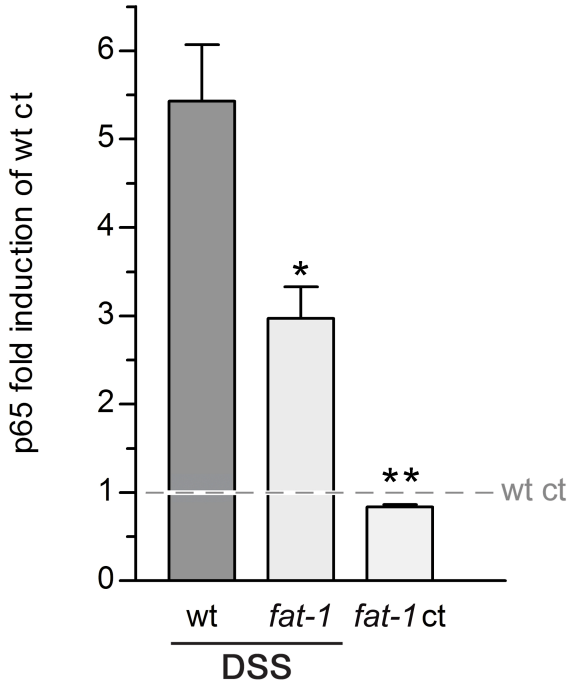

Fig. 4.4.1. *NF-κB activation assay.*
NF-κB activation reflected in p65 ELISA activity shows significant differences in control baselines and in disease between WT and *fat-1* mice. *n*=3 for each group.

*****, *P*=0.05 versus WT DSS;

******, *P* =0.05 versus WT control.

4.4.2 Tumor necrosis factor alpha

Semiquantitative real-time PCR studies of TNFα mRNA levels in colons of *fat-1* and WT mice at day 8 revealed a significantly reduced induction of this proinflammatory cytokine in the transgenic group (6.67 ± 0.75 in WT DSS vs 2.88 ± 0.43 in *fat-1* DSS). There was no siginficant difference seen between *fat-1* control mice and WT controls (**Fig. 4.4.2**).

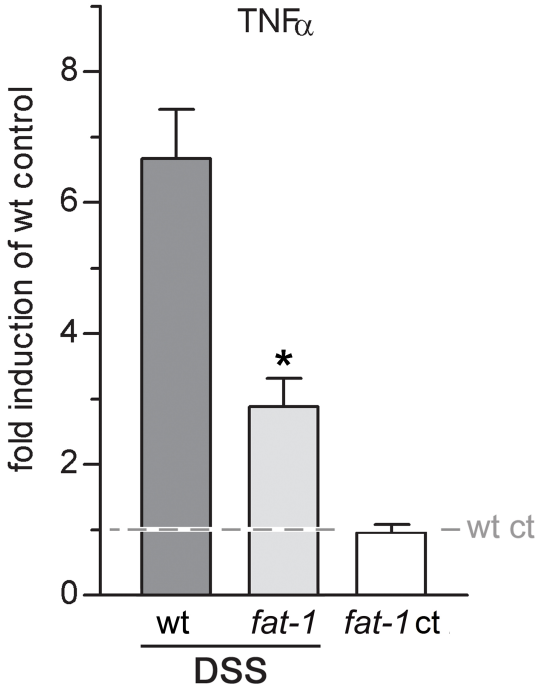

Fig. 4.4.2. *TNF alpha mRNA levels*

Semiquantitative real-time PCR analysis of mRNA expression levels of tumor necrosis factor alpha in colons of *fat-1* and WT mice after DSS exposure and *fat-1* control mice, normalized as fold increase to the baseline of WT controls (dashed line). *n*=3 for each group.

*****, *P* = 0.05 versus WT DSS;

4.4.3 Interleukin 1 beta

During colitis, transcription of interleukin 1 beta was elevated in both *fat-1* (12.70 ± 2.52 fold induction) and WT mice (97.20 ± 23.9 fold induction) compared to WT controls. There was no significant difference in mRNA expression levels between *fat-1* controls and WT controls (**Fig. 4.4.3**). However, IL-1 transcription was much less upregulated in *fat-1* mice compared to WT mice during colitis.

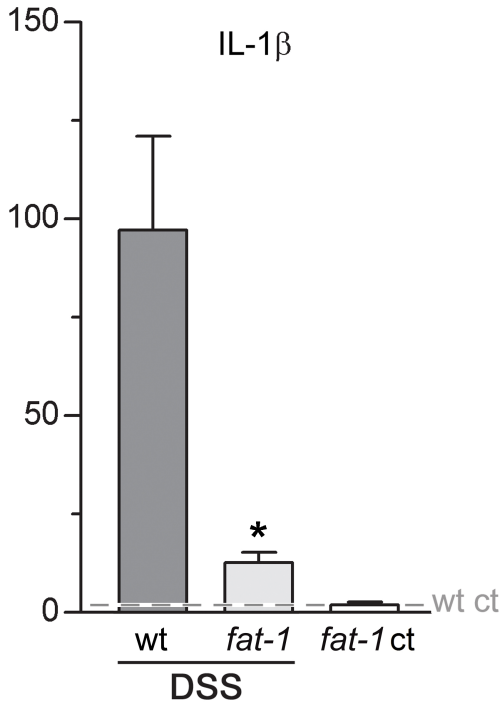

Fig. 4.4.3. *IL-1 beta mRNA levels.*

Semiquantitative real-time PCR analysis of mRNA expression levels of interleukin 1 beta in colons of *fat-1* and WT mice after DSS exposure and *fat-1* control mice, normalized as fold increase to the baseline of WT controls (dashed line). *n*=3 for each group.

*****, $P = 0.05$ versus WT DSS;

4.4.4 Inducible nitric oxide synthase

The transcription of another prominent inflammatory marker, the inducible NO synthase, was dampened in the transgenic *fat-1* mice (2.28 ± 0.59 vs. 6.30 ± 1.24 in WT DSS). No significant difference was seen between *fat-1* controls and WT controls (**Fig. 4.4.4**).

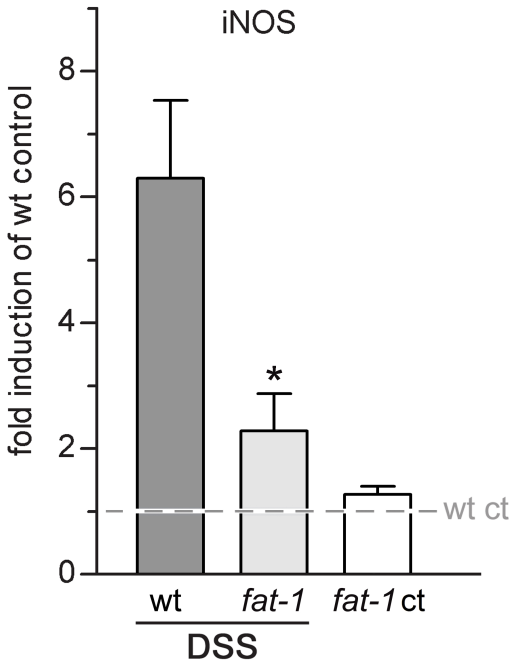

Fig 4.4.4. *iNOS mRNA levels.*
Semiquantitative real-time PCR analysis of mRNA expression levels of inducible nitric oxide synthase in colons of *fat-1* and WT mice after DSS exposure and *fat-1* control mice, normalized as fold increase to the baseline of WT controls (dashed line). *n*=3 for each group.

*****, *P* = 0.05 versus WT DSS;

4.5 Markers of mucoprotection

4.5.1 Intestinal trefoil factor

In addition, we observed that the mRNA levels of intestinal trefoil factor 3 (TFF3), a factor important in maintenance and repair of the intestinal mucosa (65), was increased in the colons of *fat-1* mice (5.69 ± 0.30 vs 3.49 ± 0.01 in WT DSS). Again, there was no significant difference in TFF3 expression levels between *fat-1* and WT controls (**Fig. 4.5.1**).

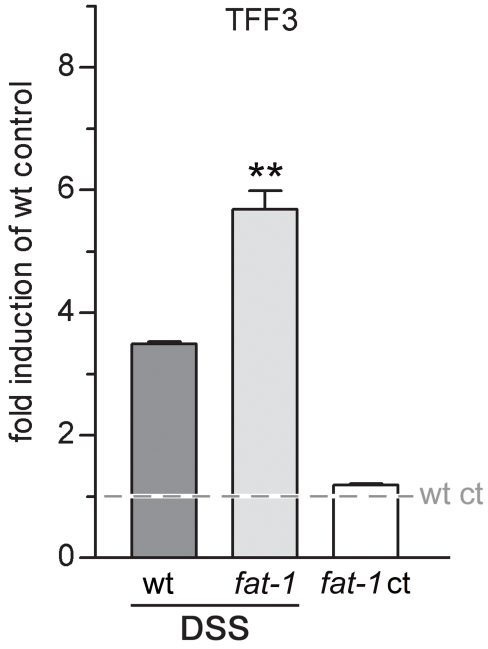

Fig. 4.5.1. *TFF-3 mRNA levels.*
Semiquantitative real-time PCR analysis of mRNA expression levels of trefoil factor 3 in colons of *fat-1* and WT mice after DSS exposure and *fat-1* control mice, normalized as fold increase to the baseline of WT controls (dashed line). *n*=3 for each group.

******, *P* = 0.01 versus WT DSS;

4.5.2 Toll interacting protein

Furthermore, mRNA levels of Toll interacting protein (Tollip), a downstream inhibitor of the Toll-like receptor pathway that mediates inflammatory response (44), were higher in *fat-1* transgenic mice (0.64 ± 0.12 vs. 0.12 ± 0.02 in WT DSS). Although not significant, Tollip expression was higher in *fat-1* control mice (1.15 ± 0.08) compared to WT controls (**Fig. 4.5.2**).

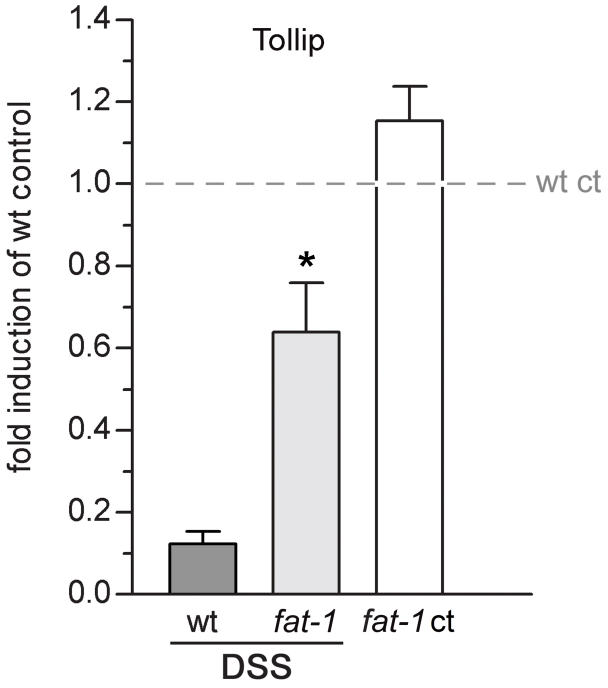

Fig. 4.5.2. *Tollip mRNA levels.* Semiquantitative real-time PCR analysis of mRNA expression levels of toll interacting protein in colons of *fat-1* and WT mice after DSS exposure and *fat-1* control mice, normalized as fold increase to the baseline of WT controls (dashed line). *n*=3 for each group.

*****, *P* = 0.05 versus WT DSS;

4.5.3 Zona occludens 1

Immunohistochemistry of full-thickness colonic sections showed that the intercellular tight junction protein zona occludens 1 (ZO-1), which is important in epithelial integrity (66), was sustained at luminal surfaces in *fat-1* transgenic animals (**Fig. 4.5.3**).

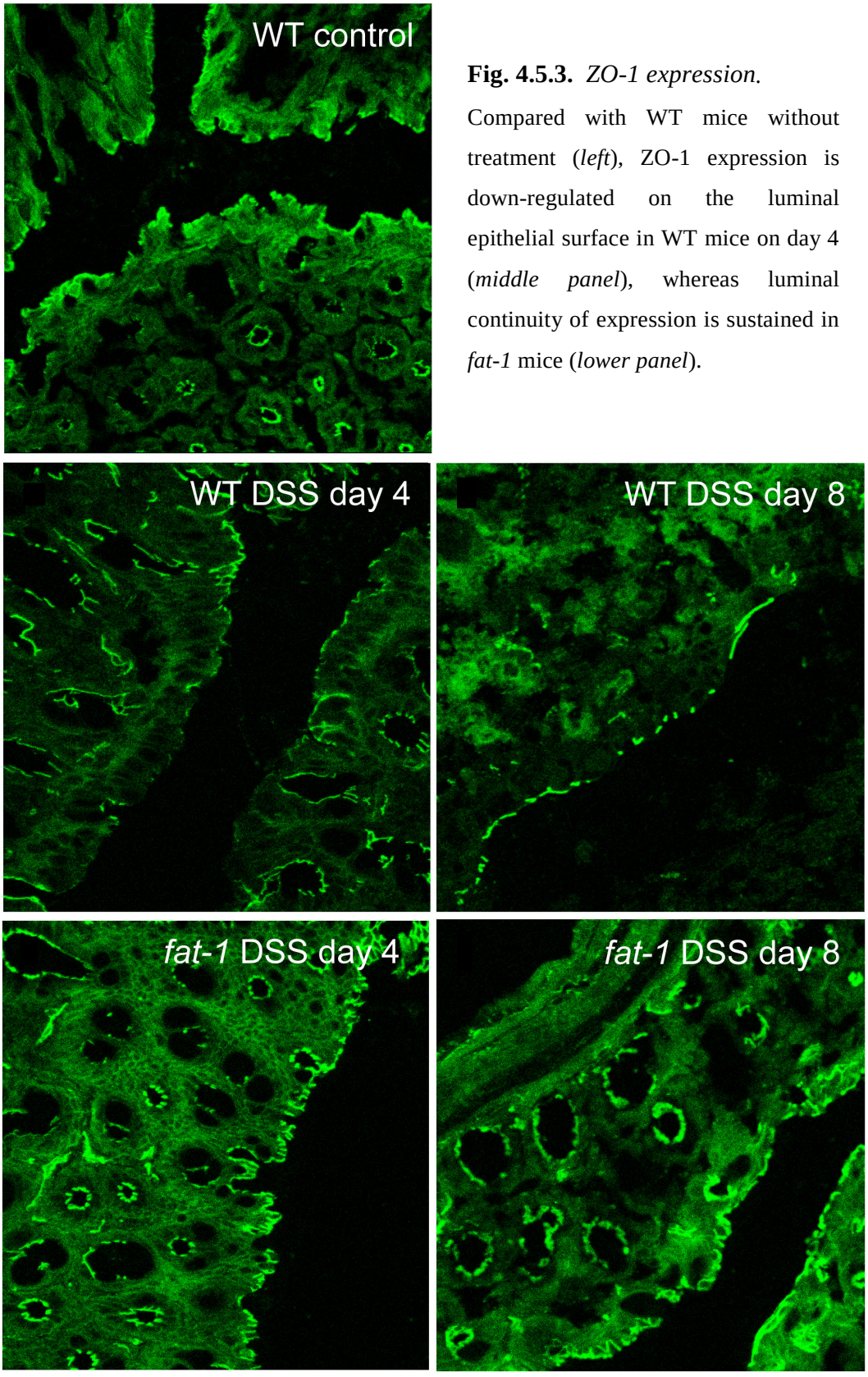

Fig. 4.5.3. *ZO-1 expression.*
Compared with WT mice without treatment (*left*), ZO-1 expression is down-regulated on the luminal epithelial surface in WT mice on day 4 (*middle panel*), whereas luminal continuity of expression is sustained in *fat-1* mice (*lower panel*).

5 Diskussion/ Discussion

5.1 Summary of results

This study is the first to investigate an experimentally-induced model of colitis in the recently engineered transgenic *fat-1* mice with an enhanced n-3 PUFA status. While similar amounts of n-6 PUFA arachidonic acid were detected in transgenic *fat-1* mice and in their wild-type littermates, significant levels of n-3 PUFA DPA, DHA and EPA were only detected in lipid profiles of *fat-1* mice. Considering the fact that all other variables in the experimental set-up had been identical in both groups, we have come to the conclusion that this n-3 PUFA status itself must have influenced the ameliorated course of disease in the transgenic mice.

Inflammation dampening was reflected in differential clinical manifestations of DSS colitis, most importantly, a less drastic loss of body weight and in improved histological and pathological results in the *fat-1* mice. We observed a smaller amount of inflammatory infiltrate, to a lesser extent, epithelial damage as well as reduced colon shortenings and strictures in the *fat-1* mice. Lipidomic analysis of inflamed colon specimens revealed that during colitis, the *fat-1* mice rather than the WT mice, generated novel n-3-derived mediators of resolution (resolvins and protectins). The protection from colitis in the transgenic group also correlated with downregulation of the proinflammatory molecules NF-κB, IL-1β, TNFα and iNOS. The protection also matched with the induction of the mucoprotective factors TFF3 and ZO-1 in the colons of these animals.

In summary, these findings suggest that an increased tissue status of n-3 fatty acids can protect against colitis due to the generation of anti-inflammatory and proresolution lipid mediators that regulate inflammatory gene expression via inhibition of the transcription factor NF-κB.

5.2 Advantages and limitations of this study

5.2.1 Transgenic *fat-1* mice

Although a number of previous studies have examined the effectiveness of n-3 fatty acids in prevention and treatment of human colitis (37, 67-70), the outcomes were inconsistent or conflicting. The discrepancy may be due to confounding factors of diet or n-3 fatty acid supplements. In fact, many variables can arise from the diets and the feeding procedure. These include the impurity or unwanted components of the oils used (e.g., fish oil versus corn oil), sensitivity to oxidation, diet storage and duration of the diet change. Finally, patient compliance is a key explanatory variable in clinical trials and critically affects their reliability. It is known that patient compliance for the intake of fish oil might be compromised by its flavor and some unpleasant side effects on the digestive system (fishy aftertaste, flatulence, indigestion, laxative effect, heartburn).

In contrast, the genetic approach using the *fat-1* gene, as presented here, only modifies the n-6/n-3 fatty acid ratio (endogenously converts n-6 to n-3) and thereby allows experimental animals (i.e., WT and transgenic littermates) to be fed with a uniform diet. In contrast to feeding studies, total amount of lipids is not altered by fatty acid conversion. Therefore, the results obtained by using this model are more reliable. It was our primary goal to elucidate the underlying mechanisms of the beneficial effects of n-3 PUFA on inflammatory conditions. While the transgenic model is uniquely useful in this regard, it is certainly open to question whether the methodic advantages can be translated to the human circumstances since mammals do not have the *fat-1* gene and supplementation of n-3 fatty acids remains the therapeutic strategy. Here, clarification of the metabolic pathways of these fatty acids to bioactive proresolutionary mediators and their phase-dynamic impact on immunoregulation and tissue protection may enhance the knowledge for future therapeutical indications (e.g. by administration of synthetic RvE1 during colitis).

5.2.2 DSS induced colitis

As with all experimental animal models, relevance of murine DSS colitis to the human disease is limited and results obtained from animal studies cannot be unquestioningly transferred to the human condition. The etiology of CD and UC is pluricausal, rendering a yet not fully understood structure of susceptibility to disease development, while experimental DSS colitis is induced by a chemical agent via a clearly defined mechanism. While DSS colitis resembles features of IBD, it is unclear at which point and to what extent its mechanism is effective in the causal chain of pathoetiological events leading to human disease. Furthermore, the 8-day course of the experimental setup can only simulate an acute state of inflammation, but it is not able to recreate the lifelong alternation of remission and relapses that are typical for human IBD. It therefore focuses more on the immediate local host responses and adaptations of the innate immune system and may not be the adequate instrument to investigate adaptive immunologic processes that accompany the chronic alternating inflammatory conditions of human IBD.

In pilot studies we found that reproducibility of the experimental course was critically determined by mouse gender (male mice were more susceptible to DSS induced injury), age, weight and strain. To minimize influence of these factors to the course of DSS colitis, only female C57BL/6 mice with a body weight of 19-21 gram and a designated age of 9-10 weeks were used for the experiments. One major criticism of the DSS model of colitis regards the question whether all mice have a comparable intake of DSS. Since DSS is fed to the mice via drinking water, it is of crucial importance that every animal has unrestricted access to the feeding bottle. Therefore each cage housed only one transgenic and one WT mouse during our experiments. It is also known that discontinuity of day and night cycles/exposition to light can alter drinking habits of mice. This concern was addressed by automatically controlled duration of day/night cycles in the animal facility. While it is impossible to exactly monitor the individual intake of drinking water for each mouse and interindividual discrepancies in DSS ingestion cannot be excluded, the homogenic responses within the transgenic and WT groups indicate that this may not be a principal limitation of the results presented here.

5.2.3 Methods of biomolecular analysis

Semiquantitative real-time PCR was employed for gene expression profiling of cytokines and mucoprotective compounds in the colitic and control colons of *fat-1* and WT mice. Compared to other methods of mRNA expression analysis (e.g. Northern Blot, RT-PCR with gelelectrophoresis), this relatively recent technique is faster, more sensitive, more reliable and has less risk of contamination. Nonetheless, the utilization of optimized primers and an adequate housekeeping gene are crucial prerequisites for the quality of this otherwise well standardized procedure. All primer sequences were obtained from Primer Bank of the Department of Molecular Biology at Harvard Medical School and have been extensively tested for real-time PCR quantification (61). Housekeeping genes are necessary to normalize mRNA expression values for interindividual comparison. Therefore, these internal control genes must be expressed constitutively in all cells in a constant, comparable and independent manner. GAPDH, a key enzyme of glycolysis metabolism, is one of the most widely used normalizing standards and has been validated in a multitude of tissues and experimental conditions, including a vast corpus of colon specimens in DSS induced colitis (71-74).

5.3 The N-3/N-6 PUFA ratio

In the 5th century BC, Hippocrates states that ‘Positive health requires a knowledge of man's primary constitution and of the powers of various foods, both those natural to them and those resulting from human skill. If there is any deficiency in food or exercise, the body will fall sick.’ This concept of nutrition as a major environmental factor interacting with the human genetic constitution appreciates two fundamental susceptibility factors of disease, that have become exquisitely prominent in recent decades, especially with the many of civilizational maladies plaguing our modern industrialized societies.

Studies of the paleolithic diet suggest that our ancestors evolved on a diet containing roughly equal amounts of n-6 and n-3 fatty acids (75). During civilization and most importantly, the advent of agriculture with dependence on grain diets and animal husbandry, this balanced n-6/n-3 ratio underwent a drastic change in favor of total saturated fats and n-6 PUFA. Industrialization, appearance of agribusiness with processed foods, grain-fattened livestock and hydrogenation of vegetable fats have been implicated in the further diminution of n-3 fatty acids from foodstuff.

Today, modern western diets have a n-6/n-3 ratio of ~15:1 (36). With the human genetic profile being established on a 1:1 ratio during the Paleolithic period 40.000 years ago, this shift appears to be conflicting since the body may not adjust its genetic pattern so swiftly to adapt to the new ratio. In fact, with an estimated spontaneous mutation rate for nuclear DNA at 0.5% per million years, the vastly changed dietary habits of humans during the last 100 years, including the loss of n-3 PUFA intake, face a potential adaptive modification in genes of about 0.005% during the last 10.000 years .

The experimental design of this study allows us to simulate a balanced essential fatty acid composition in the *fat-1* mice. In contrast, WT mice are almost entirely deplete of n-3 PUFA. Therefore, given the small amounts of n-3 PUFA actually present in human foodstuff of the western world today, the WT group in our study may even have extrapolated the effects of this nutritional shortcoming in the present results.

5.4 A Paradigm change

5.4.1 Leukotrienes and Prostaglandins

The n-3 fatty acids may exert an anti-inflammatory effect via competitive inhibition of the n-6 (arachidonic acid)-derived proinflammatory eicosanoids, most notably LTB_4 and PGE_2, and this has been the mechanism mostly proposed in the past to explain the biological effectiveness of n-3 fatty acids (76, 77).

LTB_4 is suggested to play a pivotal role in the genesis of colitis (78), and decreased rectal dialysate levels of LTB_4 have been found in patients with UC who were treated with fish oil and who exhibited improved histological findings and weight gain (68). However, the disruption of LTB_4 synthesis by specific inhibition of the 5-LOX resulted in decreased LTB_4 levels in the colonic mucosa of UC patients without any beneficial impact on the course of the disease (79). This leads to the conclusion that a decrease in LTB_4 production is probably not the primary mechanism by which fish oil mediates its beneficial effects. Even more intriguing, this observation indicates that the 5-LOX is a requisite for the protective activity of fish oil. In fact, the 5-LOX is critically involved in the generation of newly discovered n-3 derived resolvins and protectins.

In this study, contrary to our expectations, we found no significant differences in the content of arachidonic acid, LTB_4, and PGE_2 between *fat-1* transgenic and WT mice. Instead, there was a remarkable difference in the amounts of n-3 fatty acids (EPA, DHA, and precursors) and their potent bioactive products, the resolvins and protectins (RvE1, RvD3 and PD1/NPD1), as well as the n-3 PUFA-derived LTB_5 and PGE_3 (Fig. 3). It is possible that LTB_5 and PGE_3 could exert an anti-inflammatory effect through competition with LTB_4 and PGE_2. However, given the much higher absolute concentrations of LTB_4 and PGE_2 present in the *fat-1* mice this may not be the major underlying mechanism.

5.4.2 New n-3 derived lipid mediators of resolution

While inflammation is a vital reaction to the host defense, self-limitation of the acute inflammatory response is necessary to protect tissues and to return to homeostasis after the pathogen has been eliminated. This resolution phase comprises an active termination program governed by a lipid mediator class-switch from the amplificatory n-6 derived eicosanoids to the recently stereochemically assigned lipoxins and resolvins (27).

The n-3-derived resolvins (RvE1-2, RvD1-6) and protectins (PD1) are potent anti-inflammatory compounds that have been identified in the resolution phase of dorsal skin pouchitis in mice. They have been shown to be protective in various settings of acute inflammation (26, 27, 30, 64). In a model of trinitrobenzene sulfonate colitis, treatment with synthetic RvE1 resulted in an ameliorated course of disease and was accompanied by decreased formation of the proinflammatory molecules TNFα, IL-12 p40, iNOS and COX-2 (64). Our data confirm decreased levels of cytokines also in the *fat-1* mice with DSS-colitis, which may be due to the documented lower NF-κB activity in *fat-1* mice shown here.

5.4.3 Mechanism of RvE1 action and NF-κB regulation

Indeed, Makoto Arita et al. have shown that RvE1 inhibits NF-κB activation through its specific G protein-coupled receptor, ChemR23. ChemR23 is mostly expressed on antigen presenting cells like macrophages and dendritic cells and regulates migration and cytokine production of these cells in acute inflammation (29). Campbell et al. found that, besides attenuating transepithelial migration of PMN, ChemR23 signalling also increased their clearance from the mucosal surface by upregulating the anti-adhesive CD55 (80). Furthermore RvE1 blocks LTB_4-induced calcium mobilization and NF-κB activation in PMN by binding to the LTB_4-receptor BLT1 as a partial agonist (81). Thus, RvE1 dampens propagation of proinflammatory signals mediated by cytokines and eicosanoids and actively promotes resolution of the acute inflammatory response.

Our data are consistent with this mechanism of action. Furthermore, our study is the first to show that protectins and resolvins, including RvE1, are endogenously generated in vivo from the n-3 PUFA DHA and EPA in the transgenic *fat-1* mice in inflamed colonic tissue. During the 8-day course of acute experimental colitis, biologically relevant amounts of RvE1, RvD3 and PD1 were synthesized in these animals, while they were absent in the WT group. Accordingly, NF-κB activity, measured by a p65/RelA ELISA assay, was significantly dampened in colonic specimens from transgenic mice compared to their WT littermates at day 8.

Fat-1 mice exhibited similar clinical signs of DSS colitis compared to the WT group in the early phase of the present experiment, representing the acute inflammatory amplification phase in response to the induced toxic disruption of the epithelial structure by dextran molecules. Nearly all animals (of both groups), had a positive hemocult, indicating occult blood in stools, at day 1, exhibited diarrhea and progressively lost body weight. Nonetheless, the extent of these features appeared to be less severe in the *fat-1* group and loss of body weight stagnated from day 7 (2 days after cessation of DSS feeding), which may account for a beginning recovery.

WT mice, suffering from an ongoing aggravated course of disease without signs of recovery, were devoid of the active resolution-phase interaction compounds found in the transgenic group, as quantified in liquid chromatography-tandem mass spectrometric lipid mediator analysis. In view of these results, the presence n-3 fatty acid-derived mediators documented in the *fat-1* transgenic mice link an enhanced n-3 PUFA status to inflammation dampening and resolution.

5.5 Proinflammatory cytokines and oxidative stress

Human IBD is characterized by an imbalance between pro- and anti-inflammatory cytokines that leads to a pathologic immune response. Although the pathogenesis of DSS colitis appears to be T cell-independent (82), cytokine production plays a key role in the development of colitis in this model (11, 12). In fact, cytokines are important mediators of inflammation (83) and increased production of proinflammatory cytokines such as TNFα, IL-1β, IL-12, and IFN-γ is found in inflamed colons from patients with IBD (84) as well as DSS colitis animals (11, 12).

Interestingly, Schreiber et al. report that increased serum levels of TNFα and IL-1β precede relapses in CD, suggesting a correlation between overproduction of these inflammatory mediators and clinically symptomatic disease progression (85). Further evidence for the involvement of these cytokines came from the observations that antibodies against TNFα and IL-12 reduced the severity of the disease in the animal model of DSS-induced colitis (86, 87) as well as patients with Crohn's disease (88). Thus, reduction of proinflammatory cytokines appears to be an effective approach to the prevention and treatment of IBD.

5.5.1 Tumor necrosis factor alpha

During experimental colitis the colons of *fat-1* mice, rich in n-3 fatty acids, exhibited significantly lower amounts of the proinflammatory cytokine TNFα than those from WT animals. Many animal studies have shown that TNFα activity correlates with severity of the disease and its overexpression is associated with an IBD-like phenotype in mice (89). Also, it is well appreciated that TNFα is a critical pathogenic player in human IBD and the central role of anti-TNFα therapy in exacerbated disease further highlights its importance (2). It must be noted that the effect of Infliximab appears to be primarily mediated by its proapoptotic signalling, inducing programmed cell death in TNF secernating cells, such as T-lymphocytes and monocytic cells. Isolated reduction of soluble TNFα, as accomplished with the TNF-binding TNFR/IgG fusion protein Etanercept, did not show a significant effect in CD (90).

In this light, the documented efficacy of n-3 PUFA in blocking macrophage/monocytic TNFα production as observed in vitro (91), in human trials (92, 93) and in the experimental setup of this study, may link to the inhibition of NF-κB (mediated by its anti-inflammatory derivates, the resolvins via the specific receptor Chem23) and the subsequent induction of apoptosis in these cells.

5.5.2 Interleukin 1 beta

Synergism of TNFα and IL-1β is well known and could be observed also in the present study. As with TNFα, IL-1β levels were upregulated during colitis in all experimental groups compared to control animals. In *fat-1* mice, this upregulation was significantly less pronounced.

Cell culture studies showed that EPA and DHA can inhibit monocytic synthesis of IL-1β and decrease the ex-vivo production of IL-1β by murine macrophages. Decreased production of IL-1 has also been shown in IBD patients treated with fish oil in healthy and colitic conditions (92, 93) and in inflamed colon specimens incubated in fish oil (94).

This finding suggests that the inflammation protection observed in *fat-1* mice may be, in part, the result of the reduction in cytokine production and action by n-3-derived resolvins.

5.5.3 Inducible nitric oxide synthase and respiratory stress

Elevated iNOS expression levels found in WT and *fat-1* mice during colitis are consistent with a number of experimental animal studies as well as with findings in colons of IBD patients. Ljung et al. investigated the NO levels in rectal biopsies from Children with CD and UC. They found that, while NO was greatly increased in children with active disease, low NO concentrations with no significant differences to healthy controls were measured in quiescent CD and UC. Thus it seems that NO activity is closely associated with the state of the disease and highlights its detrimental role in the pathophysiology of IBD (95). Beck et al. performed immunohistochemical studies during experimental DSS colitis, revealing that iNOS expression was mainly upregulated within inflammatory cell infiltrates of the lamina propria and the submucosa in areas of ulceration and inflammation. In these experiments, iNOS-/- knock out mice had less severe features of DSS-induced colitis, while nNOS-/- and eNOS-/- knock outs were not protected (96). This supports the role of the iNOS isoform as the critical source for exacerbated NO production during inflammatory events.

In this study, *fat-1* mice expressed significantly lower levels of iNOS than their WT littermates and had a significantly ameliorated course of disease. It appears that this protection was in part afforded by inhibition of an exacerbated NO production as an executive agent of respiratory stress and subsequent tissue damage.

5.6 Enhanced mucoprotection

The results of the present study also support a role for n-3 fatty acids in the maintenance of intestinal integrity, as demonstrated by higher levels of the mucosal protective factors Tollip, TFF3, and ZO-1 in the colon tissues of *fat-1* transgenic mice.

5.6.1 Intestinal Trefoil factor 3

Intestinal TFF3 is secreted by goblet cells throughout the entire colon onto the luminal surface under physiological conditions. Under disease conditions TFF3 promotes epithelial cell migration into damaged areas to help in the reestablishment of mucosal integrity. In this context, mice lacking intestinal trefoil factor may suffer from an impaired epithelial defense and are more vulnerable to inflammatory injury (65). Conversely, Vandenbroucke et al. used a genetically altered lactococcus to actively deliver trefoil factors to the colonic mucosa and observed protection from DSS-induced epithelial tissue damage (97).

Our results show a favorable effect of n-3 fatty acids on this system, as evidenced by the stronger upregulation of TFF3 in *fat-1* transgenic mice during experimental colitis. While there was some induction of TFF3 expression in wild type mice when challenged with DSS, this was significantly less pronounced than in the *fat-1* group. This indicates that the increased TFF3 activation in *fat-1* mice is a primary regulatory event afforded by the differential fatty acid composition of these animals, leading to an ameliorated course of disease.

Furthermore, this finding is also concordant with recent expression profiles gathered from Danish patients with ulcerative colitis and Crohn's disease. Here, increased TFF3 levels in active disease, correlated with disease activity indices and appeared to reflect the protective/restitutional capabilities to confront the challenge of intestinal damage caused by the disease (98). In this light, an enhanced n-3 PUFA status may strengthen the host protection by increasing the expression of intestinal trefoil factors.

5.6.2 Toll interacting protein

The innate immune system maintains a steady state of benign physiologic inflammation in coexistence with the luminal commensal bacteria. Toll-like receptors sense components of these micro-organisms (e.g., LPS) and lead to a delicately regulated downstream signalling cascade that balances an appropriate mucosal response by production of protective factors (39) or inflammatory mediators (42).

In the uninflamed colon a state of reduced sensitivity to bacterial products like LPS inhibits an exaggerated activation of the transcriptional factor NF-κB and the consecutive proinflammatory stimuli. Otte et al. showed that expression of Tollip, a downstream regulator of the Toll-like receptor pathway, was directly correlated with the luminal bacterial load and inversely correlated with NF-κB activation after repeated exposure of an intestinal epithelial cell line to LPS (44). Overexpression of Tollip results in inhibition of IL-1R, TLR-2 and TLR-4 mediated NF-κB activation (43, 99). Tollip appears to play an exquisite role in maintaining mucosal hyporesponsiveness to the luminal commensal bacteria. Dysregulation of this balancing system may contribute to the onset and chronification of intestinal inflammation.

Further evidence for the immunoregulatory role of Tollip and its implication in IBD pathoetiology comes from a recent gene expression cluster analysis in peripheral blood monocytes from Japanese patients with Crohn's disease. Here, decreased Tollip expression before and after lipopolysaccharide stimulation was detected compared to control patients (100).

It is possible that n-3 fatty acids may preserve this system, because our results showed that, during experimental colitis, the inhibitory Tollip was markedly reduced in WT mice but sustained in *fat-1* transgenic mice. Although not significant, it is worthy of notice that even healthy *fat-1* control mice without treatment expressed higher amounts of Tollip compared to WT controls. This finding is corresponding to the differing baseline NF- κB levels measured in the same animals.

Tollip features a C2-like domain in the N-terminus that might be important for its inhibitory function. It is known that C2 domains in other proteins act as binding sites for various phospholipids. Li et al. observed a preferred binding of the Tollip C2-like domain to phosphatidyl-inositol-3-phosphate (PI). In Tollip K150E mutants, exchange of a lysine residue for glutamic acid within the C2 domain abolishes this lipid binding and Tollip K150E mutants fail to inhibit LPS-induced NF-κB activation (101). This indicates that phospholipid binding may be an obligatory prerequisite to the regulatory action of Tollip and leads to the intriguing hypothesis that the nature of fatty acid (n-6 vs. n-3) incorporated into PI might modulate its inhibitory potency. However, from the observations made in this study it is not possible to identify the exact mechanism by which n-3 fatty acids influence the Toll/IL-1 mediated downstream cascade and if the documented sustenance of Tollip expression during inflammatory conditions is a primary event afforded by lipid mediator signalling.

5.6.3 Zona occludens 1

Diarrhea is a main hallmark of both Crohn's disease and ulcerative colitis and an increased intestinal permeability has been found in patients with CD and UC and their asymptomatic at-risk relatives (102-104). In acutely inflamed or chronically damaged areas of patients with IBD a defective epithelial barrier function was associated with an altered tight junction (TJ) structure (105). In fact, altered expression patterns of TJ proteins were found in colon specimens of IBD patients, exhibiting a decrease in occludin and ZO-1 (66, 106). In an *in vitro* study with human T84 intestinal epithelial monolayers, Nash et al. showed that PMN migration occurs via a paracellular route (107).

Nonetheless, perturbations of tight junctions caused by PMN were rapidly reversible as demonstrated in the same cell line by Nusrat et al., suggesting a transient event of epithelial barrier opening without permanent morphological discontinuities (108). Ma et al. found that, in Caco-2 monolayers, TNFα increases tight junction permeability in a time- and dose-dependent manner, which is associated with activation of NF-κB and loss of ZO-1. Inhibition of NF-κB restored Caco-2 tight junction function (109).

Immunofluorescent stainings of WT and *fat-1* colons were assessed at day 4 and day 8 to evaluate protein level and localization of ZO-1. In DSS colitis, the loss of ZO-1 protein and concomitant increase in epithelial permeability occur as an early event after DSS administration, prior to histological signs of inflammation and clinical manifestations of colitis (110). On this account, we chose to set two timepoints of evaluation to be able to discriminate changes of protein levels during inflammation from a simple loss of cellular structure by ulcerous tissue destruction. Concordant with the findings described above, we found that the tight junction protein ZO-1 was globally downregulated at the epithelial luminal surface in colons from WT mice during DSS-induced colitis at day 4, while its expression was sustained in *fat-1* mice given the same treatment. Reepithelialisation processes can be recognized on an ulcerated area in WT mice at day 8.

Taken together with our other findings, this supports the hypothesis that rather than being a secondary event to the inflammatory process, active modulation of paracellular epithelial permeability represents a prerequisite for the initiation of the inflammatory response. Its pathologic increase, compromising the intestinal epithelial barrier sealing leads to the sustained exposure of immunocompetent cells of the lamina propria to normally excluded luminal antigens and results in selfperpetuating inflammation.

Thus, it seems that up-regulation or maintenance of mucoprotective factors (TFF3, ZO-1 and Tollip) in the n-3 PUFA-enriched tissues may be one of the underlying mechanisms for the observed protection against colitis in *fat-1* mice. However, the molecular mechanisms remain for further study.

5.7 Conclusion and clinical relevance

In short, the present results demonstrate that colon tissue with an increased n-3 PUFA status endogenously generates higher levels of bioactive n-3 PUFA-derived lipid mediators (resolvins and protectins), which may, on the one hand, suppress the inflammatory response and, on the other hand, enhance mucoprotection (defense of intestinal mucosa) and is thereby protected against inflammation and injury.

It would be of great interest to carefully evaluate the n-3/n-6 PUFA status and lipid mediator profiles in patients with IBD before and during therapeutical intervention with n-3 PUFA. Furthermore, clinical studies to investigate the therapeutical power of the newly identified resolvins and protectins in chronic inflammatory conditions like IBD are warranted (a stable analogue of Resolvin E1 was recently described). The active process of resolution provides previously unappreciated targets and approaches to novel therapeutic interventions to cope with the challenges of these severe diseases. The crucial role of n-3 PUFA in resolution is now emerging and recognizing the importance of a balanced fatty acid status in immunohomeostasis offers the chance to recover an evolutionary evolved inherent physiologic system that may have been disrupted by our own civilizatory progress.

6 Zusammenfassung/ Abstract

Omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFA) are the precursors of potent lipid mediators and play an important role in the regulation of inflammation. Generally, n-6 PUFA promote inflammation whereas n-3 PUFA have anti-inflammatory properties, traditionally attributed to their ability to inhibit the formation of n-6 PUFA-derived proinflammatory eicosanoids. Recently discovered resolvins and protectins are potent anti-inflammatory lipid mediators derived directly from n-3 PUFA with distinct pathways of action. However, the role of the n-3 PUFA tissue status in the formation of these anti-inflammatory mediators has not been addressed. Here we show that an increased n-3 PUFA tissue status in transgenic mice that endogenously biosynthesize n-3 PUFA from n-6 PUFA leads to significant formation of anti-inflammatory resolvins and effective reduction of inflammation and tissue injury in colitis. The endogenous increase in n-3 PUFA and related products did not decrease n-6 PUFA-derived lipid mediators such as leukotriene B4 and prostaglandin E2. The observed inflammation protection might result from decreased NF-κB activity and expression of TNFα, inducible NO synthase, and IL-1β, with enhanced mucoprotection probably because of the higher expression of Trefoil Factor 3, Toll-interacting protein, and Zonula occludens-1. These results thus establish the *fat-1* transgenic mouse as a new experimental model for the study of n-3 PUFA-derived lipid mediators. They add insight into the molecular mechanisms of inflammation protection afforded by n-3 PUFA through formation of resolvins and protectins other than inhibition of n-6 PUFA-derived eicosanoid formation (111).

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8 Selbständigkeitserklärung/ Statement

„Ich, Christian Hudert, erkläre an Eides statt, dass ich die vorgelegte Dissertationsschrift mit dem Thema: „Transgenic mice rich in endogenous omega-3 fatty acids are protected from colitis“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

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10 Lebenslauf/ Curriculum vitae

Mein Lebenslauf wird aus Datenschutzgründen in der elektronischen Version meiner Arbeit nicht mit veröffentlicht.