Polyunsaturated Fatty Acids in Inflammatory Bowel Diseases: A Reappraisal of Effects and Therapeutic Approaches

Rachel Marion-Letellier, PhD,* Guillaume Savoye, MD,**† Paul L. Beck, MD,‡ Remo Panaccione, MD,‡ and Subrata Ghosh, MD‡

Recent epidemiological studies highlight the key role of the type of consumed unsaturated fatty acid and the development of ulcerative colitis (UC). We aimed to review the potential mechanisms behind the antiinflammatory effects of unsaturated fatty acids on intestinal inflammation, to discuss their potential limitations, and to propose a new reappraisal of polyunsaturated fatty acids (PUFAs) in the pathophysiology of inflammatory bowel disease (IBD). A literature search using PubMed was carried out to identify relevant studies (basic science, epidemiological studies, or clinical trials) with unsaturated fatty acids and IBD. Only articles published in English were included. IBD patients exhibit an altered lipid metabolism. While in vitro and in vivo studies have demonstrated the antiinflammatory properties of n-3 polyunsaturated fatty acids in experimental models IBD, results of clinical trials have been disappointing. In addition, the impact of fatty acid on innate immunity as an alternative therapeutic approach is explored. This may offer insight into therapeutic avenues for designing n-3 PUFA diet therapy for IBD.

(Key Words: innate immunity, Crohn’s disease, polyunsaturated fatty acids, PPARγ, TLR, ulcerative colitis)

Fatty acids are essential components of the intestinal inflammatory response. Indeed, they modulate inflammatory processes by acting as signaling molecules and targeting numerous nuclear receptors to modulate gene expression. Pivotal roles of fatty acids in inflammatory bowel disease (IBD) also include modification of cell membrane composition. Interestingly, IBD patients exhibit an altered lipid metabolism (Table 1). The purpose of the present review is to describe the potential mechanisms by which fatty acids influence IBD with a particular focus on fatty acid modulation of innate immunity. The results of recent epidemiological studies about fatty acids intake and IBD risk and the disappointing results concerning n-3 polyunsaturated fatty acid (PUFA) clinical trials in IBD are highlighted.

Epidemiological Evidence of Dietary Influence of PUFA in IBD Development

Environment, in particular dietary habits, has long been suspected to contribute to the development of IBD. An increased incidence of IBD has been associated with diets high in animal protein. Shoda et al2 reported this association between dietary pattern (fat/protein) and Crohn’s disease (CD) risk in Japan while increased consumption of animal protein has been associated with higher IBD risk in France.3 Very recently, a systematic review demonstrated that this Western dietary pattern (high fat, high n-6 PUFA, high meat) is associated with an increased IBD risk. n-3 and n-6 PUFA are essential in human nutrition and a Western diet is also characterized by an unbalanced ratio of both types of PUFA (n-3/n-6 ratio). Linoleic acid (LA, n-6 PUFA) consumption has markedly increased (3-fold throughout the 20th century).5 Recently, epidemiological studies by Hart and colleagues6 highlighted the role of dietary intake of monounsaturated fatty acids (MUFA) or PUFA in ulcerative colitis (UC) development (Table 1). Higher intake of LA, an n-6 PUFA, is associated with an increased risk of UC, while oleic acid7 (n-9 MUFA) or docosahexaenoic acid (DHA) (n-3 PUFA)8 consumption is beneficial.

Mechanisms

Fatty Acids Derivatives

n-3 and n-6 PUFA are defined by the position of the first double bond in the carbon chain. Conventionally, n-6 PUFA are considered proinflammatory compounds because linoleic acid (LA, 18:2n-6), the major vegetal dietary PUFA, is a precursor.
for arachidonic acid (AA, 20:4n-6), a precursor of inflammatory mediators such as prostaglandins and leukotrienes. Increased production of AA-derived eicosanoids such as prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) have been well described in IBD (Table 1). In contrast, n-3 PUFAs appear to be regulators of inflammation. α-Linolenic acid (ALA, 18:3n-3) is the major vegetal n-3 PUFA and is a precursor for long chain n-3 PUFA such as eicosapentaenoic acid (EPA, 20:5n-3) and DHA (22:5n-3). Anti-inflammatory effects of n-3 PUFA may be mediated by competition with the n-6 PUFAs because n-3 PUFA acts as a competitive substrate for the n-6 PUFA metabolism (Fig. 1).

In vivo and in vitro evidence suggest that nutritional intervention with n-3 PUFA decreased AA-derived eicosanoids while it increased cell content of n-3 PUFA (Table 2). We have

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<th>Intake</th>
<th>Crohn’s</th>
<th>Ulcerative colitis</th>
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<td></td>
<td>Correlation of increased CD incidence with dietary intake of n-6 PUFA (P &lt; 0.001, r = 0.883) (127)</td>
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<td></td>
<td>Preventive effect of high dietary oleic acid intake [OR = 0.33, 95% CI = 0.12-0.93, P = 0.04] (7)</td>
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<td>Association of decreased UC risk with high dietary intake of DHA [OR = 0.43, 95% CI = 0.22-0.86, P = 0.02] (128)</td>
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<th>Metabolism</th>
<th>Ulcerative colitis</th>
<th>Ulcerative colitis &amp; Crohn’s</th>
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<tr>
<td></td>
<td>Decreased expression of fatty acid synthase in UC patients (P &lt; 0.001 versus control) (129)</td>
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<td>Increased concentration of n-6 PUFA in cell membrane (130, 131)</td>
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<td></td>
<td>Increased COX-2 activation (132) and increased production of PGE2 (10, 133)</td>
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<td>Increased production of LTB4 (11) and decreased LTB4 omega-hydroxylase activity (134)</td>
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<td>Impaired gene expression of PPARγ (43, 50)</td>
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<td>Decreased gene expression of HNF-4α in UC and CD patients (P &lt; 0.001 and P &lt; 0.05, respectively) (50)</td>
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<td>Altered LTB4 metabolism (134)</td>
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<td>Decreased mRNA levels of LRH-1 (135)</td>
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<th>Anatomical landmarks</th>
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<td>Visceral fat (136)</td>
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TABLE 2. The Good (ω3), the Evil (ω6): Unexpected Effects of Unsaturated Fatty Acids in IBD

<table>
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<tr>
<th>Effects</th>
<th>Proposed Mechanisms</th>
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<tr>
<td>ω3 Exacerbated inflammatory response by fish oil in SMAD3 mice with <em>Helicobacter hepaticus</em>-induced colitis (137)</td>
<td>Altered innate immune response?</td>
</tr>
<tr>
<td>ω3 Exacerbated inflammatory response by fish oil in IL-10 knockout mice (138)</td>
<td>Unknown</td>
</tr>
<tr>
<td>ω3 Exacerbation of chemically induced colitis by n-3 PUFA (139)</td>
<td>Decreased adiponectin in colonic subepithelial myofibroblasts</td>
</tr>
<tr>
<td>ω6 Linoleic acid–rich formula more effective than an oleic acid–rich formula in inducing remission in CD (59)</td>
<td>Differential induction of PPARγ?</td>
</tr>
<tr>
<td>ω6 Anti-inflammatory properties of n-6 PUFA metabolites (Lipoxin-A4 (20), nitrolinoleic acid (21))</td>
<td>Lipoxin-A4: Inhibition of NF-κB activation</td>
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<tr>
<td>ω6 Reduction of oxidative stress by dietary arachidonic acid in IL-10 knockout mice (140)</td>
<td>Nitrolinoleic acid: PPARγ induction</td>
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previously shown that nutritional intervention with ALA-rich formula decreased colon expression of LTB4 and increased red blood cell content in EPA in rats with 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis. COX-2 is responsible for the overproduction of PGE2 from AA during the inflammatory processes and 4-hour incubation with fish oil, the dietary source of DHA, downregulates COX-2 expression in both the intestinal epithelial cell lines Caco-2 and HT-29. We also previously reported that DHA pretreatment led to an altered expression of COX-2 and an inhibition of LTB4 and PGE2 release in human intestinal microvascular endothelial cells (HIMEC) in response to IL-1β.

Recently, two new classes of antiinflammatory lipid mediators have been identified: lipoxins and resolvins. These compounds are derived respectively from n-6 and n-3 PUFA and promote resolution of the inflammatory process. Both compounds are able to inhibit dendritic cell function and LTβ production in human neutrophils in response to the antimicrobial peptide LL-37. In vitro and in colitis models, the analog of lipoxin A4 downregulates intestinal inflammation. Similarly, in mice with TNBS-induced colitis, intraperitoneal injection of RvE1 (EPA derivative) before the induction of colitis protects against colitis.

Nitrate fatty acids are derived from reactions of unsaturated fatty and nitric oxide have also been described as antiinflammatory mediators. Nitrolinoleic acid and nitrooleic acid (derivatives of LA and OA, respectively) inhibit proinflammatory cytokines in LPS-stimulated macrophages. Both also inhibit vascular inflammation by inhibiting adhesion molecule expression, monocyte rolling, and adhesion. In conclusion, n-6 PUFA are generally solely recognized as proinflammatory molecules, while their signaling is more complex (Table 2).

Chemotactism

Dietary n-3 PUFA can modulate adaptive immune response. In vitro studies have reported an inhibition of T-cell proliferation and a decreased antigen presentation by n-3 PUFA treatment. Dietary fatty acids are able to modulate chemotactism of immune cells. During inflammation, epithelial and endothelial cells participate in innate defense by secreting chemotactic factors; the migration of leukocytes from the intravascular compartment into the inflammatory tissue is a key process that involves adhesion molecules such as ICAM-1 and VCAM-1. n-3 PUFA inhibits chemokine production such as interleukin-8. In vitro, AA increased production of adhesion molecules ICAM-1 in enterocyte-like Caco-2 cells. In addition, it has been shown that DHA pretreatment decreased chemokine production of interleukin (IL)-8 and VCAM-1 in IL-1-treated HIMEC. In human aortic endothelial cells, treatment with oleic acid inhibited the stearic acid-induced ICAM-1 expression by inhibiting nuclear factor κB activation. Nitrovasodilators have also been described as antiinflammatory molecules, while their signaling is more complex (Table 2).

Angiogenesis

Angiogenesis is a key process that is enhanced in IBD. Since inhibition of COX-2 leads to an inhibition of angiogenesis, consumption of n-3 PUFA could inhibit angiogenesis. VEGFA, a key angiogenic mediator and its receptor VEGFR2 have been demonstrated to have important roles in IBD patients and colitic mice. An upregulation of VEGFR2 in response to a proinflammatory stimulus (IL-1β) in HIMEC and in colitic rats has also been demonstrated. Interestingly, DHA pretreatment leads to a downregulation of VEGFR2 in HIMEC in response to IL-1β. We also confirmed this finding in colitic rats, where dietary intervention with fish oil rich in EPA and DHA normalized endothelial VEGFR2 levels.

Pain

DHA can modulate pain including inflammatory and postinflammatory pain. A significant proportion of IBD patients...
continue to complain of abdominal pain despite their disease being otherwise quiescent. This may have multiple potential explanations, one of which may be due to the transient receptor potential (TRP) channel family. TRP has been associated with having a key role in visceral hypersensitivity.\textsuperscript{30} We demonstrated the role of TRPV1 in quiescent IBD with abdominal pain.\textsuperscript{31} Interestingly, n-3 PUFA can directly interact with TRPV1.\textsuperscript{32} Other TRP with a demonstrated role in intestinal inflammation...
TRPV4 are targets of ResolvinD1, a DHA derivative. Further studies are required to elucidate the putative antinociceptive role of DHA during intestinal inflammation.

**Molecular Targets of PUFA**

Dietary PUFAs modulate gene expression involved in intestinal inflammation by regulating activity of transcription factors. The best-described molecular targets of PUFAs are NF-kB and peroxisome proliferator-activated receptor gamma (PPARγ) (Fig. 2). NF-kB is a key transcription factor in the inflammatory process. NF-kB is sequestrated in the cytosol bound to its inhibitory protein IκB, which, upon stimulation, is phosphorylated and degraded. Free NF-kB translocates to the nucleus and regulates the expression of its target genes. ALA, EPA, and DHA have all demonstrated their ability to inhibit the NF-kB pathway in experimental models of IBD. Recently, we demonstrated that dietary ALA-rich formula inhibited NF-kB activation in colitic rats. The effect of oleic acid on NF-kB activation is not documented in IBD models but its effect has been demonstrated in human aortic endothelial cells.

The nuclear receptors are considered dietary lipid sensors. Nuclear receptors ligands have demonstrated antiinflammatory properties in IBD models (Fig. 2) and are involved in gut barrier preservation. The PPAR belong to the nuclear receptor family and are activated by fatty acids and their derivatives. NF-kB and PPAR are highly expressed in the colon and regulates inflammation by inhibiting NF-kB. A reduced expression of PPARγ has been reported in UC patients but not in CD. PPARγ has been identified as a susceptibility gene in CD. PPARγ is highly expressed in the colon and regulates inflammation in IBD patients. Interestingly, dietary CLA, a natural ligand of PPARγ, was beneficial in colitis. PPARγ is expressed in the enterocyte-like Caco-2 cells in response to IL-1β, and found that DHA and EPA were the most potent PUFAs to inhibit cytokine production through PPARγ. In a randomized, placebo-controlled trial, rosiglitazone, a synthetic PPARγ ligand, has shown beneficial effects for the treatment of patients with mild-to-moderately active UC. After binding to a ligand, PPARγ forms a heterodimer with the retinoid X receptor (RXR) to activate transcription. It has been recently demonstrated that PPARγ binds the promoter of gene encoding for a subset of antimicrobial peptides and thereby maintains the constitutive epithelial expression of β-defensin in the colon. RXR−/− mice are more sensitive to colitis and RXR agonists were equally effective as PPARγ agonists in reducing intestinal inflammation.

Recent studies emphasized the role of the transcription factor hepatocyte nuclear factor-4α (HNF4-α) in the intestine. Its endogenous ligand has been recently discovered: LA. HNF4-α expression is decreased in intestinal tissues from IBD patients and in colitic mice. Its inactivation in enterocyte-like Caco-2 leads to a downregulation of tight junction proteins. HNF4-α is also a key regulator of gene expression during enterocyte differentiation. Other nuclear X-receptors such as farnesoid X receptor (FXR), liver X receptor (LXR), and pregnane X receptor (PXR) can be activated by lipids and have been studied in IBD. Genetic polymorphism of PXR and LXR have been associated with UC. UC patients exhibited a low expression of PXR and PXR-deficient mice are more sensitive to intestinal inflammation. The synthetic FXR agonist (INT-747) ameliorates intestinal inflammation in two models of murine colitis. There are numerous crosstalks between these nuclear receptors. For example, administration of LXR and PXR agonists leads to an upregulated PPARα gene expression in the mouse small intestine PPAR.

A potential molecular mechanism of fatty acids is their binding with G-protein-coupled receptors (GPR) to modulate inflammation. GPR20 is expressed in the gut, adipose tissue, and macrophages and its induction by n-3 PUFA causes antiinflammatory effects in macrophages.

**CLINICAL TRIALS**

Nutritional intervention with n-3 PUFA has shown mixed results in IBD patients (Fig. 1). Bamba et al have shown that the antiinflammatory effects of defined formula diets in IBD may be dependent on the fatty acid composition of the diet. In this clinical trial, active CD patients received elemental diets containing different fat percentages and they found a remission rate after 4 weeks in each group of 80%, 40%, and 25% in the low-, medium-, and high-fat groups, respectively. As dietary fat may influence the efficacy of enteral diets, Gassull et al. performed a clinical trial to evaluate the efficacy of diets varying in their fat composition (oleic acid vs. LA). They observed that an LA-rich composition was more effective than an oleic acid-rich formula in inducing remission in CD (remission rates: 52% (12/23) vs. 20% (4/20)), which challenges the notion that such antiinflammatory effects are solely mediated via modulation of AA precursors. A recent small clinical trial has demonstrated that enteral feeding changes in plasma fatty acid profiles in pediatric CD. Two clinical trials from the Epanova Program in Crohn’s Studies (EPIC-1 and -2) have been conducted to evaluate the effects of n-3 fatty acids therapy for the maintenance of CD. These large multicenter, randomized, double-blind, placebo controlled-studies were conducted in Europe, Israel, Canada, and the United States and included 363 (EPIC 1) and 375 patients (EPIC 2) with quiescent CD. In these studies, CD patients were randomized to receive either 4 g of n-3 free PUFA (DHA and EPA mix) or an isolipidic placebo containing medium chain triglyceride oil daily. No benefit was demonstrated of the n-3 free PUFA over placebo on clinical relapse (EPIC-1: 31.6% vs. 35.7%, P = 0.30; EPIC-2: 47.8% vs. 48.8%, P = 0.48). There was a significant decrease in serum triglyceride concentration in patients who received n-3 therapy. Taken on its own, these results do not support that n-3 therapy is better than placebo.
for maintaining remission in CD. 
Recent meta-analyses studying the effects of n-3 PUFAs for induction of remission in UC or maintenance of remission in IBD concluded that current data are insufficient to make recommendations for the use of n-3 PUFA in clinical practice. Large studies evaluating n-3 PUFA are still lacking in UC. Further research may be needed in different patient populations to correctly define if PUFAs have a therapeutic role in IBD.

PUFA Studies in IBD: Limitations and Considerations

The disparate results between the clinical trials and in vitro and murine studies deserve further exploration (Fig. 3). It is important to distinguish the difference between the effects of an individual fatty acid from oils. Indeed, in vitro studies commonly tested the effect of single fatty acids while oil used in clinical trials contains a mix of fatty acid and other compounds. For example, an ALA-rich formula tested in rats with TNBS-induced colitis only contains a third of ALA in total fatty acid composition. 

Nonfatty acid compounds such as vitamin E or D can also confound the observed effects. Choosing an adequate lipid control is also crucial in the experimental design. Oleic acid is generally considered as a good neutral lipid in experimental models. However, why has oleic acid been associated with lower IBD risk? In addition, oleic acid may have effects on the jejunal brake and therefore increase small bowel transit time, confounding results. The next question is, is the fatty acid effect dependent on a single fatty or an unbalanced ratio of fatty acids? Third, increasingly studies have demonstrated different genotypes may be associated with different responses to fatty acids. Fourth, timing of nutritional intervention may also be crucial. The discrepancy between clinical trials and experimental studies could result from the timing of the intervention. In colitis models, nutritional intervention started before the onset of colitis. We thus speculated that treatment time is a crucial point and nutritional intervention with fatty acid should be preventive and not curative. As dietary intake of PUFA modifies IBD risk, identification of their potential mechanisms is required. Three main mechanisms may be involved: alteration of eicosanoids mechanisms, cell membrane fluidity, and modulation of gene expression.

To date, clinical relapse has been the main outcome in the evaluation of n-3 clinical trials. Other patient populations may warrant study including mild CD, mild UC, or the postoperative patient. Also of interest would be the long-term effect of nutritional intervention with n-3 PUFA on the prevention of inflammation-driven colorectal cancer. The effects of n-3 PUFA on colorectal cancer has been recently reviewed by Cockbain et al.

Increasingly, studies have demonstrated that different genotypes may be associated with different responses to fatty acids. For example, genetic polymorphisms of tumor necrosis factor alpha (TNF-α) and PPARα have been associated with an altered response to nutritional intervention with fatty acids. Two studies from Portugal have recently reported that different genotypes may be associated with different responses to fatty acids.
types of fat intake interact with polymorphism of IL-6, TNFα, and PPARγ to modulate CD activity.70,71 In addition, genetic polymorphisms of other nuclear receptors are often associated with IBD (Fig. 3).

Timing of nutritional intervention may also be crucial. The discrepancy between clinical trials and experimental studies could result from the timing of the intervention. In colitis models, nutritional intervention started before the onset of colitis.12,14,72,73 In addition, Collie-Duguid et al74 demonstrated that DHA pre-treatment decreased IL-1-induced inflammatory response in endothelial cells, while DHA treatment did not. We thus speculated that treatment time is a crucial point and nutritional intervention with fatty acid should be preventive and not curative. PUFAs are not associated with an exacerbation of symptoms or a worsened form of IBD while dietary intake of PUFA modifies IBD risk.

**INNATE IMMUNITY**

**Diet and Innate Immunity**

Dietary PUFA may influence intestinal inflammation by acting on the innate immune response. The etiology of IBD remains elusive but it is well established that environment, genetics, and the immune system interact to contribute to IBD development. A gene–environment interaction has been demonstrated by genetic studies showing the association between genetic polymorphism in the gene coding for NOD2, an intracellular bacterial sensor and CD risk.75 In addition, alteration of another family of bacterial sensor, Toll-like receptors (TLR), is well described in IBD studies.76 These primary defects in innate immunity could contribute to IBD pathogenesis by enabling a tolerance breakdown to intestinal microbial flora in genetically predisposed individuals. Compelling studies have confirmed the concept that IBD results from an unbalanced relationship within the triad: environment, genetic, and innate immunity. This demonstrated the innate immunity control of adaptive immune response and the crucial role of the gene–environment interplay in IBD induction. Until now, this prevailing theory did not take into account the role of the diet in IBD development. There is considerable interest in modulating IBD development by dietary therapy in an attempt to rectify the innate immunity defect resulting from genetic mutations. Except for probiotics, no dietary approach was performed to influence IBD development. However, a recent case–control study demonstrated that more IBD patients than controls use probiotics to manage their health.77 While probiotic studies were promising in experimental models of IBD, clinical trials have failed to prove their efficacy for postoperative prophylaxis.78,79

Given this, how may fatty acids affect innate immunity and does this offer an alternative therapeutic approach?

**Intestinal Barrier Function and Gut Microbiota**

IBD patients exhibit an abnormal composition and activity of gut microbiota.80 Interactions between intestinal microbes and food may influence IBD genesis.81 Compelling studies demonstrate the role of diet on shaping gut microbiota.82 In mouse models, consumption of high-fat diet was associated with changes in the gut microbiota.83,84 While the influence of a Western diet on gut microbiota is established,85 more research is required to identify the potential mechanism as to how dietary fatty acids can modify intestinal microflora. A metabolomics study recently reported that metabolites produced by the gut microbiota can be correlated with CD and they found a strong correlation between the most abundant bacteria in ileal CD and fatty acids.86 Dietary PUFA altered diversity of cecal bacteria in mice87 and IL-10-deficient mice.88 In vitro and in vivo studies have shown that dietary fats increased small intestinal permeability through three mechanisms: 1) alteration of tight junction protein in rats89,90; 2) modification of cellular membrane composition in T84 IEC90; and 3) through AA-derived eicosanoids production.91 In contrast, n-3 PUFA prevent this disruption of barrier function.92

Bacterial compounds are recognized by a variety of receptors, including TLRs and nucleotide-binding oligomerization domain (NOD, a family of intracellular bacterial sensors) and are potent stimuli of innate immune responses. In vitro evidence suggests that types of fatty acids modulate NOD- or TLR-mediated inflammation: activation by saturated fatty acids and inhibition by DHA in several cell types (IEC,93 macrophages,94 and dendritic cells95). Dietary fats modulate cellular lipids and lead to a differential activation of membrane receptors and we have recently shown that DHA inhibits TLR4 expression in IL-1-treated HIMEC.14 Similarly, nutritional intervention with PUFA in a necrotizing enterocolitis rat model led to a downregulation of TLR4.96 Bertin et al97 have elegantly documented that visceral fat is an anatomical landmark shared between CD and obese patients. In addition, obese IBD patients exhibited a more severe disease course.98 A high concentration of AA in adipose tissue is associated with an increased risk of UC.99 As TLR4 is expressed in visceral adipose tissue,100 dietary lipids may play a role in maintaining the gut in a state of controlled activation. As dietary intervention with n-3 PUFA prevents insulin resistance in high-fat diet-induced obese mice via modulation of adipose tissue inflammation,101 similar results can be expected in IBD models. For example, fat-1 mice, which are rich in endogenous n-3 PUFA, are less susceptible to colitis102 but also have an improved glucose tolerance compared to wildtype.103

In conclusion, a Western diet may exacerbate intestinal inflammation through changes in gut microflora leading to an upregulation of TLR- and NOD-pathways and an increased intestinal permeability resulting from an alteration of tight junction proteins. Interestingly, these Western diet-induced inflammatory pathways are also described as n-3 PUFA targets to prevent intestinal inflammation.

**Autophagy**

Key genetic mutations associated with CD such as NOD2 (CARD15), ATG16L1, or endoplasmic reticulum stress gene XBP1 have been associated with defective autophagy and
microbial clearance. There is considerable interest in modulating autophagy pathways to enhance microbial clearance in CD, in an attempt to rectify the defect resulting from genetic mutations. Given that autophagy can be activated by free fatty acids in hepatocytes, we hypothesized that dietary lipids may affect autophagy and CD can be potentially considered as a target disease for such nutrient modulation approaches to ameliorating intestinal inflammation.

Inflammasome

A family of intracellular danger sensing molecules called NLRs (NOD-like receptors or nucleotide-binding domain leucine-rich repeat-containing genes) have been implicated in the pathogenesis of IBD. Within this family are the NODs, IPAF (ICE protease activating factor), and NLRP (pyrin domain containing NLR) subfamilies. The link between NOD2 mutation and CD has been well established and is one of the most commonly found gene variants associated with CD. Recently, NLRPs, the main functional components of a signaling complex referred to as the inflammasome, have been linked to IBD. Genome-wide association studies have found that hypofunctional mutations of NLRP3 are associated with an increased risk of CD. Loss of NLRP3 also increases the severity of intestinal inflammation in dextran sodium sulfate (DSS) and TNBS murine models of colitis, although the precise mechanisms are unclear. The loss of NLRP3 causes broad changes in innate immune function and alterations in the ability of the intestinal mucosal to respond to bacteria, resulting in altered intestinal microbiota. Similar findings have been recently reported with the loss of NLRP6. Although there are 14 NLRPs that have been described, only NLRP3 and NLRP6 have been assessed in intestinal disease states.

One of the main functions of the NLRP inflammasome is to cleave pro-forms of IL-1β and IL-18 in to their active secreted forms. Several agents can activate the inflammasome, including monosodium urate (MSU) crystals, alum, ATP, viruses, bacteria, and bacterial toxins as well as fatty acids. In MSU-induced gouty arthritis, fatty acids can act through the TLR2 receptor to increase IL-1β release, a process dependent on an intact NLRP inflammasome. Recently, fatty acids have been found to activate the NLRP3 inflammasome and IL-1β and IL-18 release which interferes with insulin signaling. In this study, Wen et al clearly showed that the saturated fatty acid palmitate activates the NLRP3 inflammasome but the unsaturated fatty acid oleate has no effect. This further defines the observation by Tschopp and colleagues that showed a role for NLRP3 in glucose regulation. Others have also shown that NLRP3 and IL-1β expression is increased in adipose tissue and that these changes correlate with severity of obesity and insulin resistance. Interestingly, it appears that fatty acid mediated inflammasome activation in the liver and this may represent the underlying mechanism involved in the development of nonalcoholic steatohepatitis (NASH), one of the most common causes of chronic liver disease in the Western world. Inflammasome activation and altered signaling has been implicated in numerous disease states involving not only the gastrointestinal tract and liver but also in the heart, central nervous system, kidneys, and lung. These new and exciting observations directly link how diet and obesity lead to activation of the innate immune system, inflammation, and end organ dysfunction. Recent studies linked dietary lipids to inflammasome in regulating innate immunity. A high-fat diet activated the NRLP3 inflammasome in macrophages and in hepatocytes. In addition, SREBP-a1, a lipogenic transcription factor, plays a key role of innate immunity by regulating inflammasome in macrophages.

**FUTURE DIRECTIONS**

We thus hypothesized that modulation of innate immunity may be fatty-driven in IBD development (Table 3). A proof of concept study is required but we cannot directly target IBD physiopathology with a nutritional intervention before the IBD diagnosis. A first step to evaluate the efficacy of n-3 PUFA in the earliest phase of the disease will be to select CD postoperative patients. CD patients mostly undergo surgery during the course of their disease and postoperative recurrence is a frequent feature. The postoperative state is thus considered a perfect time to evaluate predisposing factors to disease recurrence and many studies have been designed to modulate CD natural history to maintain a sustained postoperative remission. In our study design, we should take into account the issues listed above concerning n-3 PUFA therapies. For example, as different genotypes can be associated with different responses to fatty acids, we have to characterize CD patient’s genotypes for previously described cytokine receptors or nuclear receptors involved in fatty acid response in order to identify potential patients eligible for n-3 PUFA therapies.

IBD patients commonly use restrictive diets to avoid a relapse without a scientific rationale. For example, Jowett et al did not observe an association between increased intake of milk and dairy products, contrary to frequent IBD patient’s beliefs. A recent Japanese study evaluated the efficacy of n-3 diet therapy for IBD patients and demonstrated that the n-3/n-6 ratio was higher in the remission group than in the relapse group. In addition, a Norwegian study demonstrated the potential of n-3 rich PUFA diet in a small pilot study. In this 8-week intervention study, the authors evaluated the effect of 600 g of salmon consumption weekly in 12 UC patients and they observed a decreased clinical

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**TABLE 3. Targets of Innate Immunity by PUFA**

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<th>Autophagy</th>
<th>Barrier function</th>
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<th>Inflammasome</th>
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score and a decreased plasma AA concentration. A better understanding of the mechanisms behind the effects of dietary fatty acids on IBD risk may provide a scientific basis to offer dietary advice to IBD patients.

Proof of concept studies are mandatory to offer further therapeutic avenues for designing diets beneficial in IBD to modulate early inflammatory states and innate immunity, especially in the prevention setting in IBD relative at risk, in the prevention of relapse in UC patients, or after surgery in CD patients.

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