HYPOTHESIS

Inflammatory bowel disease as a disorder of an imbalance between pro- and anti-inflammatory molecules and deficiency of resolution bioactive lipids

Undurti N. Das¹,²

Abstract

The inflammatory process seen in inflammatory bowel disease (IBD) is due to excess production of pro-inflammatory cytokines interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α), interferons (IFNs), macrophage migration inhibitory factor (MIF), HMGB1 (high mobility group B1) and possibly, a reduction in anti-inflammatory cytokines IL-10, IL-4, and transforming growth factor-β (TGF-β). These pro-inflammatory molecules lead to increased production of reactive oxygen species (ROS) including nitric oxide resulting in target tissue damage. I propose that inadequate production of inflammation resolving molecules lipoxins, resolvins, protectins, maresins and nitrolipids that suppress inflammation, ROS production, enhance wound healing and have cytoprotective properties results in inappropriate inflammation, delay in healing/repair process and so target tissue/organ damage continues in IBD. Hence, suggested therapeutic approach could include administration of stable synthetic analogues of lipoxins, resolvins, protectins, maresins and nitrolipids may be used to detect the onset, progression and response to treatment of IBD.

Keywords: Inflammatory bowel disease, Lipoxins, Polyunsaturated fatty acids, Cytokines, Free radicals, Resolvins, Protectins

Background

Although genetic, environmental and sex hormonal factors have been implicated in the pathogenesis of autoimmune diseases, including inflammatory bowel disease (IBD), it is known that pro-inflammatory cytokines, nitric oxide (NO), free radicals, a deranged immune system, a deficient anti-oxidant defenses, Toll-like receptors and microbiota play a significant role both in the initiation and perpetuation of the inflammatory process observed. Once the inflammatory process is triggered, this leads to the production of a variety of pro-inflammatory molecules and possibly, a reduction in the elaboration of anti-inflammatory molecules. This imbalance between the pro- and anti-inflammatory molecules would ultimately cause target tissue/organ damage seen in these conditions. Despite many advances in the understanding of the pathobiology of IBD, it is still not clear what triggers the onset of IBD and which factors are needed to induce resolution of the same.

Hypothesis

I propose that continued inflammatory events seen in IBD are due to failure of the resolution of inflammation. Thus, the balance between inflammation and resolution is disturbed more in favor of pro-inflammatory events and/or failure of resolution inducing molecules to be produced at the most appropriate time leading to non-resolution of inflammation. In other words, even after the inciting agent responsible for the initiation of inflammation is removed; inappropriate inflammation continues simply because resolution failed to occur. This leads to delay in the healing/repair process and so tissue/organ damage continues. This may explain as to why
even when these patients are continuing to take anti-inflammatory and immunosuppressors medicines, target organ damage continues. In view of this, it is suggested that institution of pro-resolution-inducing agents could induce remission and restore normal physiological function of the target tissues/organisms in IBD and other autoimmune diseases. Such endogenous pro-resolution-inducing molecules include: lipoxins, resolvins, protectins, maresins, nitric oxide, nitrolipids, 15-deoxy-

Based on this hypothesis, it is suggested that progression and flares of IBD are not only due to increased production of pro-inflammatory molecules IL-6, TNF-α, MIF (macrophage migration inhibitory factor), HMGB1 (high mobility group box 1), free radicals and lipid mediators such as prostaglandins (PGs), leukotrienes (LTs) but also as a result of decreased formation and release of anti-inflammatory molecules: IL-4, IL-10, TGF-β, and lipoxins, resolvins, protectins, maresins and nitrolipids.

**Lipoxin A₄ is detectable in urine**

LXA₄, generated by lipoxygenase (LO) transformation of AA possess potent anti-inflammatory activity in vivo, and temporal biosynthesis of LXA₄, concurrent with spontaneous resolution, has been observed during exudate formation [1–3]. LXs, resolvins, protectins and maresins, which are anti-inflammatory bioactive lipids formed from polyunsaturated fatty acids: arachidonic acid (AA), the product of AA lipoxygenase, can suppress inflammation in autoimmune diseases by acting on GPCRs. Such endogenous pro-inflammatory agents could induce recruitment and inflammation. G protein-coupled receptors (GPCRs) that play a significant role in leukocyte recruitment and inflammation. G protein-coupled receptor 120 (GPR120) that functions as a ω-3 fatty acid receptor/sensor that when stimulated with ω-3 fatty acids (EPA and DHA) induced broad anti-inflammatory effects in monocytes RAW 264.7 cells and in primary intraperitoneal macrophages that were abrogated by GPR120 knockdown [14]. Thus, PUFAs and their anti-inflammatory bioactive lipids may suppress inflammation in autoimmune diseases by acting on GPCRs.

**LXA₄ and LTs ratio determines the degree of inflammation**

In animal models of glomerulonephritis and other renal pathologic states, LTs exerted adverse effects in the glomerulus. LTB₄ augments neutrophil infiltration, and LTC₄ and LTD₄ mediate potent vasoconstrictor effects on the glomerular microcirculation. Selective blockade of the 5-lipoxygenase pathway produced a significant amelioration of the deterioration of renal hemodynamic and structural parameters. On the other hand, 15-S-hydroxyeicosatetraenoic acid (15-S-HETE), the immediate product of arachidonate 15-lipoxygenase, and the lipoxins, which are produced by sequential 15- and 5- or 5- and 12-lipoxygenation of AA are also generated in the course of glomerular injury that antagonize leukotriene-induced neutrophil chemotaxis and lipoxin A₄ antagonized the effects of LTD₄ and LTC₄ on the glomerular microcirculation. Thus, the contrasting effects of 5- and 15-lipoxygenase products represent endogenous pro- and anti-inflammatory influences that ultimately determine and regulate the extent and severity of glomerular inflammation [9–12].

These results are in favor of the proposal that deficiencies of anti-inflammatory bioactive lipids may play a significant role in autoimmune diseases including IBD.

**PUFAs and lipoxins bind to GPCR and suppress inflammation**

Monocytes and macrophages express an extensive repertoire of G protein-coupled receptors (GPCRs) that regulate inflammation and immunity [13]. PUFAs, especially AA, EPA and DHA and their metabolites such as eicosanoids, lipoxins, resolvins, protectins and maresins function as agonists at a number of G protein-coupled receptors (GPCRs) that play a significant role in leukocyte recruitment and inflammation. G protein-coupled receptor 120 (GPR120) that functions as a ω-3 fatty acid receptor/sensor that when stimulated with ω-3 fatty acids (EPA and DHA) induced broad anti-inflammatory effects in monocytes RAW 264.7 cells and in primary intraperitoneal macrophages that were abrogated by GPR120 knockdown [14]. Thus, PUFAs and their anti-inflammatory bioactive lipids may suppress inflammation in autoimmune diseases by acting on GPCRs.

Based on the preceding discussion, I suggest that increased production of lipoxins, resolvins, protectins and maresins at sites of inflammation in IBD may suppress the disease process. Failure to produce adequate amounts of these anti-inflammatory lipoxins, resolvins, protectins, maresins and nitrolipids could render the inflammatory process to continue unabated. Hence, methods designed to enhance the production of lipoxins, resolvins, protectins and maresins and/or administration of synthetic analogues of lipoxins, resolvins, protectins
Fig. 1 (See legend on next page.)
and maresins will aid in the prevention and management of IBD and other autoimmune diseases. This proposal is supported by the recent report [15] that β-Cell destruction produced by multiple low-doses streptozotocin was completely prevented in fat-1 mice (transgenic fat-1 mice carry a C. Elegans gene, fat-1, encoding an n-3 fatty acid desaturase catalyzing the conversion of n-6 to n-3 PUFA) and hence, these animals have high tissue levels of n-3 PUFA that was associated with increased levels of the anti-inflammatory lipoxin A4 and the 18-hydroxyecosapentaenoic acid, precursor of the anti-inflammatory resolvin E1.

Testing the hypothesis
The hypothesis presented here can be tested by performing relevant in vitro, in vivo and human studies. (1) In vitro studies could be performed using colon epithelial cells. For instance, one can use HCoEpiC cells from ScienCell Research laboratories (USA) which are isolated from human colon tissue. These cells can process and present antigens to T cells in vitro, and can be stimulated to express HLA class II and intercellular adhesion molecules in vivo [16]. They also respond to a broad array of cytokines with altered gene expression and growth characteristics [17]. These HCoEpiC cells could be exposed to LPS (lipopolysaccharide) and various pro- and anti-inflammatory cytokines and their ability to secrete pro- and anti-inflammatory bioactive lipids such as prostaglandins, leukotrienes, thromboxanes, lipoxins, resolvins, protectins, maresins and nitrolipids could be measured. It is anticipated that in response to pro-inflammatory stimuli, HCoEpiC cells would produce increased amounts of prostaglandins, leukotrienes, thromboxanes and IL-6 and TNF-α and reduced concentrations of anti-inflammatory bioactive lipids such as lipoxins, resolvins, protectins and maresins and IL-10, IL-12 and TGF-β. In contrast, when HCoEpiC cells are supplemented with anti-inflammatory cytokines would secrete higher amounts of lipoxins, resolvins, protectins, maresins and nitrolipids and less of prostaglandins, leukotrienes and thromboxanes.

(2) In vivo studies could include colonic instillation of the hapten, trinitrobenzene sulphonatic acid (TNB) that induces ulceration and inflammation which can persist for 3 weeks [18]. Another IBD model that could be studied includes the guinea pig acetic acid-induced colonic inflammation model that shows characteristics of IBD including PMN infiltration, edema, ulceration and necrosis [19]. Both acute and chronic colitis can also be induced by feeding female Swiss-Webster mice with 5% DSS (dextran sulfate sodium 30,000–40,000 mol wt) for 5 or 7 d, respectively [20]. This IBD model could be used to study prophylaxis, prophylaxis plus therapy and therapeutic effect of lipoxins, resolvins, protectins,
nitrolipids (all as singly or in combination) during the acute and chronic phase of the disease and therapeutically during the chronic phase of the disease. Thus, a variety of IBD models could be used to study the balance between pro- and anti-inflammatory bioactive lipids and changes in their concentrations during the onset of the disease, progression of the disease and remission of IBD to understand the dynamics of their changes in various phases of the disease. In these animal models of IBD, plasma, tissue (colonic secretions and colonic tissue) and urinary levels of prostaglandins, leukotrienes, thromboxanes, lipoxins, resolvins, protectins, maresins, nitrolipids and various cytokines could be measured to ascertain their role in the disease process. Similarly, lipoxins, resolvins, protectins, maresins and nitrolipids can be administered (including their more stable synthetic analogues) to know their protective action against IBD.

(3) Most important studies that need to be performed to firmly establish the role of bioactive lipids will be to measure the plasma, stool and urinary levels (and if possible colonic concentrations) of anti-inflammatory lipoxins, resolvins, protectins, maresins and nitrolipids and pro-inflammatory prostaglandins, leukotrienes and thromboxanes and various cytokines in subjects who are having acute, chronic and subacute features of IBD. If the hypothesis presented here is correct, it is expected that plasma, colonic, urinary and stool concentrations of lipoxins, resolvins, protectins, maresins and nitrolipids and anti-inflammatory cytokines will be low whereas those of pro-inflammatory bioactive lipids and cytokines will be high during the acute and subacute and chronic phases of IBD (though the degree of elevation could be variable). It is likely that all pro- and anti-inflammatory bioactive lipids and cytokines need not be altered and the alteration may be seen only in one or more than one of the biomolecules. If such a study is in support of the hypothesis presented here, then it is worthwhile to study the preventive and curative action of more stable and synthetic analogues of lipoxins, resolvins, protectins, maresins and nitrolipids in patients with IBD.

Implications of the hypothesis

If the hypothesis presented here is true, it will usher in a new era in our approach to IBD, which predisposes to the development of colon cancer, in the form of use of lipoxins, resolvins, protectins, maresins and nitrolipids in its prevention and treatment. It is possible that more stable and orally active synthetic analogues of lipoxins, resolvins, protectins, maresins and nitrolipids could be developed for IBD. Furthermore, if such an approach of use of lipoxins, resolvins, protectins, maresins and nitrolipids in the prevention and management of IBD becomes successful, it will pave way to treat other autoimmune diseases such as lupus, rheumatoid arthritis, multiple sclerosis and type 1 diabetes mellitus.

Conclusions

Based on the preceding discussion, it is likely that administration of stable synthetic analogues of lipoxins, resolvins, protectins and maresins may form a new approach in the prevention and treatment of IBD. Measurement of urine, stool and plasma levels of lipoxins, resolvins, protectins, maresins and leukotrienes would help in determining whether IBD is in remission, continuing or resolving. For instance, if the levels of lipoxins, resolvins, protectins, maresins and nitrolipids are lower than normal and those of leukotrienes are high it indicates that the disease is continuing and/or not responding to the therapy instituted. In those subjects who are on therapy for IBD if the levels of lipoxins, resolvins, protectins, maresins and nitrolipids are not increasing and/or if the levels of leukotrienes are increasing it indicates that the disease is continuing and remission is yet to set in. On the other hand, if the plasma, urine and stool levels of lipoxins, resolvins, protectins, maresins and nitrolipids are increasing and those of leukotrienes are decreasing it indicates that the underlying IBD is entering remission phase and that the patient is responding to the therapy instituted. Thus, serial measurements of lipoxins, resolvins, protectins, maresins, nitrolipids and leukotrienes will give an indication as to the progress and response to therapy. It is likely that changes in the plasma, urine and stool levels of lipoxins, resolvins, protectins, maresins, nitrolipids and leukotrienes will precede the clinical remission or progression IBD. It is also envisaged that by determining the plasma, stool and urinary levels of lipoxins, resolvins, protectins, maresins and nitrolipids in those with IBD, one will be able to determine which of these bioactive lipids is needed for appropriate therapy. For instance, in those with IBD who show only a decrease in lipoxins need to be given lipoxins; those with deficiency of resolvins need only resolvins; those who show low levels of protectins need to get only protectins and similarly those who have decreased levels of maresins need to be administered only maresins. It is anticipated though lipoxins, resolvins, protectins, maresins and nitrolipids are all anti-inflammatory and resolution inducing molecules, all patients with IBD may not have deficiency of all these five bioactive lipids. It is likely that patients with IBD may show deficiency of only one, two, three or all five of these molecules. This heterogeneity in the deficiency of these bioactive lipid molecules could due to genetic heterogeneity in the synthesis and action of pro-resolution bioactive lipids (namely lipoxins, resolvins,
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that undesirable immunosuppression does not set in. In view of these interactions, administration of adequate amounts of lipoxins, resolvins, protectins and maresins are expected to be of benefit in the prevention and management of IBD.

Competing interests
The author declares that he has no competing interests.

Authors’ contributions
UND solely contributed to this work.

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protectins and maresins), polymorphism of 5-, 12- 15-lipoxygenases and cyclooxygenases. Such genetic heterogeneity and polymorphism of lipoxygenases and cyclooxygenases may explain innumerable variations in the histological and clinical presentation of IBD and their response to therapies employed.

The recent observation that the cholinergic anti-inflammatory pathway based on vagus nerve activity regulates macrophage and dendritic cell responses in the spleen through alpha-7 nicotinic acetylcholine receptor (a7nAChR) signaling and IBD patients have dysautonomia with decreased vagus nerve activity, dendritic cell and T cell over-activation [21] can also be attributed to alterations in the production of lipoxins, resolvins, protectins and maresins. For instance, it was reported that rats with metabolic syndrome have ineffective inflammation-resolution mechanisms that was associated with 120 % decrease in plasma LXA4 levels [22]. These results suggest a close interaction between the cholinergic anti-inflammatory pathway and lipoxins, resolvins, protectins and maresins. Furthermore, it was reported that electroacupuncture at the sciatic nerve controlled systemic inflammation by inducing vagal activation leading to the production of dopamine in the adrenal medulla. Dopamine inhibited cytokine production via dopamine type 1 (D1) receptors and D1 receptor agonists suppressed systemic inflammation and rescued mice from polymicrobial peritonitis. These results suggest that sciatric and vagus nerves modulate the production of catecholamines [23]. Since acetylcholine is able to enhance the production of lipoxins, resolvins, protectins and maresins (and possibly, nitrolipids), it is likely that dopamine is also able to enhance the production of these anti-inflammatory bioactive lipids. Though no studies have been performed with serotonin and its possible influence on the production of lipoxins, resolvins, protectins, maresins and nitrolipids, I propose that a close interaction exists between them. It is known that serotonin plays a significant role in the inflammatory events of IBD [24]. Studies showed that nicotine, which mediates its actions via a7-nicotine acetylcholine receptor (a7-nAChR), attenuated both IL-1β and 5-hydroxytryptamine (5-HT, serotonin)-evoked Ca2+ transients [25], suggesting that cholinergic anti-inflammatory pathway has a regulatory role on serotonin release and its actions. These evidences indicate that anti-inflammatory bioactive lipids, anti-inflammatory cholinergic pathway, serotonin and dopamine interact among them to regulate immune response and inflammation and thus, ultimately in the pathogenesis of IBD.

Furthermore, LXA4 seems to have a negative regulatory role on cholinergic regulatory pathway by elaborating nitric oxide. This implies that once the cholinergic anti-inflammatory pathway has reached its optimum, its action is negated by acetylcholine-induced LXA4 generation so...
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