Moving Beyond “Good Fat, Bad Fat”: The Complex Roles of Dietary Lipids in Cellular Function and Health

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ABSTRACT

The International Life Science Institute North America and the American Society for Nutrition annual Functional Foods for Health Symposium was held 9 April 2011. Evidence that foods and their components offer health benefits beyond basic nutrition continues to captivate the interest of the scientific community, government agencies, and the general public. This paper is comprised of extended abstracts from the session and addresses issues related to emerging lipid nutrition science, including active roles of lipids in modulating physiological pathways. Identified pathways underlie the development of obesity, cognitive development, and inflammation, the latter of which is thought to relate to multiple disease processes. These data point to a new way of thinking about the role of lipids in health and disease. Adv. Nutr. 3: 60–68, 2012.

Session Abstracts

A similar pathway may mediate cd36 involvement in fat taste perception and inflammation

Nada A. Abumrad

The membrane scavenger receptor FAT/CD36 has broad specificity and can recognize a number of ligands. It plays an important role in long chain FA9 metabolism in both rodents and humans (1). In CD36-deficient mice and rats, studies of FA biodistribution in vivo documented that CD36 contributes a major part of FA uptake into the heart, skeletal muscle, and adipose tissues. CD36 has been implicated in mediating fat taste perception. Localized on the apical face of taste bud cells, the protein, after interaction with long chain FA, transduces signals that increase intracellular calcium and associate with neurotransmitter release (2). CD36 also functions in the uptake of oxidized LDL by macrophages, promoting their conversion into foam cells, and its deletion has a protective antiatherosclerotic effect (3).

As a result, altered CD36 function has been proposed to contribute to multiple abnormalities of lipid metabolism. Variations in the CD36 gene are common in humans and SNPs (frequency between 10 and 40%) associate with blood

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4 Abbreviations used: AA, arachidonic acid; AD, Alzheimer’s disease; ADAS-Cog, Alzheimer’s Disease Assessment Scale; ADCS, Alzheimer’s Disease Cooperative Study group; ARCD, age-related cognitive decline; Cer, ceramide; cPLA2, cytoplasmic phospholipase A2; DG, diglyceride; ER, endoplasmic reticulum; FA, fatty acid; GPCR, g-protein coupled receptor; MCI, mild cognitive impairment; MIDAS, Memory Improvement with DHA Study; miRNA, microRNA; MMSE, Mini-Mental State Exam; OEA, oleoylethanolamide; PAF, platelet-activating factor; PAL, Paired Associate Learning; RvD1, resolvins; SNP, single nucleotide polymorphism; SPM, specialized proresolving mediator.
lipid levels, positively with VLDL and negatively with HDL (4). A common haplotype in the CD36 gene associates with blood FFA (5) and CD36 SNPs influence individual variability in response of blood lipids to (n-3) FA supplementation (6). CD36 SNPs associate with risk of coronary heart disease in diabetic individuals with risk of metabolic syndrome and stroke (5,4,7).

In addition to its functions in substrate uptake, CD36 interacts with src kinases and participates in intracellular signal transduction (3, 8). This signaling function was shown to be important for fat taste perception in taste bud cells. In studies with mice, it was shown that CD36 deletion impairs spontaneous fat preference and the mechanism involved cellular calcium dynamics with the subsequent release of neurotransmitters (9). CD36 signaling is also postulated to mediate the role of the protein in inflammation. It has been well established that in several cell types, including macrophages’ excess supply of the CD36 ligands, FA and oxidized LDL can promote oxidative and ER stress and signaling to stress kinases. As a result, CD36 is implicated in the inflammation associated with diabetes and atherosclerosis (3).

Our recent work documents that one important mechanism for CD36’s role in inflammation may involve a pathway similar to that functioning in fat taste perception. We show that CD36 is required for membrane calcium influx in response to ER stress and that one consequence of this role is the regulated release of AA from cellular membranes by cPLA2 (8). Release of AA is the trigger for the formation of eicosanoids, bioactive molecules with pleiotropic effects in acute inflammation. Eicosanoids derived from AA, an (n-6) PUFA, have proinflammatory functions in contrast to (n-3) PUFA-derived eicosanoids, which have antiinflammatory properties (10). Our findings would explain at least some of the proinflammatory effects of CD36, especially those related to the acute inflammatory response. This novel function of CD36 integrates regulation of FA utilization, cellular calcium homeostasis, and inflammation. Further studies of how this is accomplished are important to our understanding of the etiology of diet-induced inflammatory processes and their contribution to disease.

Various factors (e.g. purinergic receptor agonists, ATP and UTP) or events (e.g. cellular stress) release calcium from the ER. Inhibition of the ER calcium pump SERCA2 is one common method to deplete ER calcium by preventing its reuptake (Fig. 1). The increase in cytosolic calcium drives CD36 to the plasma membrane (possibly by inhibiting its internalization). CD36 at the membrane regulates activation of store-operated calcium channels (channels responsive to ER calcium depletion) via its interaction with the Src family kinase Fyn. The sustained rise in intracellular calcium translocates cPLA2 to nuclear and ER membranes, where it acts to release AA from the sn-2 position of phospholipids. The enzyme is also activated via CD36-dependent phosphorylation by the MAP kinase ERK1/2. The AA produced by cPLA2 is converted by cyclooxygenases into eicosanoids, which have pleiotropic effects in inflammation. One of the major products formed from AA by the cyclooxygenase pathway is PGE2.

**Figure 1** CD36 involvement in calcium influx and activation of cPLA2 for releasing arachidonic acid (AA) and production of AA derived eicosanoids.

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### Literature Cited


### Roles of FA and their derivatives in satiety

**Daniele Piomelli**

Mammals have an adaptive advantage in seeking fat-rich foods, which are nutritionally essential but scarce in most...
natural habitats. This innate preference can become maladaptive, however, when it is not limited by environmental constraints. Indeed, the unrestricted availability of fatty foods, which characterizes diets of industrialized societies, is considered a key contributing factor to obesity, diabetes, and cardiovascular disease. Despite its theoretical and practical importance, fat preference is still incompletely understood. Our laboratory has investigated the role of locally acting lipid hormones, such as OEA and the endocannabinoids, in the control of dietary fat intake.

OEA, the naturally occurring amide of ethanolamine and oleic acid, is produced in the upper small intestine of mammals and other vertebrates, and its biosynthesis is regulated by feeding status (1–3). Supporting the possibility that feeding-dependent fluctuations in OEA production represent a satiety signal, experiments in rodents have shown that administration of OEA delays meal initiation and prolongs the interval between successive meals, resulting in a persistent inhibition of food intake (4, 5). The anorectic effects of OEA are substantially different from those produced by peptide satiety factors, such as cholecystokinin, which reduce meal size without affecting the interval between meals. Moreover, the hypophagic actions of OEA differ from those exerted by glucagon-like peptide-1 and corticotropin-releasing factor in that they are not accompanied by behavioral signs of malaise and anxiety or changes in circulating levels of the stress hormone corticosterone (4).

The molecular mechanisms through which OEA regulates feeding have been elucidated in some detail. OEA is a high-affinity agonist for the nuclear PPARα (5), which has been implicated in the control of energy balance and lipid metabolism. OEA binds to the purified ligand-binding domain of PPARα with a $K_D$ of 40 nmol/L and activates this receptor in cell-based assays with an EC$_{50}$ of 120 nmol/L (5). Additionally, in vivo studies have demonstrated that PPARα deletion in mice abrogates the anorectic effects of OEA without changing the effects of cholecystokinin (5). Finally, synthetic PPARα agonists, but not agonists of PPARδ and PPARγ, produce a hypophagic response that is behaviorally indistinguishable from that elicited by OEA and is absent in mutant mice lacking PPARα (5). A plausible interpretation of these data is that OEA regulates food intake in rodents by selectively activating intestinal PPARα. Interestingly, OEA is also produced in adipocytes and myocytes, where it engages PPARα to stimulate lipolysis and FA oxidation, respectively (6, 7).

We examined whether select macronutrients, such as fat, carbohydrate, or protein, might be responsible for initiating OEA biosynthesis in the small intestine. We found that infusion of a fat emulsion into the rat duodenum stimulates OEA production in the jejunum, whereas infusions of carbohydrate or protein solutions have no such effect (8). OEA biosynthesis utilizes dietary oleic acid as a substrate and is disrupted in mutant mice lacking the membrane FA transporter CD36. Targeted disruption of CD36 or PPARα abrogates the satiety response induced by fat, providing strong evidence that activation of small intestinal OEA mobilization, enabled by CD36-mediated uptake of dietary oleic acid, serves as a molecular sensor linking fat ingestion to satiety (8).

Are central and peripheral neural circuits involved in regulating the satiety effects of OEA? To address this question, we utilized a combination of morphological and pharmacological approaches. We found that systemic administration of OEA increases expression of oxytocin in magnocellular neurons of the paraventricular nucleus and supraoptic nucleus of the hypothalamus and, secondly, that blockade of brain oxytocin receptors prevents the anorectic effects of OEA (9). These results suggest that OEA suppresses feeding by activating central oxytocin transmission. In a more recent series of experiments, we found that surgical resection of the sympathetic celiac superior mesenteric ganglion, which sends projections to the upper gut, abolishes feeding-induced OEA production in rat small intestinal cells (10). These effects are accounted for by suppression of OEA biosynthesis and are mimicked by administration of a selective $\beta_2$-adrenergic antagonist. We further observed that sympathetic ganglionectomy increases meal frequency and lowers satiety ratio and these effects are corrected by pharmacological administration of OEA. The results suggest that sympathetic activity controls fat-induced satiety by enabling OEA production in the small intestine (10).

**Literature Cited**


Cognitive effects of DHA in ARCD and mild to moderate AD

Karin Yurko-Mauro

DHA, the principle (n-3) FA in brain, plays an important role in multiple neuronal functions (1). Community-based cohort studies continue to demonstrate that higher DHA (n-3) intake and DHA plasma levels are significantly correlated with lower risk of cognitive decline, all-cause dementia, and AD (2,3,4). As many as 5.4 million older Americans are estimated to have cognitive impairment without dementia and ~12% of these elders will develop dementia annually (5). Thus, gradual memory loss and diagnosis of probable AD is a major health concern of older adults.

Preclinical studies have shown that algal-DHA supplementation reduces β-amyloid, plaques, and τ in transgenic AD mouse models (6,7). Some clinical trials have demonstrated less cognitive decline with fish oil (DHA + EPA) supplementation in those with MCI (8) or in a very mild AD subgroup (9) but no benefit in those with mild to moderate AD. These results may indicate that mild, age-related memory loss is responsive to DHA (n-3) supplementation. The sole effects of algal DHA as a nutritional supplement for ARCD and as a potential therapy for mild to moderate AD were recently examined in 2 clinical trials.

Both trials were randomized, double blind, placebo controlled, and multi-centered. The MIDAS determined effects of 900 mg/d algal DHA on improving cognitive functions in healthy elderly with ARCD over 6 mo (10). The DHA AD study conducted by the ADCS examined the rate of cognitive and functional decline in mild to moderate AD over 18 mo with 2 g/d algal DHA (11).

In MIDAS, 485 participants (242 DHA; 243 placebo) with baseline MMSE scores ≥ 26 and Wechsler Memory Scale Logical Memory score ≥ 1 SD below younger adults were randomized. The primary outcome measure was a change from baseline in the Cambridge Automated Neuropsychological Test Automated Battery PAL, a test of visuospatial learning and memory. Other Cambridge Automated Neuropsychological Test Automated Battery cognitive tests, self-assessment scales, and plasma phospholipid FA analyses were secondary outcomes along with safety assessments. The MIDAS completion rate was 90%, with 59% female, a mean age of 70 ± 9 y, and mean education of 14.6 ± 2.6 y. An intention to treat analysis demonstrated fewer PAL errors with DHA at 6 mo (diff. score = −1.63 ± 0.76; P = 0.03) (Fig. 2). Verbal Recognition Memory showed greater immediate and delayed responses with DHA administration (P = 0.02). Executive function and working memory tests showed no differences between groups. The plasma phospholipid DHA levels doubled (from 3.2 to 6.4 weight %; P < 0.001) in the supplemented group as expected and plasma DHA levels were correlated with the change in the PAL response (r = −0.11; P = 0.02). Dietary intake of (n-3) FA (~100 mg/d) did not differ between groups or over the course of the study. No changes in blood pressure were detected; however, a decrease in heart rate (~3.2 ± 0.59 bpm; P = 0.03) was shown with DHA supplementation. Algal DHA supplementation was well tolerated with no difference between groups in the incidence of treatment-emergent adverse events.

The second DHA Alzheimer’s ADCS study randomized 402 patients (238 DHA, 164 placebo) with MMSE scores of 14–26. The coprimary outcomes in this study were the rate of change in the ADAS-Cog and the Clinical Dementia Rating-sum of boxes, a global outcome scale. The DHA ADCS study had similar demographics, although the mean MMSE was 20.7 ± 3.6 and the mean age (76 ± 8.7 y) was older than MIDAS, with 58% being ApoEe4 carriers. The majority of the sample population (85.8%) was taking approved antidementia medications. The overall completion rate of the study was 74%. No significant differences with DHA treatment on the rate of change on ADAS-Cog or Clinical Dementia Rating-sum of boxes were observed overall. However, in a planned secondary analysis, the ApoEe4 negative subgroup on algal DHA had a lower rate of ADAS-Cog decline (P = 0.028) and a lower MMSE score (P = 0.03) compared to placebo. There were no significant changes with DHA on other outcome measures or in the ApoEe4 positive group. As in MIDAS, plasma DHA levels significantly increased with DHA treatment and adverse events were equivalent across groups.

In summary, in ARCD, supplementation of 900 mg/d algal DHA improved episodic memory and learning over 6 mo. A significant 2-fold reduction in the number of visuospatial memory errors was shown with DHA compared to placebo and these changes were correlated with changes in plasma DHA. Compared to age-related normative data on the PAL test, DHA supplementation yielded a net 3.4-y improvement in memory function, thereby providing benefit to aging adults with early mild memory loss. Significant decreases in heart rate were also seen, substantiating the cardiovascular benefits of DHA (n-3) FA in older adults. DHA treatment did not benefit patients with established mild to moderate AD overall. However, the positive results in...
ApoE4 negative AD patients suggest that DHA may be potentially useful in this subgroup and should be confirmed in a large, randomized trial. Previous clinical studies have shown some benefits of long-chain (n-3) FA in patients with MCI or mild AD. However, 2 recent randomized controlled studies in cognitively healthy elderly participants (12, 13) found no cognitive benefits with fish oil supplementation. Differences in study designs, especially baseline cognitive variability, baseline dietary (n-3) FA intake, DHA dose, and sensitivity of cognitive measures likely contributed to the null results in these studies compared to MIDAS. The long-term effects of DHA on rates of cognitive decline or conversion to MCI have not been studied; thus, early MCI or predementia patients remain an important clinical group to target for further research with DHA. Based on epidemiological (2,14) and clinical data such as MIDAS, higher DHA intake or DHA supplementation has significant positive cognitive effects on older adults with age-related memory loss and may serve as a nutritional neuroprotective supplement for healthy brain aging.

Literature Cited


Effects of diet and other factors on brain sphingolipids and neuronal function

Al Merrill Jr

Sphingolipids are a very diverse family of compounds that participate in biological structure and inter- and intra-cellular signaling, as summarized in Figure 3 (1). Although considerable progress has been made in understanding their biochemical functions, much remains to be learned about their relationship to nutrition, both with respect to how dietary sphingolipids are utilized (or not) and how diet affects sphingolipid metabolism and functions.

Figure 3 represents a cellular membrane comprised of 4 categories of sphingolipids: the Cer backbone is depicted by a sphingoid base in blue and amide-linked FA in grey; sphingomyelins by a black headgroup; lactosylCer by blue and yellow circles representing glucose and galactose, respectively; gangliosides GM3 (with sialic acid in purple) and GM1 (with a yellow square for N-acetylgalactosamine); the signaling backbones (in box, left to right) Cer-1-phosphate, Cer, sphingosine, and sphingosine 1-phosphate; and 1-deoxyCer (lower left, at *). Complex sphingolipids define membrane microdomains (rafts) and bind to cellular and extracellular proteins and the backbones regulate multiple processes, as indicated. Sphingolipids that are taken up from exogenous sources can be recycled and/or degraded; most of the cellular sphingolipids are usually made de novo. Biosynthesis de novo is affected by many dietary factors, including the availability of the precursors (e.g. palmitate, serine, alanine, and glycine) and mycotoxins and other compounds.

Sphingolipids are relatively minor components of the diet, but humans consume >100 g/y, which is in the ballpark where they have been found to have biological activity (2). Most of the dietary sphingolipids (sphingomyelins, Cer, glycosphingolipids, and smaller amounts of other species) undergo some degree of hydrolysis to the backbone sphingoid bases by a combination of intestinal enzymes and gastrointestinal microflora. The resulting sphingoid bases are readily taken up by intestinal cells and can suppress colon carcinogenesis, with normalization of β-catenin signaling as one of the plausible mechanisms of action (3). Most of the absorbed sphingoid bases are degraded in the intestine rather than transported to other tissues, although this might not be the case for all of the sphingoid bases that have been discovered, especially ones that lack a 1-hydroxyl-group and...
have been found to suppress prostate cancer (4, 5). Dietary gangliosides have been shown also to protect the gastrointestinal tract from bacteria and inflammation and have been discussed as possibly important factors in neonatal brain development (6, 7).

At the other end of the spectrum, diet affects endogenous sphingolipid biosynthesis in many ways, with the most clear-cut relationship to health being damage to liver, kidney, brain, and other organs (toxicity and carcinogenicity) upon consumption of fumonisins, mycotoxins that disrupt sphingolipid biosynthesis (8). More subtle relationships are also emerging, such as that de novo sphingolipid biosynthesis is enhanced by palmitic acid and this has been suggested to play a role in metabolic syndrome (9) and production of bioactive intermediates of sphingolipid metabolism, namely (dihydro)ceramides, appear to mediate some of the effects of drugs and other agents (10). Two novel and highly bioactive categories of sphingolipids (1-deoxy-sphinganine and 1-deoxydihydroceramides, biosynthesized by mammalian cell lines and animals) are produced by the first enzyme of de novo sphingolipid biosynthesis, serine palmitoyltransferase, when it utilizes alanine and glycine, respectively, in place of serine (10,11). These “atypical” sphingolipids have been found to be elevated in animals exposed to fumonisins, human neuropathies, and other toxic sphingolipids. J Biol Chem. 2009;284:4786–95.

The findings summarize here illustrate that there are many links between sphingolipids, nutrition, and health that warrant further investigation and there are undoubtedly more waiting to be discovered.

**Literature Cited**


Impact of gangliosides in acute inflammation
M. Tom Clandinin

Membrane microdomains rich in cholesterol and sphingolipids, including gangliosides, are known to be important regions for cell signaling and binding sites for various pathogens. Cholesterol depletion inhibits the cellular entry of pathogens and also reduces inflammatory signals by disrupting micro-domain structure. Our studies show that dietary gangliosides increase total ganglioside incorporation while decreasing cholesterol in the intestinal mucosa. We hypothesized that diet-induced reduction in cholesterol content in the intestinal mucosa disrupts microdomain structure, resulting in reduced proinflammatory signals. Male weanling Sprague-Dawley rats were fed semipurified diets for 2 wk. Experimental diets were formulated to include either ganglioside-enriched lipid [ganglioside diet, 0.02% gangliosides (wt:wt of diet)] or PUFA (PUFA diet, 1% AA and 0.5% DHA, wt:wt of total fat) in a control diet containing 20% fat (1). Levels of cholesterol, ganglioside, caveolin, PAF, and DG were measured in the micro-domain isolated from the intestinal brush border. The ganglioside diet increased total gangliosides by 50% with a relative increase in GD3 and a relative decrease in GM3. The cholesterol content was also reduced by 23% in the intestinal microdomain. These changes resulted in a significant decrease in the ratio of cholesterol:ganglioside. The ganglioside and PUFA diets were both associated with reduced caveolin, PAF, and DG content in microdomains, whereas no change occurred in the ganglioside profile of animals fed the PUFA diet. Dietary gangliosides decrease the cholesterol:ganglioside ratio, caveolin, PAF, and DG content in microdomains, thus exerting a potential antiinflammatory effect during gut development.

LPS or inflammatory cytokines, TNFα, and IL-1β induce expression of NO and decrease expression of tight junction proteins. Dietary ganglioside shows antiinflammatory signals in inhibiting TNFα and IL-1β in response to LPS exposure, suggesting that antiinflammatory effects of dietary ganglioside may protect gut tight junction in LPS exposure. I hypothesized that antiinflammatory effects of dietary ganglioside will inhibit production of NO and increase IL-10 in response to LPS, resulting in protection of gut occluding tight junction protein.

Rats were fed semipurified diets with or without (control) ganglioside (0.1% wt:wt of total lipid). After 2 wk of feeding, animals from each diet group were i.p. injected with saline or LPS [3 mg/(mL-Kg)]. Intestinal tissue, mucosa, and blood were collected after 6 h of LPS exposure. The effect of dietary ganglioside on the production or expression of IL-10, NO, inducible NO synthase, and occludin protein was determined (2).

Dietary ganglioside increased IL-10 content in intestinal mucosa by 32-fold ($P < 0.0001$) and in plasma by 2.4-fold ($P < 0.0001$). Feeding animals the ganglioside diet decreased total NO content in intestinal mucosa and plasma by 44 and 30%, respectively, in response to LPS compared to that of control animals. Inducible NO synthase expression was also remarkably inhibited in the intestines of animals fed the ganglioside diet. Degradation of occludin tight junction protein in response to LPS was significantly reduced by dietary ganglioside and the treatment suggests potential for the prevention of increased gut permeability in epithelial cells during acute inflammation or inflammatory bowel disease (1). Necrotizing enterocolitis has high morbidity in premature infants. Hypoxia- ischemia, infection, and enteral feeding are risk factors, whereas feeding human milk is protective. Vasoactive and inflammatory mediators in necrotizing enterocolitis remain elusive. An infant bowel model of necrotizing enterocolitis was developed to test the hypothesis that gangliosides modulate inflammatory response to infection and hypoxia. (3) Viable, noninflamed bowel was obtained from 9 infants between 28 and 42 wk of gestational age. Infant bowel was treated in culture with Escherichia coli LPS and hypoxia in the presence or absence of preexposure to gangliosides. Bowel necrosis and the production of NO, endothelin-1, serotonin, eicosanoids, H2O2, and pro-inflammatory cytokines were measured. Results indicated that ganglioside preexposure reduced bowel necrosis and endothelin-1 production in response to LPS. Gangliosides suppressed infant bowel production of NO, ITB3, PGE2, H2O2, IL-1β, IL-6, and IL-8 in response to LPS exposure and hypoxia. We concluded that a bowel protective effect of ganglioside is indicated by modulation of vasoactive mediators and proinflammatory signal suppression, providing rationale for ganglioside use in food products to treat inflammatory bowel disease.

Literature Cited

Resolvins and protectins in inflammation resolution
Charles N. Serhan, Antonio Recchiuti, Sriram Krishnamoorthy, Gabrielle Fredman, and Nan Chiang

An acute inflammatory reaction in response to infection or tissue damage, in general, is characterized at least at the gross level by the classic cardinal signs of inflammation (heat, redness, swelling, and pain) (1). Using a systems approach with self-limited acute inflammatory exudates to track and map tissue events, cell traffic, and identification of protein and chemical mediators, we identified novel families of potent bioactive lipid-derived mediators, coined the resolvins and protectins, in resolving exudates (2). Each of these novel proresolving mediators controls the duration and magnitude of acute inflammation in vivo with stereospecific actions in the pico- to nanogram range and is biosynthesized from both (n-3) and (n-6) essential FA (2-4).
The mapping of these endogenous resolution circuits provides new avenues to appreciate the molecular basis of many widely occurring diseases that are characterized by un-governed inflammation (2–8). Importantly, because the precursors of the lipid mediators engaged in the initiation and termination of the acute inflammatory response are essential PUFAs, the tissue status of both (n-3) and (n-6) PUFAs is of interest in evaluating the impact of nutrition in healthy individuals as well as in diseases associated with uncontrolled inflammation.

The temporal relationships of the acute inflammatory response are well established, namely, edema and the accumulation of leukocytes, specifically polymorphonuclear leukocytes, followed by the nonphlogistic accumulation of monocytes and macrophages (1, 9, 10). These events in self-limited or resolving inflammatory reactions are coupled with the release of local factors that prevent or limit further or excessive trafficking of leukocytes, allowing for resolution. Early in the inflammatory response, proinflammatory mediators such as PG and leukotrienes, which are biosynthesized locally from the (n-6) AA, play an important role.

Many of the widely used antiinflammatory therapies are currently directed toward the inhibition of key enzymes and/or antagonism of receptors involved in the inflammatory response. Selective cyclooxygenase inhibitors and anti-TNFα are examples of this therapeutic approach that are used with the goal of blocking production of local proinflammatory chemical mediators (11). Research in the author’s laboratory focusing on profiling and mapping of self-limited inflammation to mine the ideal outcome of the inflammatory response has uncovered novel mechanisms that terminate the local acute inflammatory response as well as stimulate resolution and return of the tissue to homeostasis. Identification of these biochemical and cellular processes indicates that resolution of acute inflammation is an active programmed process at the tissue level (2, 4). Therefore, rather than targeting inhibition or antagonism of inflammation, our research addresses the potential use of endogenous agonists of resolution to stimulate key regulatory points that naturally resolve inflammation.

The progression from an acute inflammation to chronic inflammation, as in many widely occurring human diseases such as arthritis, periodontal disease, and cardiovascular disease, is widely viewed as an excess of proinflammatory mediators (1). Although mononuclear cells can sometimes contribute to proinflammatory responses, they are also critical in wound healing, tissue repair, and remodeling in a noninflammatory and/or nonphlogistic manner. Hence, it is possible that resolution pathway defects or deficiencies in (n-3) FA substrate levels associated with mounting endogenous proresolving circuits and local autacoids in the host could underlie some of the aberrant mechanisms that lead to chronic unresolved inflammation (2–4).

Complete resolution of an acute inflammatory response and the return of local tissues to homeostasis is the ideal response and is necessary for ongoing health. Removal of leukocytes from tissues involved in the inflammatory response without leaving remnants of the host defenses and combat between leukocytes, invading microbes, and/or other initiators of inflammation is an ideal outcome. In our laboratory, we focused our efforts to address the endogenous molecules and mechanisms that regulate the acute inflammatory response in vivo. It is widely thought that simple dilution of local proinflammatory mediators is sufficient to “burn out” inflammation, with the subsequent responses ending passively (1). We found that on initiation of inflammation, e.g. with TNFα, there was a typical acute phase response characterized by rapid neutrophil (polymorphonuclear) infiltration preceded by both local PG and leukotrienes. Unexpectedly, the eicosanoids then underwent temporal changes in vivo that we termed the “lipid mediator class switch.” As the exudate evolved, the eicosanoid profiles switched and the lipid mediators made within this milieu changed with time from proinflammatory to proresolving (12, 13). Leukotriene B4, a potent leukocyte chemotactant, is metabolically inactivated and the transcriptional regulation of enzymes required for lipoxin and resolvins production is activated. This in turn attracts mononuclear cells and stimulates macrophages to take up apoptotic neutrophils within the contained inflammatory exudate site (2–4).

We recently have made advances on the biosynthesis and functions of this novel genus of SPM. These new families of chemical mediators were originally identified in murine exudates captured during the natural self-limited phase. As of today, the SPM include 3 structurally distinct chemical mediator families: resolvins, protectins, and, the most recent, maresins, as well as the aspirin-triggered epimeric forms of the lipoxins, resolvins, and protectins, which are biosynthesized from essential FA. Lipoxins are produced from (n-6) AA and resolvins and protectins are biosynthesized from (n-3) EPA and DHA (2). Specific members of each family possess potent, multipronged, antiinflammatory, proresolving (4), and antimicrobial actions in animal models of sepsis (7). The actions of SPM proved to be potent, cell type specific, and stereoselective with human cells and in many experimental animal diseases. These diseases include periodontal disease, skin inflammation, peritonitis, colitis, and ocular inflammation (4). For example, in mice, overexpression of the murine 12/15-lipoxygenase protects from atherosclerosis and the associated vascular inflammation via local production of local SPM, including lipoxin A4, resolvin D1, and protectin D1 (5). It is important to note that the murine 12/15-lipoxygenase or, precisely, its enzymatic activity in humans is carried out by 2 separate enzymes, 15-LO type I and 12-LO, which are each expressed separately in cell type-specific distribution throughout the body. These are key enzymes in the biosynthetic routes to SPM that can also include transcellular lipid mediator biosynthesis within contained inflammatory exudates in vivo (14).

Toward human direct translation of these findings with SPM, we used microfluidics in addition to animal models to monitor the single cell–targeted actions of SPM in tandem with LC-MS/MS–based lipid mediator lipidomics. This new
Microfluidic chambers have bioassay compartments of ~1 μL³ volume and can isolate leukocytes in <5 min from whole blood rather than the routine 2–3 h (6). Endogenous formation of resolvins and protectins and their protective roles were recently confirmed and extended, e.g. in murine ischemic renal injury (15) and obesity-induced insulin resistance and liver disease (16). In addition, the resolvins (e.g. RvE1 and RvD1) are potent analgesics possessing direct actions within the central nervous system and reduce peripheral inflammatory pain (17). Hence, the identification of endogenous SPMs that are turned on and biosynthesized during inflammation resolution indicates that the resolution of acute inflammation is an active programmed process at the tissue level (2). This changes the century-old concept that resolution of acute inflammation is a passive process (1).

To further address the molecular mechanisms involved in the actions of resolvins, we recently obtained evidence for the role of miRNA in self-limited, acute, inflammatory murine exudates and their regulation by (n-3) FA-derived resolvins (18). Using real-time PCR analysis and LC-MS-MS, we found that in resolving exudates, RvD1 is produced from DHA during the resolution phase and that miR-21, miR-146b, and miR-203, miR-142, miR-302, and miR-219 are selectively regulated (P < 0.05) in self-limited murine sterile peritonitis. The regulation of these miR by resolin D1 g-protein coupled receptors (GPCRs) identified in murine and human systems was confirmed with isolated human macrophages.

For example, RvD1 given in nanogram amounts or mouse-reduced inflammation reduced neutrophil infiltration 25–50% into the peritoneum as well as shortened the resolution interval by ~4 h. In murine peritonitis at 12 h, RvD1 upregulates miR-21, miR-146b, and miR-219 and downregulates miR-208a in vivo (18). With human macrophages overexpressing recombinant human RvD1 receptors, which include both ALX/FPR2 and GPR32, we found that these miRNA were also regulated by RvD1 (P <0.05), which activates these miRNA at concentrations as low as 10 nmol/L in a GPCR-dependent manner. Hence, isolated human macrophages exposed to RvD1 recapitulate the in vivo circuit identified during the resolution of murine peritonitis. In addition, RvD1-miRNA identified in these experiments target cytokines and protein networks known to be involved in the immune system, e.g. miR-146b targeted NF-κB signaling and miR-219 targeted 5-lipoxygenase (18) that in turn reduced leukotriene production, as monitored by LC-MS-MS-dependent lipid mediator lipodomics (3,19). These recent results indicate that resolvins regulate specific miRNA target genes involved in resolution. They establish a novel resolution circuit that involves (n-3) FA-derived RvD1 and its receptor-dependent regulation of specific miRNA in vitro and in vivo. Taken together, these findings indicate that natural resolution pathways may underlie many prevalent diseases associated with uncontrolled inflammation and open the potential for resolution-based pharmacology. Moreover, they demonstrate the ability of proresolving GPCR to regulate miR that affect inflammation resolution via the nutritionally essential PUFA lipid mediator metabolome.

Literature Cited