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Omega-3 INSIGHTS

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Special Report

Specialized Pro-Resolving Lipid Mediators

Omega-3s have long been indicated in reducing inflammation throughout the body, potentially aiding conditions such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), vascular inflammation and traumatic brain injury. Recent discoveries point to the use of EPA- and DHA-derived lipid mediators and marine-sourced oils to more consciously modulate inflammation resolution in the human body.

by Gerard Bannenberg, Ph.D.

Specialized Pro-Resolving Lipid Mediators

Understanding the Mechanism of Action of EPA and DHA

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A Central Role for Omega-3 in the Resolution of Inflammation

The understanding is relatively new that the resolution of inflammation—the termination and disappearance of an inflammatory lesion—is an actively regulated process. The term "resolution" has been used for more than a century, but the awareness that the regulation of resolution of inflammation is mediated by specific physiological signals is a very recent discovery made just a decade ago. Of particular relevance to the food and dietary supplement ingredient sector, omega-3 fatty acids have been instrumental in this new awareness since they are central to regulating resolution. An avalanche of knowledge generation on omega-3-derived bioactive lipid mediators is unfolding right now. For businesses interested in omega-3s, there is a new and developing area with ample possibilities to begin using marine and eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA)-rich oils to more consciously modulate resolution as a fundamental process in human physiology.

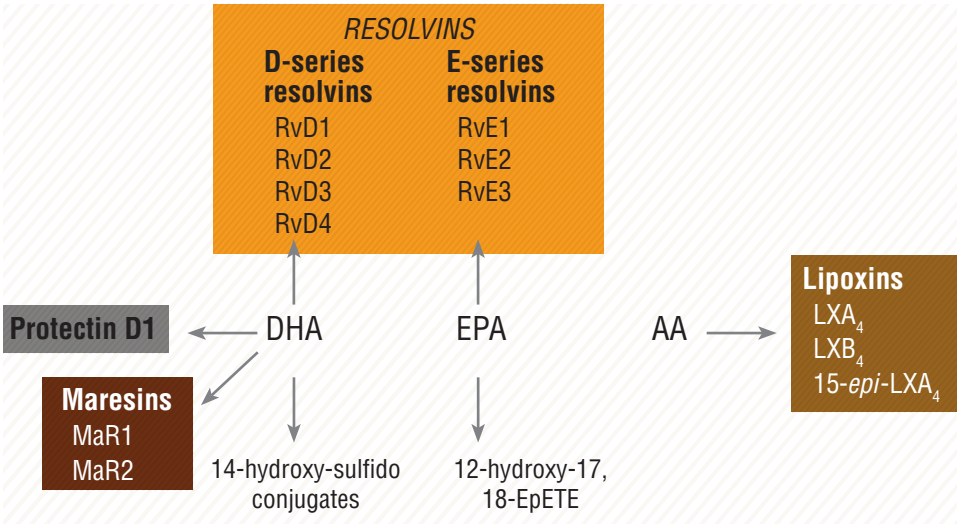
Specialized Pro-Resolving Mediators (SPMs)

During the past 15 years, a range of endogenous signaling substances possessing potent anti-inflammatory and resolution-promoting activities was discovered that can be formed by specific enzymes within all tissues in the body from the long-chain omega-3 polyunsaturated fatty acid (omega-3 LCPUFA) EPA and DHA. These newly recognized lipid mediators are broadly grouped into several families named resolvins, maresins and protectins (see Figure 1). These are chemically structurally different from the prostanoids discovered in the 1970s, in that they do not contain a cyclic structure, but are rather characterized by two or more hydroxyl and epoxide functional groups that are found in specific positions of the omega-3 LCPUFA structure (positional isomers) in combination with very specific double-bond configurations. Another characteristic is that the newly identified substances are often formed during the resolution of an inflammatory response and have potent local hormone-like activities that drive an inflammatory response toward its end. Plainly speaking, without resolution, there is inflammation. The acute inflammatory response is protective in that the response helps to rid the body of invading microbes and injured tissues. But without a controlled stop, this response can turn into chronic painful inflammation as seen in many chronic diseases in humans.

Collectively, these specialized pro-resolving mediators (SPMs) are viewed as a new broad class of counter-regulatory local hormones (autacoids) that help tissues turn off inflammation and permit recovery of normal tissue function after some localized tissue injury has occurred. Since many of their actions impinge on cells of the immune system, they have also been termed immuno-resolvents.

Not only have EPA and DHA been found to be used as the substrates for the formation of SPMs, but also arachidonic acid (AA), an omega-6 LCPUFA, is now recognized to contribute to the formation of potent endogenous anti-inflammatory and pro-resolving lipid mediators. This is an important point to remember since many people like to denote omega-6 fatty acids as merely "pro-inflammatory." This notion should be abandoned and replaced by a more balanced view that conveys the idea that both the omega-6 PUFA AA, as well as EPA and DHA, are important for the mounting as well as the counter-regulation and resolution of inflammation. Being able to initiate an inflammatory response is essential for good health—without it, we would rapidly succumb to bacterial, fungal and viral infections, and we would be incapable of starting to restore an injured tissue. But we also need to be able to control the extent and duration of inflammation, to avoid chronic inflammatory disease when the initial stimulus that triggered inflammation is long gone. That is the newly appreciated role of SPMs, and the need for sufficiency in omega-3 LCPUFA. Essentiality of fatty acids refers to both omega-6 and omega-3 PUFA, and their dietary sufficiency is critical for attaining and maintaining good health.

Figure 1: Specialized Pro-Resolving Lipid Mediators Derived from EPA, DHA and AA



Specialized pro-resolving lipid mediators, or SPMs, are local hormones with signaling functions in the resolution of inflammation. SPMs are formed by cells that express the enzymatic pathways that use eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA) as substrates. Some SPM classes are shown here. DHA-derived SPMs consist of D-series resolvins, protectin D1 (also called neuroprotectin D1 when formed in neuronal tissue), maresins, and recently identified 14-hydroxy-sulfido conjugates. EPA-derived SPMs include E-series resolvins and recently identified 12-hydroxy-17,18-epoxy-eicosatetraenoic acid. AA-derived lipoxins, which were the first recognized endogenous anti-inflammatory lipid mediators derived from polyunsaturated fatty acids, are now also viewed as endogenous signaling substances that participate in the resolution of inflammation.

How precisely dietary sufficiency of essential fatty acids (EFAs) relates to optimal tissue responses in nutritional prevention and therapeutic responsiveness toward disease is a topic of much past and current research activity. It is believed now that an adequate balance of EFAs in their role as substrate for SPM formation makes a critical contribution to many aspects of health, and to the lowering of risk of inflammatory disease. In a period of about 15 years, more than 20 distinct biologically active fatty acid derivatives have been discovered that are formed through enzymatic pathways and promote resolution at very low local tissue concentrations (picomolar to low nanomolar). These are composed of *i*) resolvins, which are named after their initial description when found to be formed via interactions of different cell types that bring closely together the required enzymatic activities for their formation, i.e., **resol**ution-**in**teraction products, *ii*) protectins, named for their ability to mediate tissue-**protect**ive responses, and *iii*) maresins, which are named for their recognition to be formed by **ma**crophages during **res**olution. A range of SPMs is now known, with E-series resolvins being derived from EPA and D-series resolvins, maresins and protectin D1 derived from DHA (see Figure 1). An example of the chemical structure and enzymatic formation of a recently discovered resolvin, resolvin E3 (RvE3), is shown in Figure 2. Not only do EPA and DHA lend themselves to the formation of SPMs, but resolution-promoting lipid mediators derived from both omega-3 docosapentaenoic acid (clupanodonic acid or DPA) and omega-6 docosapentaenoic acid (also known as osbond acid) have also been described.

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SPMs in the Resolution of Inflammation

SPMs exert potent agonistic activities on dedicated receptor proteins found on a variety of cell types, thereby activating a variety of cellular processes needed for the orderly return to tissue homeostasis. There are several key resolution-promoting activities that distinguish SPMs from substances that are merely anti-inflammatory, and from agents classified as immune-suppressing. These are their ability to stimulate phagocytosis by professional phagocytes, which permits the clearance of microbes, of dying phagocytes that have terminated their function in clearing microbes, and of cellular debris produced during inflammation. SPMs have the ability to accelerate the pace of resolution (disappearance of the neutrophil-rich inflammatory exudate), while down-regulating the biochemical and cellular events necessary to sustain inflammation.

This leads to another notion that needs to be dispelled, which is that the dynamic down-regulation of polypeptide cytokines such as interleukin-1 and tumor necrosis factor by omega-3 PUFA supplementation would negatively

affect the capacity for microbial clearance. Such suspected "immune-suppressive" effects are now better viewed as immune-modulatory in the light of recent discoveries that resolvin E1 (RvE1), resolvin D1 (RvD1), RvD5 and protectin D1 (PD1) are potent activators of microbial clearance in models of bacterial, viral and fungal infections. Promotion of microbial clearance is mediated through improved delivery (migration) of phagocytes to an infected tissue, increased phagocytic rate and augmented microbicidal activity of phagocytes, a shorter resolution period, and improved survival of animals that would succumb to otherwise poorly controlled microbial infection. SPMs are not immune-suppressive in fighting inflammation, and in fact may improve antibiotic therapies.

Notably, a greater number of resolution-promoting lipid mediators derived from PUFA are now known than prostanoids involved in initiating inflammation. This suggests that the down-regulation of the extent of inflammation, and the capacity to regulate the onset and speed of resolution, may be under more stringent control than inflammation itself. The wide variation in counter-regulatory signals may point at the need to be able to activate multiple different signaling pathways to bring under control distinct types of inflammation that deal with a variation in harmful stimuli, and the particular organs and tissues in which inflammatory responses operate.

Modulating the Outcome of Inflammation – Addressing Deregulated SPM Formation

Several variables determine optimal tissue defenses and the outcome of an inflammatory response. First, sufficiency in dietary intake of LC omega-3 and omega-6 PUFA is required for attaining appropriately high concentrations within cellular membranes of different cell types. Phospholipase enzymes that selectively release particular PUFA from membranes are now known, but insufficiency in specific PUFA within the available sites for membrane esterification relates strongly to increased risk for inflammatory disorders, such as chronic cardiovascular disease (CVD)—atherosclerosis, heart failure, sudden death. Dietary imbalances in the relative intake of omega-6 and omega-3 have been identified in Western societies and predispose to chronic inflammatory disorders. Such dietary imbalances need to be reoriented to improve relative and absolute increases in omega-3 intake accompanied with a decrease in linoleic acid intake. Second, interindividual variability operates at the regulation of omega-3 absorption, distribution and metabolic fate within the body, the limited endogenous biosynthetic capacity to form EPA and DHA from alpha-linolenic acid, and the enzymatic formation and degradation of SPMs. Third, it is conceivable that multiple ways exist in which lifestyle affects SPM formation and function. For example, the intake of other biologically active nutrients, pharmaceutical therapies and behavioral habits may modulate how efficiently

SPMs are formed and act, on a background of individual enzyme expression required for their formation and breakdown.

The study of deregulation of SPM formation and action in human disease and its relation to dietary LCPUFA sufficiency is now taking center stage in understanding why LCPUFAs are important to good health. Understanding the factors that determine SPM bioactivity in specific physiological and pathophysiological settings in humans is expected to point us in new directions to address improved recommendations in nutrition and in directed therapeutic options to address prevalent disorders such as obesity, diabetes, cardiovascular disease, depression, neurodegeneration and cancer—all of which have been reported to have modifiable outcomes related to EFA intake. Understanding and regaining control over inflammation through any of the three variables may be suitable approaches to overcome poorly controlled and chronically non-resolving inflammation at the basis of a range of diseases typical of a Westernized lifestyle.

Key Lessons Learned

Some key aspects of SPM biology have already been appreciated and provide a foundation to advance future studies and applications. First of all, consider that SPMs are produced in a temporally-defined order during an inflammatory response. A so-called lipid mediator "class switching" appears to operate in which sequential formation of specific SPMs conveys information on the stage in which the inflammatory response develops and in which each lipid mediator activates new cell-specific activities that drive the inflammatory process forward to resolution. SPMs are typically formed in a cell-specific fashion, act locally on receptors in neighboring cells and thereafter degrade rapidly. Resolution of inflammation involves multiple activities such as limiting the infiltration of neutrophils, facilitating phagocytic removal of infections in a manner that limits tissue damage, promoting recruitment of monocytes/macrophage that regulate the clearance of the early phagocytes that removed infectious microbes, restoring the inflamed tissue parenchyma without the formation of scars, restoring appropriate vascular regrowth, regulating adaptive immune responses in draining lymph nodes, and repopulating tissues with immune cells that execute immune surveillance to alert tissues on new tissue insults. The inflammatory response is a process that requires temporal control over many different cell types. In "healthy" inflammation, this process operates without lasting consequences to proper tissue functioning, due largely to the availability of omega-3 LCPUFA and capacity to form SPMs.

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FIGURE 2

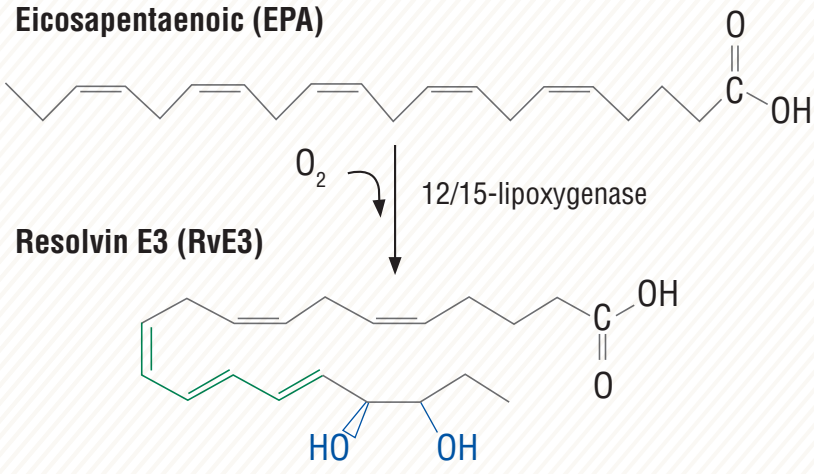

FIGURE 2 - Resolvin E3 (RvE3 or 17,18-dihydroxy-eicosapentaenoic acid: *bottom*) has been found to be produced during the process of inflammation resolution. As an example, the chemical structure of an SPM, and its enzymatic formation is shown here. RvE3 is formed by oxygenation of eicosapentaenoic acid (EPA: *top*) by an enzyme called 12/15-lipoxygenase. It is a potent endogenous inhibitor of inflammation and promotes the resolution of inflammation.

The chemical structure of RvE3 is characterized by a *vicinal* (neighboring) *diol* (two hydroxyl groups located next to each other; blue color) and a conjugated *triene* (three double bonds that are located closely together; green color).

SPMs in Human Disease

At this stage, we need to better understand which mediators should be restored to functional levels in specific pathologies, and how to achieve this with respect to dosage and timing of intake of particular EFA substrates or SPMs directly. Recent studies have indicated SPMs play a role in human subjects, and the number of studies reporting the measurement of SPMs in different conditions is increasing. Human studies rely on their measurement in accessible fluids, such as blood (plasma, serum), urine and broncho-alveolar lavage fluid. Since the concentrations of SPMs in vivo are generally very low (picomolar to low nanomolar) and their metabolic inactivation is considered to happen fast in local tissues, technical challenges exist in reliably extracting and measuring SPMs. Recent studies have pointed out that some resolvins derived from EPA and DHA can be reliably detected in plasma, and RvE1 is responsive to changes in omega-3 supplementation within a few days in healthy human subjects. Vascular formation of specific epoxide-containing derivatives of DHA has been demonstrated to increase upon increases in EPA/DHA intake, and may also contribute to the blood pressure-lowering activity of increased omega-3 LCPUFA intake. To mention a few examples, deregulated SPM formation has been measured in atherosclerotic plaques, vascular smooth muscle hyperproliferation in vascular inflammation,

inflammatory airway disorders, chronic kidney disease, neurodegenerative diseases such as Alzheimer's disease, and post-heart attack heart failure.

Often in tissues that are measured for SPM level, higher levels of biosynthetic intermediates of SPMs are found. These enzymatically oxygenation products of EPA and DHA can be interpreted as gauges for SPM biosynthesis, and provide valuable information on the activity of specific enzymatic pathways involved in SPM biosynthesis, as well as the utilization of particular PUFA, even when SPM levels are hardly detectable due to their rapid elimination in tissues. However, the interpretation is not always straightforward; for example, higher plasma levels of 14-hydroxy-DHA (14-HDHA) observed in plasma after a few days of omega-3 supplementation in healthy adults may signify maresin biosynthesis is more active than other biosynthetic routes, that 14-HDHA is relatively more resistant from metabolic inactivation, or that 14-HDHA represents an accumulation of singly oxygenated and reduced product from DHA that is not further oxygenated to maresins.

Resolution Pharmacology and Prospects in Nutrition

A new concept termed "resolution pharmacology" has emerged that aims to determine how pro-resolving substances can be employed to actively "turn off" inflammatory disease by stimulating resolution of inflammation. Of interest, the oldest synthetic drug we know, aspirin, was found in the 1980s to trigger the enzymatic formation of so-called 15-epi-lipoxins, derivatives of AA with potent anti-inflammatory activity. Aspirin, while blocking prostanoid biosynthesis, can change the activity of the type-2 cyclooxygenase to an enzymatic activity that catalyzes the incorporation of oxygen into AA with a stereochemical orientation that was inverse ("epimeric") to the commonly known stereochemistry of such reactions, typically carried out by another enzyme class called lipoxygenases. The 15-epi-lipoxins proved to activate receptors on immune cells that turn off inflammation. From this perspective, aspirin is an unusual nonsteroidal anti-inflammatory drug because it also actively participates in triggering the resolution of inflammation. Importantly, such epimeric oxygenation was subsequently confirmed to also operate with omega-3 LCPUFA, with the discovery of the first resolvin, RvE1. Non-epimeric resolvins were subsequently also shown to be formed, either by fatty acid oxygenases that incorporate oxygen with the "normal" S stereochemistry, or because cyclooxygenase can form both epimeric and non-epimeric forms. For many SPMs, both S and R stereoisomers are now known to exist, depending on the route of enzymatic formation. Several pharmaceutical developments

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exist that develop chemical analogues of omega-3 LCPUFA derivatives that better resist metabolic degradation. These include applications in ocular inflammation, atrial fibrillation and in neurodegenerative disorders such as amyotrophic lateral sclerosis and hearing loss.

Interesting prospects await while further documenting the when and where of resolvins, protectins, maresins and other PUFA-derived lipid mediators. SPMs have been found in multicellular organisms ranging from planarians, fish, rodents and humans—suggesting a conserved role in immune regulation, tissue protection and restoration of tissue function after injury. Resolvins are present in human breast milk, and dietary supplementation with EPA/DHA during pregnancy increases SPM biosynthesis in the placenta. Since EFAs are not only essential for people, but are transferred and used throughout the entire marine to terrestrial food chain, it may be prudent to seek greater understanding regarding the impact of environment and nutrition in determining our defenses. One example is researching why SPMs in fish may be a variable for success of omega-3s in some but not all clinical trials. ❑

Gerard Bannenberg, Ph.D., is director of compliance and healthcare outreach at GOED.

Readings of Interest

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