# Controlling inflammation: a fat chance?

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**The inflammatory response protects the body against infection and injury but can itself become deregulated with deleterious consequences to the host. It is now clear that several endogenous biochemical pathways activated during defense reactions can counterregulate inflammation. New experimental evidence adds resolvin E1 to this group of endogenous inhibitors and provides further rationale for the beneficial effects of dietary supplementation with fish oils. It also highlights an unexpected twist in the pharmacology of aspirin.**

## Polyunsaturated fatty acids and diet

The discovery in the 1930s by George and Mildred Burr (1) that certain polyunsaturated fatty acids were "essential" to the health of mammals begged the question of why they were so crucial. Initially it was thought that their importance lay in their unique viscotropic effect on biological membranes, but the further discovery in the 1960s that all essential fatty acids were also substrates for prostaglandin synthesis by the cyclooxygenase enzymes (2) lead to the realization that, in addition to being important structural components of the cell, these lipids were the precursors of potent hormones with widespread effects on the cardiovascular and immune systems. The subsequent demonstration that other mediators such as the leukotrienes (derived from the 5′-lipoxygenase [3]) and, more recently, that the endocannabinoids (4) could also be derived from these same precursors, further highlighted this unusual property of these versatile lipids.

The essential fatty acids, which include arachidonic and eicosapentaenoic acids, cannot be synthesized by mammals de novo but must be supplied in the diet either as the native lipids or as immediate precursors, such as linoleic or α–linolenic acids, which are then converted by chain elongation and desaturation reactions into the required end product. Arachidonic acid is a 20-carbon fatty acid with 4 unsaturated double bonds located at positions (all cis) 5, 8, 11, and 14 in the hydrocarbon chain (counting from C1, the COOH terminal). Arachidonic acid belongs to a group of fatty acids sometimes known as ω-6 fatty acids, so called because of the location of the final double bond from C20. Since the main source of essential fatty acids is foodstuffs, it follows that the actual composition of essential fatty acids in the body reflects to a large extent the nature of the diet. Although arachidonic acid is abundant in the tissues of many land-dwelling animals, fish and marine mammals have a preponderance of the closely related eicosapentaenoic acid with five double bonds arranged at positions 5, 8, 11, 14, and 17 (thus belonging to the ω-3 group). It has been suggested (5) that mankind evolved on a diet where the ratio of ω-6:ω-3 fatty acids was ∼1:1, as opposed to the prevailing ratio (at least in Western societies) of 10–20:1. The implication is that the onset and progress of many inflammatory and other diseases may be exacerbated by this shift in dietary habits.

When oxidized by the cyclooxygenase enzyme systems, arachidonic acid gives rise to the "2" series of prostaglandins such as $PGE_2$, $PGF_{2\alpha}$, and so on, because of the loss of two unsaturated bonds during the cyclooxygenase reaction, and to the "4" series of leukotrienes, such as $LTB_4$. However the properties of eicosapentaenoic acid in this respect are quite different. To begin with, eicosapentaenoic acid is not a particularly good substrate for the cyclooxygenase and actually competitively inhibits arachidonic acid oxidation in vitro (6). $PGE_3$ is produced from eicosapentaenoic acid by the cyclooxygenase but is less active than $PGE_2$ in producing various biological effects relevant to inflammation (7). Eicosapentaenoic acid is, however, a good substrate for lipoxygenases, although $LTB_5$ is ∼30 times less active as an activator of neutrophils than $LTB_4$ (8).

It had been deduced from epidemiological and dietary studies of different populations, such as the Greenland Eskimos (9), that a preponderance of fish in the diet was generally associated with a reduced incidence of inflammatory and cardiovascular disease. Over the years, a great number of studies have tested extracts of fish oil (which usually contain a mixture of eicosapentaenoic acid together with other associated fatty acids such as docosahexaenoic acid) as dietary supplements, finding a beneficial effect in a wide range of human inflammatory conditions including rheumatoid arthritis (10), cystic fibrosis (11), ulcerative colitis (12), UV-induced skin damage (13), septic shock (14), and asthma (15). Patients fed diets rich in eicosapentaenoic acid have been shown to express fewer inflammatory biomarkers (16), reduced leukocyte activation and mobility (17), and diminished production of prostaglandins and platelet-activating factor ex vivo (14); similar effects have been seen in many animal studies (18). Eicosapentaenoic acid is, therefore, one of the few "nutriceuticals" for which there is compelling evidence of efficacy, although the optimum dosage has perhaps yet to be established.

## Explaining the beneficial effects

The most widely accepted explanation for the efficacy of eicosapentaenoic acid was that increasing proportions of this fatty acid incorporated into the cellular phospholipid pool reduces the net fraction of arachidonic acid released during cell activation, leading to less arachi-

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donic acid oxidation overall and to the production of a different panel of lipid mediators. In man, a dose-related replacement of membrane fatty acids occurs after ingestion of up to 1.6 g eicosapentaenoic acid/day (10), an effect more pronounced when the amount of arachidonic acid in the diet was concomitantly restricted (19). In animal studies, feeding increased amounts of eicosapentaenoic acid reduced the amount of arachidonic acid present in cells, and led to a reduction in the production of prostaglandins such as $PGE_2$ in experimental lesions (18).

But other explanations for the efficacy of eicosapentaenoic acid have also been suggested. It has been postulated, for example, that 15-lipoxygenase products of eicosapentaenoic acid themselves can interfere with the activation of the transcription factor NF-κB and thus prevent the activation of inflammatory genes (20), or that eicosapentaenoic acid blocks the terminal stages of arachidonic acid synthesis from its precursors in vivo (21). But in this issue (page 713), Arita et al. have come up with another fascinating observation which relates directly to the efficacy of eicosapentaenoic acid as a potential antiinflammatory in man and, interestingly enough, implicates another popular therapeutic agent, aspirin, in a unique joint antiinflammatory mechanism (22).

The work described by Arita et al. (22) follows earlier discoveries by this team, lead by Charlie Serhan, of other groups of potent lipid mediators derived from arachidonic acid, including the lipoxins, resolvins, docosatrienes, and neuroprotectins (23). In this issue, Arita et al. describe the generation, by the aspirin-treated cyclooxygenase (COX)-2, of a 15-epi product of eicosapentaenoic acid (5S, 12R, 18R-trihydroxy-6Z, 8E, 10E, 14Z, 16E-eicoasapentaenoic acid) which is subsequently transformed to resolvin E1, a compound previously found by the group to be present during the resolution phase of murine inflammation (24). By extending his observations into man, Serhan's group has now placed the whole idea of antiinflammatory lipids on a new and more relevant therapeutic footing.

The mechanism described here is an interesting one for several reasons. In contrast to its action on COX-1, aspirin does not totally inhibit the oxidation of arachidonic acid (or other polyunsaturated fatty acid substrates) by COX-2 (25), and although the aspirin-inhibited enzyme cannot produce prostaglandins it retains the ability to generate a monohydroxy fatty acid species. The most likely source of the COX-2 in this instance is the endothelial cell. In the presence of eicosapentaenoic acid and when "inhibited" by aspirin, COX-2 can release the 18R-hydroxy eicosapentaenoic acid precursor of resolvin E1. However, this moiety cannot be further metabolized by the endothelial cell itself, and its transformation to resolvin E1 depends on the presence of the 5-lipoxygenase enzyme in adjacent leukocytes that are presumably adherent to the vessel wall (Fig. 1). The resolvin E1 product was measured in bioactive concentrations in the plasma of volunteers taking both eicosapentaenoic acid (1 g) and low dose aspirin (160 mg).

### Versatility of G protein–coupled receptors

The striking antiinflammatory activity of lipoxin $A_4$ ($LXA_4$) as an inhibitor of leukocyte activation, as earlier described by Serhan's group (26), was rather surprisingly manifested through interaction with a member of the formyl peptide receptor (FPR) family termed ALXR (or FPR-like 1). This finding was unexpected, as this family of receptors, which comprises at least three subtypes in humans, is generally considered to be a promoter rather than an inhibitor of leukocyte chemotaxis and activation (27). However, the recent notion that another endogenous antiinflammatory protein, annexin 1, also acts through ALXR reinforces the concept that this receptor may also have a protective antiinflammatory function (28).

As in the case of $LXA_4$, Serhan's group has found that resolvin E1 exerts its antiinflammatory effects by acting through a G protein–coupled receptor to down-regulate NF-κB activation (22). This receptor (subsequently referred to as ChemR23), which seems fairly widely distributed in human tissues, is related to ALXR and was origi-

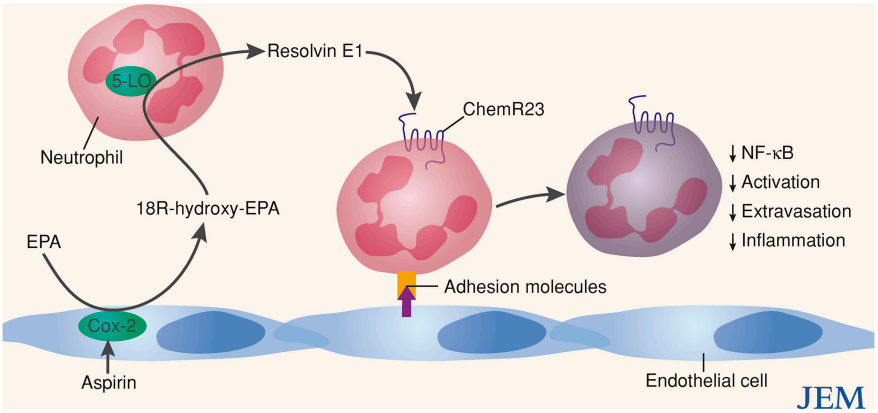


**Figure 1. Resolvin E1 and its receptor; a novel antiinflammatory circuit.** Transcellular synthesis of resolvin E1 from diet-ingested eicosapentaenoic acid (EPA) occurs within the microcirculation by the concerted action of endothelial cell COX-2 and neutrophil 5-lipoxygenase (5-LO). After aspirin treatment, resolvin E1 synthesis occurs even in the absence of inflammation. Aspirin inactivates COX-2 but permits continuing generation of the intermediate 18*R*-hydroxy-EPA which is converted to resolvin E1 (or 5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid) by the 5′ lipoxygenase in adjacent neutrophils. This lipid can then act in a paracrine or autocrine fashion on a specific seven-transmembrane G protein–coupled receptor, termed ChemR23, to bring about inhibitory effects on leukocyte activation presumably with reduced synthesis and reduced release of proinflammatory mediators: the end point of resolvin E1–ChemR23 mediated effects is a reduced flux of blood-borne cells into the site of inflammation.

nally described as a receptor for a chemotactic peptide called chemerin. The promiscuity of the FPR family of seven-transmembrane G protein–coupled receptors may be gauged by the number and diversity of the ligands they recognize, which include lipids, peptides, proteins, bile acids, and even enzymes. It seems that Serhan's group has uncovered an additional example of a series of lipids that exert their activities by binding to a receptor that might, under other circumstances, actually promote leukocyte chemotaxis (22). It is likely that receptors such as FPR, ALXR, and ChemR23 can assume ligand-specific conformations, hence transducing signals specific to each agonist. This concept has been advanced for several G protein–coupled receptors (29), including those of the FPR family (30).

### The lure of endogenous antiinflammatories

The notion that the inflammatory response generates its own regulators in tandem with the better known proinflammatory mediators such as prostaglandins and leukotrienes makes sense from the cybernetic viewpoint as it is easier to control a process with both positive and negative regulatory inputs. Indeed, several other instances of endogenous regulators of the inflammatory response (31) have been characterized recently (32), adding support to the idea that this is a widely employed mechanism. Clearly, disturbances in such counterregulatory circuits could lead to exacerbated inflammatory responses just as effectively (although perhaps less obviously) than excessive activation of the proinflammatory cascades.

### Aspirin: more than one mechanism?

This study also throws into sharp relief yet another previously unsuspected action of aspirin—its ability to facilitate the generation of antiinflammatory lipids from eicosapentaenoic acid and arachidonic acid (33). This property, which Serhan's group has noted may be shared by indomethacin and acetaminophen (24), can now be added to the catalog of antiinflammatory effects of aspirin which have recently been the focus of other investigations (34). It is a sobering thought that aspirin, the first and arguably the simplest in chemical terms of all the modern antiinflammatory drugs, should have such complex and profound effects.

### Clinical horizons

So where does this leave us in terms of practical therapeutics? It would seem that the antiinflammatory effects of eicosapentaenoic acid might be radically enhanced with low dose aspirin, and a priority should now be a formal clinical trial designed to test the additive action of these two agents in an inflammatory disease—perhaps rheumatoid arthritis—by monitoring plasma resolvin E1 levels and disease outcome. We also need to know whether resolvin E1 is found in vivo in the absence of aspirin treatment (and how) and whether other nonsteroidal antiinflammatory drugs such as indomethacin also promote its synthesis. But we should not overlook the cardiovascular implications of this work. Aspirin itself is, of course, used already in low doses for the prophylactic treatment of patients at risk from myocardial infarction and other cardiovascular pathologies, and eicosapentaenoic acid has been shown already to be beneficial in these conditions as well. For example, the data reported in the GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico) study (35), quoted by the authors, demonstrates a beneficial effect of eicosapentaenoic acid in patients at risk for myocardial infarct, many of whom were taking aspirin. In the light of the evidence presented by Serhan's group (22), we should now also take a closer look at the aspirin–eicosapentaenoic acid interactions in the cardiovascular arena. We need to discover any direct effects resolvin E1 may have on platelet function and to investigate whether any beneficial effects that may be seen in cardiovascular disease depend on the antiinflammatory effects of this compound or on other actions.

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