persistently activated in a human disease called anaplastic large cell lymphoma[10]. Chromosomal translocations join the nucleoplasmin gene to the gene that encodes ALK kinase, producing the persistently active NMP-ALK oncogene.

To investigate the role of STAT3 in malignant transformation by NMP-ALK and in cancer cell survival, Chiarle *et al.*[5] for the first time have studied bone marrow–derived cancer cells in which the *STAT3* gene has been deleted in bone-marrow derived cancer cells. They first showed that fibroblasts containing one functional *STAT3* allele could be transformed by the NMP-ALK oncogene, as measured by growth in soft agar. In contrast, STAT3-deficient fibroblasts could not be transformed.

NMP-ALK activity can be expressed in lymphocytes both in cultured cells and in the whole animal. The authors studied animals bearing NPM-ALK and molecularly marked *STAT3* genes capable of being deleted specifically in lymphocytes (a Cre recombinase driven by the T cell–specific CD4 promoter and homozygously *lox*P-marked *STAT3*).

T lymphocytes retaining a single *STAT3* allele and T lymphocytes lacking both *STAT3* alleles formed NMP-ALK–induced lymphomas that killed the animals in about 40 weeks.

In culture, however, the lymphomas without *STAT3* grew less well than those still retaining one *STAT3* allele. Most important, in the NMP-ALK–bearing animals with a single *STAT3* allele, every one of the induced lymphomas had persistently active, tyrosine phosphorylated STAT3—and when the lymphoma cells in these animals were engineered to lose the *STAT3* gene, the cells promptly underwent apoptosis.

The authors went on to show that NMP-ALK can induce myeloma, a B cell–specific tumor. In mice that had *STAT3* ablated in about 80% of B cells, all NMP-ALK–induced myeloma cells still had *STAT3*—and *STAT3* was persistently activated. These genetic experiments indicate clearly that the presence of persistently active *STAT3* favors the development of T- and B-cell tumors and is required for their maintenance.

In addition, Chairle *et al.* injected antisense oligonucleotides to *STAT3* at a site distant from transplanted lymphoma or myeloma tissue. This treatment successfully restricted growth of either T-cell lymphomas or B-cell myelomas. It is noteworthy that the antisense oligonucleotides against *STAT3* killed tumor cells but had little effect on the animal.

These experiments with hematologic tumors build on other studies suggesting that anti-STAT3 therapy would limit tumor growth. For instance, tumors in mice resulting from transplantation of human head and neck squamous carcinoma cells shrink when injected directly with an antisense *STAT3* synthetic DNA derivative[11].

All of these results—together with the requirement of STAT3 for survival in many tumor cells in culture—promise that whereas all tumors may not have persistently active STAT3, the very large number that do may well respond to anti-STAT3 therapy. To this end, efforts are underway to discover small molecules capable of directly inhibiting STAT3. Early success has been reported with platinum compounds[12] that block the DNA binding of phosphorylated STAT3 and with natural products that block STAT3 activation[13] or possibly even disrupt STAT3 phosphodimers[14].

Given the preliminary success in developing small molecules—plus the evidence from several labs, including the current report of effective antisense treatment in whole animals—it is not at all unreasonable to hope that effective anticancer therapy can come form inhibiting persistently active STAT3.

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Fish oil fix

Stephen M Prescott & William F Stenson

Fish oil has anti-inflammatory properties, but for years the mechanism has remained obscure. That mechanism now begins to come to light—and aspirin may feed into the system by promoting the production of lipid mediators derived from the oil.

The phrase "the hair of the dog that bit you" refers to a common hangover 'treatment'—imbibing more alcohol. But in ancient practice, physicians included some hair from the offending dog into a bandage that treated the bite, an example reflecting our universal sense that processes naturally incorporate their opposite.

The inflammatory process is a perfect example of the need for opposing actions; inflammation must be robust to protect us from infections and to repair damage from trauma, but it also must be limited to prevent damage to normal tissues. Dysregulated inflammation—too much at the wrong time or place—underlies many human diseases. Two recent studies by Charles Serhan and colleagues describe an 'off' switch for inflammation that has the unusual property of being derived from the same pathways that generate proinflammatory signals. Even more intriguing is that the messenger supporting resolution is derived from a fatty acid found in fish oil, which has a variety of favorable health effects.

Lipid mediators—such as prostaglandins and leukotrines—that are derived from polyunsaturated fatty acids typically promote inflammation, and a common therapeutic goal is to inhibit their synthesis or actions. Serhan and collaborators have pursued a contrarian view and report on a compound that they named resolvin E1 (RvE1).

They identified this lipid in studies of the effects of aspirin in inflammation[1]. They first

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found that inflammatory exudates from mice, or human endothelial cells, that had been treated with aspirin contained oxidized derivatives of eicosapentaenoic acid (EPA). EPA is an essential fatty acid enriched in oils of fish and marine mammals and in some plants. RvE1 was one of the products and was synthesized in a two-step process that required 5-lipoxygenase (5-LOX) and cyclooxygenase (COX)-2 that had been partially inhibited by treatment with aspirin.

These oxidized products of EPA had anti-inflammatory properties even though they had been synthesized in part by the 5-LOX pathway, which typically yields the proinflammatory leukotriene B_4 (LTB_4). In a recent issue of the *Journal of Experimental Medicine*, Serhan's group completed the structural characterization of RvE1, including determining the precise stereochemistry[2]. This enabled the total chemical synthesis of the molecule, which in turn led to the identification of a receptor for RvE1, a G-protein-coupled cellular receptor named ChemR23 that was previously shown to interact with peptide ligands. From cellular studies, the researchers concluded that RvE1 resolves inflammation by suppressing the activation of NF-κB and the consequent synthesis of cytokines and chemokines (**Fig. 1**).

In a recent issue of the *Proceedings of the National Academy of Sciences*, the authors extend their observations to an animal model in which severe inflammatory bowel disease was induced by exposure to 2,4,6-trinitrobenzene sulfonic acid (TNBS)[3]. Pretreatment of the mice with RvE1 resulted in lower mortality, decreased weight loss and less inflammation. These effects were accompanied by improved histological appearance, fewer leukocytes and a reduced amount of mRNA for some endogenous inflammatory mediators such as tumor necrosis factor-α, COX-2 and interleukin-12.

Fish oil and products derived from it are beneficial for treatment of a variety of human diseases, but the basis for this effect is not known. In one study, individuals with ulcerative colitis who received fish oil improved, as reflected by weight gain, decreased use of steroids and more favorable histology[4]. Another group found that fish oil prevented relapses from Crohn disease—setting in motion a series of experiments that began to lead to an understanding of how fish oil works[5].

One proposed mechanism was a decrease in LTB_4, derived from arachidonic acid, which has been suggested to be a key mediator in colitis[6]. This decrease would occur because EPA competes for the synthetic pathway, which results in less leukotriene synthesis overall and a product (LTB_5) that is much less potent than LTB_4[7]. In individuals with colitis who are on the fish-oil diet, LTB_4 synthesis was inhibited and, thus, the mechanism seemed plausible. But patients don't like taking large doses of fish oil—it tastes like, well, fish oil, and there are side effects, including diarrhea. Thus, investigators disrupted LTB_4 synthesis with an inhibitor of the initial enzyme, 5-LOX[8]. This lowered the LTB_4 in the colon of individuals with ulcerative colitis, but there was no favorable effect on the course of the disease.

These observations and those of Serhan's group are compatible with the following explanation: the fish-oil diet works because it results in synthesis of anti-inflammatory compounds such as RvE1, and the 5-LOX inhibitor does not help the disease because it blocks the synthesis of such anti-inflammatory compounds.

These observations are intriguing and raise even more questions. To begin, we need more information on the genesis of RvE1. It seems unlikely that humans evolved an important mechanism for limiting inflammation that requires partial inhibition of COX activity by aspirin. More probable is that there is another, endogenous, enzyme for the initial step of production of RvE1; bacterial cytochrome P450 can catalyze the conversion of EPA to 18 *R-HEPE*, and perhaps this mechanism is active in the colon. In other tissues a mammalian P450 may serve this role.

Next, we need to know how much RvE1 is made. Arita *et al.*[2] report about 0.2–1.0 nM RvE1 in the plasma of six individuals given EPA and aspirin, but systematic assessment with and without aspirin treatment is needed.

Once made, how does RvE1 act? These studies describe a role for ChemR23, implying that the lipid and peptide ligands of this receptor have different effects on signaling[2]. This is unusual, and it either means that there are unappreciated mechanisms for bifurcating signals after the message is transmitted through a G-protein-coupled receptor or that there is a confounding variable in these experiments. One powerful test would be to ask whether TNBS-treated mice that lack ChemR23 respond to RvE1. An alternative mechanism of action for RvE1, which is structurally similar to LTB_4, is that it blocks the binding of LTB_4 to its receptor and prevents the propagation of a proinflammatory signal (**Fig. 1**).

A final question is the physiological role of RvE1 in inflammation. In the TNBS study, RvE1 was given before the induction of injury, prior to when it would be produced naturally. To address the question of whether RvE1 has a role as an endogenous brake on the inflammatory response, one approach would be to study colitis in animals that are given EPA and then are either treated with aspirin or not. Another arm of the study could test whether the effects are eliminated by an inhibitor of 5-LOX. The role of RvE1 in a genetically engineered mouse model of spontaneous colitis such as the IL-10 knockout might be a better test than the TNBS model of whether RvE1 is involved in resolution of inflammation.

The Serhan studies of RvE1 in inflammation point out the importance of understanding the endogenous mechanisms by which inflammation is controlled and the potential of manipulating those endogenous mechanisms to prevent or treat inflamma-

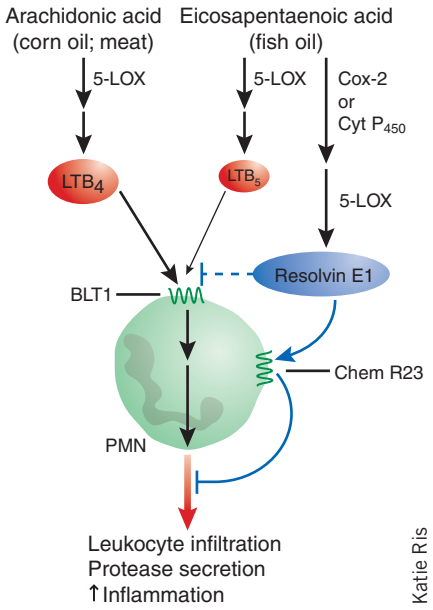

Figure 1 Fish oil functionality. LTB_4 is a central mediator of inflammation; it attracts and activates polymorphonuclear leukocytes. LTB_4 is synthesized from the essential fatty acid arachidonic acid. Another essential fatty acid, eicosopentanoic acid, which is derived mostly from fish oils, can be processed by the same pathway to yield LTB_5, although LTB_5 is made in smaller quantities and is less potent than LTB_4. LTB_5 acts at the same G-protein-coupled receptor as LTB_4 and, given its lower potency, LTB_5 can function as a competitive antagonist. Serhan and colleagues have described another product derived from EPA, resolvin E1, and have concluded that it has intrinsic anti-inflammatory properties that are exerted through another receptor, Chem R23.

tory diseases. This interesting product of fish oil seems to have marked pharmacological actions and future studies should tell us whether it is a physiological brake—that is, a dog bite treated with the hair of the dog.

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Less stress, longer life

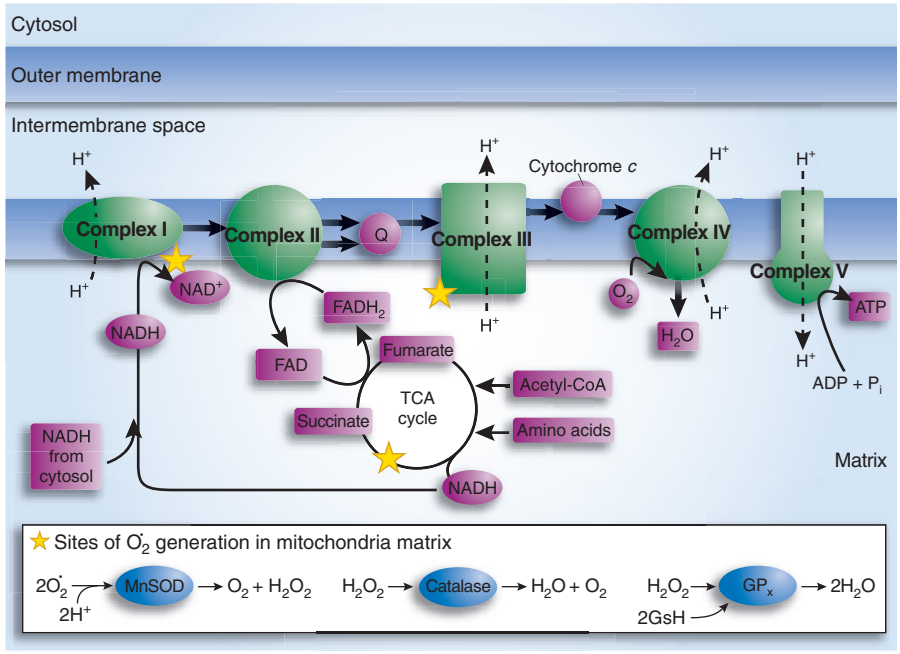
M Flint Beal

The theory that oxidative stress limits lifespan and causes age-related disease rests on experiments in invertebrates and correlative evidence from studies in mammals. This theory now gains a strong experimental basis in mammals.

The oxidative stress theory of aging holds that the slow, steady accumulation of oxidative damage to macromolecules causes age-associated reductions in physiologic functions and reduces life expectancy. Reactive oxygen species are generated in large part from single electrons escaping from the electron transport chain, suggesting that mitochondria are the major target of attack by free radicals. A corollary of the theory is that the rate of aging should be retarded by attenuation of oxidative damage. In strong support of this theory, Schriner *et al.* now show that overexpression of the antioxidant enzyme catalase in mitochondria extends lifespan in mice[1].

Several lines of correlative evidence already provided strong support for this hypothesis. There are age-dependent increases in oxidative damage to lipids, protein and DNA. Oxidative damage and mitochondrial DNA point mutations accumulate as humans age[2–5]. Moreover, a threefold increase in the number of mitochondrial DNA point mutations, which correlates negatively with cytochrome oxidase activity, occurs in brains of elderly subjects as compared to younger subjects[6].

Another study examined whether mitochondrial mutations contribute to normal aging by examining genetically engineered mice with mutations in a mitochondrial DNA polymerase involved in copying and proofreading mitochondrial DNA[7]. The polymerase was rendered error prone by eliminating its proofreading activity. This resulted in a marked accumulation of mitochondrial DNA mutations and accelerated aging. By 25 weeks of age, the mice stopped gaining weight, became bald and developed osteoporosis. Half of the mice died by 48–61 weeks, which is much earlier than the typical mouse, which lives to about two years.



Katie Ris

Figure 1 Powering up lifespan. The electron transport chain of mitochondria is the major source of free radicals in the cell. Because of electron leak, free radicals react with oxygen (O_2) to generate superoxide radicals ($O_2^{\cdot}$). The major sites of generation includes the iron-sulfur clusters of complex I, coenzyme Q associated with complex III, and components of the tricarboxylic acid cycle, including α-ketoglutarate dehydrogenase. Superoxide radicals are dismutated by manganese superoxide dismutase in the mitochondrial matrix to generate O_2 and hydrogen peroxide (H_2O_2). H_2O_2 is then converted to H_2O by either catalase or glutathione peroxidase (GPx) which uses glutathione (GSH). Aging is associated with increased mitochondrial production of H_2O_2, leading to oxidative damage and mitochondrial DNA mutations. Schriner *et al.* find that they can substantially extend lifespan in mice and reduce age-associated disease by overexpressing catalase in mitochondria in mice.

Other researchers have examined effects of oxidative damage on gene expression in frontal cortex of human aged 26–106 years[8]. They found downregulation of genes involved in synaptic plasticity, vesicular transport and mitochondrial function, and upregulation of genes involved stress response, antioxidant defenses and DNA repair. The genes that were downregulated showed selective oxidation of their promoters, which could be replicated by oxidative stress in cultured human neurons.

Altered expression of other antioxidant enzymes also has effects on lifespan. In *Drosophila*, overexpression of manganese superoxide dismutase, which is confined to mitochondria, extends lifespan. Overexpression of methionine sulfoxide reductase in *Drosophila*

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