

# PROSTAGLANDINS LEUKOTRIENES AND ESSENTIAL FATTY ACIDS

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## Fatty Acids, Inflammation and Immune Responses

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**ABSTRACT.** Evidence obtained from experiments *in vitro* and *in vivo* suggests that certain unsaturated fatty acids (FA) may be safe and effective antiinflammatory and immunomodulatory agents. Generation of a unique eicosanoid profile with different biological effects by administration of FA precursors other than arachidonic acid is one approach under investigation. In addition to their role as eicosanoid precursors, FA are of major importance in maintaining cell membrane structure, are key determinants of membrane bound enzyme activity and receptor expression. FA can exert these functions directly and therefore may themselves be important regulators of immune responses. For example, certain FA influence cytokine production and proliferation of human T lymphocytes in a manner that is direct and not due to their conversion to eicosanoids. The observations indicate that FA can modulate immune responses by acting directly on T-cells and suggest that alteration of cellular FA may be a worthwhile approach to control of inflammation.

Essential fatty acids (EFA) are 'essential' not only because of their physiological importance, but because they must be derived in either direct or partially elaborated form from the diet. Thus, these acids may be classified as vitamins (indeed they were once called vitamin F).

Two groups of fatty acids (FA) are essential to the body: the  $\omega 6$  (n6) series, derived from linoleic acid (18:2 n-6) and the  $\omega 3$  (n3) series, derived from  $\alpha$ -linolenic acid (18:3 n-3). FA provide energy, are an integral part of cell membranes, and certain ones are precursors for prostaglandins (PG), thromboxanes (TX), and leukotrienes (LT), collectively termed eicosanoids. Abundant experimental evidence supports the view that eicosanoids participate in development and regulation of immunological and inflammatory responses (1). Because rheumatoid arthritis (RA) is characterized by inflammation, disordered immune regulation and tissue injury, there is much interest in the role of eicosanoids in regulation of host defenses in RA patients. As the detrimental effects of therapy for RA may be more difficult to manage than the disease itself, there is a need for new, safe approaches to the treatment of these patients.

Generation of a unique eicosanoid profile with different biological effects by administration of FA precursors other than arachidonic acid (AA) is one approach under investigation. Although changes in eicosanoid production owing to alteration of FA intake form the basis of the current hypothesis for the antiinflammatory effects of this type of treatment, it is likely that the precursor FA

themselves alter immune responses. The n-3 FA eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic (DHA; 22:6), prominent in fish oil lipids, inhibit formation of cyclooxygenase and lipoxygenase products derived from AA (2, 3). Diets enriched in fish oil reduce generation of platelet activating factor by peripheral blood monocytes (4) and reduce production of interleukin-1 (IL-1) and tumor necrosis factor (TNF) by stimulated peripheral blood mononuclear cells (5). Fish oil supplements have therefore been used with modest success to suppress inflammation in experimental animal models (6, 7) and in patients with RA (8, 9).

Evidence obtained from experiments *in vitro* and *in vivo* suggests that other novel FA may be safe and effective antiinflammatory and immunomodulatory agents. For example, certain botanical lipids, notably those extracted from seeds of the evening primrose and borage plants, contain relatively large amounts of gamma-linolenic acid (GLA; 18:3 n-6) which can be converted rapidly to dihomo-gamma-linolenic acid (DGLA; 20:3 n-6), the FA precursor of the monoenoic PG. PGE<sub>1</sub> is such an eicosanoid and it has known antiinflammatory and immunoregulating properties (10-12). These include suppression of diverse T lymphocyte functions such as proliferation, cytotoxicity and IL-2 production. PGE<sub>1</sub> also suppresses polymorphonuclear leucocyte and monocyte activation.

An approach to PGE<sub>1</sub> therapy, first suggested by Willis (13), is provision of PGE<sub>1</sub> precursors such as GLA or DGLA. The extremely short half-life of natural

PG allows moment to moment regulation of cell function in response to external stimuli and internal messengers. Therefore, enrichment of cells with DGLA should enable PGE<sub>1</sub> production to be increased as needed without overriding the physiological controls which modulate rapid changes in its synthesis and degradation.

DGLA competes with arachidonate for oxidative enzymes, thereby reducing production of cyclooxygenase products derived from arachidonate. In addition, DGLA cannot be converted to inflammatory LT by 5-lipoxygenase. Instead, it is converted to 15-hydroxy-DGLA, which can inhibit 5-lipoxygenase activity (14). GLA enrichment of diet suppresses acute and chronic inflammation as well as joint tissue injury in several experimental animal models (15, 16). In animals treated with evening primrose or borage seed oils, cells from inflammatory exudate are enriched in GLA and DGLA, exudate PGE<sub>2</sub> and LTB<sub>4</sub> concentrations are reduced and leucocyte effector functions (chemotaxis, lysosomal enzyme release) are suppressed. Enrichment with DGLA of synovial cells in culture leads to a marked reduction in PGE<sub>2</sub> synthesis, a substantial increase in PGE<sub>1</sub> production, and reduction of IL-1 induced synovial cell proliferation (17). Addition to cultures of AA (which increases PGE<sub>2</sub> substantially) or EPA does not modify synovial cell proliferation. The antiproliferative effect of DGLA is prevented by indomethacin. Thus botanical lipids have antiinflammatory actions due to their ability to reduce synthesis of those oxygenation products of AA which are potent mediators of inflammation.

In addition to their role as eicosanoid precursors, FA are of major importance in maintaining cell membrane structure and are key determinants of the behavior of membrane bound enzymes and receptors (18). FA can exert these functions directly and therefore may themselves be important regulators of immune responses. DGLA suppresses IL-2 production by human PBMC in vitro, suppresses proliferation of IL-2 dependent human T lymphocytes, and reduces expression of activation markers on T lymphocytes directly, in a manner which is independent of its conversion to a PG (19,20). These observations indicate that FA can modulate immune responses by acting directly on T-cells and suggest that alteration of cellular FA may be a worthwhile approach to control of inflammation.

This article reviews data which indicate that FA have *direct* effects on cell function which are independent of products formed through the actions of the cyclooxygenase, lipoxygenase and cytochrome p450 pathways.

Several lines of evidence indicate that FA have anti-inflammatory effects. Enrichment of diets with DGLA and EPA reduces inflammation and tissue injury in animal models (21). Administration of GLA in primrose oil reduces pain and the need for antiinflammatory drugs in patients with RA (22). Dietary supplementation with fish oil reduces inflammation in patients with psoriasis (23). Endres et al (5) have shown that the synthesis of IL-1 and TNF, principal polypeptide mediators of inflamma-

tion, can be suppressed by dietary supplementation with fish oil. The immunoregulatory effects of dietary FA are thought to occur through their oxygenation products, the eicosanoids. However, FA constitute an important component of the cell membrane structure and therefore may influence many of these defense functions directly in a manner independent of the role of essential FA as eicosanoid precursors.

Activated T lymphocytes appear to have a central role in the pathogenesis of many autoimmune and inflammatory diseases. Although measurements of the T lymphocyte product IL-2 in RA patients have produced conflicting results, excessive production of IL-2, as well as B-cell activation and high levels of soluble IL-2 receptors, have been observed in these patients and in patients with multiple sclerosis (MS) and systemic lupus erythematosus (24–28). IL-2 responsive activated T-cell clones have been described at the site of tissue injury in patients with RA, MS, and pulmonary sarcoidosis (29–31). Thus downregulation of IL-2 dependent T-cell proliferation by benign means might be a useful therapeutic maneuver in these patients. Indeed, gold sodium thiomalate, in concentrations attainable during chrysotherapy for RA, significantly inhibits the proliferative responses of cultured human T-cells stimulated by IL-2 (32).

DGLA and AA, and to a lesser extent EPA, inhibit IL-2 production by mitogen-stimulated human T-cells via a PGE-independent mechanism. These essential FA can also inhibit IL-2 dependent human T-cell proliferation and long-term growth without conversion into their cyclooxygenase pathway metabolites. In fact, little or no PG is produced by FA-treated T-cell cultures as compared to the untreated cultures and indomethacin, a PG synthetase inhibitor, does not reverse the antiproliferative effects of the FA (19, 20). Controversy exists regarding the capacity of human T lymphocytes to produce PGE: studies by Goldyne & Rea (33) indicate that stimulated T lymphocytes and T-cell lines do not release PGE, whereas Aussenet et al reported (34) that Jurkat cells are capable of PGE synthesis. Our own experiments indicate that when PGE measurements are corrected for PGE in the medium and for cross-reactivity of the anti-PGE antibody with AA (0.015%), no PGE production by T-cell cultures is detected (19). Thus, the effect of the FA on IL-2-dependent T-cell proliferation is direct and not due to conversion to PGE. Between 2 and 48 h treatment, a rapid rate of incorporation of these FA into cellular lipids occurs, which could account for the reduced ability of FA-treated T-cells to respond to IL-2 even after a short-term exposure to the compounds and their removal from the cultures. However, both DGLA and AA inhibit IL-2-dependent T-cell growth more efficiently when they are either present throughout the short-term proliferation assays or added weekly to long-term cultures, thus demonstrating the ability of such cultures to reconstitute a normal IL-2-responsiveness upon removal of the FA.

The effects of PGE on T-cell proliferation have been studied extensively. This agent induces a profound inhibition of T-lymphocyte activation and proliferation in mitogen-stimulated cells (35,36). The phenomenon is associated with inhibition of IL-2 production, IL-2 receptor and transferrin receptor expression, transient increases in intracellular levels of cyclic adenosine monophosphate, and modulation of protein kinase C (PKC) activation pathways which prevents the increase in intracellular calcium (37-45). The latter event occurs very early after mitogenic activation and precedes IL-2 and IL-2 receptor synthesis. All these observations point to the conclusion that PGE interferes with early signal transduction mechanisms. In a system of T-cell activation triggered by anti-CD3 monoclonal antibodies, it was shown (42) that PGE added simultaneously with anti CD3 antibody inhibits T-cells at an early step of the activation process in a direct fashion and not through suppressor T-cells nor through inhibition of accessory cell function as previously indicated by others. However, even at high doses (up to  $3 \times 10^{-6}$  M) PGE<sub>2</sub> did not affect IL-2 driven proliferation of T-cells *after* they had been activated with the anti-CD3 antibodies for 3 days. In contrast, it has been shown (19,20) that DGLA and AA can inhibit both short-term growth of cultures propagated in IL-2 for as long as 2 weeks after the initial mitogenic stimulus. Thus, these FA do not only inhibit at an early step of T-cell activation but are also capable of suppressing the IL-2 driven proliferative phase of preactivated long-term T-cell cultures.

In IL-2 propagated T cell cultures, high affinity binding to <sup>125</sup>I labeled rhIL-2 is not affected by either preincubation with or addition of FA. A marked reduction in the percentage of CD25<sup>+</sup> (Tac<sup>+</sup>) T-cells is also induced by FA in IL-2-propagated T-cell cultures. The apparent discrepancy between binding and immunofluorescence data showing, respectively, that FA do not reduce the number of IL-2 binding sites but down-modulate the Tac molecule in the IL-2 propagated T-cell cultures, can be explained based on the existence of three distinct affinities of IL-2 binding: low (Tac alone), medium (p70-75 alone), and high (the p70-Tac dimeric complex) (46, 47). The anti-Tac antibody binds both the high and low affinity receptors, and not the p70-75 receptor. As one would normally expect from T-cells that are maintained in IL-2 for 10-14 days after activation, the IL-2 propagated T-cell cultures express a relatively low number (400-500) of high affinity (K receptors =  $4 \times 10^{12}$  M), and likely a high number of low affinity IL-2 receptors. Therefore, the results suggest that FA affect the expression of low affinity (Tac<sup>+</sup>) binding sites only. It is currently accepted that the high affinity IL-2 receptor (formed by the association between p70-75 non-Tac molecule with Tac) functions in signal transduction for lymphocyte proliferation (48, 49). It appears then, that EFA and their metabolites do not inhibit IL-2-dependent T-cell growth by interfering with the expression and binding of functional IL-2 receptor molecules.

Thus, the precise mechanism by which these FA suppress the *in vitro* growth of IL-2-dependent T-cells remains to be elucidated. Since DGLA (three double bonds) and AA (four double bonds) have a similar capacity to suppress IL-2 driven T-cell proliferation, and both FA are more potent in this regard than EPA (five double bonds), it is not their degree of unsaturation per se which accounts for the antiproliferative effect of the FA. However, in the bilayer of cell membranes, the shorter chain unsaturates (such as oleic acid) and the saturated FA (such as palmitic acid) induce a high degree of order whereas the longer chain EFA might be expected to introduce a disordering effect in the region near the phospholipid head group (50). Such a change might alter expression of surface molecules and/or the response of cells to mitogenic stimulation. Another mechanism by which EFA might inhibit IL-2 driven T-cell growth would be through the induction of an inhibitory factor which is secreted into the medium, analogous to recent observations with glucocorticoids in murine T-cells (51).

More recent studies (52) indicate that DGLA selectively suppresses IL-2 induced proliferation of the murine CTL.L-2 (T lymphocyte) cell line. Unsaturated FA are important components of glycosyl-phosphatidylinositol (gly-PI) molecules which serve as membrane lipid anchors for certain cell activation antigens such as Th1 and Ly6. Results of FACS analysis show that the differential effect of DGLA on CTL.L2 growth vs AA and EPA (which do not suppress cell growth in this system) is not mediated by specific modulation of gly-PI anchored molecules.

The antiproliferative effect of DGLA is largely reversed by down regulation of PKC activity (52). Thus, DGLA appears to influence lymphocyte activation at the point of induction or inactivation of PKC activity. Indeed, our preliminary data indicate (R. Rossetti, J. Chen, R. B. Zurier, unpublished data) that DGLA, but not EPA, AA or other FA, suppresses PKC activity when cells are stimulated with phorbol ester and enhances PKC activity when cells are activated by anti CD3 antibodies. Thus, the stimulus to cell activation dictates the signal transduction pathways utilized and modulation of cell function by FA varies accordingly.

Unsaturated FA such as DGLA are incorporated into membrane phospholipids at the sn-2 position on the glycerol backbone (53). Phosphatidylinositol (PI) turnover induced by cell stimulation results in production of diacylglycerol (DAG) which stimulates PKC directly (54). Although some investigators suggest that the alkyl side chains of the fatty acyl groups of DAG provide little specificity in regard to PKC activation (53, 55, 56), results of other studies indicate that the acyl groups of DAG are critical determinants of PKC activity (57, 58). Thus, it is conceivable that DGLA enriched DAG influences PKC differently than the usual DAG acyl groups (arachidonate, linoleate).

Thus eicosanoid precursor FA likely influence lymphocyte activation and proliferation by several complex

mechanisms, including effects on signal transduction. DGLA in particular appears to act at the level of PKC activation or degradation rather than altering PI turnover or calcium mobilization. By regulating lymphocyte proliferation, DGLA and other PUFA may control the magnitude of immune responses.

Overall, the data indicate that DGLA and AA have potent suppressive effects on the IL-2 driven proliferative phase that follows T-cell activation. It is very likely that the concentrations of FA used in these in vitro studies can be obtained in vivo. For example, the concentration of DGLA in plasma from a volunteer given 1.4 g GLA/day (12 capsules of borage seed oil) for 4 weeks was found in our studies (D. DeMarco, R.B. Zurier, unpublished observations) to be 34.4  $\mu\text{g/ml}$ . Moreover, daily doses of GLA of 540 mg (22) to 1.1 g (59) appear to have a beneficial effect in patients with RA. These data indicate that minimal alteration of dietary FA may have important effects on immune responses, especially in diseases in which uncontrolled T-cell proliferation contributes (or leads) to overt autoimmune or inflammatory reactions.

Other direct effects of FA include activation of purified preparations of PKC (60) and guanylate cyclase (61) and modulation of gap junctions in lacrimal glands (62). In addition, a number of UEFA, including the saturated myristic acid and those that are not eicosanoid precursors, activate potassium channels in smooth muscle cells (63, 64). In smooth muscle cells, an increase in certain FA would result in hyperpolarization and subsequent antagonism of contractile activity. Levels of FA may be controlled by several processes including phospholipase activity (65), alterations in metabolism of UEFA (66) and fluctuations in extracellular sources of FA due to hormonal status (67). Thus the potential regulatory roles for FA in the control of ion channels range from a lipid-derived second messenger to a signal carried via the circulation.

Relatively little work has been done on the effects of FA on monocyte/macrophage function. As the chief source of IL-1 and related cytokines, monocytes are critical to regulation of many pathological processes which lead to chronic inflammation and tissue injury. EPA administration does reduce production by peripheral blood monocytes of IL-1 and TNF (5). Addition of DGLA in vitro and administration of GLA in vivo reduces TNF production (D. DeMarco, R.B. Zurier, unpublished data) but the influence of GLA and DGLA on IL-1 production is not clear. Preliminary data (68) indicate that peripheral blood monocytes from healthy human volunteers given 1.9 g GLA/day for 12 weeks exhibit decreased production of IL-1 $\beta$  when stimulated in vitro with phytohemagglutinin. However, stimulation of monocytes from GLA treated individuals with IL-1 $\beta$  results in increased production of IL-1 $\alpha$ . In addition, IL-2 stimulation increased production of IL-1 $\alpha$  and TNF compared to cells studied before GLA administration. The results emphasize the complexity of cytokine regulation.

The potential ability of particular FA to directly regulate cell activation, immune responses and inflammation is exciting to consider at the clinical, cellular and molecular levels. A better understanding of how FA modulate function of cells involved in host defense might lead to development of new, benign treatment for diseases characterized by acute and chronic inflammation.

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