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FATTY ACIDS AND IMMUNE RESPONSES—A NEW PERSPECTIVE IN SEARCHING FOR CLUES TO MECHANISM

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■ **Abstract** Dietary essential fatty acids are the precursors for eicosanoids. Among the eicosanoids derived from arachidonic acid, prostaglandin (PG) E_2 is known to possess immunosuppressive actions. Thus, it has been a prevailing hypothesis that the immuno-modulatory roles of dietary fatty acids are mediated at least in part through the alteration of PG biosynthesis. PGs exert their biological effects through their cognate receptors. There are four subtypes of PGE receptors (EP1, EP2, EP3, and EP4) so far identified. Although the association of EP receptors with G proteins coupled to adenylate cyclase and the mobilization of intracellular calcium are well documented, downstream signaling pathways for these receptors are virtually unknown. Identification of downstream signaling pathways for each subtype of EP receptors and target genes regulated by the activation of the receptor will help with our understanding of the mechanism by which dietary fatty acids affect immune responses through the modulation of PGE_2 biosynthesis. Emerging evidence suggests that fatty acids can additionally act as second messengers, regulators of signal transducing molecules or transcription factors. Acylation with long-chain fatty acids can occur on a variety of signaling molecules and can affect their membrane translocation and functions. Dietary fatty acids can alter functional properties of lipid mediators by changing the composition of acyl moieties of these molecules. Evidence accumulated recently indicates that long-chain unsaturated fatty acids and their metabolites bind and activate peroxisome proliferator–activated receptors (PPARs). PPARs are nuclear hormone receptors and transcription factors that regulate the expression of broad arrays of genes involved not only in lipid and glucose metabolism, but also in immune and inflammatory responses. PPARs may therefore be important cellular targets that mediate modulation of immune responses by dietary fatty acids. Together, it becomes clear now that multiple steps in various receptor-mediated signaling pathways can be modulated by dietary fatty acids. It will be a challenging task to quantitatively determine how different fatty acids alter functional properties of multitude of signaling components and final cellular responses. Elucidating the mechanism of actions of fatty

acids on receptor-mediated signaling pathways in immuno-competent cells will provide a new insight for understanding the immuno-modulatory roles of dietary fatty acids.

CONTENTS

INTRODUCTION

Numerous experimental observations have revealed modulatory roles of dietary fatty acids on immune responses, as summarized in many review articles (7, 15, 31, 57). However, the molecular and cellular mechanism of immuno-modulatory effects of dietary fatty acids is largely unknown, and currently, the conceptual framework for the mechanism of actions is not understood. Rapid advancement in research on receptor-mediated signal transduction pathways provides new clues toward understanding the mechanism of immuno-modulatory roles of dietary fatty acids at molecular and cellular levels. Emerging evidence indicates that fatty acids are not only the precursors of eicosanoids and other lipid mediators, but also important regulators of signaling molecules and ligands for transcription factors. In this review, potential molecular targets and signaling pathways through which dietary fatty acids can modulate immune responses are examined. The aim of this review is to identify critical gaps and to propose a new concept and direction for research that can yield significant new information in understanding the molecular and cellular mechanisms by which dietary fatty acids modulate immune responses.

METABOLIC CONVERSION OF DIETARY FATTY ACIDS

Three major groups of dietary fatty acids are oleic acid, linoleic acid, and linolenic acid. These fatty acids serve as the precursors for the biosynthesis of an independent group of polyunsaturated fatty acids (PUFA), as depicted in Figure 1. These biosynthetic pathways consist of a series of desaturation and chain elongation steps. Because desaturation occurs only toward the carboxylic end of fatty acids in mammalian tissues, there is no direct crossover among unsaturated fatty acids from one group to other. It is well documented that there is metabolic competition among these three groups of fatty acids (30, 47, 48, 58). Polyunsaturated fatty acids derived from oleate do not accumulate in tissue lipids of laboratory animals fed a balanced diet. However, when rats are fed a diet devoid of essential fatty acids, the major PUFA accumulated in tissue lipids is 20:3n-9 derived from oleate. If linoleate is included in the diet, linoleate competes with oleate for the same Δ6-desaturase, and thus, desaturation of oleate is suppressed. Desaturation of oleate is also inhibited when the animals are fed a diet containing 18:3n-6, 20:3n-6, or arachidonic acid (78). Similarly, increasing dietary n-3 fatty acids results in reduction of the level of arachidonic acid in tissue lipids by inhibiting its synthesis from linoleate. Thus, the modification of dietary fatty acids can lead to alteration of the fatty acid composition of tissue lipids and, in turn, changes in cellular responses.

MODULATION OF EICOSANOID BIOSYNTHESIS BY DIETARY FATTY ACIDS

Twenty carbon PUFAs, such as arachidonic acid (20:4n-6) and eicosapentaenoic acid (20:5n-3), can be enzymatically converted to eicosanoids, as shown in Figure 2 (59, 72). The revelation that eicosanoids as autocrine and paracrine factors possess diverse pathophysiological actions has expanded our understanding regarding the modulatory roles of different fatty acids in various cellular responses, including immune and inflammatory responses (31).

The unesterified free fatty acids are the direct precursors for eicosanoid biosynthesis. Most long-chain PUFAs are esterified in membrane phospholipids and cholesterol esters and thus cannot serve as direct substrates for eicosanoid biosynthesis. The concentrations of free arachidonic acid in unstimulated cells are considered to be extremely low. Therefore, availability of the direct precursor acid is an important limiting factor in regulating the biosynthesis of eicosanoids in animal tissues. On appropriate stimulation, the precursors acids are released from phospholipids by the actions of various lipases. Amounts and types of precursor acids released depend on the composition of fatty acids in tissue lipids, which, in turn, is influenced by the composition of dietary fatty acids. Thus, cellular responses that are sensitive to eicosanoids can be modulated by modifying the composition of dietary fatty acids. During the past two decades, this relationship has been a

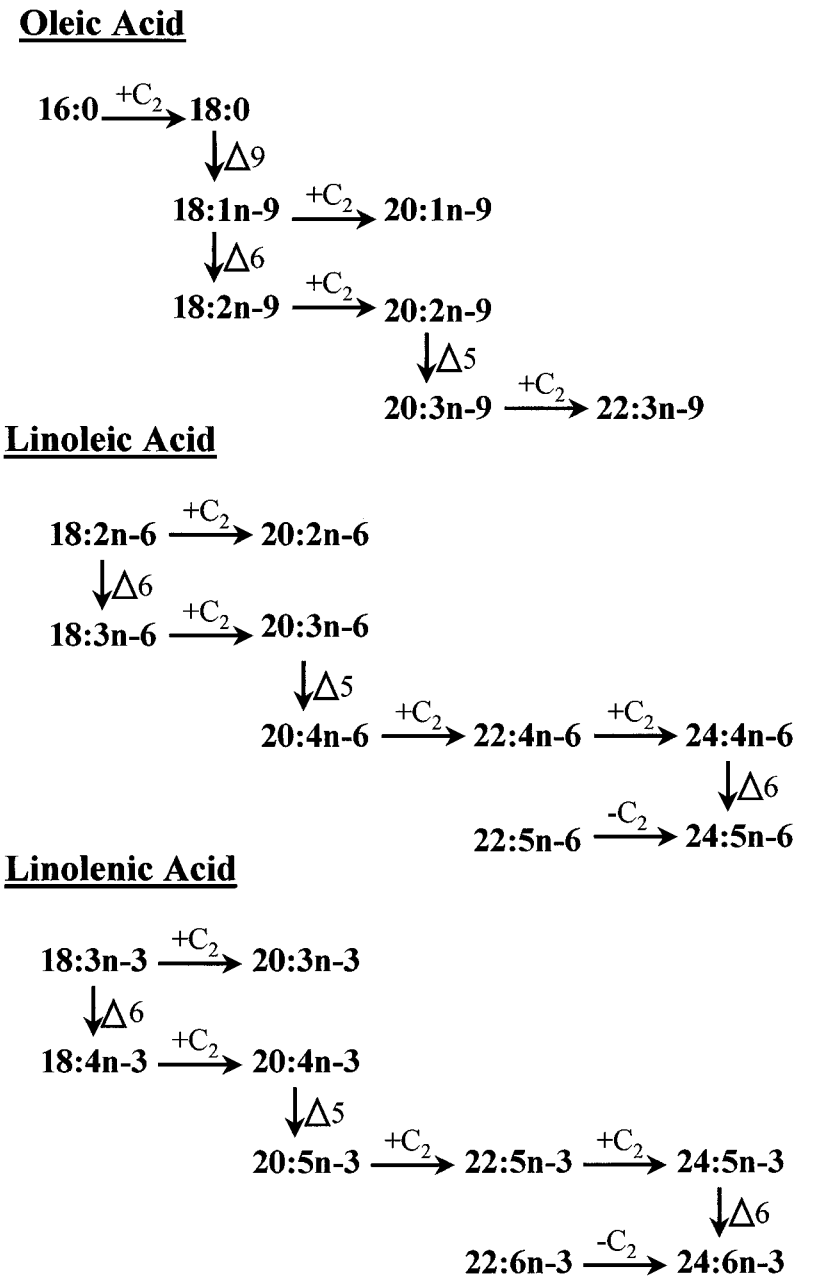


Figure 1 Metabolic conversion of three major groups of dietary fatty acids through elongation and desaturation. The first number denotes the number of carbon atoms, the number after the colon denotes the number of double bonds, and the last number denotes the position of the last double bond from the methyl end.

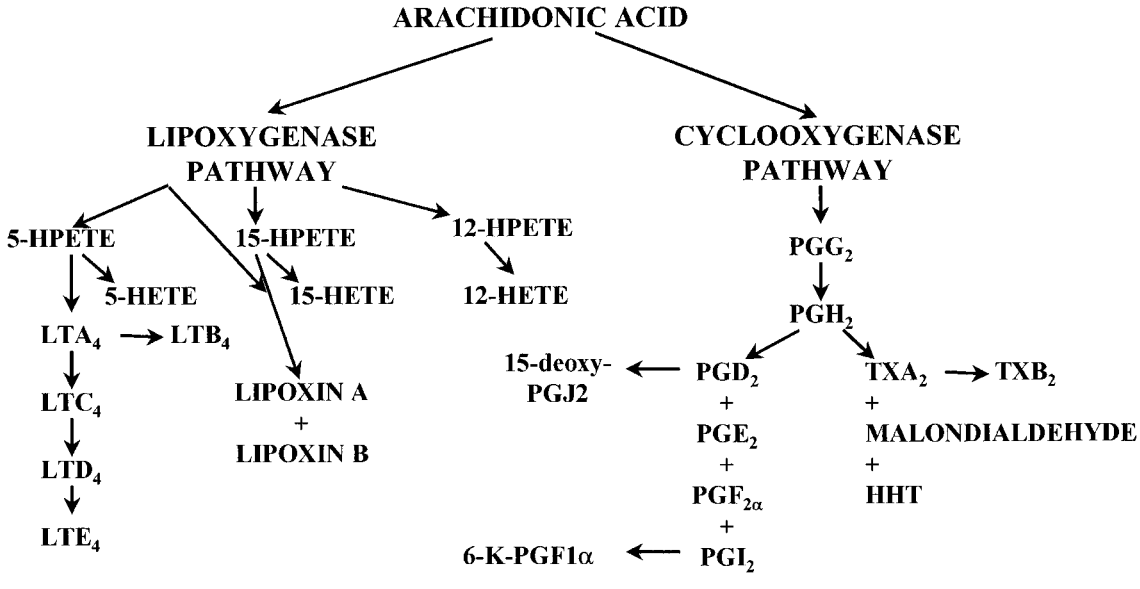


Figure 2 Biosynthesis of eicosanoids from arachidonic acid via cyclooxygenase and lipoxygenase pathways. HPETE, hydroxyperoxy-eicosatetraenoic acid; HETE, hydroxy-eicosatetraenoic acid; HHT, hydroxy-heptadecatrienoic acid; PG, prostaglandin; LT, leukotrienes; TX, thromboxanes.

major framework for understanding the modulatory roles of dietary fatty acids on risks of many chronic diseases.

IMMUNO-MODULATORY ROLES OF EICOSANOIDS

The implication that dietary essential fatty acids (EFA) can modulate immune responses was based on the observations that EFA deficiency can accentuate or improve symptoms of certain autoimmune diseases and can accelerate the skin allograft rejection (55, 79). Supplementation of linoleic acid reversed these effects of EFA deficiency. Furthermore, indomethacin, an inhibitor of cyclooxygenase (COX), abolished the effects rendered by linoleic acid supplementation. Based on these results and in vitro studies indicating that mitogen-induced blastigenesis of T lymphocytes, lymphokine production, and generation of cytotoxic cells are inhibited by prostaglandin (PG) E (77), it was suggested that COX-derived products of arachidonic acid have immunosuppressive action.

The modulatory roles of eicosanoids on immune responses were reviewed previously (31). Among eicosanoids, the immuno-modulatory actions of PGE_2 have been most extensively studied. Thus, this review focuses primarily on the modulatory roles of PGE_2. The PGE series have been shown to suppress both T and B lymphocyte functions in vitro—as assessed by antigen- or mitogen-stimulated blastogenesis, lymphokine production, and cytotoxicity of T lymphocytes—and antibody production by B lymphocyte–derived plasma cells (24, 77, 91). PGE_2 suppresses expression of tumor necrosis factor α and lymphotoxin in murine Th1, the antigen-specific major histocompatibility complex class II–restricted T cell clones (20).

The results from a recent study provided evidence that reduction in PG production by essential fatty acid deficiency leads to enhanced T lymphocyte function (1). In this study, athymic (*nu/nu*) mice were infected in foot pads with *Mycobacterium leprae* and fed a linoleic acid–free diet. These mice, and infected nu/nu mice on the control diet, were given an adoptive transfer of *M. leprae*–primed, T cell–enriched lymphocytes (Figure 3). After 2 weeks, *M. leprae* bacilli were harvested from the recipient mice, and bacterial viability was determined by the BACTEC system. *M. leprae* recovered from recipient mice fed control diets displayed little reduction in metabolic activity (Figure 4). In contrast, *M. leprae* from recipient mice fed the EFA-deficient diet exhibited markedly reduced viability (Figure 4). In addition, *M. leprae*–infected granuloma macrophages from EFA-deficient recipient *nu/nu* mice secreted significantly less PGE_2 than did granuloma macrophages from mice on control diets. These data suggest that enhanced levels of macrophages-generated PGE_2, induced by *M. leprae* or its constituents, could act as an endogenous negative modulator of the immune response occurring in the microenvironment of the lepromatous granuloma.

PGE_2 is known to regulate activation and differentiation of mature B lymphocytes. PGE_2 inhibits certain activation events, such as enlargement and hyperexpression of class II major histocompatibility complex (MHC), and it diminishes

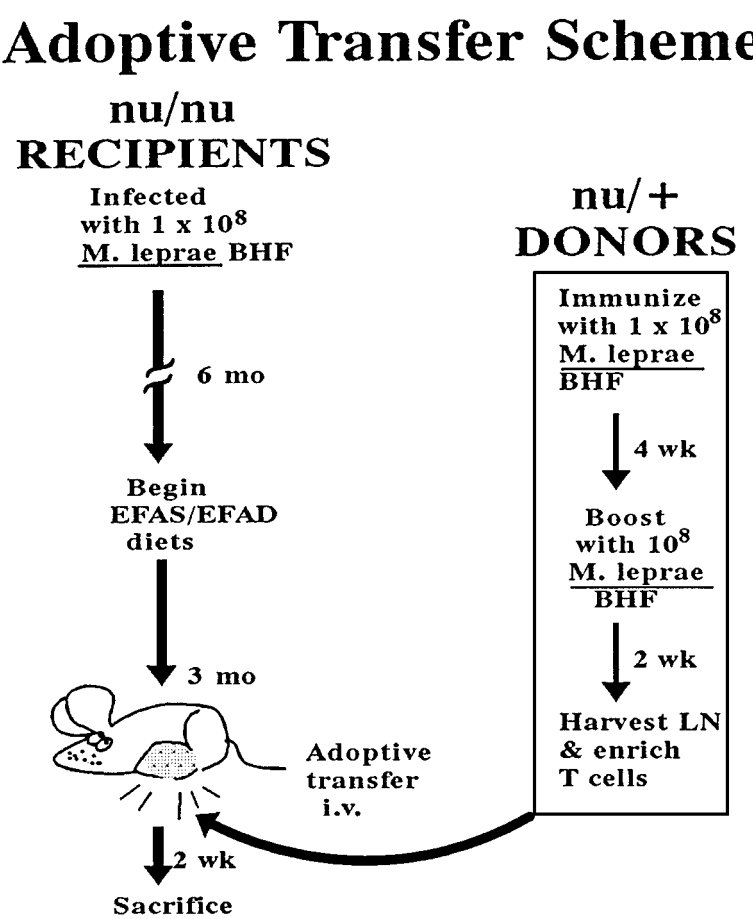
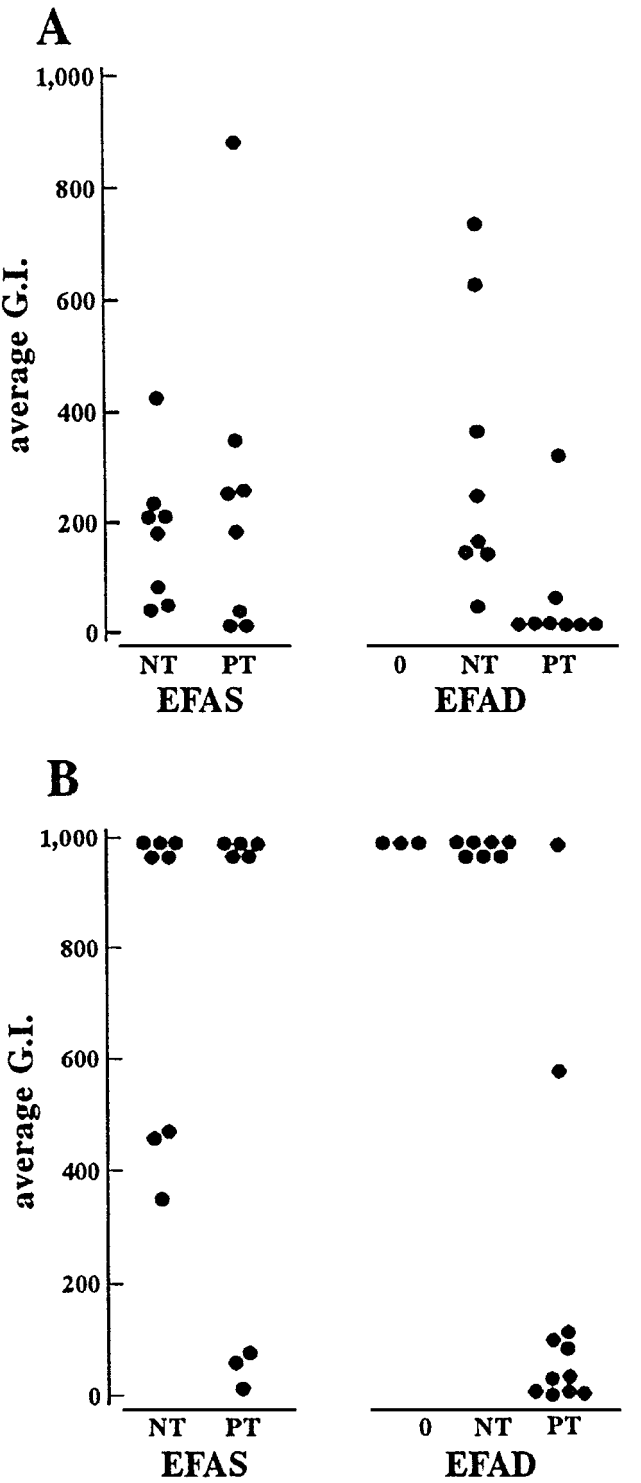


Figure 3 Protocol for the adoptive transfer of T cell–enriched cell samples into essential fatty acids (EFAs) and EFA-deficient (EFAD) *nu/nu* mice. LN, lymph nodes; EFAS, EFA sufficient. (From Reference 1.)

immunoglobulin M (IgM) production. However, PGE_2 increases production of IgE (17, 64, 69–71). PGE_2 can indirectly modulate humoral responses by modulating the production of cytokines by non-β-lineage cells. PGE_2 inhibits production of T-helper type cytokines such as interleukin (IL)-2, interferon-γ, and IL-12, whereas it can increase production of T-helper type 2 cytokines (IL-4, IL-5, and IL-10). PGE_2 can also increase the expression of interferon-γ receptors on human CD8+ lymphocytes and IgG2a Fc receptors on WEHI-3 cell (13, 96). Therefore, the effects of PGE_2 on immune responses are not limited to suppressive activities and may vary with the cell types involved and with interactions with other cytokines.



The mechanism by which PGE_2 modulates immune responses is not understood. Generally, the PGE group stimulates cAMP formation in many cell types, including leukocytes, T lymphocytes, and B lymphocytes (4). Other agents, such as cholera toxin and dibutyryl cAMP, that increase cAMP levels in B cells mimic the ability of PGE_2 to inhibit B cell enlargement, and class II major histocompatibility complex induction (70), which suggests that PGE_2 signaling occurs via cAMP.

PROSTAGLANDIN E RECEPTORS AND DOWNSTREAM SIGNALING PATHWAYS

PGs exert their biological effect through their cognate receptors. PG receptors are G protein–associated, seven-transmembrane receptors, and so far, four subtypes of PGE receptors (EP receptors) been identified: EP1, EP2, EP3, and EP4 (10). The EP1 receptor activates phospholipase (PL) C and phosphatidylinositol turnover and stimulates the release of intracellular calcium through a poorly characterized G protein–mediated signaling pathway (10). The EP_2 receptor activates adenylate cyclase via a cholera toxin–sensitive, stimulating G protein ($G\alpha s$) (10). Molecular cloning of human EP_3 subtype has revealed at least six isoforms resulting from alternative mRNA splicing of the same gene (65). Activation of all EP_3 receptor isoforms are capable of inhibiting adenylate cyclase via inhibitory G protein (Gi) and increasing the intracellular concentration of calcium (34, 60). The EP_4 receptor functions similarly to the EP_2 receptor, activating adenylate cyclase via $G\alpha s$. Lymphocytes do not synthesize PGs. However, both T and B lymphocytes express various subtypes of EP receptors (5, 18, 19). Nonlymphoid cells in the B cell microenvironment such as macrophages, fibroblast, and vascular endothelial cells can produce PGE_2 in response to various immunological and inflammatory stimuli. Thus, PGE_2 derived from nonlymphoid cells can act on lymphocytes in a paracrine fashion, as illustrated in Figure 5 (see color insert). Because the activation

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Figure 4 *Mycobacterium leprae*–infected *nu/nu* mice, fed an essential fatty acid–sufficient (EFAS) or–deficient (EFAD) diet, were given intravenous injections of T cell–enriched cell samples isolated from the spleens of uninfected mice (NT, naive T cells) or from the lymph nodes of *M. leprae*–infected mice (PT, primed T cells). Two weeks after adoptive transfer, *M. leprae* bacilli were harvested from the footpads and their viability determined by the BACTEC 460 system. Three separate adoptive transfer experiments were done. Each *datum point* represents the GI reading for an individual mouse. (*A*) Results obtained with BACTEC vials inoculated with 10^7 *M. leprae* bacilli. Statistical significance was as follows: EFAS-NT versus EFAS-PT, $P = 0.9075$; EFAD-NT versus EFAD-PT, $P = 0.0020$; EFAS-PT versus EFAD-PT, $P = 0.0182$. (*B*) Results obtained with BACTEC vials inoculated with 10^8 *M. leprae* bacilli. Statistical significance was as follows: EFAS-NT versus EFAS-PT, $P = 0.2240$; EFAD-NT versus EFAD-PT, $P = 0.0001$; EFAS-PT versus EFAD-PT, $P = 0.0058$. (From Reference 1.)

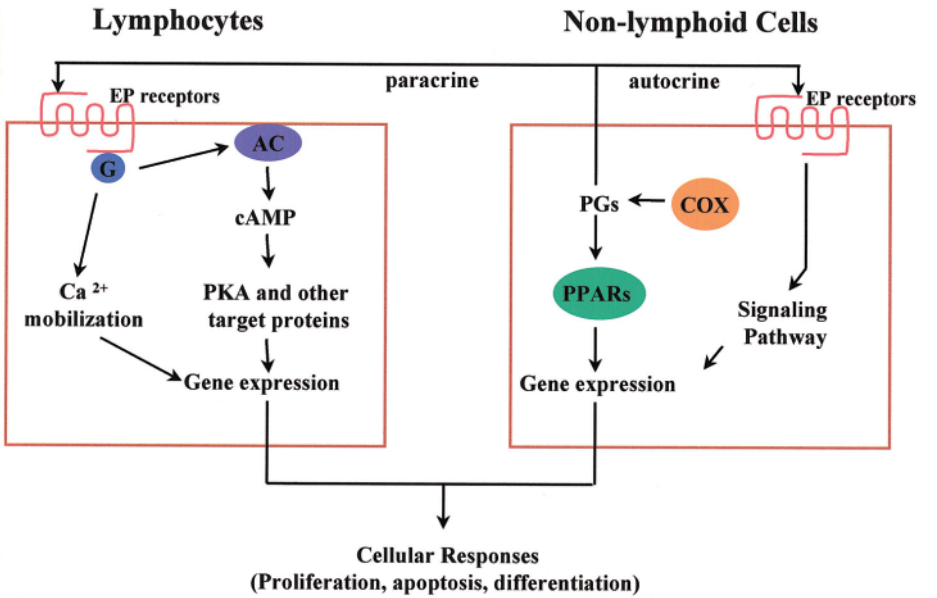

Figure 5 Signaling pathways derived from the activation of prostaglandin (PG) receptors (EP) in lymphocyte and nonlymphoid cells via autocrine and paracrine actions of PGE group. PKA, protein kinase A; COX, cyclooxygenase; PPARs, peroxisome proliferator-activated receptor.

of EP_2 and EP_3 receptors exerts opposite effects on adenylate cyclase activity, the response of lymphocytes to PGE_2 should vary with the profile and the density of different subtypes of EP receptors expressed.

Although association of G proteins, coupling to adenylate cyclase, and mobilization of intracellular calcium are well documented, proximal steps in EP receptor–mediated signaling pathways and downstream signaling pathways for these receptors are virtually unknown. Furthermore, types of gene products that are differentially expressed in response to the activation of each subtype of EP receptor are not known. Elucidation of the mechanism by which PGE_2 modulates immune responses requires identification of the downstream signaling pathways and differentially expressed genes in immuno-competent cells in response to PGE_2.

Recent advancement in identifying signaling pathways for β-adrenergic receptor provided an important framework for the basic signaling components for G protein–coupled seven-transmembrane receptors (33): They are composed of extracellular ligand binding domain, a G protein dissociating into α subunits bound to GTP, and $\beta\gamma$ subunits and an effector molecule that interacts with dissociated G protein subunits to generate second messengers such as cAMP or Ca^{2+} ion. Signaling pathways require well-coordinated homeostatic regulation for activation and deactivation (or termination). Termination of G protein–coupled receptor activation (in case of β-adrenergic receptor) is initiated by serine-threonine phosphorylation of agonist-occupied receptors by G protein–coupled receptor kinase (GRK), adenosine 3′,5′-monophosphate-dependent protein kinase, or protein kinase C (31). Arrestins, adaptor proteins, bind the phosphorylated receptor and inhibit further activation of G proteins (23). This agonist-specific or receptor specific diminution of cell response represents homologous desensitization (9). Recently, it was demonstrated that β-arrestin bound to phosphorylated β-adrenergic receptor recruits c-Src (a nonreceptor tyrosine kinase) and initiates activation of extracellular signal-regulated protein kinases (Erk_1 and Erk_2) (54). Thus, receptor activation of β-adrenergic receptor can lead to activation of both cAMP-mediated and MAPK signaling pathways. Whether similar activation and desensitization processes are operated in EP receptors remains to be determined.

Identification of downstream signaling pathways for each subtype of the EP receptors and the target genes regulated by the activation of the receptor will undoubtedly help understanding the fundamental mechanism by which dietary fatty acids affect immune responses through the modulation of PGE_2 biosynthesis.

RECEPTOR-MEDIATED SIGNALING PATHWAYS IN IMMUNOCOMPETENT CELLS—POTENTIAL TARGETS OF MODULATION BY DIETARY FATTY ACIDS

Rapidly advancing research in cell signaling pathways has unveiled the possibility that different types of fatty acids can modulate many receptor-mediated signal transduction pathways. Growing evidence now suggests that fatty acids, in addition

to their roles as structural components of membrane lipids and as precursors of eicosanoids, can act as a second messengers or regulators of signal transducing molecules.

To focus on potential targets of modulation by dietary fatty acids in receptor-mediated signaling pathways, simplified receptor-mediated signaling pathways are depicted in Figure 6 (see color insert). Signaling molecules that may be modulated by different fatty acids can be broadly divided into three groups (Figure 7, see color insert).

Signaling Molecules that Require Fatty Acid Acylation for Membrane Translocation and Functional Activation

Receptor activation by ligand binding leads to translocation of signaling molecules that can interact with the receptors or other downstream signaling molecules to initiate the propagation of the signals. The translocation of many signaling molecules can be achieved by binding between molecular binding domains, such as SH2 or SH3, and docking sites, phosphorylated tyrosine, or proline rich regions (the last two for SH2 or SH3 domain, respectively) of counterpart molecules. Recently accumulated evidence indicates that covalent attachment of long-chain fatty acids occurs on a variety of proteins and can dramatically influence translocation and function (75, 85). Many signaling molecules that play critical roles in transmitting extracellular signals are known to be acylated for their membrane translocation. Two modes of acylation for these molecules have been described. These are co-translocational myristoylation and postranslational palmitoylation.

Myristoylation occurs cotranslationally at NH_2-terminal glycine residues via a covalent amide bond formation (36). The half-life of the myristoyl moiety is equivalent to that of the polypeptide backbone, indicating that myristoylation is a "permanent" modification (67). The signaling molecules that are known to be myristoylated include GTP-binding proteins (α), cAMP-dependent protein kinase, and members of the *Src* family of nonreceptor type tyrosine kinases (75, 85). Myristoylation can increase hydrophobicity of proteins and, thus, can facilitate membrane anchorage. However, it has been suggested that myristoylation alone is not sufficient to stably anchor a myristoylated protein to the lipid bilayer (63). Additional factors may be required to promote the membrane localization of myristoylated proteins. The enzyme catalyzing the myristoylation of the glycine residue is considered to have a high degree of substrate specificity for myristoyl coenzyme A (CoA) (85), although other acyl moieties (e.g. C12:0, C14:$1^{\Delta 5}$, C14:$2^{\Delta 5,8}$) in addition to C14:0 can be covalently bound to the amino-terminal glycine residue of bovine retinal transducin and recoverin (44, 61). Other acyl CoAs significantly inhibit binding of [^{14}C]myristoyl CoA to the enzyme (84). These results suggest that acylation of N-terminal glycine can be regulated by the availability of alternative acyl chains. This, in turn, leads to the important question of whether dietary fatty acids can modulate myristoylation of signaling proteins. The fact that myristoylation is a cotranslational event and the half-life of the myristoyl moiety is the

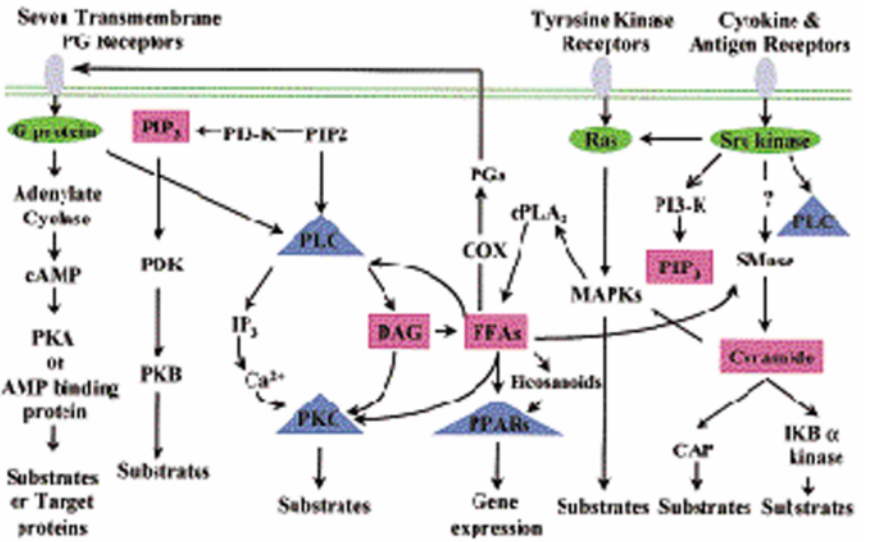

Figure 6 Potential targets that can be modulated by dietary fatty acids in receptor-mediated signaling pathways. CAP, ceramide-activated protein kinase; $cPLA_2$, cytosolic phospholipase A_2; DAG, diacylglycerol; FA, fatty acid; IκB, inhibitory subunit of NFκB; PDK, phosphoinositide-dependent protein kinase; PKA, cAMP-dependent protein kinase; PKB, proto-oncogenic protein kinase; PIP_2, phosphatidylinositol bisphosphate; PIP_3, phosphatidylinositol trisphosphate; PLC, phospholipase C; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; PPAR, peroxisome proliferator–activated receptor; SMase, sphingomyelinase. (From Reference 32.)

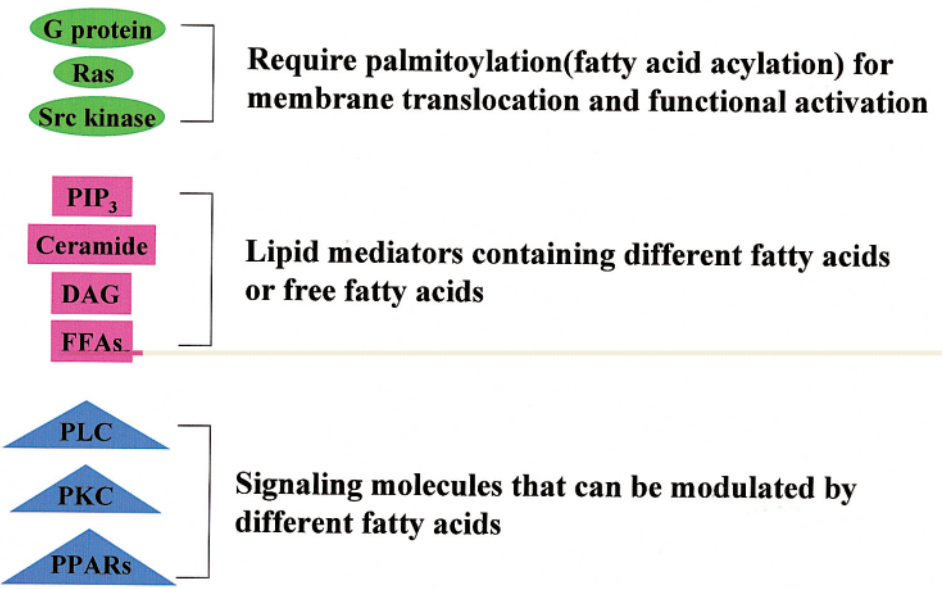

Figure 7 Targets that can be modulated by dietary fatty acids (FA) in receptor-mediated signaling pathways are divided into three groups. DAG, diacylglycerol; PIP3, phosphatidylinositol trisphosphate; PKC, protein kinase C; PLC, phospholipase C; PPAR, peroxisome proliferator–activated receptor.

same as the protein itself indicates that myristoylation is not a reversible process. Thus, myristoylation is not likely to be dynamically regulated, as in the case of reversible palmitoylation described below.

Unlike myristoylation, palmitate is acylated posttranslationally to cysteine residue(s) via a labile thioester linkage. Palmitoylation is a reversible process and is dynamically regulated. Biophysical measurements suggest that myristoylation provides barely enough binding energy to associate a protein with membrane lipids (63). Greater hydrophobicity of thioester-linked palmitate can enhance membrane association of the proteins (67). The roles for fatty acid acylation of proteins include anchoring proteins to membranes (3), stabilizing protein-protein interactions, and regulating enzymatic activities in mitochondria (3). Mutations that prevent fatty acid acylation abolish or attenuate biological function of these proteins. There are three groups of palmitoylated proteins (2). The first group includes transmembrane proteins that are acylated at cysteine residues. The second group includes the *Ras* family, in which palmitoylation occurs in the C-terminal region and requires prior isoprenylation of the cysteine residue (6, 28). The third group includes the α subunits of GTP-binding proteins and *Src* family tyrosine kinases (2). These groups of proteins are both myristoylated and palmitoylated. A comprehensive list of acylated proteins was reviewed elsewhere (75). Palmitoylation of G proteins, *Ras*, and *Src* family tyrosine kinases is of particular interest because these proteins are proximal components transducing diverse receptor-mediated signals (Figures 6 and 7, see color insert). Reversibility of palmitoylation implies that these proteins are the logical targets of modulation by dietary fatty acids. In contrast to myristoylation, the enzymology of palmitoylation is not well characterized. There are several questions that bear immense nutritional implications. Critical questions include whether fatty acids other than palmitate can acylate the cysteine residues and, if so, whether it would affect the functional properties of the proteins. Competition assays for G protein palmitoyl transferase with various unlabeled acyl CoAs indicated that palmitate is a preferred substrate for the enzyme (12). However, other acyl CoAs also significantly inhibited G protein palmitoyl transferase activity, implying that other fatty acids can either (*a*) be substrates for the acylation of cysteine residues or (*b*) inhibit palmitoylation. This leads to the possibility that dietary fatty acids can modulate fatty acid acylation of the signaling molecules. Because G proteins and *Ras* and *Src* family tyrosine kinases are proximal components in diverse receptor-mediated signaling pathways, modulation of their functional activity by dietary fatty acids can affect downstream signaling pathways in many cell types and target gene expression.

T and B cells have structurally different oligomeric antigen receptors that recognize distinct forms of antigens. Both T cell antigen receptors (TCR) and B cell antigen receptors (BCR) have separate antigen-binding and signal transduction subunits. Neither the TCR nor the BCR possess intrinsic protein tyrosine kinase (PTK) activity in their cytolasmic domains. However, it has been demonstrated that two classes of nonreceptor types of PTKs play important roles in both TCR and BCR signaling pathways (92). They are members of the Src and Syk/ZAP-70

families. Src family PTKs (lck, fyn, yes) expressed in lymphocytes require both myristaylation and palmitoylation for membrane association. In contrast, Syk and ZAP-70 are not acylated by fatty acids and, thus, are not likely to be constitutively localized in the plasma membrane. It would be interesting to determine whether acylation of Src PTKs and subsequent downstream signaling pathways for both TCR and BCR are modulated by different dietary fatty acids.

Lipid Mediators Acylated with Different Fatty Acids or Free Fatty Acids

PI 3′-Kinase–Derived Products Phosphatidylinositol (PI) 3′-kinases phosphorylate various phosphoinositides (88). PI(3,4)P_2 and PI(3,4,5)P_3 activate proto-oncogenic protein kinase (PKB) (40, 88) (Figure 8*A*, see color insert). PKB participates in the activation of the ribosomal protein S6 kinase (11) inhibits glycogene synthase kinase-3, and inhibits apoptotic cell responses (66). Results from a recent study (81) indicate that the activity of PI(3,4,5)P_3 varies with the types of fatty acids in Sn-1 and Sn-2 position. Because the composition of fatty acids in Sn-1 and Sn-2 of phosphoinsitides can be altered by dietary fatty acids, it is possible that dietary fatty acids can modulate the activities of PKB and its downstream substrates.

Ceramide Ceramide released from the hydrolysis of sphingomyelin contains one fatty acyl moiety that is linked to the sphingosine backbone by an amide bond. Whether the type of this fatty acyl moiety can be altered by different fatty acids is not known. Structure-activity relationship of ceramide containing different fatty acyl moieties has also not been determined. For most in vitro studies reported, water-soluble ceramides with short-chain fatty acyl groups have been utilized. Ceramide has emerged as an intracellular signal effector molecule with multiple downstream targets for diverse extracellular signals (82). Modulation of the activity of ceramide by dietary fatty acids would have profound nutritional implications.

Diacyl Glycerol Because the fatty acid composition of membrane phospholipids is modified by different dietary fatty acids, fatty acyl moieties of diacylglycerol (DAG) should also be altered by dietary fatty acids. The alkyl group instead of acyl group in the 1-ester bond diminishes the activity of DAG in activating protein kinase C (PKC) (95). Cho & Ziboh (8) demonstrated that DAG containing 13-hydroxyoctadecadienoic acid, a 15-lipoxygenase metabolite of linoleic acid, at the 2 position inhibits PKCβ isozymes in contrast to 1,2-dioleoylglycerol. However, the structure-activity relationship for the different acyl groups in the 1- and 2-ester bonds of DAG is largely unknown.

Free Fatty Acids Most long-chain fatty acids are esterified as a fatty acyl chain of glycerolipids in animal cells. Therefore, the intracellular concentrations of free

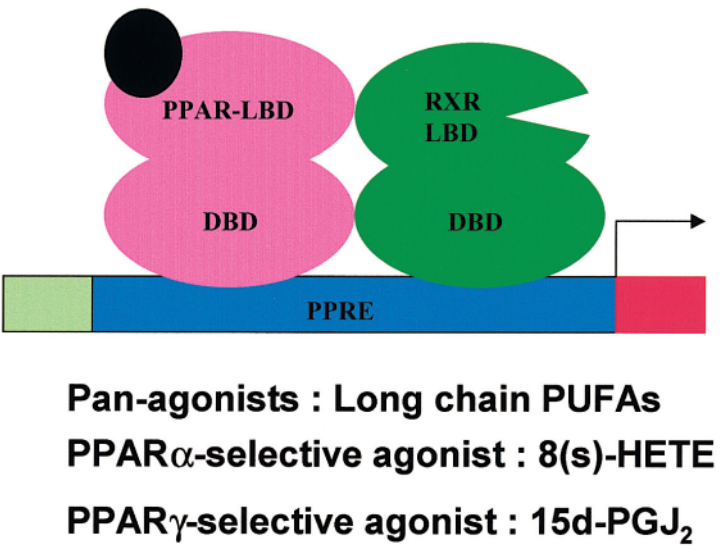

Figure 8 Fatty acids and eicosanoids are peroxisome proliferator–activated receptor (PPAR) ligands. Long-chain polyunsaturated fatty acids (PUFAs) function as ligands for all three subtypes (Pan-agonists) whereas 8(S)-hydroxy-geicosatetraenoic acid [8(S)-HETE] and 15-deoxy-prostaglandin J_2 (15d-PGJ_2) are selective agonists for PPARα and PPARγ, respectively.

acids are generally considered to be very low. However, free fatty acids are rapidly released by the activation of various PLA_2 and mono- and diacylglycerol lipases in response to diverse cellular stimulations (Figure 8*A*, see color insert). There are two major forms of mammalian PLA_2: a low-molecular-weight form (14 kDa) of secretory PLA_2 and a high-molecular-weight form (85–100 kDa) present in the cytosol PLA_2. The secretory PLA_2 are not selective for fatty acids at the Sn-2 position of phospholipid substrates. Thus, they do not lead to selective release of arachidonic acid. On the other hand, cyosolic PLA_2 has a high degree of substrate specificity for arachidonic acid in Sn-2 position of phospholipids (27, 45). Free arachidonic acid can be generated by the action of mono- and diacylglycerol lipases on DAG that is produced through receptor-mediated activation of either PLC or PLD (26). Thus, it is possible that local concentrations of intracellular free fatty acids can be significantly increased on cell stimulation. However, it is difficult to quantify such local concentrations of intracellular free fatty acids.

Arachidonic acid can be metabolized via cyclooxygenase (COX), lipoxygenase, and P-450–dependent epoxygenase pathways. COX-derived products of arachidonic acid, such as prostanoids, can activate their receptors, which are known to be G protein–coupled, seven-transmembrane receptors, in a paracrine fashion. There are two major branches of prostanoid receptors: one group activating adenyl cyclase, and a second group either stimulating phosphatidylinositol hydrolysis or inhibiting adenyl cyclase (65). It has been well documented that dietary fatty acids can modulate the biosynthesis of prostanoids by altering the availability of substrate, free arachidonic acid. This suggests that dietary fatty acids can modulate prostanoid receptor-mediated signaling pathways by altering availability of ligands.

Signaling Molecules that Can Be Modulated by Different Fatty Acids

It has been demonstrated that free fatty acids can directly activate PKC (39). This activation can be distinguished from activation of PKC by phosphatidyl serine (PS) and DAG. It was found that oleate is unable to inhibit phorbol ester binding to PKC, which suggests that fatty acids interact with PKC at a site distinct from the phorbol ester/DAG binding site (14). In addition, PKC inhibitors (sphingosine) and conditions known to inhibit PKC activity are unable to inhibit oleate-induced activation of PKC to the same extent to which they inhibit PS/DAG-induced activation. Oleate preferentially activates soluble and the calcium-independent PKCs over membrane-bound PKC (39). Unsaturated fatty acids are also shown to enhance the activation of PLC-γ in the presence of Tau proteins (52). Among the unsaturated fatty acids tested, arachidonic acid was the most potent stimulator of PLC-γ. However, no apparent difference in potency was observed among the unsaturated fatty acids tested in activating PKCs. In addition, it has been demonstrated that arachidonic acid and other free fatty acids regulate the activities of multiple cellular proteins, including ion channels and protein kinases (39).

Recently, it was demonstrated that various polyunsaturated fatty acids (PUFAs) and their metabolites are the ligands for peroxisome proliferator–activated receptors

(PPARs) that regulate the transcription of genes involved in diverse cellular responses, including metabolic, inflammatory, and immune responses. Therefore, this topic deserves further coverage.

ACTIVATION OF PEROXISOME PROLIFERATOR–ACTIVATED RECEPTORS BY FATTY ACIDS

PPARs are nuclear hormone receptors that regulate gene transcription in response to peroxisome proliferators (52). Three subtypes of PPARs (α, β, and γ) have been identified so far. These receptors are transcription factors that heterodimerize with retinoid X receptor, and that regulate glucose and lipid homeostasis through modulation of the transcription of genes whose products are involved in glucose and lipid metabolism. These receptors are activated by various peroxisome proliferators, including fibrate hypolipidemic drugs.

Evidence accumulated recently indicates that naturally occurring fatty acids, particularly long-chain unsaturated fatty acids and their metabolites (e.g. prostanoids and other lipoxygenase metabolites), bind and activate PPARs (21, 25, 43, 46, 94) (Figure 8, see color insert). Xu et al (93) reported the crystal structure demonstrating that eicosapentaenoic acid binds to the PPARδ ligand-binding domain. Both eicosapentaenoic acid and the synthetic fibrate GW 2433 interact directly with the activation function 2 helix and occupy the same space. These results unequivocally demonstrate that PUFAs have ligand binding activity for PPARs.

In addition, structural analyses of the PPARα and PPARβ/δ ligand binding domains reveal that their ligand binding pockets are roughly three times larger than those of other nuclear receptors and are sufficiently large to allow fatty acids to bind in multiple conformations (62, 86). These findings provide molecular insights into promiscuous binding properties of the PPARs for various fatty acids and their metabolites. Various nonsteroidal antiinflammatory drugs (NSAIDs) that inhibit the enzyme activity of cyclooxygenase are also known to bind and activate PPARs (51). Furthermore, the activation of PPARs inhibits macrophage activation and the production of monocyte inflammatory cytokines (35, 68, 80). These findings suggest that PPARs regulate the expression of a broad array of genes that are involved not only in lipid homeostasis but also in immune and inflammatory responses. PPARs may, therefore, be one of the important cellular targets that mediate modulation of immune responses by dietary fatty acids.

Many naturally occurring fatty acids activate PPARα (25, 38) and directly bind PPARα/δ to induce PPARα/δ-RxR complex formation, which then binds the PPAR response element (21). PPARα can be activated by saturated (14:0, 16:0, 18:0) and monounsaturated (16:1, 18:1) fatty acids and by PUFAs. However, the binding affinity of PUFAs is much higher for PPARα and -δ than for PPARα (43, 93) (Table 1). PPARγ shows most efficient binding with PUFAs and only weakly with monounsaturated fatty acids. The concentrations of PUFAs that can activate PPARs

TABLE 1 Binding of fatty acids to PPARα, -γ, and -δ[a]

Fatty acid	α	γ	δ
Capric (C10:0)	>30	>30	>30
Lauric (C12:00)	>30	>30	>30
Myristic (C14:0)	5.4 ± 0.80	21 ± 12	24 ± 6.5
Plamitic (C16:0)	1.5 ± 0.13	>30	7.4 ± 1.8
Stearic (C18:0)	1.1 ± 0.14	>30	6.0 ± 1.2
Arachidic (C22:0)	>30	>30	>30
Behenic (C22:0)	>30	>30	>30
Palmitoleic (C16:1)	1.7 ± 0.19	6.4 ± 1.2	9.6 ± 1.9
Oleic (C18:1)	0.60 ± 0.10	4.1 ± 0.70	5.3 ± 0.80
Eurcic (C22:1)	>30	>30	>30
Linolenic (C18:2)	1.1 ± 0.14	6.2 ± 1.1	10 ± 1.8
α-Linolenic (C18:3)	1.2 ± 0.19	6.0 ± 1.0	16 ± 3.2
γ-Linolenic (C18:3)	0.27 ± 0.070	2.2 ± 0.50	0.75 ± 0.15
Dihomo-γ-linolenic (C20:3)	1.4 ± 0.25	2.4 ± 0.30	4.3 ± 0.70
Arachidonic (C20:4)	1.2 ± 0.23	1.6 ± 0.20	3.1 ± 0.80
Eicosapentaenoic (C20:5)	1.1 ± 0.23	1.6 ± 0.20	4.0 ± 0.90

[a]Values shown are 50% inhibitory concentrations (in micromolars). Fatty acids were tested for their ability to compete with radioligands for binding to peroxisome proliferator–activated receptor α (PPARα), -γ, and -δ. [^{3}H]BRL49653, [^{3}H]BRL49653, and [^{3}H]GW2433 were used as radioligands for PPARα, -γ, and -δ competition binding assays, respectively. (From Reference 93.)

are in the micromolar range, as determined by transactivation assays, ligand-induced binding assays, or competition with radioligands for binding to PPARs. The concentration of intracellular free fatty acids are believed to be very low, in the nanomolar range. One study using the synthetic fluorescent PPARα ligand demonstrated effective concentrations of the intracellular free fatty acids in the nanomolar range (53). It is possible that local concentrations of intracellular free fatty acid could reach micromolar range in certain subcellular compartments when localized PLs and DAG lipases are activated.

PPARα is highly expressed in liver, kidney, heart, and muscle tissue and regulates genes involved in fatty acid degradation (41). Mice lacking functional PPARα are incapable of responding to peroxisome proliferators and fail to induce expression of genes required for fatty acid degradation (50). In smooth muscle cells in which PPARα instead of PPARγ is the major PPAR expressed, ligands for PPARα inhibit IL-1–induced IL-6 and COX-2 expression (80). Both saturated fatty acids and PUFAs and certain eicosanoids [e.g. 8-S-HETE, leukotriene B4 (LTB_4), and PGD_2] can activate PPARα, as shown in Figures 9 and 10 (21, 43, 94).

PUFAs bind and activate PPARγ (21, 38, 43, 93). A PGJ_2 metabolite, 15-deoxy-PGJ_2, was identified as an endogenous ligand for PPARγ (42). Fatty acids and

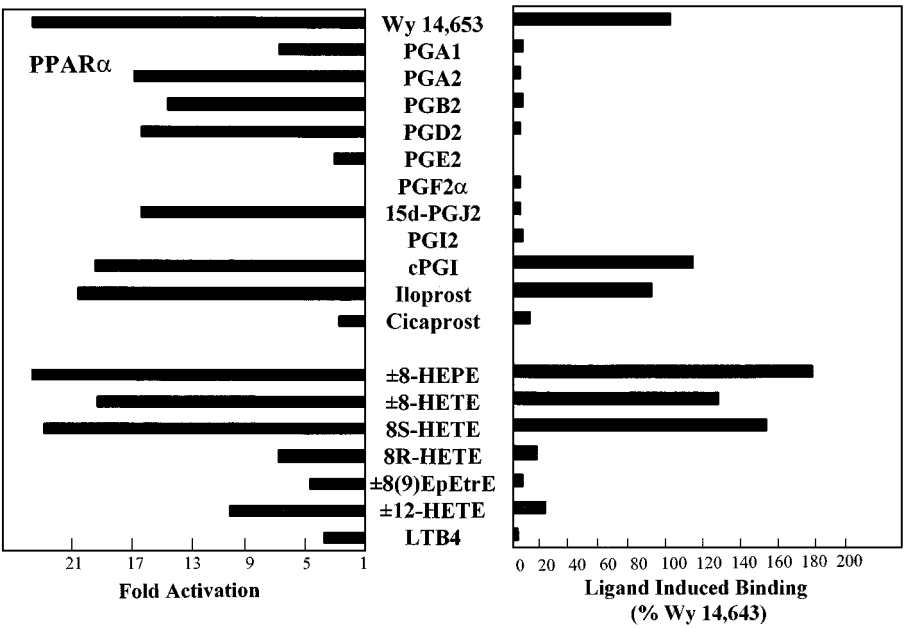

Figure 9 Identification of eicosanoid ligands for peroxisome proliferator–activated receptor alpha (PPARα). Carbaprostacyclin I (cPGI), iloprost, 8(S)-HETE, and 8(S)-HEPE transactive (*left*) and bind (*right*) PPARα. For transfections (*left*), compounds were added to cells at the following concentrations: 5 μM Wy14643; 10 μM PGA_1, PGA_2, PGB_2, PGD_2, PGE_2, and $PGF_{2\alpha}$; 3 μM 15d-J_2; 10 μM PGI_2; 1 μM cPGI and iloprost; 10 μM cicaprost; 10 μM $\pm$8-HETE ($\pm$8-hydroxy-$\Delta^{5Z,9E,11Z,14Z,17Z}$-C20:5), $\pm$8-HETE ($\pm$8-hydroxy-$\Delta^{5Z,9E,11Z,14Z}$-C20:4), $\pm$8(9)-EpEtrE [$\pm$8(9)-epoxy-$\Delta^{5Z,11Z,14Z}$-C20:3], and $\pm$12-HETE ($\pm$12-hydroxy-$\Delta^{5Z,8Z,10E,14Z}$-C20:4); 5 μM 8(S)- and 8(R)-HETE; and 10 μM LTB_4. For the ligand binding assay (*right*), compounds were added as follows: 10 μM Wy14643, PGA_1, PGA_2, PGB_2, PGD_2, PGE_2, $PGF_{2\alpha}$, 15D-J_2, and PGI_2; 2 μM cPGI, iloprost, and cicaprost; 1 μM $\pm$8-HEPE, $\pm$8-HETE, $\pm$8(9)-EpEtrE, and $\pm$12-HETE; 300 nM 8(S)-HETE and 8(R)-HETE; and 10 μM LTB_4. (From Reference 21.)

eicosanoids were shown to have binding activity for PPARβ/δ, as assessed by transactivation assay or competition binding assay (21, 43, 93, 94). PPARβ/δ is ubiquitously expressed in nearly all tissues. However, the functions of PPAR β/δ are largely unknown.

POTENTIAL ROLES OF PPARS IN IMMUNE RESPONSES

Emerging evidence indicate that PPARs regulate the expression of a diverse array of genes that are involved not only in lipid metabolism but also in cell differentiation proliferation, apoptosis, and immune and inflammatory responses. Results from recent studies demonstrated that PPARγ regulates the differentiation of monocytes

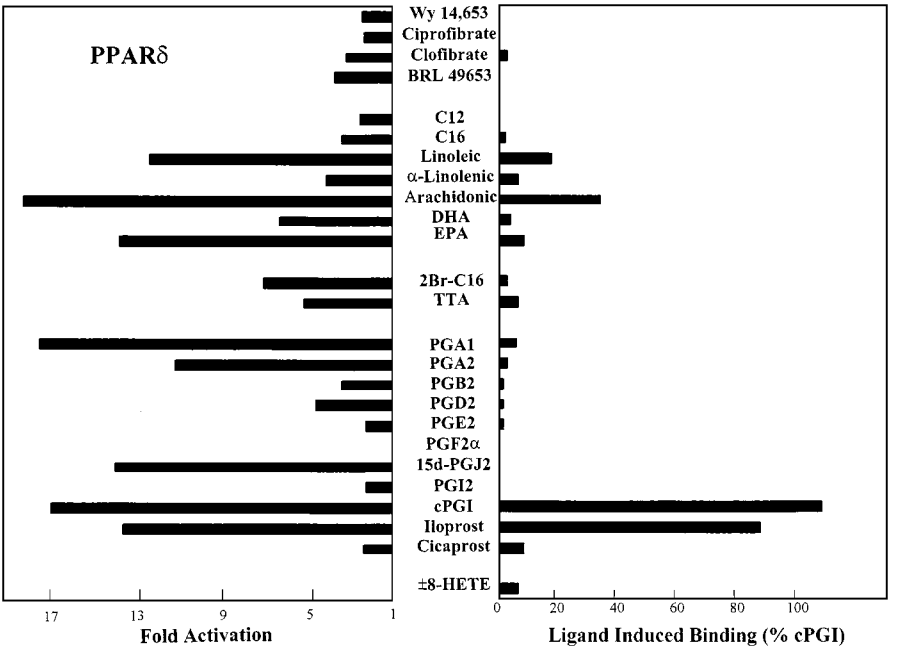

Figure 10 PPARδ displays distinct ligand response profiles. Linoleic acid, arachidonic acid, carbaprostacyclin I (cPGI), and iloprost transactivate (*left*) and bind (*right*) to peroxisome proliferator–activated receptor delta (PPARδ). After transfection (*left*), compounds were added to cells at the following concentrations: 5 μM Wy14643; 100 μM ciprofibrate; 1000 μM clofibrate; 5 μM BRL49653; 30 μM C12, C16, linoleic acid, α-linoleic, arachidonic, docosahexaenoic (DHA) (all-Z-$\Delta^{4,7,10,13,1j6,19}$-C22:6), and eicosapentaenoic (EPA) (all-Z-$\Delta^{5,8,11,14,17}$-C20:5) acids; 5 μM 2Br-C16; 30 μM tetradecylthioacetic acid (TTA); 10 μM PGA_1, PGA_2, PGB_2, PGD_2, PGE_2, and $PGF_{2\alpha}$; 3 μM 15d-J_2; 10 μM PGI_2; 1 μM cPGI and iloprost; 10 μM cicaprost; and 3 μM $\pm$ 8-HETE. For the ligand binding assay (*right*), compounds were added as follows: 5 μM Wy14643; 100 μM ciprofibrate; 1000 μM clofibrate; 50 μM BRL49653; 30 μM C12, C16, linoleic acid, α-linoleic arachidonic acids, DHA, and EPA; 10 μM 2Br-C16, TTA, PGA_1, PGA_2, PGB_2, PGD_2, PGE_2, $PGF_{2\alpha}$, 15d-J_2, PGI_2, cPGI, iloprost, and cicaprost; and 1 μM $\pm$8-HETE. (From Reference 21.)

and macrophages (83) and suppresses the expression of proinflammatory cytokines and other downstream markers of inflammation (35, 68, 80). PPARγ is abundantly expressed in adipocyte, large intestine, and cells of monocyte lineage, including macrophage. However, no PPARγ was detected in lymphocytes of murine lymph node, as assessed by immnohistochemical analysis (83). The fact that fatty acids and some of their metabolites are endogenous ligands for PPARs underlines the potential importance of PPARs as molecular targets through which dietary fatty acids can modulate immune and inflammatory responses. Although some of target genes regulated by PPARs are identified, a full spectrum of downstream genes

regulated by each PPARs subtype remains to be identified. Each subtype of PPARs regulates transcription of a different set of genes. The levels and profile of expression of each subtype vary with cell types. Thus, the types of genes expressed in response to fatty acids should vary with cell types. Although it is well documented that PUFAs are activators of each subtype of PPAR, there appears to be a lack of consistent patterns in the relative specificity of individual PUFAs in activating each subtype PPAR. This may be due to differences in assay methods assessing PPAR binding or activation.

Each study demonstrating activation or ligand binding activity of PPARs by fatty acids utilized variant transactivation assays or ligand binding assays: PPRE-luciferase or CAT reporter gene constructs cotransfected with different subtypes of PPRE (21, 38) competition assay using specific radioactive ligands for each subtype (42, 93), transactivation of pTetO-luciferase reporter gene by TetR-PPAR fusion receptors (94), transactivation of PPAR and glucocorticoid receptor that activates expression of alkaline phosphatase reporter gene (25), ligand-induced DNA binding of PPARs (21), or transactivation of UAS_5-tk-CAT or luciferase by GAL4-PPAR (43).

As shown by other steroid hormone receptors, regulation of gene expression by PPAR may also be regulated by other proteins associated with PPARs. In addition to retinoid X receptors, PPARs have been shown to be associated with heat-shock protein 70, the orphan nuclear hormone receptor (LXR), PPARα-binding protein, the co-activators SRC-1 and p300, cAMP response-element binding protein, and c-jun (87). Thus, PPAR activation by fatty acids as assessed by transactivation assay, by receptor binding to PPRE, and by binding activity of ligand binding domains of PPARs may not accurately reflect specificity of individual fatty acid in regulating the expression of the endogenous target genes. Therefore, the relative potency and specificity of individual PUFA in activating or suppressing the expression of the target genes that are regulated by PPARs need to be determined by quantifying levels of expression of endogenous target genes.

DIETARY N-3 FATTY ACIDS AND IMMUNE RESPONSES

The implication that dietary fatty acids modulate immune responses was based on the observation that essential fatty acid (EFA) deficiency can accentuate or ameliorate symptoms of certain autoimmune diseases in animals and that supplementation of EFA to animals can reverse the effects. Levels of EFAs in ordinary diets (i.e. American diets) generally exceed those required to prevent overt symptoms of EFA deficiency. Results from human and animal studies suggest that immune responses can be modulated by different types of dietary fatty acids, as reviewed elsewhere (7, 15, 31, 57).

Epidemiological, clinical, and biochemical studies have demonstrated beneficial effects of n-3 fatty acids in reducing risks of cardiovascular and inflammatory diseases (49, 74, 76). The biochemical basis for the suggested beneficial effects of

consuming n-3 PUFAs is generally considered, in part, due to the reductions in the level of tissue arachidonic acid (20:4n-6, the major precursor acid for eicosanoids) and its eicosanoid metabolites. However, many of cellular effects exerted by n-3 PUFAs cannot be explained solely on the basis of alteration of substrate availability for eicosanoid biosynthesis. Recently, it was demonstrated that consuming n-3 PUFAs resulted in a reduction in the production of cytokine (IL-1, IL-2, and tumor necrosis factor α) in human peripheral blood mononuclear cells, and in a reduction in platelet-derived growth factor in cultured endothelial cells (16, 22, 73, 90). Diminution of T cell–derived IL-2 production by the intake of n-3 PUFAs has also been demonstrated in humans and mice (37, 89). The expression of the early response genes (cytokines, growth factors, etc) induced by mitogens does not require intervening protein synthesis (29). This suggests that suppression of cytokine production by dietary n-3 PUFAs is mediated through the modulation of signaling pathways, leading to the expression of cytokine genes. In addition, consuming diets supplemented with fish oil leads to the suppression of mitogen-induced proliferation of T cell– and delayed-type hypersensitivity skin responses, which suggests a generalized decrease in T cell–mediated immune responses (16, 56, 89).

Because n-3 PUFAs suppress the tissue levels of arachidonic acid and production of PGE_2 possessing immunosuppressive effects, increased intake of n-3 PUFAs would lead to enhancement instead of suppression of immune responses. This fact indicates that suppression of cytokine production by n-3 PUFAs is mediated through signaling pathways other than the modulatory effects on PG biosynthesis. Abbreviated receptor-mediated signaling pathways depicted in Figures 6 and 7 (see color insert) present a conceptual framework for identifying potential targets of modulation by dietary fatty acids.

An important question is what are the target signaling molecules that mediate rather specific suppression of cytokine production by n-3 PUFAs as compared with n-6 PUFAs. To answer this question, the comparative efficacy of n-3 PUFAs in modulating the signaling molecules (Figures 6 and 7, see color insert) needs to be determined. N-3 PUFAs can modulate both signaling pathways leading to cytokine production and downstream signaling pathways derived from the activation of cytokine receptors. The signaling molecules that are potential targets of modulation by fatty acids depicted in Figure 6 (see color insert) are important components in both T cell and B cell receptor–mediated and cytokine receptor–mediated signaling pathways. Recently, it was demonstrated that PPARγ is a negative regulator of macrophage activation (68) and that PPARγ agonists inhibit production of monocyte inflammatory cytokines (35). Most naturally occurring PUFAs and some of their metabolites are activators and ligands for PPARs (see Figures 9 and 10 and Table 1). However, there is no evidence that suggests that the efficacy of n-3 PUFAs and their metabolites is different from that of n-6 PUFAs in activating each subtype of PPAR. Most studies that aimed to determine the efficacy of each PUFA and its metabolites in activating PPARs employed transactivation assays using reporter plasmids or competition assay with radioactive ligands. Each subtype of PPAR appears to possess promiscuously broad affinity for different

PUFAs and their metabolites. PPARs in cells are exposed in the milieu of different fatty acids and their metabolites, whose composition can be modified by different types of dietary fat. Thus, activation of PPARs and their effects on expression of endogenous target genes need to be quantified in the context of overall composition of cellular PUFAs. Accordingly, it would be challenging to quantify how changes in the composition of cellular fatty acids caused by intake of n-3 PUFAs modulate (*a*) activities of the multitudes of signaling molecules (Figure 6, see color insert), (*b*) expression of target genes, and (*c*) final cellular responses.

SUMMARY

The modulation of immune responses by different dietary fatty acids has been demonstrated in both human and animal studies. It has been generally considered that this immuno-modulatory role of dietary fatty acids is at least in part mediated through alteration in eicosanoid production. In particular, the immunosuppressive effects of the PGE group have been demonstrated in both in vivo and in vitro studies. PGs exert their biological effects through their receptors. PG receptors are G protein–associated, seven-transmembrane receptors. Downstream signaling pathways and target genes regulated by the activation of each subtype of PG receptor are not understood. Thus, the mechanism by which PGE group suppresses immune responses is still not known. Recent advancement in cell signaling research revealed that fatty acids can modulate the functional properties of signaling molecules involved in a diverse array of receptor-mediated signal transduction pathways in immunocompetent cells, as summarized in Figure 6 (see color insert). Determining how changes in the fatty acid composition of tissues lipids can affect the receptor-mediated signaling pathways, expression of target genes, and subsequent cellular responses will help us to understand the mechanism by which dietary fatty acids modulate immune and inflammatory responses.

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