REGULATION OF HUMAN IMMUNE AND INFLAMMATORY RESPONSES BY DIETARY FATTY ACIDS

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I. INTRODUCTION

The development, maintenance, and optimal functioning of the immune system are dependent on balanced and adequate nutrition. However, either a deficiency or an excess of a number of nutrients can have adverse effects. The nutrients with the most pronounced effects in humans include amount and type of dietary fatty acids (FAs), protein energy malnutrition, vitamins A, B6, B12, C, and E, and minerals including zinc, copper, selenium, and iron. Multiple rather than single nutrient deficiencies

ADVANCES IN FOOD AND NUTRITION RESEARCH VOL 50 ISSN: 1043-4526

DOI: 10.1016/S1043-4526(05)50004-4

are often the cause for a compromised immune system. A number of excellent review articles and books deal with the nutritional regulation of immune functions (Amati *et al.*, 2003; Calder and Kew, 2002; Field *et al.*, 2002).

Dietary fat can have diverse effects on human health based on the amounts consumed and, more importantly, on the types consumed. Dietary fat may also differentially affect certain cells, tissues, and organs depending on their stage of development. The FA composition of human tissues and organs can vary depending on the types of FAs that are consumed in the diet; that composition has been used as a biomarker for correlation with immunity and risk with disease. In addition, some dietary FAs can be transformed into potent biological mediators that have been shown to initiate or alter numerous processes in the body. For example, linoleic acid (LA, 18:2, n-6), a common component of some vegetable oils, can be converted by a number of cell types into arachidonic acid (AA, 20:4, n-6), a major precursor for the potent immunomodulatory agents, prostaglandin (PG)E_2 and leukotriene (LT)B_4, which are produced from AA by the enzymes cyclooxygenase and 5-lipoxygenase, respectively. Other 20-carbon FAs, eicosapentaenoic acid (EPA, 20:5 n-3), and dihomo-gamma linolenic (DGLA, 20:3, n-6) compete with AA as substrates for these enzymes and, thus, can decrease the production of PGE_2 and LTB_4. The eicosanoids produced from EPA and DGLA consequently have only weak effects on cells of the immune system. Docosahexaenoic acid (DHA, 22:6, n-3) is not a substrate for the cyclooxygenase and lipoxygenase; however, it can inhibit the synthesis of the n-6 eicosanoids by inhibiting the release of membrane AA (Martin, 1998). It can also be retroconverted to EPA. Because PGE_2 and LTB_4 have been linked to alterations in the immune system and to specific pathological processes, dietary fat intake has the potential to alter human disease. Reduced production of inflammatory eicosanoids by DHA, EPA, and DGLA, therefore, forms the basis for their use in the management of inflammatory diseases.

In this chapter, we provide a brief introduction to immune system and FA nomenclature. The effects of dietary FAs on the number and activity of the cells of the immune system is a main focal point. We emphasize the results of human studies and discuss animal studies as a means to understand some of the proposed mechanisms by which FAs alter immune functions. The amount of published information for various topics can be quite different, and thus, the amount of detail given varies. Further details can be found in individual papers cited or other reviews regarding the effect of dietary FAs on immune functions (Calder and Grimble, 2002; De Pablo and De Cienfuegos, 2000; Harbige, 2003; Yaqoob and Calder, 2003).

II. IMMUNE SYSTEM AND ITS RESPONSE

The human immune system is very complex and involves a number of organs, cell types, and molecules that are scattered throughout the body. The primary organs associated with the immune system include bone marrow, thymus, spleen, lymph nodes, liver, and small intestine. The cells of the immune system are mainly leukocytes or white blood cells (WBCs), a component of the hematopoietic system. In adult humans, all WBCs originate from the bone marrow, with their maturation occurring in other tissues as well. Based on the shape of their nuclei, WBCs can be divided into two major groups: mononuclear or segmented nuclei, that is, polymorphonuclear. The mononuclear cells include monocytes/macrophages and lymphocytes. The macrophages differentiate from bloodborne monocytes upon migration into tissues. They play a central role in nonspecific immunity and processes such as phagocytosis, bactericidal and tumoricidal activity, inflammation, and tissue repair. These cells are a major source of inflammatory cytokines and eicosanoids and have specific roles in regulating the activity of other immune cells (T, B, and natural killer [NK]) through those mediators. Macrophages also process and present antigens to T and B cells.

Depending on the site of maturation, specific surface markers, and functions, the lymphocytes are divided into T (thymus), B (bone marrow or bursa), NK, and LAK cells. T lymphocytes mature in the thymus and are involved in DTH and in anticancer and antifungal immunity. T helper (Th) and T suppressor (Ts) cells play a central role in immune regulation. The NK cells provide spontaneous and nonspecific immunity against tumor cells, virally infected, and chemically modified cells. The LAK cells are the primary blood or spleen lymphocytes that acquire the ability to kill certain tumors *in vitro,* when cultured with interleukin (IL)-2. They have been reported to cause regression of solid tumors when administered to experimental animals or patients with cancer and suppress the formation of metastases.

The PMN cells include neutrophils, eosinophils, and basophils. Neutrophils make up 60–75% of circulating WBCs and provide the first line of defense against microbes that penetrate the normal barriers of skin. They are extremely efficient phagocytes and are a source of inflammatory cytokines and lipid mediators such as PGs, LTs, and platelet-activating factor. Eosinophils are involved in allergy and provide protection against parasites. Basophils contain vasoactive amines such as histamine, serotonin, and heparin, as well as precursors for PGs and LTs. Release of these pharmacological materials by the basophils is responsible for the anaphylactic reaction. These factors also serve as chemoattractants for neutrophils and eosinophils to sites of inflammation.

Immunity is a state of resistance or protection from pathogenic microorganisms. There are two major types of immunity: innate and adaptive. Innate immunity is present at all times in most individuals and is thus fully functional before infectious pathogens enter the body. It does not distinguish between pathogens of different species, and the response time does not change with repeated exposures. Examples of innate immunity include mechanical barriers such as skin and the epithelial linings of lungs and gut; secreted products, such as saliva and tears; and cells including macrophages, neutrophils, and the NK cells. The adaptive immunity or specific immune response is the reaction to challenge by a specific immunogen. It is activated after the pathogen has evaded the innate response and has entered the body. It is specific for the attacking microbes, and the response time is reduced during repeated exposures. Adaptive immunity has two major classes: humoral immunity mediated by B lymphocytes and cell-mediated immunity mediated by T lymphocytes and activated macrophages. The cells of the immune system synthesize and recognize various molecules, including antibodies, complement proteins, cytokines and their receptors, adhesion molecules, growth factors, and many others. Many of these molecules are also synthesized by a diverse array of cells not belonging to the immune system. These molecules, which can act in an autocrine or paracrine manner, have pleiotropic and synergistic effects. Thus, the overall protection against the pathogen is provided by an interaction between the various cells and molecules of the immune system.

III. ALLERGY AND INFLAMMATION

Allergy or hypersensitivity is an immunologically mediated reaction to a foreign antigen (allergen), causing tissue inflammation and organ dysfunction. It generally represents the undesirable aspects of an immune reaction, whereas immunity implies the desirable effects. The immune response may be a local reaction at sites such as skin, respiratory, vasculature, gastrointestinal tract, or other visceral organs or a systemic reaction such as anaphylaxis or serum sickness. Immunologically induced inflammation may involve the reaction of the antigen with T cells and with the products of B cells (antibodies). Only antibodies of immunoglobulin (Ig) E, IgG, and IgM types are known to be involved in allergic reactions. Types I, II, and III hypersensitivity reactions are immunoglobulin mediated, whereas type IV is T-cell mediated. Type I hypersensitivity involves IgE-mediated release of vasoactive amines, AA metabolites, and cytokines from mast cells. Examples include anaphylaxis, rhinitis, asthma, allergic gastroenteropathy, and atopic dermatitis. Type II reactions involve the binding of either IgG or IgM antibodies specific for tissue or cell

surface antigens, which activates complement cascade and destroys the cells to which the antigen is bound. Examples include immune hemolytic anemia, Rh hemolytic disease, and autoimmune hyperthyroidism. In type III or immune complex–mediated hypersensitivity, antibodies of the IgG or IgM isotype form circulating immune complexes with the allergen and thereby activate complement to generate mediators of inflammation. Examples include the Arthus reaction and serum sickness. Type IV hypersensitivity is caused by T-cell activation and direct target cell lysis by cytotoxic T cells; examples include DTH and tuberculin reactions.

Inflammation is a local protective response to infection or injury whereby cells and proteins in the blood enter to remove the pathogens and repair the damaged tissue. Edema, redness, pain, and heat are the four cardinal symptoms of inflammation. Extent of reactions is determined by inflammatory mechanisms mediated by serum protein or cellular systems. Serum protein systems include complement, coagulation, fibrinolysis, and kinin; cellular systems include PMN cells, mast cells, platelets, eosinophils, lymphocytes, macrophages, and reticuloendothelial system. Insufficient responses result in immunodeficiency leading to cancer and infections; excessive responses are the cause of a number of chronic diseases like diabetes, cardiovascular disease, rheumatoid arthritis, multiple sclerosis, and Alzheimer's disease (Tracey, 2002).

The early phase mediators of inflammation are histamine and serotonin released from basophils, and the late-phase mediators include AA metabolites, neutrophil lysosomal enzymes, lymphokines, and monokines. PMN cells are involved in acute inflammation, whereas mononuclear cells are involved in chronic inflammation. Cytokines produced by the Th1 cells such as IL-2 and interferon-γ (IFN-γ) favor the development of inflammatory reactions and suppress the allergic responses, whereas cytokines produced by the Th2 cells such as IL-4, IL-5, IL-6, and IL-10 lead to IgE production and activation or production of eosinophils and mast cells (Kidd, 2003). Under normal conditions, a balance is maintained between the activity of the Th1 and Th2 cells; however, an imbalance can lead to inflammatory or allergic diseases. In addition to the cytokines produced by the T cells, those produced by other cells also regulate the immune and inflammatory responses. IL-1, IL-6, IL-8, IL-18, tumor necrosis factor-α (TNF-α), and IFN-γ and IFN-β are considered proinflammatory, whereas IL-4, IL-10, IL-1 receptor antagonist (IL-Ra), and transforming growth factor-β (TGF-β) are considered antiinflammatory. Thus, cytokines play an important role in the pathogenesis of allergic and inflammatory diseases. Commonly used markers to evaluate the degree of chronic inflammation include determination of serum inflammatory proteins (C-reactive protein [CRP], serum amyloid protein [SAA], fibrinogen), inflammatory cytokines, and eicosanoids discussed earlier, as well as adhesion molecules (intercellular

adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1], E-selectin).

IV. DIETARY LIPIDS

Dietary lipids are composed mainly of triglycerides with only small amounts of phospholipids, cholesterol, and other sterols. Chemically, triglycerides are the triacylglycerols or a glycerol molecule esterified with three FAs. Saturated FAs have no double bonds in their carbon chain; examples include palmitic (16:0) and stearic acid (18:0). Unsaturated FAs have one (monounsaturated) or more (polyunsaturated) double bonds in the carbon chain. Depending on the position of the first double bond from the methyl end, these FAs are divided into n-9, n-6, or n-3 (also called ω-9, ω-6, or ω-3) series, with the first double bonds being between carbon 9 and 10, carbon 6 and 7, and carbon 3 and 4, respectively. Common sources of different dietary FAs are shown in Table I. Animal fats are a rich source of saturated

TABLE I
MAJOR DIETARY SOURCES OF COMMON FATTY ACIDS

Fatty acid	Source
Saturated	
Lauric (12:0)	Coconut and palm oils
Myristic (14:0)	Coconut and palm kernel oils
Palmitic (16:0)	Palm oil, milk fat, beef tallow, lard, chicken fat, cottonseed oil, chocolate
Stearic (18:0)	Beef tallow, lard, milk fat, cashew nut butter, chocolate
Omega-9 or n-9	
Oleic (OA,18:1n-9)	Olive, peanut, rapeseed, palm oils, chicken, turkey, lard, tallow
Omega-6 or n-6	
Linoleic (LA, 18:2n-6)	Sunflower, safflower, corn, soybean, cottonseed, walnut oils
Conjugated linoleic (CLA)	Hydrogenated oils, margarines, milk fat, ruminant meats
Gamma linolenic (GLA, 18:3n-6)	Borage, evening primrose, black currant oils
Arachidonic (AA, 20:4n-6)	Organ meats, eggs, fish
Omega-3 or n-3	
α-linolenic (ALA, 18:3n-3)	Flaxseed, perilla, rapeseed, walnut oils
Eicosapentaenoic (EPA, 20:5n-3)	Fish and fish oils (salmon, menhaden, mackerel, tuna)
Docosahexaenoic (DHA, 22:6n-3)	Fish and fish oils (salmon, menhaden, mackerel, tuna)

medium-chain FAs and oleic acid (18:1n-9). Plant seed oils are good sources of LA and contain only trace amounts of n-6 FAs with more than 18 carbons; animal tissues contain significant amounts of AA. Conjugated LA (CLA) and gamma LA (GLA) (18:3n-6) are other important n-6 FAs that are found in relatively small amounts in the diets but have significant physiological effects. *CLA* is a collective term for isomers of LA that have conjugated double bonds. Depending on the position and geometry of the double bonds, more than a dozen isomers of CLA have been reported; two of those isomers, cis 9, trans 11-CLA (*c*9, *t*11-CLA) and trans 10, cis 12-CLA (*t*10, *c*12-CLA), have been studied regarding their health effects. The major dietary sources of *c*9, *t*11-CLA are dairy products and ruminant meat, whereas that of *t*10, *c*12-CLA are partially hydrogenated vegetable oils from margarines and shortenings. Primrose, borage, and black currant seed oils contain considerable amounts of GLA. Dietary FAs of the n-3 series include ALA (18:3n-3), EPA, and DHA (22:6n-3). These FAs are found only in plant oils, because the animals lack the enzyme 15-delta desaturase required for inserting the double bond at n-3 carbon of LA. Flax and perilla seed oils are very rich sources of ALA, whereas deep ocean fish and the fish oils are good sources of EPA and DHA. Another good source of DHA–triglyceride is the oil from genetically engineered algae (Martek Corporation) in which DHA represents approximately 50% of the total FAs.

The three classes of unsaturated FAs are not interconvertable; however, they are metabolized by a common series of elongases and desaturases (Figure 1). A competitive interaction between FAs exists so that FAs of the 18:3n-3 family suppress the metabolism of the 18:2n-6 family and those of the 18:2n-6 family suppress the metabolism of the 18:3n-3 family, though less strongly. Both 18:2n-6 and 18:3n-3 FAs suppress the metabolism of the 18:1n-9 FAs. FA composition of the diet plays a critical role in determining the tissue FA composition; the ratios between different classes of FAs, rather than their absolute amounts, are more important.

Humans cannot insert the first double bond at C3 or C6 but can elongate and desaturate LA and ALA. Therefore, FAs with 18, 20, or 22 carbons, with two to six double bonds in *cis* configuration, and the first double bond between C3 and C4, or C6 and C7, are considered essential FAs (EFAs). In addition to their many physiological roles in processes such as growth, reproduction, and brain and eye functions, EFAs are structural components of cell membranes, involved in signal transduction, as well as ligands for nuclear receptors, required for the growth and maintenance of immune cells. In addition, they are produced and secreted during immune cell activation.

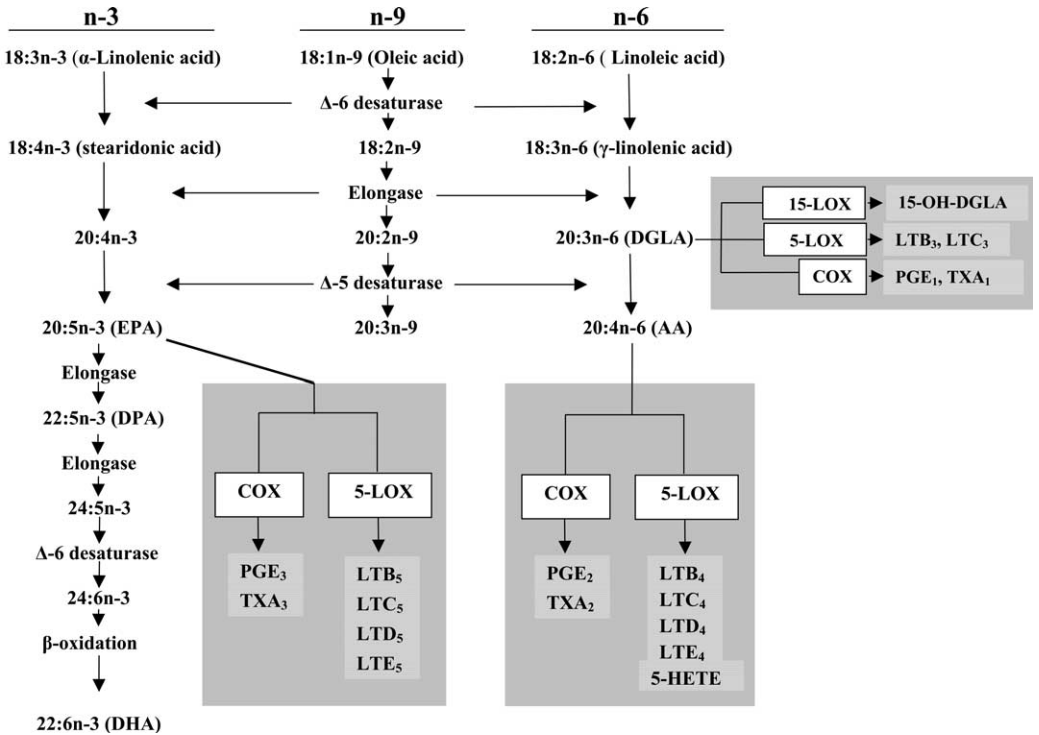

FIG. 1 Metabolism of n-3, n-6, and n-9 fatty acids and their conversion to various eicosanoids. AA, arachidonic acid; COX, cyclooxygenase; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; TX, thromboxane.

V. EFFECTS OF DIETARY FATTY ACIDS ON IMMUNE AND INFLAMMATORY RESPONSES

Both the total fat intake and the ratios between FAs of different classes influence the activity of immune cells. Such information was initially obtained through epidemiological human studies, and studies conducted with cultured cells and animal models. These studies showed that EFAs are required for the growth and maintenance of the immune cells, and free FAs are produced and secreted during the activation of these cells. A number of intervention studies regarding the effects of the amount and composition of dietary fat on human immune response have been conducted, results of which are discussed in the following sections.

A. AMOUNT OF DIETARY FAT AND IMMUNE RESPONSE

Eight published studies have examined the effects of reduction in energy from fat intake on different indices of immune response (Table II). Age of the study participants and the amount and duration of change in dietary fat are the most critical factors in determining modulation of immune functions by dietary fat; this information is included in Tables II–VII. In the studies that focused on the amount of dietary fat and its effect on immune responses, total calories, and calories from proteins were held constant, whereas the amount of calories from fat was adjusted with calories from carbohydrates. Reduction in fat intake ranged from 8 to 16 energy % (en%) for a duration of 4–11 weeks. Seven of the eight studies reported an increase in some aspect of immune response when the percentage of energy from fat was decreased. Four studies reported an increase in peripheral blood mononuclear cell (PBMC) proliferation (Han *et al*., 2003; Kelley *et al*., 1989, 1992; Meydani *et al*., 1993), two reported an increase in NK cell activity (Barone *et al*., 1989) or numbers (Rasmussen *et al*., 1994), two reported an increase in the DTH response (Han *et al*., 2003; Meydani *et al*., 1993), and one reported an increase in cytokine production by monocytes (Meydani *et al*., 1993). One study (Rasmussen *et al*., 1994) reported a 20% increase in the number of circulating NK cells but no change in their activity, whereas another (Barone *et al*., 1989) found a 50% increase in NK cell activity. This discrepancy may be due to use of elderly subjects, and the increase in the polyunsaturated FA (PUFA) content of the diet; P:S ratios were 0.70 for low-fat diets and 0.37 for high-fat diets (Rasmussen *et al*., 1994). Only one report found no change in immune functions with a reduction in fat intake (Venkatraman *et al*., 1997). In that study, increasing the fat content of the diet from 32 to 41 en% for endurance athletes who ran more than 40 miles/wk did not alter lymphocyte proliferation, IL-2 secretion, or the number of circulating mononuclear

TABLE II

EFFECTS OF TOTAL FAT INTAKE ON HUMAN IMMUNE RESPONSE

Immune response and magnitude	Subjects age and sex (n)	Change in energy % from fat	Duration (wk)	Reference
PBMC P, 50–60% increase	29–40 M (8)	41 to 25	11	Kelley *et al.*, 1989
PBMC P, 80–100 % increase	30–65 W (7)	41 to 31 and 41 to 26	9	Kelley *et al.*, 1992
NK activity, 50% increase	Mean 31 M (14)	30 to 22	8	Barone *et al.*, 1989
NK number, increase 20%, NC in activity	65–81 M (13)	40 to 29	5	Rasmussen *et al.*, 1994
PBMC, P, and DTH, increase 28%	>50 M and W (17)	38 to 28	4.5	Han *et al.*, 2003
DTH, 50% increase	>40 M and W (10)	35 to 15	6	Santos *et al.*, 2003
PBMC, P, DTH, IL-1, TNF 30–60% increase	>40 M and W (10)	36 to 27	6	Meydani *et al.*, 1993
PBMC, P, and cytokine production NC	Mean 35 M and W (6–8)	41 to 32	4	Venkatraman *et al.*, 1997

Note: DTH, delayed hypersensitivity skin response; NC, no change; NK, natural killer cell; PBMC, peripheral blood mononuclear cells; P, proliferation; M, men; W, women; *mean* pertains to mean age.

cells when preexercise blood samples were tested. However, the postexercise number of total circulating mononuclear cells increased by approximately 50% in men with increase in fat intake but did not change in women; the number of circulating NK cells following exercise increased with increases in fat intake by 100% in women but did not change in men. Authors concluded that a high percentage of calories from fat did not have an adverse effect on the immune system of well-trained athletes. This may be a valid interpretation. The inconsistency in the results between men and women regarding the number of circulating mononuclear and NK cells may be due to gender differences or the small number of subjects in the study (n = 6–8).

Overall, results from all these studies suggest that the long-term reduction in fat consumption improved both *in vivo* and *ex vivo* measures of immune functions. Increased response in these immune functions will generally indicate increased protection that may be of clinical significance. Only one study has compared the effects of lipid infusion on *ex vivo* measures of immune functions and the *in vivo* markers for diseases (van der Poll *et al.*, 1995). In this study, intralipid infusion caused up to a 70% reduction in the *in vitro* secretion of IL-1β, IL-6, and TNF-α; however, it did not influence inflammatory responses to endotoxin (fever, leukocytosis, release of TNF and its receptor) and even potentiated plasma endotoxin responses (release of IL-6 and IL-8 and neutrophil degranulation). The authors concluded that hypertriglyceridemia does not inhibit the *in vivo* responses to endotoxin.

B. EFFECTS OF DIETARY N-3 FATTY ACIDS ON IMMUNE AND INFLAMMATORY RESPONSES

The source of n-3 PUFAs used in human feeding trials has been either flaxseed or linseed oil as a source of ALA, fish and fish oils as sources of EPA and DHA, purified esters of EPA and DHA, or DHA triglycerides from genetically engineered algae. There are only a few human studies in which flaxseed or fish was fed, whereas there are several dozen studies with fish oils only. Results from these studies are summarized in the following section.

1. α-linolenic acid

Results from seven studies that examined the effects of dietary ALA on indices of human immune response have been published (Table III). The amount of ALA in these studies ranged from 2 to 18 g/day for a duration of 4–24 weeks. Three of these studies (Caughey *et al.*, 1996; Kelley *et al.*, 1991; Rallidis *et al.*, 2003) reported a 20–50% reduction in various indices of immune response such as levels of serum CRP, serum amyloid protein A, and IL-6; *in vitro* secretion of IL-1β and TNF-α; or DTH response. The

TABLE III

EFFECTS OF α-LINOLENIC ACID ON HUMAN IMMUNE FUNCTIONS

Response and magnitude	Subjects age and sex (n)	ALA g/day	Duration (wk)	Reference
PBMC, P, and DTH, 20–50% decrease	21–37, M (9–10)	18	8	Kelley *et al.*, 1991
IL-1 and TNF, 30% decrease	26–36, M (13)	14	4	Caughey *et al.*, 1996
CRP, SAA, IL-6, 25–30% decrease	Mean 51, M (50)	8.0	12	Rallidis *et al.*, 2003
PBMC P, lymphocyte and monocyte cytokines, DTH, NC	25–72 M and W (29–32)	4.5 and 9.0	24	Kew *et al.*, 2003
PMN chemotaxis and superoxide production, NC	>40, M (8)	4.0	12	Healy *et al.*, 2000
PBMC P, cytokines, DTH NC	18–39, M (8)	3.5	12	Wallace *et al.*, 2003
PBMC P, NK activity, cytokines, NC	55–75 M and W (8)	2.0	12	Thies *et al.*, 2001a,b,c

Note: DTH, delayed hypersensitivity skin response; NC, no change; NK, natural killer cell; PBMC, peripheral blood mononuclear cells; P, proliferation; M, men; SAA, serum amyloid protein A; W, women; *mean* pertains to mean age.

amount of ALA in those studies ranged from 8 to 18 g/day for a period of 4–12 weeks; the LA:ALA ratios ranged from 0.6 to 1.3. Even at the highest amount of ALA (18 g/day for 8 weeks; Kelley *et al*., 1991), a number of other indices of immune status did not change, including the number of circulating WBCs, granulocytes, monocytes, lymphocytes, and their subsets; serum concentrations of IgG, IgA, C3, or C4; *ex vivo* secretion of IL-2 and IL-2R; and mitogen-induced B-cell proliferation. Thus, dietary ALA can differentially alter parameters of immune status.

The four other studies (Kew *et al*., 2003; Thies *et al*., 2001a,b,c; Wallace *et al*., 2003) reported no effect of dietary ALA on several indices of immune response tested, such as DTH, PBMC proliferation, cytokines produced by lymphocytes and monocytes, PMN chemotaxis, and superoxide production. The amount of ALA used in these studies varied from 2 to 9 g/day for 12–24 weeks. No significant differences in these studies were most likely due to the small amounts of ALA in the diets; this may explain the results from the studies by three groups (Healy *et al*., 2000; Thies *et al*., 2001a,b,c; Wallace *et al*., 2003), but not those of others (Kew *et al*., 2003), who fed ALA at 9 g/day for 24 weeks. In this study, the ratios of LA:ALA in the low and high ALA diets were 8.0 and 3.0, respectively. The amount and duration of ALA in the diets do not seem to be the sole factors that determine whether ALA will inhibit indices of immune status, because one group of investigators (Rallidis *et al*., 2003) reported inhibition with ALA at 8.0 g/day for 12 weeks and another group (Kew *et al*., 2003) did not detect inhibition with a greater amount and longer duration (ALA 9 g/day for 24 weeks). The LA:ALA ratios were 1.3 (Rallidis *et al*., 2003) and 3.0 (Thies *et al*., 2001a,b), which may explain the lack of an effect of ALA in one study (Thies *et al*., 2001a,b,c). Collectively, it appears that ALA does inhibit lymphocyte and monocyte functions, the magnitude of which depends on the amount and duration of ALA supplementation and the ratio between LA:ALA. Another factor that is important and is discussed in the following section is the concentration of antioxidants. Feeding ALA increased both the ALA (0.2–0.6%) and the EPA (0.2–0.4%) concentration of PBMC lipids but did not alter their DHA concentration (Kelley *et al*., 1993). It also increased the serum concentration of ALA from 0.5 to 3.2%. It is, therefore, difficult to determine whether the effects of dietary ALA observed in this study were caused by ALA itself or by one of its elongation products.

2. Fish

There are only two published studies in which the effects of fish consumption on indices of immune response were tested. In one study, 500 g/day of salmon containing 2.3 g of EPA and 3.6 g/day of DHA was fed to nine healthy young

men for 40 days (Kelley *et al.*, 1992). The study had a crossover design and was conducted at a metabolic unit where the diet and activity levels were strictly controlled. The salmon diet contained 3.6% less calories from fat, which were replaced by an equivalent amount of calories from carbohydrates. Feeding the salmon diet did not have any effect on a number of indices of immune response, including DTH, PBMC proliferation, and serum Ig concentration. The lack of an effect on immune status in this study was most likely due to short duration, reduction in energy from fat in the salmon diet, and supplementation of both diets with additional vitamin E. Another study was conducted with 22 men and women older than 40 years in a parallel design for 24 weeks (Meydani *et al.*, 1993). Eleven subjects consumed a high fish National Cholesterol Education Program Step 2 diet (120–188 g/day fish containing 1.2 g EPA plus DHA), and the remaining subjects received the same diet with only one-fourth the amount of fish. Lymphocyte proliferation, *ex vivo* production of IL-1β, IL-6, and TNF-α , and DTH were significantly reduced in the group fed the high fish diets. The duration of fish consumption, age of the subjects, and the lack of additional vitamin E supplementation in this study may be some of the reasons the results differ from those of other investigators (Kelley *et al.*, 1992). Additional studies are needed to establish whether there are any risks associated with increased fish consumption. From the available data, it appears that two to three servings of fish per week should be safe even without vitamin E supplementation, but that amount may not substantially alter immune response.

3. *Fish oils, eicosapentaenoic, and docosahexaenoic acids*

Most of the studies with EPA and DHA have used fish oils that contain both of these FAs; however, a few other studies have examined the effects of these FAs individually. Several studies have examined the effects of fish oil supplementation on *ex vivo* neutrophil and monocyte chemotaxis, superoxide production, phagocytosis, lymphocyte and monocyte cytokine production, lymphocyte proliferation, and *in vivo* indices of immune response. The amount of fish oil supplemented ranged from 2 to 30 g/day containing 0.55–8.0 g/day EPA plus DHA for 4–52 weeks. Many of these studies were longitudinal in which the fish oils were added to the usual diets and did not include parallel control groups. This study design increased not only the intake of EPA and DHA, but also that of total fat. Most newly published studies included placebo control groups, and the total fat intake between the experimental and placebo groups was comparable. Some studies supplemented variable amounts of vitamin E, which itself affects many indices of immune response, and others did not. Results of fish oil supplementation on immune response have varied extensively.

a. Effects of EPA and DHA on number of circulating white blood cells. Four studies have examined the effects of EPA and/or DHA on the number of circulating WBCs (Kelley *et al.*, 1998b; Meydani *et al.*, 1991b; Thies *et al.*, 2001b; Yaqoob *et al.*, 2000). The amount of EPA and/or DHA ranged from 0.7 to 6.0 g/day for 12–13 weeks; one study supplemented these FAs with a large dose of 200 mg/day α-tocopherol (Yaqoob *et al.*, 2000), and one study (Kelley *et al.*, 1998b) supplemented the diets with 6 g/day DHA only for 13 weeks. Only one of these studies found alterations in the number of circulating WBCs or their specific subsets (Kelley *et al.*, 1998b). Kelley *et al.* (1998b) found a 10% reduction in the number of circulating WBCs at the end of 13 weeks of DHA supplementation, and there was no change in the control group. This decrease in WBCs was primarily due to a reduction (21%) in the number of granulocytes and was detectable as early as 7 weeks after DHA supplementation. One reason for the lack of this effect in other studies may be the low amount of DHA in the supplements. High levels of vitamin E in one study (Yaqoob *et al.*, 2000) may have also contributed to those results. A reduction in the number of circulating granulocytes by DHA (Kelley *et al.*, 1998b) was similar to that found in rats (Atkinson *et al.*, 1997; Ohhashi *et al.*, 1998) and human patients with adult respiratory distress syndrome after feeding fish oils (Mayer *et al.*, 2002).Whether this reduction in circulating granulocytes associated with dietary n-3 PUFA was due to altered margination, proliferation, differentiation, or apoptosis needs to be investigated.

b. Respiratory burst, phagocytosis, and chemotaxis. Fifteen studies have reported the effects of EPA and DHA on neutrophil and/or monocytes chemotaxis, phagocytosis, and superoxide production (Table IV). Amount of EPA and DHA in these studies ranged from 0.6 to 14.4 g/day for 3–24 weeks. Seven of these studies included placebo controls where the total fat intake matched that of the experimental groups, and in the remaining eight studies fish oils were added to the usual diets. Nine of these studies reported a 25–70% reduction in neutrophil and/or monocyte functions with the consumption of EPA and DHA. The lowest amount of EPA and DHA that showed inhibition of neutrophil superoxide production was 2.2 g/day for 4 weeks (Thompson *et al.*, 1991), and the highest amount of EPA or DHA that failed to inhibit neutrophil and monocyte phagocytosis was 4.7 g/day for 12 weeks (Kew *et al.*, 2004). All other studies that have used EPA and DHA at more than 4 g/day, except two (Halvorsen *et al.*, 1997; Kew *et al.*, 2004), demonstrate a 30–70% reduction in phagocytosis and chemotaxis. In one of the studies (Halvorsen *et al.*, 1997), cells were stored frozen before phagocytosis assays, a possible reason for the lack of effect. However, another study (Kew *et al.*, 2004) used freshly isolated cells for a flow cytometric method to determine phagocytosis; the reasons for the

TABLE IV

EFFECT OF EPA AND DHA ON CHEMOTAXIS, PHAGOCYTOSIS, AND SUPEROXIDE PRODUCTION BY HUMAN PMN AND MONOCYTES

Function and magnitude of change	Subjects age and sex (n)	EPA–DHA (g/day)	Duration (wk)	Reference and study type
PMN chemotaxis, 25–60% decrease	25–59, M and W (8)	14	3	Sperling *et al.*, 1993
PMN chemotaxis, decreased with increase in EPA–DHA	28–66, M and W (8)	1.3, 4, 9	6	Schmidt *et al.*, 1991
PMN chemotaxis, 30–50% decrease	41–60, M and W (8)	8.6	3	Luostarinen *et al.*, 1991
Monocytes chemotaxis, superoxide production, 45–60% decrease	29–54, M and W (9)	6	6	Fisher *et al.*, 1990
PMN chemotaxis, 70% decrease	22–53, M (7)	5.4	6	Lee *et al.*, 1985
PMN chemotaxis, 30% decrease	29–49, M (12)	5.3	6	Schmidt *et al.*, 1989
PMN phagocytosis, NC	23–65, M and W (10)	4.7	12	Kew *et al.*, 2004
PMN chemotaxis, 70% decrease	M and W (4–5)	4.0	8	Payan *et al.*, 1986
Monocytes respiratory burst and phagocytosis, NC	36–56, M and W (19)	4.0 EPA, DHA	7	Halvorsen *et al.*, 1997, PC
PMN chemiluminescence, superoxide production, 27–64% decrease	M and W (6)	3.6	6	Fisher *et al.*, 1986, PC
PMN chemotaxis and superoxide production, NC	>40, M (8)	2.2	12	Healy *et al.*, 2000, PC
PMN superoxide production, 50% decrease	20–30, M and W (6)	2.2	4	Thompson *et al.*, 1991, PC
PMN/monocytes phagocytosis and respiratory burst, NC	55–75, M and W (8)	1.0	12	Thies *et al.*, 2001a, PC
PMN/monocytes phagocytosis, NC	25–72, M and W (29–32)	0.8, 1.7	24	Kew *et al.*, 2003, PC
Monocyte chemotaxis, NC	24–52, M and W (16)	0.6	12	Schmidt *et al.*, 1996, PC

Note: Studies marked *PC* (placebo control) included a control group, and others did not; PMN, polymorphonuclear leukocytes; NC, no change; M, men; W, women.

discrepancy between results from this and many other studies are not clear. The shortest intervention to demonstrate inhibition of PMN chemotaxis was 3 weeks, in a study that supplemented the usual diets with fish oils and provided 8.6 g/day of EPA and DHA (Luostarinen *et al*., 1991). Normally, high intakes of vitamin E protect from the inhibitory effects of fish oils, but in this study, PMN chemotaxis was equally inhibited in subjects provided with 9 or 45 IU of vitamin E per day. That indicates that 45 IU/day was not adequate to protect from the inhibition caused by the high intake of fish oil. After a short period of feeding EPA and DHA concentrations of less than 1.7 g/day, no inhibition of neutrophil and monocyte phagocytosis, chemotaxis, or superoxide production was observed; the amounts needed to inhibit these responses were generally similar (Table IV). Genetic variation of the subjects studied also seemed important in determining the response to EPA and DHA as one study reported a reduction in monocyte chemotaxis in type IIa but not type IV hyperlipidemic subjects (Schmidt *et al*., 1991). Long-term studies are needed to determine the effects of genetic polymorphisms, antioxidants, and the amounts and types of dietary PUFAs on monocyte and neutrophil functions discussed here.

c. Cytokine production by monocytes. Nineteen studies have investigated the effects of dietary EPA and DHA on production of inflammatory cytokines by PBMCs isolated from human volunteers fed diets containing EPA and DHA, and then stimulated with mitogens *in vitro*; two additional studies examined the effects of these FAs on serum/plasma cytokines (Table V). The amount of EPA and DHA intake varied from 0.3 to 6.0 g/day for 4–52 weeks. EPA+DHA supplementation caused a 20–90% reduction in the *ex vivo* secretion of inflammatory cytokines such as IL-1, IL-6, or TNF-α in 8 of 19 studies (Abbate *et al*., 1996; Caughey *et al*., 1996; Endres *et al*., 1989; Gallai *et al*., 1995; Grimble *et al*., 2002; Kelley *et al*., 1999; Meydani *et al*., 1991b, 1993); these FAs also caused a 10–20% reduction in the serum concentrations of CRP and IL-6 in one of two studies (Ciubotaru *et al*., 2003). It seems the reduction in cytokine production was dependent on the dose of fish oil, as 7 of the 11 studies that failed to detect a decrease used supplements with less than 2 g/day EPA plus DHA. Studies that failed to detect a reduction in cytokine production with EPA and DHA consumption of greater than 2 g/day supplemented the FAs either for a short 4-week duration (Kew *et al*., 2004) or co-supplemented with a high dose of 205 mg/day vitamin E (Yaqoob *et al*., 2000). Similarly, no reduction in plasma CRP or cytokines was detected when the diets of type II diabetic subjects were supplemented with 4 g/day DHA for only 6 weeks (Mori *et al*., 2003). The lack of an effect in that study may be due to a combination of short duration and elevated serum lipids. The importance of the duration of supplementation was

TABLE V

INHIBITION OF HUMAN MONOCYTE CYTOKINE PRODUCTION BY EPA AND DHA

Function	Subjects age and sex (n)	EPA–DHA (g/day)	Duration (wk)	Reference
IL-1β, TNFα 30–45% decrease	Mean 33, M (7)	6 (DHA)	13	Kelley *et al.*, 1999, PC
IL-1β, TNFα 50–80% decrease	20–50, M and W (15)	5.2	24	Gallai *et al.*, 1995, PC
IL-1β, IL-6, IL-8, TNF-α NC	23–65, M and W (14)	4.7	12	Kew *et al.*, 2004, PC
IL-1β, TNF-α 20–60% decrease	21–39, M and W (9)	4.6	6	Endres *et al.*, 1989
Plasma CRP, IL-6, TNF-α NC	40–75, M and W (16)	4.0	6	Mori *et al.*, 2003, PC
IL-6, 50% decrease	M and W (9)	4.0	18	Abbate *et al.*, 1996
IL-1β, TNF-α, NC	Mean 25, M and W (9)	4.0	7	Molvig *et al.*, 1991
IL-1β, TNF-α, NC	Mean 45, M and W (8)	3.2	12	Yaqoob *et al.*, 2000, PC
IL-1β, IL-1Rα, TNF-α NC	21–81 M (58)	1.1–3.1	13–52	Blok *et al.*, 1997
IL-1β, IL-6, 85–90% decrease	18–36, M and W (6–8)	2.8	8	Cooper *et al.*, 1993, PC
IL-1β, TNF-α 70–80% decrease	26–36, M (13)	2.7	4	Caughey *et al.*, 1996, PC
IL-1β, IL-6, TNF-α 50–80% decrease	23–33, W (5) 51–68 W (6)	2.4	12	Meydani *et al.*, 1991b
Serum CRP, IL-6, 10–20% decrease	Mean 60, W (10)	1.3, 2.6	5	Ciubotaru *et al.*, 2003, PC
IL-6, TNF-α >50% decrease	M (15)	0.3, 1, 2	4 each	Trebble *et al.*, 2003a
IL-1β, NC	18–39, M and W (8)	0.4–1.9	12	Wallace *et al.*, 2003, PC
TNF-α decrease or increase based on phenotype	Mean 37, M (37)	1.8	12	Grimble *et al.*, 2002
IL-1β, IL-6, TNF-α, NC	25–72, M and W (30)	0.8 or 1.7	24	Kew *et al.*, 2003
IL-1β, IL-6, TNF-α, NC	>40, W (10)	1.2	24	Meydani *et al.*, 1993, PC
IL-1β, IL-6, TNF-α, NC	55–75, M and W (8)	1.0	12	Thies *et al.*, 2001a
IL-1β, IL-6, TNF-α, NC	W (27)	0.4 or 0.7	4	Hawkes *et al.*, 2002, PC
IL-1β, IL-6, TNF-α, NC	24–52, M and W (16)	0.6	12	Schmidt *et al.*, 1996, PC

Note: Studies marked *PC* (placebo control) included a control group, and others did not; CRP, C reactive protein; IL, interleukin; TNF-α, tumor necrosis factor-α; NC, no change.

demonstrated in two studies. Although supplementation of DHA did not significantly alter immune response at 55 days, after 91 days, secreted cytokine levels were reduced by 30–45% (Kelley *et al*., 1999). Similarly, other investigators (Endres *et al*., 1989) reported a 20–45% reduction in cytokine secretion 6 weeks after the start of fish oil supplementation, whereas it was reduced by 61% at 10 weeks after discontinuing the supplement. In that study, IL-1β and TNF-α secretion was restored to the presupplement levels 20 weeks after discontinuing the supplement. One study did not detect a reduction in cytokine secretion even with a EPA–DHA supplement of 3.1 g/day for 52 weeks. However, cytokine secretion was not assessed before the start of the supplements, and the first assessment was made 13 weeks after the start of supplements (Blok *et al*., 1997).

In addition to the amount and duration of the n-3 PUFA supplementation, the amount of antioxidant nutrients and the cytokine genes polymorphism are also important factors that determine the cytokine response to n-3 PUFA. Thus, vitamin E supplements of 200 mg/day or more prevented the inhibition of cytokine secretion by monocytes (Yaqoob *et al*., 2000) and proliferation of lymphocytes cultured with Con A (Kramer *et al*., 1991); however, 30 mg/day of vitamin E supplements did not prevent the inhibition of cytokine secretion by fish oil supplements (Trebble *et al*., 2003a). Likewise, 45 IU/day failed to prevent the inhibition of PMN chemotaxis by dietary fish oil (Luostarinen *et al*., 1991). The amount of vitamin E needed to prevent the inhibition of monocyte responses by n-3 PUFA appears to depend on the amount of the n-3 PUFA, and it is several-fold greater than the usual intake of vitamin E. Furthermore, the reduction in TNF secretion in response to n-3 PUFA consumption was dependent on the polymorphism in the TNF gene (Grimble *et al*., 2002). Although further studies are needed to resolve some of the inconsistencies regarding the effects of n-3 PUFA on the secretion of inflammatory cytokines, results from the studies discussed here indicate that amounts greater than 3 g/day, particularly in the absence of high intakes of vitamin E, will inhibit monocyte and PMN functions.

d. Lymphocyte functions. Currently, 14 studies have investigated the effects of EPA–DHA supplementation on lymphocyte functions, including NK cell activity, lymphocyte proliferation, and cytokine production (Table VI). NK cell activity was examined in three studies after supplementing with EPA or DHA (Kelley *et al*., 1999; Thies *et al*., 2001a; Yaqoob *et al*., 2000) or infusion with EPA–triglyceride (Yamashita *et al*., 1991). One study used 6 g/day of DHA and found no change in NK cell activity at 8 weeks after the start of DHA supplementation, although it was significantly reduced at 13 weeks (Kelley *et al*., 1999). NK cell activity in the placebo group remained unchanged at 8 and 13 weeks. A 48% reduction in NK cell

TABLE VI
EFFECTS OF DHA AND EPA ON HUMAN LYMPHOCYTE FUNCTIONS

Lymphocyte function	Subjects age and sex (n)	EPA–DHA (g/day)	Duration (wk)	Reference & study type
P, 80% decrease	24–57, M (35)	7	10	Kramer *et al.*, 1991, PC
P, NC, NK activity 20% decrease	Mean 33, M (7)	6 (DHA)	13	Kelley *et al.*, 1998b, PC
IL-2 and IFN-γ, 25–30% decrease	20–50, M and W (15)	5.2	24	Gallai *et al.*, 1995, PC
CD25 expression, 13% decrease	M & W Psorotic patients (10)	5.1	16	Soyland *et al.*, 1994, PC
IL-2, 4, 5, and IFN-γ, NC; DHA not EPA, decreased T-cell activation	23–65, M and W (14)	4.7	4	Kew *et al.*, 2004, PC
P and IL-2, NC; 65% decrease 10 wk postsupplementation	Mean 28, M (9)	4.6	6	Endres *et al.*, 1993
P 15–33% decrease	Mean 25, M and W (9)	2.0, 4.0	7	Molvig *et al.*, 1991, PC
P, IL-2, and IFN-γ, NK activity, NC	Mean 45, M and W (8)	3.2	12	Yaqoob *et al.*, 2000, PC
P and IL-2, 30–36% decrease	23–33 and 51–68, W (5 and 6)	2.4	12	Meydani *et al.*, 1991a
P, 24 % decrease	>40, W (10)	1.2	24	Meydani *et al.*, 1993
P, IL-2, 4, 10, and IFN-γ, NC	25–72 M and W (30)	0.8, 1.7	24	Kew *et al.*, 2003, PC
IL-2, 4, 10, and IFN-γ, NC	55–75 M (8)	0.4, 0.9, 1.9	12	Wallace *et al.*, 2003, PC
P, IFN-γ, increased twofold to threefold	M (15)	0.3, 1, 2	4	Trebble *et al.*, 2003a, PC
P and NK activity 48–65% decrease; IL-2 and IFN-γ, NC	55–75 M and W (8)	1	12	Thies *et al.*, 2001b, PC

Note: Studies marked *PC* (placebo control) included a control group, and others did not. CD, cluster designation; IL, interleukin; IFN, interferon; M, men; NC, no change; NK, natural killer; P, proliferation; W, women.

activity after 12 weeks of supplementation with fish oil (EPA 720 mg + DHA 280 mg/day), but not in the group that was supplemented with 700 mg/day of DHA alone, was observed (Thies *et al*., 2001b). Results of that study suggest that EPA may be more potent in inhibiting NK cell activity than DHA. In contrast to the inhibition of NK cell activity reported with 1-g/day fish oil supplement (Thies *et al*., 2001b), another study from the same laboratory failed to detect inhibition of NK cell activity even when the fish oil supplement provided 3.2 g/day of EPA–DHA for 12 weeks (Yaqoob *et al*., 2000). The lack of inhibition of NK cell activity by fish oil in that study was most likely due to concomitant intakes of 205 mg/day of vitamin E. Infusing 30 ml of EPA–triglyceride 24 hours before the isolation of PBMCs to determine NK cell activity reduced NK cell activity by more than 50% (Yamashita *et al*., 1991). Together, these studies suggest that both EPA and DHA inhibit NK cell activity; however, results have been variable because of variance in experimental designs.

Ten studies examined the effects of EPA–DHA supplementation on the *ex vivo* proliferation of lymphocytes cultured with mitogens (Table VI). The amount of n-3 PUFA supplemented ranged from 0.3 to 7 g/day for 4–24 weeks. Six of these studies found a 24–80% reduction in lymphocyte proliferation after EPA–DHA supplementation, three found no change (Kelley *et al*., 1998b; Kew *et al*., 2003; Yaqoob *et al*., 2000), and one reported a twofold increase (Trebble *et al*., 2003b). The lack of lymphocyte proliferation in one study (Kelley *et al*., 1998b) was most likely due to the lack of EPA in the supplement. Another study (Yaqoob *et al*., 2000) included a vitamin E supplement of 205 mg/day, which may be the reason for the lack of inhibition of lymphocyte proliferation by n-3 PUFA. This interpretation was supported by another study (Kramer *et al*., 1991), which indicated that concurrent supplementation with 200 mg/day of vitamin E prevented the inhibition of lymphocyte proliferation by an EPA–DHA supplement of 7 g/day; in the absence of extra vitamin E, inhibition of proliferation by fish oil supplementation ranged from 0 to 80% depending on the concentration of Con A. The lack of an effect on lymphocyte proliferation in another study (Kew *et al*., 2003) may simply be due to a relatively low amount of 1.7 g/day of n-3 PUFA and a modest supplementation with 15 mg/day of vitamin E. These results suggest that n-3 PUFA intakes of up to 1.7 g/day may be consumed without inhibiting lymphocyte proliferation as long as there is modest supplementation of vitamin E. The increase in lymphocyte proliferation after fish oil supplementation was found in only one study (Kew *et al*., 2003). The experimental design involved concurrent supplementation with a cocktail of antioxidant nutrients including vitamins A, C, and E, selenium, and magnesium. The increase in lymphocyte proliferation in this case was most likely the result of antioxidants and not fish oils.

Eight published studies examined the effects of EPA–DHA supplementation on the *ex vivo* secretion of cytokines by lymphocytes cultured with mitogens (Table VI). Fish oil supplementation caused a 25–35% reduction in the secretion of IL-2 and IFN-γ in only two of these studies (Gallai *et al*., 1995; Meydani *et al*., 1991b). One study found no change in secreted IL-2 with an EPA–DHA supplementation of 4.6 g/day for 6 weeks; however, 10 weeks after supplementation with fish oil, IL-2 secretion was reduced by 65% (Endres *et al*., 1993). The remaining five studies that did not find a reduction in lymphocyte cytokine secretion after fish oil supplementation supplemented either with a modest amount (<2 g/day) of n-3 PUFA (Kew *et al*., 2003; Thies *et al*., 2001a; Wallace *et al*., 2003) or with the fish oil for only 4 weeks (Kew *et al*., 2004) or concurrently supplemented with high amounts of vitamin E (Yaqoob *et al*., 2000). One study that concurrently supplemented with a cocktail of antioxidants involving vitamins A, C, and E, selenium, and magnesium actually reported a threefold increase in IFN-γ secretion after fish oil supplementation (Trebble *et al*., 2003a). As stated earlier, this increase was most likely due to the antioxidants rather than the n-3 PUFA. In addition to the cytokines secreted, one study examined the effect of fish oil supplementation on lymphocyte surface expression of the receptor for IL-2 (CD25) and reported a 13% reduction after supplementing the diets with EPA–DHA at 5.1 g/day for 16 weeks (Soyland *et al*., 1994). The lowest concentration of EPA–DHA that was associated with inhibition of lymphocyte function was 1.2 g/day fed for 24 weeks (Meydani *et al*., 1993), and the highest concentration that did not inhibit was 3.2 g/day for 12 weeks (Yaqoob *et al*., 2000). Overall, the results from these studies generally demonstrate that fish oils inhibit lymphocyte functions, and the responses vary with the amount, duration, and overall composition of the diet, as well as the function tested. Long-term studies are needed to determine the concentrations of fish oils that can be consumed without compromising the immune response.

e. Reasons for inconsistencies regarding the effects of n-3 PUFA on immune functions. Most of the results presented in this chapter indicate that n-3 FAs can inhibit the immune and inflammatory responses; however, there are several exceptions. The extensive variation in the study protocols and methods used has probably contributed to the discrepancies in results from different studies. Potentially important factors related to the study protocol include antioxidant nutrient content of the diets, total fat and FA composition, ratio between n-6 and n-3 PUFAs, amount and duration of supplementation, the daily amount of EPA and DHA, age, sex, and health of the subjects, and inclusion/exclusion of a control or placebo group. Methods and cells used for assessments have varied greatly and add to the discrepancies. For example, isolated PBMCs or whole blood cells cultured in

autologous sera or fetal calf serum have been used with a wide variety of physiological and nonphysiological agents to stimulate cells and monitor their responses. Some of the assays used may have little relationship to human immunity *in vivo*. Most critical among these factors seem to be the ratio between the amounts of n-3 PUFA and vitamin E, as the latter blocks the inhibition by n-3 PUFA, as well as the amount and duration of supplementation. Interaction between n-3 PUFA and vitamin E may involve protein kinase C alpha, as EPA and DHA stimulate this enzyme, whereas α-tocopherol inhibits its activation (Madani *et al*., 2001). Responses also vary with the immune function being tested; for example, mitogen-induced lymphocyte proliferation in contrast to cytokine secretion appears to be more sensitive to n-3 PUFA. Despite these differences, n-3 FAs have been successfully used in the management and prevention of several inflammatory and allergic diseases.

C. EFFECTS OF DIETARY N-6 FATTY ACIDS ON IMMUNE AND INFLAMMATORY RESPONSES

Most of the human studies dealing with the effects of n-6 FAs on immune and inflammatory responses were conducted by altering the intake of oils rich in LA. However, a few studies have also used oils rich in GLA or supplemented diets with purified CLA or AA. Effects of these FAs are discussed individually in the following sections.

1. Linoleic acid and immune response

In two studies, men and women consumed diets in which total fat and LA intake were altered (Kelley *et al*., 1989, 1991). Reduction in total fat was correlated with increased lymphocyte proliferation and DTH response (discussed in Section V.A). Changing the consumption of LA from 3 to 9 en% or 3 to 13 en% in these studies did not alter lymphocyte proliferation, DTH response, serum concentrations of immunoglobulins, and the number of circulating WBCs (Kelley *et al*., 1989, 1991), even when the excretion of urinary PGE_2 in the high LA group was significantly increased (Blair *et al*., 1993). Another study reported that adding 9 g/day of sunflower oil for 12 weeks to the diets of healthy subjects did not alter lymphocyte proliferation, NK cell activity, or the production of cytokines by lymphocytes and monocytes (Yaqoob *et al*., 2000). Similarly, NK cell activity did not differ between two groups of healthy men when their diets were supplemented with 15 g/day of either an oil rich in saturated FAs such as coconut oil or LA (safflower oil) for 2 months (Hebert *et al*., 1990). As discussed earlier, NK cell activity in this study was increased with a reduction in total fat intake. In contrast,

results from a study with elderly Danish men with a mean age of 71 years showed that NK cell activity was inversely related to the serum LA concentration (Rasmussen *et al.*, 1994). Poor health status of the elderly and a wide range in the P:S ratio of the diets may have made it possible to detect the negative effect of LA on NK cell activity.

FA composition of the adipose tissue reflects the long-term FA composition of the diet. In a group of 94 healthy American men, the P:S ratio for adipose tissue FAs ranged between 0.54 and 1.01. Within this range, the P:S ratio had no effect on a number of indices of immune response tested (Berry *et al.*, 1987). Results of the animal studies dealing with LA feeding have been reviewed (Crevel and Saul, 1992) and are generally consistent with what has been reported in humans, but in some studies, unrealistically high levels of LA were found to inhibit lymphocyte proliferation and DTH response. Taken together, these studies do not suggest a suppression of human immune response by modest increases in the consumption of LA, even if these changes were adequate to increase the levels of urinary PGE_2. An increase in the production of proinflammatory eicosanoids with increased consumption of LA may be a concern in the exacerbation of inflammatory diseases.

2. *CLA and human immune response*

There are only four published reports in which the effects of CLA supplementation on immune cell functions in humans have been examined. The first was a metabolic unit study that was conducted with 17 healthy women (Kelley *et al.*, 2000, 2001). For the first 30 days of the study, all subjects consumed a basal diet supplemented with 6 g/day of sunflower oil (standardization diet). The supplement for 10 women was replaced with 6 g/day of Tonalin, which provided 3.9 g of a mixture of CLA isomers for the ensuing 63 days; 7 women received a placebo supplement, which served as a control throughout the study. Immune cell functions were evaluated with fasting blood samples. Delayed hypersensitivity skin response to a battery of seven recall antigens was determined at the start and end of the intervention. All subjects were immunized with influenza vaccine on study day 65, and the serum antibody titers were determined using the blood samples drawn before (preimmunization) and 92 (postimmunization). The total CLA concentration in circulating WBCs at the end of the study increased eightfold when compared to the concentration on study day 30; however, it did not significantly alter the concentration of other FAs. CLA supplementation did not modify the number of circulating total WBCs, lymphocytes, granulocytes, or monocytes. It had no effect on proliferation of lymphocytes cultured with PHA, Con A, or influenza vaccine, *in vitro* production of IL-2, IFN-γ, IL-1, or TNF-α, as well as the production of inflammatory eicosanoids, PGE_2,

and LTB_4 when cells were cultured with mitogens in medium containing 10% autologous sera. Serum influenza antibody titers and DTH responses were also not affected by CLA supplementation. Overall, results of this study suggest that short-term supplementation with a modest level of CLA to healthy adult women was well tolerated but had no beneficial effect on the measured parameters of human immune response. This is consistent with the observed CLA effects on body composition and energy metabolism (Zambell, 2000). Results from another study indicate no change in serum concentrations of TNF-α and IL-6 when overweight/obese volunteers supplemented their diets with a 3.4-g/day isomer mixture of CLA or purified t10, c12-CLA for 12 weeks or an equivalent amount of olive oil (Riserus *et al.*, 2002). On the other hand, concentrations of serum CRP and urinary PGF_2-α were doubled at the end of t10, c12-CLA supplementation compared to the other two groups. These results suggest that t10, c12-CLA may exacerbate oxidative stress and inflammatory response. In a study with experienced resistance-trained men, supplementing their diet with a mixture of 6 g/day of CLA isomers for 4 weeks caused a 25% decrease in the ratio of circulating neutrophils to lymphocytes, whereas the ratio did not change in an olive oil–supplemented group (Kreider *et al.*, 2002). From the available data, it is not possible to determine whether the reduction in this ratio was due to a decrease in neutrophil numbers or an increase in lymphocyte numbers. In this study, CLA supplementation also did not significantly affect gains in bench- or leg-press weight. Whether higher amounts or longer duration of CLA would affect human immune cell functions or increase health risk remains to be determined.

In a preliminary human study, the effects of two mixtures of c9, t11 and t10, c12 CLA isomers (80:20, or a 50:50 mixture) on antibody response to hepatitis B vaccination, delayed hypersensitivity skin response, and other indices of immune cell functions in healthy men were examined (Albers *et al.*, 2003). The amount of CLA supplemented was 1.7 g/day for 84 days. CLA supplementation did not alter delayed hypersensitivity skin response or many other indices of immune cell functions tested. Mean antibody titers against hepatitis B did not differ significantly between the three groups; however, the number of subjects attaining antibody titers higher than 10 IU/L was significantly greater in the 50:50 group compared to the 80:20 or placebo groups. The validity of such arbitrary titers as seroprotective may be questionable, particularly when they are only twofold to threefold higher than the preimmunization titers. Because the 50:50 mixture contained more of the t10, c12-CLA isomer than the 80:20 mixture, the authors concluded that t10, c12-CLA enhanced the antibody response to hepatitis B, whereas the c9, t11-CLA did not alter that response. Those results are not consistent with the observed effects of CLA on influenza antibody titers (Kelley *et al.*,

2000). Whether that was antigen specific, sex specific, or the result of differences in CLA isomers used needs to be addressed in studies. Even if t10, c12-CLA enhances antibody response to specific antigens, this benefit will have to be balanced against the risks of increased lipid peroxidation and inflammation caused by this isomer (Kelley and Erickson, 2003).

3. GLA and human immune response

Results from eight studies have been published regarding the effects of GLA supplementation on human immune functions (Table VII); only three of those studies had a placebo control (Thies *et al.*, 2001c; Wu *et al.*, 1999; Yaqoob *et al.*, 2000). In seven of these studies, GLA was provided orally, and in the eighth study, GLA was infused (DeLuca *et al.*, 1999). Sources of GLA in these studies included borage, black currant, evening primrose seed oils, or a synthetic triacylglycerol; amounts of GLA ranged from 0.7 to 3.3 g/day for a maximum period of 24 weeks.

PBMC proliferation was examined in five studies; it was reduced by 65–80% in two studies (Rossetti *et al.*, 1997; Thies *et al.*, 2001c) and did not change in two studies (Fisher and Harbige, 1997; Yaqoob *et al.*, 2000). In the fifth study, lymphocyte proliferation in response to PHA increased significantly (30–60%) in both the GLA and the placebo group (Wu *et al.*, 1999). The increase in response to PHA was not significantly different between the GLA and control groups. Furthermore, GLA had no effect on lymphocyte proliferation when the cells were stimulated with Con A. Thus, only two of the five studies showed inhibition of lymphocyte proliferation. In one report (Rossetti *et al.*, 1997), the study included only two men; one supplemented his diet with 10 g of borage oil (GLA 2.4 g/day). Blood samples were taken at 5, 12, and 24 weeks after the start of GLA supplementation. Lymphocyte proliferation in response to antibodies against CD3 and CD4 progressively decreased with time, with greater than 80% inhibition at 24 weeks. The other individual in the study received similar supplements for 6, 8, and 11 weeks. Lymphocyte proliferation was again decreased, remained decreased 4 weeks after discontinuation, and returned to the presupplement levels 12 weeks after discontinuation. Even if the study included only two subjects, the data are quite convincing regarding the inhibitory effect of GLA. It is possible that the increase in total fat intake of 10 g/day rather than GLA or presenting the results as stimulation indices has contributed to some of the effects observed in this study. Results from another study (Thies *et al.*, 2001c) are even more difficult to interpret. This was the only placebo-controlled study, and it reported a 65% reduction in lymphocyte proliferation with only a GLA supplement of 700 mg/day for 12 weeks. The supplement was a blend of 21:5:74 of palm oil, sunflower seed oil, and a GLA-rich triacylglycerol.

TABLE VII

EFFECTS OF γ-LINOLENIC ACID ON HUMAN IMMUNE FUNCTIONS

Function and effect	Subjects age and sex (n)	GLA (g/day)	Duration (wk)	Reference and study type
P, IFN-γ, TNF-α, NC; IL-4 and IL-10, 50% decrease wk 8 and 12; TGF 300% increase wk 12	Adult M and W (8)	3.3	4, 8, 12	Fisher and Harbige, 1997
P 80% decrease wk 11	24–40, M (2)	2.4	6, 8, 11	Rossetti *et al.*, 1997
Auto-induction of IL-1β 75% decrease wk 8; 17% after LPS stimulation	23–63, M and W (4–5)	1.8	4, 8	Furse *et al.*, 2002
P, NK activity, IL-1, 2, 10, IFN-γ, TNF-α, NC	Mean 45, M and W (8)	1.1	4, 8, 12	Yaqoob *et al.*, 2000, PC
P, 65% decrease wk 12; NK activity, IL-1, 6, 10, NC	55–75, M and W (8)	0.8	4, 8, 12	Thies *et al.*, 2001b, PC
P, 30–60% increase; IL-1β, IL-2, NC	>65, M and W(15)	0.7	8	Wu *et al.*, 1999, PC
IL-1β, IL-6, TNF-α, 20–25% decrease	Mean 20, M and W (10)	0.5	6	Watson *et al.*, 1993
IL-1β, TNF-α 40% decrease	29–46, W (4)	2.4	24 hr	DeLuca *et al.*, 1999

Note: Studies marked *PC* (placebo control) included a control group, and others did not. IL, interleukin; IFN, interferon; LPS, lipopolysaccharide; NC, not changed; P, proliferation; TNF, tumor necrosis factor; TGF, transforming growth factor.

Inhibition of lymphocyte proliferation was observed after PBMCs were stimulated with Con A in a medium supplemented with 10% autologous plasma. It is possible that GLA in this supplement was more readily available than that from natural oils, or some other component of the mixture contributed to the inhibition of lymphocyte proliferation. In another study (Fisher and Harbige, 1997), GLA was associated with a 30% decrease in PBMC proliferation, but the results did not attain statistical significance. Even if the inhibition of lymphocyte proliferation was significantly reduced, it could not definitely be attributed to GLA, because the study did not have a placebo group, although the response was tested before and after supplementation with 15 g/day borage oil. Another study that failed to detect inhibition of lymphocyte proliferation was placebo controlled and involved a supplement of 9 g/day of evening primrose oil (GLA 1.1 g/day) for 12 weeks (Yaqoob *et al.*, 2000). The most likely reason for the lack of an effect in this study was a high concomitant supplement with 205 mg/day of vitamin E. GLA supplementation did not alter NK cell activity (Thies *et al.*, 2001c; Yaqoob *et al.*, 2000) or the secretion of IFN and IL-2 (Fisher and Harbige, 1997; Thies *et al.*, 2001c; Yaqoob *et al.*, 2000), but it significantly reduced the secretion of IL-4 and IL-10 (Fisher and Harbige, 1997). GLA supplementation, however, caused a threefold increase in the secretion of TGF-β (Fisher and Harbige, 1997). Thus, the effects of GLA supplementation on lymphocyte functions have been quite variable. This may be due to the differences in experimental design, methods, amount, source, and duration of GLA supplementation, composition of the basal diets, and age and health status of the study subjects. Studies need to address these issues.

Six studies examined the effects of oral GLA supplementation on the *ex vivo* production of inflammatory cytokines by monocytes (Table VII); only two of these studies found a modest reduction in the secretion of IL-1, IL-6, or TNF (Furse *et al.*, 2001; Watson *et al.*, 1993), whereas the remaining four studies found no change in the secretion of these cytokines. One study that used both lipopolysaccharide (LPS) and IL-1α to stimulate the production of IL-1β found that GLA supplementation reduced the LPS-induced pro-IL-1β mRNA by only 17%, and it reduced the IL-1α–induced pro-IL-1β mRNA by 75% (Furse *et al.*, 2002). In this study, GLA supplementation also increased the secretion of IL-1 receptor antagonist. It is possible that some of these studies failed to detect the effect of GLA on the secretion of inflammatory cytokines simply because they did not use the appropriate stimulus. Overall, the effects of GLA on lymphocyte and monocyte functions appear much weaker compared with those of fish oils. Even if the effects of GLA are modest, it would be of great interest to determine whether GLA would enhance the antiinflammatory effects of EPA and DHA.

4. *Arachidonic acid and immune response*

There are only two published reports in which the effects of AA supplementation on human immune functions were examined. In one crossover study (Kelley *et al*., 1997), the diets of young healthy men were supplemented with 1.5 g/day of AA for 50 days. AA supplementation did not alter a number of indices of immune response tested, including DTH response, NK cell activity, mitogen-induced lymphocyte proliferation, or *in vitro* secretion of IL-1, IL-2, and TNF-α (Kelley *et al*., 1997, 1998a). However, it significantly increased (25–100%) the number of circulating neutrophils, the *ex vivo* production of PGE_2 and LTB_4 by the PBMC stimulated with LPS, or the secondary response to influenza vaccine. These results indicate that increased intake of AA may increase the inflammatory and allergic disorders. In the second study, AA was found to have no effect on NK cell activity when the diets of men and women older than 55 years were supplemented with 700 mg/day of AA for 12 weeks (Thies *et al*., 2001b). Moreover, it did not alter the number of circulating leukocytes, lymphocytes, and T or NK cells. Thus, the results from the two studies are consistent regarding the lack of an effect on NK cell activity, but the higher amounts used in one study (Kelley *et al*., 1998b) did increase the number of circulating granulocytes in young healthy men, which was not the case when one-half the amount was used in elderly subjects (Thies *et al*., 2001b). These results are generally consistent with those discussed for LA earlier, indicating that a modest increase in consumption of these FAs does not have adverse effects in human immune response, whereas higher concentrations may increase the inflammatory response.

D. EFFECTS OF MONOUNSATURATED, SATURATED, AND TRANS FATTY ACIDS ON IMMUNE AND INFLAMMATORY RESPONSES

There are only two studies in which the effects of diets rich in monounsaturated FAs (MUFAs) on human immune functions have been examined. In one study, we compared the effects of feeding diets containing safflower oils with either 75% oleic acid or LA to young healthy men for 80 days (Kelley *et al*., 1989). The daily intake of oleic acid in the low and high MUFA diets was 24 and 51 g, whereas that of LA was 36 and 15 g. Lymphocyte proliferation and serum concentrations of immunoglobulins and complement proteins did not differ between the two diets. In another study, the effects of refined olive oil on indices of immune response in healthy men were examined (Yaqoob *et al*., 1998). The MUFA content of the two diets was 18.4 and 11.3 en%, and the diets were fed for 8 weeks. Lymphocyte proliferation and NK cell activity did not differ between the subjects fed the high and low

MUFA diets. Results from these two studies indicate that moderate changes in the consumption of MUFAs do not have any adverse or beneficial effects on several indices of immune response as long as the total fat content of the diets are comparable. The effects may differ significantly if MUFA is being replaced by n-3 PUFA, but those should be attributed to the changes in n-3 PUFA and not MUFA.

There is only one published report that examined the effects of feeding diets enriched in saturated and trans FAs on human functions (Han *et al.*, 2002). Three diets containing 30 en% from different fat sources were fed to healthy adults for 32 days; two-thirds of the fat was supplied by either PUFAs from soybean oil, trans FAs from hydrogenated soybean oil, or saturated FAs from butter. Many indices including lymphocyte proliferation and secretion of IL-2 and PGE_2 did not differ between the three diets. Secretion of IL-6 and TNF-α did not differ between the saturated FA and the PUFA group, but these were increased by 36 and 58%, respectively, in the group fed the hydrogenated fat. Results of this study suggest an increased inflammatory response by trans FAs. This notion is supported by the results from an epidemiological study that examined the association between serum concentrations of trans FAs and markers for inflammation (Mozaffarian *et al.*, 2004). In this study, the concentration of soluble TNF receptors 1 and 2 were positively correlated with dietary concentrations of trans FAs. Concentrations of IL-6 and CRP were not associated with those of trans FAs if all subjects were included; however, positive associations were found in subjects with higher body mass index. Overall, these results are consistent with the hypothesis that trans FAs increase inflammatory response and the risk for inflammatory diseases. Further studies are needed to quantify the risk from trans FAs and to find ways to mitigate this risk.

E. MECHANISMS OF ACTION OF PUFA

While several years of research have led to the description of clear as well as ambiguous phenomenological observations with respect to effects of dietary fat on the human immune system, few if any studies have specifically focused on mechanisms. However, many animal model and cell culture studies have been done in an attempt to describe the possible mechanisms involved in FA effects on immune cells and the immune system. In addition, studies on other cell types can be used to infer possible mechanistic effects by dietary fat in animal models or humans. In the following sections, we discuss selected mechanisms of how FAs may alter immune cells and ultimately the immune system. The list is by no means exhaustive, but it is a focused approach.

1. *Structural membrane alterations: effects on lipid rafts*

Lipid rafts have gained considerable attention because of their importance as sites for signal transduction initiation in many cells including those of the immune system (Rao *et al*., 2004). Structurally, lipid rafts are patched domains within the lipid bilayer of the plasma membrane whose composition differs from the bulk for the usual bilayer (Pizzo and Viola, 2004). Operationally, they are described as detergent-resistant domains (DRMs) within the plasma membrane (Pizzo and Viola, 2004). The DRMs are rich in cholesterol and sphingolipids, which have longer and more saturated FA chains compared to the relatively more unsaturated FA chains of the typical membrane phospholipids (Pizzo and Viola, 2004). That means that sphingolipids and cholesterol are more attracted to each other and that changes in FA composition, such as from the diet, may alter DRM. Pizzo and Viola (2004) have discussed the involvement of lipid rafts in lymphocyte activation. At this time, it is suggested that during lymphocyte activation and chemotaxis, lipid rafts act as platforms to compartmentalize signaling and facilitate specific interactions (Pizzo and Viola, 2004). Two other studies (Diaz *et al*., 2002; Switzer *et al*., 2004) have shown that DHA can affect lymphocyte activation by alteration of lipid rafts. In one study, phospholipase D1 was excluded from lipid rafts after DHA treatment, thus, relocating it and allowing for its activation (Diaz *et al*., 2002). That phospholipase D activation might be responsible for the immunosuppressive effect of DHA because it is known to transmit antiproliferative signals in lymphoid cells (Diaz *et al*., 2002). In another study, n-3 PUFA enhanced the polarization and deletion of proinflammatory Th1 cells, possibly as a result of alterations in membrane microdomain (lipid raft) FA composition (Switzer *et al*., 2004).

2. *Alteration of lipid mediators*

The alteration of lipid mediators by PUFA has been reviewed in great detail (Stulnig, 2003) and is mentioned only briefly here. The function of cells of the immune system can be exquisitely sensitive to lipid mediators such as prostaglandins and leukotrienes. Those mediators are normally produced by the action of specific enzyme systems on the AA substrate that is released from membrane phospholipids pools. Thus, cyclooxygenase (COX-1 or COX-2) converts AA to prostaglandins, and lipoxygenase (e.g., 5-LOX) converts AA to HETEs and leukotrienes. The amount of substrate, the activity of the enzymes, and the amount and potency of the lipid mediators appear to be regulated by FA composition and, thus, the dietary intake of PUFA. Specifically, EPA is and DHA is not a substrate for COX and LOX and can competitively inhibit AA metabolism. In addition, DHA can inhibit

the release of AA and, thus, decrease the amount available for conversion to eicosanoids (Martin, 1998). There is also some evidence that EPA and DHA can alter PG and LT synthesis at the level of gene expression by regulating COX-2 and 5-LOX (Stulnig, 2003). Although these potent lipid mediators are continuously regarded as a link between intake of PUFA and subsequent immune system alterations, there is still debate over whether eicosanoid interference is a viable mechanism.

3. *Effects on gene expression*

Literature is replete with studies describing the role of peroxisome proliferator–activated receptor (PPAR) in metabolism and immune function. These are a family of nuclear receptors that can bind various lipophilic metabolites including FAs and their derivatives. After ligand binding, PPAR activity is involved in the regulation of many genes including those involved in adipogenesis and adipocyte function, as well as lymphocyte activation and the promotion of macrophage differentiation (Stulnig, 2003). Thus, FAs have a means of altering the expression of genes important for maintenance and modulation of the immune response. For example, agonists of the specific PPAR, PPARγ, can inhibit TNF-α, IL-6, and IL-1β expression in monocytes (Stulnig, 2003). Although PUFAs have been shown to alter PPAR activity or genes that are normally regulated by PPAR, very few studies can prove that it is a direct result of binding and activation of the nuclear receptor. Far more studies are required to elucidate how PUFAs effect PPAR signaling. Other nuclear receptors that PUFA may be involved in mediating include retinoid X receptors and liver X receptors.

4. *Effects on differentiation, proliferation, and apoptosis*

FA regulation of cellular differentiation and apoptosis has been reviewed (Rudolph *et al.*, 2001). In general, differentiation was promoted by PUFAs such as AA, EPA, and DHA and metabolites such as PGE_1, PGE_2, and LTD_4. The effect of PUFA on apoptosis was less clear-cut, with AA and GLA increasing apoptosis, whereas effects of EPA, DHA, and eicosanoids varied from stimulation to inhibition (Rudolph *et al.*, 2001).

With respect to proliferation, DHA may be responsible for arresting cells, making them susceptible to apoptosis. In one study, DHA induced cell cycle arrest in Jurkat leukemic cells, perhaps by inhibiting of pRb phosphorylation (Siddiqui *et al.*, 2003). In that study, DHA greatly reduced the level of cyclin A while increasing the level of p21 WAF1, a cellular inhibitor of cyclin A/cyclin-dependent kinase 2 (cdk2) activity. In another study with HT-29 tumor cells, DHA inhibited the phosphorylation of pRb and DNA-binding

activity of E2F-1 and prevented entry of cells to S phase, and the antioxidant butylated hydroxytoluene was able to reverse the inhibition of activation of cyclin A/cdk by DHA in a dose-dependent manner, suggesting that endogenous oxidative stress produced by lipid peroxidation in HT-29 cells may be involved in the control of cell cycle progression (Chen and Istfan, 2001). One or more of these proposed mechanisms or additional ones not outlined here may account for how FAs alter human immune response. Future studies need to be designed that elucidate a series of molecular events, which will define how FAs function in alteration of immunity.

ACKNOWLEDGMENTS

Supported by U.S. Department of Agriculture (D. S. K.) and a grant from America's Beef Producers (N. E. H. and K. L. E.).

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