

MINIREVIEWS

Biological and Clinical Significance of Lipids as Modulators of Immune System Functions

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It is widely recognized that malnutrition is the commonest cause of immunodeficiency worldwide (14). In fact, a recent investigation has determined that food intake increases the levels of gamma interferon (IFN- γ) but not interleukin-4 (IL-4) production, whereas starvation enhances the IL-4 response but not IFN- γ levels of T lymphocytes (76). Therefore, the interactions between certain nutrients and immunity exert a crucial role that should be analyzed from a biological and a clinical point of view. In recent years, a large number of studies have been conducted to investigate the relevance of certain fatty acids in the alteration of immune system functions in both humans and animals. At the beginning, the epidemiological findings contributed to demonstrating that certain fatty acids supplied in their diets (particularly long-chain *n*-3 polyunsaturated fatty acids contained in fish oil) affect the immune responses of Greenland Eskimos, as indicated by the low prevalence of inflammatory disorders in this population (11, 36, 47). Subsequently, on the basis of these epidemiological studies, experimental investigations determined the main processes by which several polyunsaturated or monounsaturated fatty acids are capable of altering the immune system, as well as the mechanisms of action involved in the regulation of immune functions.

At least four modes of action have been proposed to explain the potential action of fatty acids on the modulation of immune system in both animals and humans. Accordingly, immune system modulation by dietary lipids may be attributed to changes in the composition of membrane phospholipids, lipid peroxidation, alteration of gene expression, or eicosanoid production. In addition, recent *in vitro* and *ex vivo* studies have demonstrated the involvement of several fatty acids (such as eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA], arachidonic acid [AA], or palmitic acid [PA]) in the induction or inhibition of programmed cell death or apoptosis (21, 22). The participation of different fatty acids in apoptosis opens new approaches for the study of the properties that different fatty acids exert as modulators of immune system functions.

In this review, we summarize the effects of dietary lipids on

the immune functions and the mechanisms responsible for these alterations as well as the crucial aspects that relate fatty acids to the triggering of apoptotic mechanisms. Similarly, we analyze the clinical consequences derived from dietary lipid administration, the effects of these substances on tumor promotion, and finally, the adverse effects that several dietary lipids may exhibit as a direct consequence of an immunosuppressive process (Fig. 1).

TYPES OF FATTY ACIDS THAT ALTER IMMUNE SYSTEM FUNCTIONS

Overall, the integrated action of several polyunsaturated or monounsaturated fatty acids on the immune system includes the alteration of different immune functions in both humans and animals. Therefore, lymphocyte proliferation (30, 53, 81), production of cytokines (30, 79), modification of cell surface molecules (38), changes in phagocytic activity (12, 23), and alteration of natural killer (NK) cell activity (24, 43, 80) are susceptible to be modulated by dietary lipid administration or by culture of cells in the presence of several free fatty acids. However, it is important to note that not all of the fatty acids have the same immunomodulatory properties. Thus, long-chain *n*-3 polyunsaturated fatty acids, such as EPA (20:5*n*-3) or DHA (22:6*n*-3) (which belong to *n*-3 fatty acid series derived from fish oil) are particularly known to exert a profound influence on the immune system, reducing many of these functions previously described for both humans and animals (30, 53, 81). Other fatty acids belonging to *n*-6 fatty acid series such as AA (20:4*n*-6) have also been reported to affect the immune system; although AA has not been shown to produce an immunosuppression as relevant as long-chain *n*-3 fatty acids, it is also considered an immunosuppressive agent. Nevertheless, both *n*-6 polyunsaturated fatty acids and saturated fatty acids have been associated to a chemopromotive role in cancer, probably due in part to a suppression of apoptosis (64). However, oleic acid (a monounsaturated fatty acid [18:1*n*-9] that is the main component of olive oil belonging to *n*-9 fatty acid series) is also capable of acting as a modulator of immune functions (23, 24, 40, 83). Accordingly, olive oil (a traditional and essential component of the Mediterranean diet) also plays an important role in the modulation of immune functions, and therefore it has been efficiently applied in the alleviation of the symptomatology caused by rheumatoid arthritis (44, 48).

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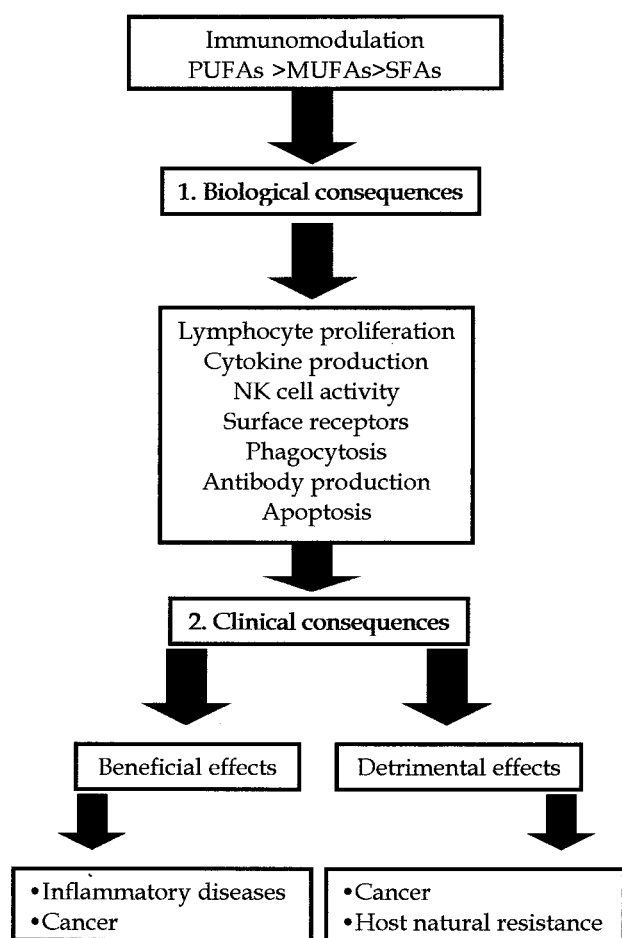


FIG. 1. Schematic representation of potential role of dietary lipids and biological and clinical consequences of the administration of several dietary lipids. PUFAs, polyunsaturated fatty acids; MUFAs, mono-unsaturated fatty acids; SFAs, saturated fatty acids.

BIOLOGICAL CONSEQUENCES ON IMMUNE FUNCTIONS ATTRIBUTED TO FATTY ACID ADMINISTRATION

Differential properties of fatty acids and their role in the modulation of immune system functions. The changes attributed to fatty acid administration include the reduction of lymphocyte proliferation, which is modified by polyunsaturated fatty acids (*n*-3 or *n*-6) or monounsaturated fatty acids (*n*-9). Studies carried out in both humans and animals have revealed that the administration of high levels of dietary *n*-3 polyunsaturated fatty acids or the inclusion in parenteral regimens of lipid emulsions rich in *n*-3 or *n*-9 fatty acids are related to the reduction of lymphocyte proliferation during the supplementation (30, 35, 53, 56, 81). Thus, concanavalin-A or lipopolysaccharide-stimulated lymphocytes have reduced the cellular proliferation in assays carried out *in vitro* or *ex vivo* in the presence of free fatty acids or in cell cultures from both animals and humans fed dietary lipids, respectively (10, 30). Cytokine production is reduced by the action of *n*-3 polyunsaturated fatty acids or *n*-9 monounsaturated fatty acids (79), cytokine receptor expression is also affected (56), natural killer

(NK) activity is significantly suppressed (24, 43, 80), phagocytic activity of macrophages is modified (12, 23), and the antigen-presenting function of human monocytes is inhibited (7, 20). Based on these experimental observations, we can affirm that *n*-3 polyunsaturated fatty acids rather than *n*-6 polyunsaturated or *n*-9 monounsaturated fatty acids are directly associated to the alteration of immune and inflammatory response. This modulation depends on different factors, such as the nature of fatty acids added to diets, the concentration of fatty acids, the duration of supplementation with dietary lipids, or differences among animal species fed dietary lipids (5).

Mechanisms of action. As mentioned previously, for several years numerous studies have attempted to elucidate the mechanisms by which some dietary lipids produce a potential effect on immune system functions (52). Several hypotheses have been suggested as possible mechanisms. Experimental investigations have confirmed that several fatty acids exert changes in the phospholipids of plasma membrane which affect the membrane fluidity (81); they also alter eicosanoid production (34), produce lipid peroxides (1), or regulate the transcription factors (42). Overall, long-chain *n*-3 polyunsaturated fatty acids, such as EPA, are incorporated into cell membranes, replacing AA (the most important of the eicosanoid precursors). This process reduces the production of a biological mediator as prostaglandin E₂ responsible for inhibiting IL-1 and tumor necrosis factor (TNF) production (30). Similarly, EPA inhibits gene transcription because it reduces the translocation of NF-κB (50). In addition to these important considerations, a crucial role of lymphocyte subsets has been suggested to explain the effects of different dietary polyunsaturated fatty acids on the immune system, because they alter the number of lymphocyte subsets as well as the proliferation of these cells (41, 78). Thus, antigen presentation and the proportion of T-cell subsets are modified after dietary lipid manipulation. In fact, recent studies have determined that polyunsaturated fatty acids inhibit the surface expression of major histocompatibility complex (MHC) class II molecules, as well as some adhesion molecules on human monocytes (38, 39). Similarly, consumption of a monounsaturated fatty acid-rich diet by humans is also involved in the reduction of adhesion molecules from peripheral blood mononuclear cells (83). Overall, these actions may explain the different expression of CD4 or CD8 on the T-cell surfaces of peripheral blood mononuclear cells of mice that were fed diets supplemented with DHA (69). The biological consequences of these changes are still unclear. It is probable that the alteration experimented by the lymphocyte population enhances the host susceptibility against opportunistic infections. In other words, the modulation of lymphocyte subsets may lead in part to a modification of cytokine production, NK cell activity, antibody production, etc. Accordingly, Th1-type cytokines such as IL-2 are more susceptible to polyunsaturated fatty acid effects than are Th2-type cytokines, such as IL-4, which indicates that these fatty acids are capable of altering the balance of Th1- and Th2-type cytokines in mice (77). As a direct consequence of a reduction of IL-2 production, Th1 cell activation is suppressed, whereas an increase of Th2 cell activation due to an enhancement of IL-4 production suppresses Th1 cell proliferation in animals fed a diet containing fish oil (3). Therefore, the anti-inflammatory effects derived from long-chain *n*-3 polyunsaturated fatty acid administration

may be the result of these two interconnected mechanisms. Hence, it is obvious that these alterations may affect significantly the interaction between host natural resistance and infectious agents due to the crucial importance of Th1-mediated response in the infectious processes promoted by viruses or bacteria (54).

Although results from epidemiological, experimental, or clinical studies have demonstrated an important association between *n*-3 polyunsaturated fatty acids and immunosuppressive properties in both humans and animals, their effects are complex and still not clearly known. In fact, some discrepant results have suggested that *n*-3 polyunsaturated fatty acids have stimulatory effects on TNF and IL-1 production (57).

Evidence for the involvement of fatty acids in apoptosis modulation. Programmed cell death or apoptosis has been defined as an essential mechanism responsible for the regulation of homeostasis, tissue development, or immune functions. Thus, damaged, aberrant, or unnecessary cells must be eliminated to ensure the correct development of multicellular organisms. Irrespective of its crucial role in normal cell control, apoptosis regulates pathological processes including human clinical disorders such as cancer, autoimmune diseases, viral or bacterial infections, and neurodegenerative disorders. Several biological factors (many viruses, bacteria, or parasites), chemical factors (glucocorticoids, ceramides, etc.), and physical factors (irradiation) are responsible for apoptosis induction (60). Moreover, different studies with both animals and humans, involving *in vitro* assays or administration of dietary lipids, have recently described an important role of several fatty acids in the induction or inhibition of apoptosis. As a result, different mechanisms of action have been proposed in order to explain the action of fatty acids on apoptosis modulation. Thus, polyunsaturated fatty acids, such as EPA and DHA, saturated fatty acids, such as PA, or fats such as fish oil administered in the diets have been defined as substances capable of inducing cell death via a mitochondrial process (27) or by downregulation of Bcl-2 (67). Different mechanisms have been proposed in order to explain the induction of apoptosis by certain fatty acids. Thus, PA (a saturated fatty acid) added to cellular cultures in *in vitro* assays induces apoptosis via a direct effect on mitochondria, because it causes a dissipation of the mitochondrial transmembrane potential ($\Delta\Psi_m$), an event that precedes nuclear apoptosis (27). Another possible partial explanation for the modulation of apoptosis by long-chain *n*-3 polyunsaturated fatty acids is that it is caused by direct action of these substances on the cells and by activation of the caspase cascade through cytochrome *c* release coupled with a modulation of mitochondrial membrane depolarization (2). Recent studies have also determined the crucial importance of dietary fatty acids in the reduction of Bcl-2 expression as well as an increase of Fas ligand (Fas-L) expression. Therefore, when the concentration of polyunsaturated fatty acids augments, Bcl-2 expression is reduced and cell death occurs (67, 75). In other words, long-chain fatty acids contained in fish oil trigger apoptosis by suppressing Bcl-2 expression and increasing Fas-L expression. On the other hand, it has been recently determined that Ras membrane localization (a protein that plays a critical role in the cell growth and apoptosis) is reduced after administration of fish oil, which indicates that dietary fish oil may play a protective role against colon cancer development (19). Finally,

it is important to note that lipid peroxidation is of crucial importance in apoptosis induction. This argument suggests that the effects of polyunsaturated fatty acids can be directly associated to specific alterations on the expression of Bcl-2, Fas, and Ras (21).

CLINICAL CONSEQUENCES OF FATTY ACID ADMINISTRATION

Early studies revealed the importance of *n*-3 polyunsaturated fatty acids as possible therapeutic substances capable of reducing the incidence of inflammatory disorders in humans (11, 47). Autoimmune diseases such as rheumatoid arthritis, psoriasis, or systemic lupus erythematosus are characterized by the production of an inflammatory response due to a marked increase of proinflammatory cytokines. In recent years, numerous epidemiological studies have described widely the reduction in the incidence of rheumatoid arthritis in populations who consume fish oil or olive oil in their diets, which are related to the prevention of inflammatory disorder incidence, although these events occur after a prolonged ingestion of these fats (38, 44–46, 48, 70). Nevertheless, in spite of the importance of these substances as biological mediators in the inflammation and their application in the resolution of inflammatory diseases, in the present review we will summarize other important features of fatty acids from a clinical point of view, focusing on the potential effect of fatty acids in cancer incidence and the involvement of these substances in the alteration of host natural resistance against infectious agents.

Crucial role of fatty acids in the incidence of cancer. Many lines of evidence have indicated the essential role of fish oil in the incidence of cancer (13). Numerous reports have suggested that high intakes of long-chain *n*-3 fatty acids or *n*-9 fatty acids (the most important fatty acid contained in olive oil) may reduce the risk of breast cancer (51, 72) in both humans and animals. Thus, *n*-3 polyunsaturated fatty acids may inhibit colon cancer in rats (65) as well as reduce the risk of colorectal cancer development (13). In contrast, *n*-6 fatty acids or saturated fatty acids may be involved in the increase of mammary or colon tumorigenesis by altering membrane phospholipid turnover. Accordingly, AA is released from plasma membrane that produces an alteration in the synthesis of prostaglandins via cyclooxygenase enzyme (64).

Epidemiological studies have determined the protective role of *n*-3 polyunsaturated fatty acids against colon cancer in populations of Alaskan and Greenland Eskimos (7, 28). Subsequently, experimental studies demonstrated that a fish oil diet appears to exert a protective effect against experimentally induced colon cancer (66). Similarly, a recent study has determined that olive oil also exerts a protective role against the development of colorectal cancer (71). Although the mechanisms that contribute to this protective effect have not been elucidated, a recent investigation has reported that fish oil diets are able to increase apoptosis and cell differentiation in induced rat colon tumorigenesis (17). Based on current knowledge, an interesting study has recently determined that the Western diet (which consists predominantly of a mixture of saturated, monounsaturated, and polyunsaturated fats) produces dysplastic lesions in the colon, which is indicative of a tumorigenic process. As a result, the administration of a diet

containing mixed lipids and high levels of saturated fatty acids (similar to the lipids contained in the Western diet) induces colon carcinogenesis in mice by an unknown mechanism (68). Nevertheless, the most interesting results from this investigation reveal that an alteration of cyclooxygenase activity as well as apoptosis suppression appears to play a crucial role in the colon tumorigenesis induced by this diet in animal models (64). In addition, our experimental observations have indicated that the survival of mice fed a diet containing fish oil and transplanted with a T-lymphoma cell line is reduced in comparison to that of mice fed a diet containing low fat. We speculate that this event may be attributed to the impairment in the function of T cells or NK cells due to polyunsaturated fatty acid administration, which reduces the cytotoxic activity of NK cells (61). Recently, an interesting investigation has shown that *n*-3 fatty acids alter cell transformation in a mouse epidermal cell line because DHA or EPA inhibited transcription activator protein AP-1 whereas AA did not produce any effect on AP-1 activity. Therefore, this finding confirms previous reports of the chemoprotective role of diets containing *n*-3 fatty acids (49). In contrast, the most significant relationship between fatty acids and reduction of cancer incidence is found in the Mediterranean diet, which could decrease the development of cancer by up to 10 to 15% in the populations of highly developed Western countries (73).

In spite of the wide information currently available, the potential role of dietary lipids in cancer etiology remains controversial. In fact, although most of the studies have shown that diets containing *n*-3 polyunsaturated fatty acids might be beneficial during inflammatory conditions, their effects are not elucidated because they promote an impairment of the host defense to infection. Therefore, future studies are needed in order to confirm or refute the different hypotheses and arguments suggested hitherto.

Relevance of dietary lipids in the reduction of host natural resistance and susceptibility to infection. As mentioned above, nutrient intake is considered a critical determinant of immunocompetence. Many investigations have reported the modulatory functions exerted by fatty acids and the clinical benefits of dietary lipid supplementation with fish oil or olive oil in both humans and animals. As a consequence, this dietary supplementation has been applied in the treatment of patients suffering from inflammatory diseases, because unsaturated fatty acids (mainly *n*-3 or *n*-9 fatty acids) reduce the levels of many biological mediators associated to the promotion of the inflammatory events that participate in an inappropriate immune response. However, the reported reduction of immune response caused by the administration in the diet of some fatty acids may exert a detrimental effect, and therefore, they may impair the cellular immunity to pathogenic agents (26).

For obvious reasons, the altered resistance to bacterial infection has been analyzed in animal models in which the administration of diets containing fish oil reduces the clearance of bacteria from liver or spleen and significantly reduces the survival during the course of *Listeria monocytogenes* infection. As a result, the elimination of pathogenic agents (bacteria, viruses, or parasites) is more difficult. Different reports have described the clinical consequences of dietary supplementation rich in *n*-3 fatty acids that suppress the immune system functions. Thus, a significant reduction of survival percentage after

feeding experimental mice with a diet containing fish oil has been determined (25, 31) whereas an increase of survival percentage was observed after challenge with *L. monocytogenes* in animals fed diets containing either lard oil, hydrogenated coconut oil, or palm oil (15, 25, 31). These mice were inoculated with a lethal dose of a virulent *L. monocytogenes* strain, a facultative intracellularly growing bacterium that has been used as a model of infectious and pathogenic processes. After the administration of this diet different results were observed. Bacterial clearance from liver or spleen was increased in these animals (25, 31), bactericidal activity of peritoneal cells was significantly reduced (62), and cytotoxic effects due to bacterial infection were increased (63), whereas the susceptibility of cells to adhesion or invasion by *L. monocytogenes* infection was substantially modified (63). These observations indicate the loss of capacity of the immune system from animals fed a diet containing fish oil to destroy and eliminate the infectious agents (25, 31, 62, 63). A recent investigation has contributed to explaining in part the reasons for which *n*-3 polyunsaturated fatty acids reduce host defense against *L. monocytogenes*: consumption of EPA or DHA (both contained in fish oil) impairs the production of IL-12 and IFN- γ , cytokines that play an essential role in the innate and adaptive responses of host immune system (32). Hence, the reduction of IL-12 levels may explain the impaired bacterial clearance and the reduction of survival to *L. monocytogenes* infection (33). Another possible explanation for the reduction of host resistance is based on the inhibition of the expression of MHC class II (called Ia in mice) that is reduced in mice fed a fish oil diet and infected with *L. monocytogenes* (37, 55). Similarly, it is important to note that these effects may be associated with the reported increase of reactive oxygen species, whose numbers were significantly increased in mice after the animals were experimentally infected with this bacterium and fed a diet containing hydrogenated coconut oil (63). In fact, the role of cellular oxidative processes requires considerable attention because they play an important role in the elimination of infectious agents.

However, not all polyunsaturated fatty acids are capable of exerting the same effects, since many studies are not in accordance with the previously described arguments. Thus, a recent investigation has determined that conjugated linoleic acid does not alter the resistance of mice after *L. monocytogenes* infection (74). Similarly, a previous study indicated the irrelevant effect of dietary lipid manipulation on the survival of mice in two models of peritonitis, one with *Pseudomonas aeruginosa* and the other with *Salmonella enterica* serovar Typhimurium (18).

Irrespective of these findings, other experimental studies have demonstrated the adverse effects of dietary lipids containing *n*-3 or *n*-6 polyunsaturated fatty acids in the impairment of immune functions in animals experimentally infected with different pathogenic agents such as *S. enterica* serovar Typhimurium (16, 29), *P. aeruginosa* (59), *Staphylococcus aureus* (20), or *Mycobacterium tuberculosis* (58). By contrast, it has been reported that the administration of diets containing fish oil does not reduce the survival percentage after experimental infection with *Klebsiella pneumoniae* (4). In fact, dietary fish oil supplementation increased resistance to infection due to an enhancement of IL-1 and TNF production by peritoneal cells in mice (5, 6). It is probable that the discrepancy in these

findings is due to different factors that include the duration of the supplementation period, the concentration of fatty acids used, or the cell population affected by fatty acid administration.

Finally, experimental observations with viruses have also demonstrated that influenza virus infection in animals fed fish oil diet delays the clearance due to an impairment of primary virus-specific T-cell cytotoxicity but has no effect on NK cytotoxicity (8, 9).

CONCLUDING REMARKS

Nutritional status is generally recognized as an essential factor involved in the modulation of immune response, which may be determinant in the development of the clinical effects derived from a malnutrition process (14). Thus, in recent years the effects that different dietary lipids exert upon immune functions have received considerable attention, because different functions of immune system are altered after dietary lipid administration. Hence, lymphocyte proliferation, cytokine production, phagocytic activity, adhesion molecule expression, and NK cell activity are susceptible to modification by the action of certain lipids in both animals and humans. Different mechanisms of action have been proposed to be involved in these processes, such as lipid peroxidation, changes in the plasma membrane, eicosanoid production, or alteration of gene expression. Both epidemiological and experimental studies have applied the beneficial properties associated to a modulation of the immune functions. Thus, different fats such as fish oil or olive oil have been applied in the amelioration of symptoms related to rheumatoid arthritis and other inflammatory disorders due to their anti-inflammatory properties. Similarly, they have also been applied as biological mediators capable of exerting a protective effect against colon or breast cancer, whereas other fatty acids such as *n*-6 polyunsaturated fatty acids exert a chemopromotive role in tumorigenic processes. In addition, these effects may be related to the ability of several fatty acids to modulate apoptosis by different pathways. Nevertheless, the immunosuppressant role associated with dietary polyunsaturated fatty acids containing fish oil may produce adverse effects because they reduce the host's natural resistance against pathogenic agents. Therefore, in the light of current experimental observations, the benefits of administering these dietary lipids should be weighed before they are applied as immunosuppression factors in order to prevent detrimental or adverse effects caused by an excessive suppression of immune functions.

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