Review

Interactions of lipids with immune function II: Experimental and clinical studies of lipids and immunity

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Dietary lipids, through the degrees and patterns of fatty acyl unsaturation, have the ability to suppress or enhance the immune response. The basic biochemical mechanisms by which these lipids alter the structure and function of plasma membranes have previously been reviewed.[1] In this second review, the diverse effects of lipids in experimental animal models and clinical human studies will be discussed. The immune modulating effects of lipids can be understood only if all the known effects of lipids on cellular function are taken into account, including (1) changes in membranes fluidity, (2) production of lipid peroxides, (3) regulation of eicosanoid metabolism, and (4) direct interactions with cellular activation. Explanations of the observed effects of lipids based on only one of these mechanisms, such as changes in eicosanoid production, are simplistic and do not explain the often contradictory findings noted. (J. Nutr. Biochem. 5:514–521, 1994.)

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Review of effects on immune function

Cytokines

Cytokines are soluble polypeptide or glycoprotein mediators produced by cells during activation of the immune system.[2] Because constitutive production is usually absent or at very low levels, and the production and secretion of most cytokines is of limited duration, it is likely that cytokine activity is exerted over a very short distance, either on the cell of origin (autocrine) or on neighboring target cells (paracrine). All cytokines exert their action by binding to specific surface receptors and initiating second messenger systems. These second messenger systems result in alterations in gene expression, leading to changes in cellular activity such as proliferation, differentiation, or function. In addition, cytokines may influence both the production and action of other cytokines.

Tumor necrosis factor (TNF) is the first cytokine released in response to bacterial endotoxin.[3] Monocytes and macrophages are the primary sources of TNF production, although several other cells, including lymphocytes and mast cells, are also capable of producing TNF. At low levels, TNF serves several functions: it activates neutrophils and monocytes to initiate microbial killing; it stimulates T- and B-cell function and up-regulates expression of major histocompatibility molecules; it stimulates the production of other pro-inflammatory cytokines such as IL-1 and IL-6; and it mediates the systemic effects of inflammation, including fever and hepatic synthesis of acute phase proteins. Although production of small amounts of TNF are beneficial in the response to infection, overproduction can be dangerous. Higher quantities of TNF unfortunately lead to adverse systemic responses that lead to the clinical syndrome known as septic shock. Thus, down-regulation may be beneficial to survival.

IL-1 is most likely the second cytokine released in response to inflammatory stimuli and shares many of the pro-inflammatory effects of TNF.[4] Similarly, IL-1 release occurs with endotoxin stimulation of monocytes and macrophages, but it is also released from a variety of other cell types (such as epithelial and endothelial cells) due to a variety of other stimuli. For example, IL-1 stimulates proliferation of helper T cells and B cells and also stimulates the release of other cytokines, including IL-2 and IL-6. Like TNF, IL-1 produces fevers and stimulates the acute phase response.

IL-6 is also produced by monocytes and macrophages in response to IL-1 and TNF. IL-6 has a broad spectrum of activities that overlaps those of TNF and IL-1; in addition, it stimulates B cells to differentiate and secrete immunoglobulins. Unlike TNF and IL-1, however, IL-6 is consistently found in the serum of patients suffering from severe stress, such as trauma, burns, or sepsis, and correlates moderately well with outcome in these patients.[5,6]

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Many studies have shown that polyunsaturated fatty acids (PUFA) depress cytokine production. For example, Santoli and Zurier found that lymphocytes cultured with arachidonic (AA), di-gamma linolenic, and eicosapentaenoic (EPA) acids suppressed IL-2 synthesis.[7] Venkatraman and Fernandes showed that the suppression of IL-2 production can be associated with an increase in malondialdehyde (MDA) because arachidonic acid both suppresses IL-2 production and increases MDA in EL-4 cells.[8] Similarly, Billiar et al. found that both fish oil and safflower oil fed to rats suppressed Kupffer cell (hepatic macrophage) production of IL-1 and TNF.[9] In contrast, Ertel et al. showed that fish oil restored the release of IL-1 and IL-2 in hemorrhagic-shocked mice.[10]

The effect of lipids on cytokine production, however, varies from species to species. Although fish oil given to laboratory animals increases TNF production by lipopolysaccharide (LPS)-stimulated resident peritoneal macrophages due to the suppression of PGE_2 synthesis,[11,12] fish oil supplements in healthy men and women suppress peripheral blood mononuclear cell production of not only TNF, but also of IL-1, IL-2, and IL-6.[13,14] Interestingly, this suppression of cytokine production by fish oil supplements persists up to 10 weeks after the supplements have been discontinued.[13]

In some cases indomethacin reverses the effects of fatty acids on cytokine production,[11,15] but variations in prostaglandin production are not the only factors responsible for the modulation of cytokine production by fatty acids. For example, Karmiol and associates found that IL-6 production by human lung fibroblasts cultured with oleic, linoleic, or linolenic acids is stimulated primarily by linoleic acid, to a lesser extent by linolenic, and then by oleic acids.[15] Furthermore, while indomethacin suppresses IL-6 production by all fatty acids, the pattern remains the same, suggesting that the suppression of cyclooxygenase products such as PGE_2 is not only factor responsible for IL-6 production in the cells. Santoli and Zurier found that the ability of fatty acids to suppress IL-2 production in cultured lymphocytes is independent of prostaglandin synthesis.[7] Similarly, Hardardottir and Kinsella found that although indomethacin increased and exogenous PGE_2 decreased TNF production from peritoneal macrophages in mice fed sardine oil, those mice produced more TNF than mice fed safflower oil, again suggesting that the modulation of TNF by lipids is multifactorial.[11]

Thus, a consistent pattern of the effects of lipids on cytokine production is difficult to describe. Both inhibition and stimulation of cytokine production have been noted. The mechanism responsible for these effects is clearly not simply due to changes in eicosanoid production, because often the degree of unsaturation of the fatty acid treatments determines the magnitude of the change in cytokine production. Finally, the changes in cytokine production due to lipids may depend on the disease process, because hemorrhagic shock appears to change the response to fish oil feedings.[10]

Phagocytes

Phagocytosis, the engulfment of microbes, is one of a number of nonspecific antimicrobial systems that constitute innate immunity. Accumulation of phagocytes at sites of acute inflammation is generally not affected by lipids.[16–18] although ω-3 PUFA increase both the influx of polymorphonuclear cells (PMNs) in response to zymosan[19] and the number of PMNs and mononuclear phagocytes in chronic inflammation.[16] Once phagocytes arrive at the site of inflammation, however, their function may be altered by lipids. For example, phagocytosis is suppressed by saturated fats,[20,21] although the type of PUFA to which phagocytes are exposed, whether ω-6 or ω-3 PUFA, has no effect on phagocytosis.[17,22]

The effect of dietary lipids on bactericidal killing by phagocytes is minimal. We found that PMNs harvested from burned rats fed a variety of natural triglycerides showed no differences in bactericidal index,[23] and Blonk et al. have shown that healthy human males supplemented with EPA/DHA capsules for 12 weeks have PMNs that kill *S. aureus* at a normal rate ex vivo.[24]

Nonetheless, lipids effect distinct changes in the oxygen-dependent intracellular killing mechanisms, which include the generation of oxygen free radicals. Unsaturated fatty acids in culture media suppress the FMLP-induced degranulation and respiratory burst of phagocytes.[25–27] On the other hand, unsaturated fatty acids increase hydrogen peroxide production and enhance superoxide release.[17,18,28] Although suppression of the respiratory burst is associated with increases in intracellular cAMP levels,[26] indomethacin has no effect on alterations of chemiluminescence of peritoneal macrophages incubated with either AA or EPA,[27] suggesting that these changes are not related to alterations in eicosanoid synthesis. In fact, Costa Rosa et al. found that mononuclear intracellular concentrations of hexokinase, glutathione peroxidase, and citrate synthetase are modified by dietary PUFA, suggesting regulation of protein synthesis.[18] Phagocytes from injured patients may not respond to dietary lipid manipulation; as Gadd and Hansbrough have shown, the PMN oxidative burst, suppressed by burn injury, is not ameliorated by the addition of fish oil to the diet of mice.[29]

Thus, it appears that some functions of phagocytes are clearly affected by lipids. For example, phagocytosis is suppressed by saturated fats, and there are changes in intracellular killing mechanisms with unsaturated fatty acids. Again, because blockade of cyclooxygenase has no effect on fatty acid-induced changes in chemiluminescence, and because these changes are seen either with fatty acids of ω-6 or ω-3 families, it does not appear that the effects are mediated primarily through modulation of eicosanoid synthesis.

T-lymphocytes

Cell-mediated immunity, effected by T-lymphocytes, begins with proliferation of lymphocyte populations following appropriate ligand binding to cell surface receptors. Lymphocyte proliferation (or blastogenesis) can be induced experimentally by mitogens, such as plant lectins, or in culture with allogeneic lymphocytes in the mixed lymphocyte response. The ability of lymphocytes to respond to stimuli such as mitogens has been used as a measurement of lymphocyte responsiveness and activation. Lipids, introduced exogenously both through the diet and as free fatty acids bound to carriers in cell culture media, affect lymphocyte proliferation.

Diets high in fat suppress blastogenesis.[21,30–34] In fact, growing rats fed diets with 15% (wt/wt) fat, compared with 3% in controls, develop thymic involution after 3 months.[35]

The suppressive effects of high fat diets appear consistent in all studies when T cells are used as the responding population but become inconsistent when B cells are studied.[21,32] The degree of unsaturation of the dietary fatty acids appears to be a less potent regulator of lymphocyte proliferation than high amounts of dietary fat because safflower and corn oil have the same effect as lard and coconut oil when diets with 20 en% are fed to mice.[21,33] Splenic lymphocytes from septic animals do not, however, share this sensitivity to high fat diets.[36]

When high amounts of dietary fat are not the major determinant of lymphocyte responsiveness, the degree of unsaturation (specifically the PUFA family) can be a powerful modulator of blastogenesis. Diets that are high in ω-6 PUFA, such as safflower or corn oils, lead to suppression of lymphocyte blastogenesis and are associated with increases in PGE_2 production.[31–33,37–39] In contrast, diets enriched with ω-3 PUFA enhance lymphocyte proliferation and are associated with a decrease in PGE_2 production.[40–42] Fish oil, however, does not enhance lymphocyte blastogenesis in burned mice.[29,43] In addition to suppressing PGE_2 release, ω-3 PUFA may alter PG receptor expression or intracellular cAMP to cGMP ratios; it has been observed that splenocytes from mice fed ω-3–enriched diets are less responsive to PGE-induced changes in proliferation.[44]

Delayed-type hypersensitivity (DTH) reactions result from interaction of T lymphocytes with antigens, peaking at 2 to 3 days. Antigen is encountered and initially processed by macrophages, which are activated to release cytokines such as IL-1 and TNF. The macrophages present the processed antigen to T lymphocytes, which then produce other cytokines such as IL-2 and interferon. DTH reactions are probably important for host defense against intracellular pathogens such as *Mycobacteria, Listeria,* and *Salmonella* species of bacteria, in addition to viruses. Although a few studies have shown that the type or amount of dietary fat does not affect DTH responses in experimental animals,[21,40,45] the majority of studies have shown that lipids can significantly suppress the DTH response.

Dietary PUFA suppress the DTH response, whether they are from the ω-6[46,47] or the ω-3 family.[16,42] Some studies, however, have noted that fish oil diets increase the DTH response in septic or burned guinea pigs.[36,48] Because the former studies were conducted with animals that were not stressed, these findings suggest that the stress of infection or injury may permit the eicosanoid-modulating properties of fish oil to improve the DTH response.

B-lymphocytes

B lymphocytes produce antibodies, which are glycoproteins that bind to antigens and are the primary component of humoral immunity. Blastogenesis of B cells can be increased by feeding rats diets that include soybean oil.[35] Production of antibodies by B cells, however, may not be affected by dietary lipids.[16,43,49] The inconsistent results from studies that have shown that dietary lipids do affect antibody production do not allow formulation of any general conclusion. For example, corn oil diets, which increase PGE_2 production, suppress antibody production,[31,33,37,46] while diets deficient in essential fatty acids and fish oil (which decrease PGE_2 production) depress antibody production.[41,49] Further confusion results from observations that antibody production can be increased by corn oil,[21] linseed oil,[40] and coconut oil,[33] all of which vary strikingly in their polyunsaturated to saturated ratio and in the their families of unsaturated fatty acids. It is tempting to view these discrepancies as due to differences in animal models or in the assays used to measure antibody production, but the fact remains that the mechanism by which dietary lipids modulate B cell populations and antibody production is unknown.

Although some specific mechanisms remain unclear, lipids have a profound effect on lymphocyte function. For the most part, diets high in fat suppress lymphocytes, as do diets high in ω-3 PUFA. In addition, ω-3 fatty acids appear to enhance lymphocyte activity, as measured either by blastogenesis or in the DTH response.

Cytotoxicity

Another major effector of the immune system is the lysis or killing of one cell type by another, including macrophages, natural killer (NK) cells, and cytotoxic T lymphocytes (CTLs). These cells are involved in protecting the body against viruses and intracellular bacteria; surveying the body for tumor cells; mediating the rejection of allograft tissues after transplantation; and initiating the chronic self-destruction of autoimmune diseases. These killing mechanisms often involve macrophages acting as antigen-presenting cells (APCs) in bringing antigen to the lymphocytes. Both the antigen-presenting capability and the tumoricidal properties can be up-regulated by interferon-γ (IFN-γ).

Generally, diets high in fat suppress cytolysis,[33,50] although this suppression also occurs in diets that are deficient in essential fatty acids and in diets with PUFA.[33,50] In some studies, unsaturated fatty acids in culture inhibit cytolysis,[51] while in others it is enhanced.[52,53] The effect is due to more than changes in membrane fluidity alone, as *trans* 18:1 (with a melting point of 53°F) has a higher cytotoxic index than 15:0 (with a melting point of 52°F).[52] Because lymphocytes in culture do not have the enzymes necessary to elongate and desaturate 18 carbon fatty acids from the medium into eicosanoid by-products, it is unlikely that this effect is due to changes in eicosanoid metabolism.

Ω-3 PUFA have mixed effects on cytotoxicity. Meydani et al. found that young mice respond to fish oil diets by suppressing NK cell activity (although this effect is not seen in old mice),[54] and Yamashita et al. found that EPA emulsions also suppress NK cell activity.[55] DHA suppresses macrophage tumoricidal activity, although bacterial phagocytosis and intracellular killing by macrophages are not affected.[56] Higher concentrations of IFN-γ can overcome the suppression of macrophage killing of mastocytoma cells in mice.[57] In contrast, Fernandes and associates found that fish oil diets restore NK cell activity in mice prone to autoimmune disease.[40] Similarly, Schlager and associates showed that incubation with α-linolenic acid (ALA) enhances cytotoxicity of murine macrophages against fibrosarcoma cells.[58] Nonetheless, not all studies have shown an effect of dietary lipids on NK cell or CTL activity.[47]

Antigen presentation by accessory cells such as macrophages may also be modulated by lipids. Long-chain ω-3

PUFA depress APC function in normal mice,[59] an effect that may be due to suppression of IFN release.[21] The opposite effect, however, is seen in hemorrhagic-shocked mice, in which fish oil restores APC function.[10]

Lipids may exert their effects on tumor surveillance by altering the susceptibility of tumor cells to attachment and lysis by cytotoxic host cells. Although some investigators have not confirmed that alterations in target cell membrane phospholipid fatty acid profiles are associated with changes in susceptibility to cytolysis,[60] others have found that unsaturated fatty acids introduced to the cells in the culture media are associated with increased lysis.[61,62] The increases in susceptibility to lysis appear to parallel the increase in unsaturation of the fatty acids, as well as the increase in membrane fluidity.[63]

Review of effects on disease or injury

As anticipated from the multiple effects of lipids on cellular function, the effects on immune function are quite varied and sometimes even contradictory. It is not unexpected, as shown below, that the results from studies of specific disease processes are also at variance with each other. The disease processes reviewed below have in common some aspect of alteration in immune function, either as a primary causative pathophysiologic factor (such as autoimmune disease or cancer), as a direct consequence of either the injury or infectious process, or iatrogenic immunosuppression (such as that used in transplantation).

Essential fatty acid deficiency

Essential fatty acid deficiency (EFAD) is a clinical syndrome that is rare in humans. Nonetheless, experimentally induced EFAD in mice and rats permits useful studies of the role of PUFA in the immune system. As predicted when its essential precursors are eliminated from the diet, eicosanoid production is lower in cells from EFAD mice and rats.[64–67] Function of some inflammatory cells is depressed by this suppression of eicosanoid synthesis. For example, PMN oxidative burst, chemotaxis, and aggregation are impaired by EFAD in rats and associated with decreased LTB_4 production; normal function of PMNs can be restored by addition of exogenous LTB_4 in culture.[68]

Not all of the anti-inflammatory effects of EFAD are related to decreased production of eicosanoids. On the one hand, Lefkowith et al. have found that EFAD impairs the capacity of resident peritoneal macrophages to spread and adhere.[69] This defect in adherence, however, cannot be pharmacologically induced in normal cells with cyclooxygenase blockade, lipoxygenase blockade, or a platelet-activating factor receptor antagonist, suggesting that the decreased production of the metabolites of AA is not responsible. On the other hand, the addition of phospholipase inhibitor in normal cells was able to induce a spreading and adherence defect similar to that seen with EFAD. The authors suggest that AA, the release of which is prevented by phospholipase inhibitors, may be an intracellular mediator of macrophage adherence.[69]

Autoimmune disease

Fish oil supplements have been shown to slow the progression of autoimmune disease. Diets enriched with fish oil prolong the survival of autoimmune-prone mice and may do so by reducing the production of pro-inflammatory prostaglandins and leukotrienes.[70,71] For example, studies done in NZB × NZW F_1 mice, which are prone to autoimmune glomerulonephritis and early death, showed that fish oil diets are associated with a lower population of autoantibody-producing lymphocytes ($Ly1B^+$) and lower levels of circulating anti-DNA antibodies. In addition, the mice fed fish oil have less proteinuria, less severe glomerulosclerosis and renal periarteritis, and longer survival than mice fed corn oil or lard.[41]

The results from clinical studies, however, have been mixed. For example, Kremer and associates have shown in a nonrandomized, double-blinded, placebo-controlled crossover trial that fish oil supplements (15 mg MaxEPA capsules per day) decrease PMN LTB_4 production and improve clinical symptoms.[72] Others, however, have shown no clinical improvement associated with fish oil in patients with psoriasis[73] or systemic lupus nephritis.[74]

Transplantation

Mertin et al. found that subcutaneous injection with certain PUFA prolongs the survival of skin allografts in mice.[75–77] Injections of LA, ALA, or AA three times a week in mice increase the median survival time of skin allografts. Interestingly, they found that the increase in median survival times of allografts was directly correlated with the degree of unsaturation of the fatty acids used, which suggests that this effect was related more to the physical properties of PUFA (such as an increase in membrane fluidity or lipid peroxidation) than to the differential production of eicosanoid metabolites.[75] These investigators also found that primary and subsequent memory cytotoxic allograft responses decreased in animals treated with LA. The immunosuppressant effects of LA, however, disappear in splenectomized mice, suggesting that this is not a direct effect on lymphocytes themselves, but rather mediated through other effector cells in the immune system.[77]

In support of the concept that increased allograft survival is not due solely to increased PGE_2 synthesis, others have found that ω-3 PUFA are immunosuppressive. For example, Kelley et al. found that cyclosporine given in a fish oil vehicle compared with olive oil increased the survival of rat heart transplants, and that delayed-type hypersensitivity is also suppressed.[78] The results of a nonblinded, noncontrolled trial of fish oil supplements in renal transplant patients have also shown a decrease in the rate of deterioration of renal allograft function.[79] These observations indicate that ω-3 PUFA, through an unspecified immunosuppressive effect, prolong allograft survival, as do ω-6 PUFA. Because both families of PUFA have the same effect on allograft survival, the effect is more likely due to the shared physical properties of PUFA on cell membranes rather than the production of divergent eicosanoid metabolites with differing biologic potency.

Cancer

Both the amount and type of dietary lipid affect neoplastic disease in laboratory animals. Diets that are high in fat lead to increases in the incidence and number of dimethyl hydrazine-induced colon carcinomas in rats,[38] decreased

tumor latency and lymphocyte cytotoxicity in mice with B16 melanoma,[50] and increased incidence and growth rate of DMBA-induced mammary carcinoma in rats.[80,81] These experimental data parallel epidemiologic studies that suggest that high fat diets increase the risk of neoplastic disease in humans.[82]

PUFA have also been shown to facilitate tumor growth. For example, both King and Kollmorgen found that diets with 20% (wt/wt) corn oil increase the incidence and growth rate of DMBA-induced mammary carcinoma in rats.[80,81] Mertin and Hunt showed that EFAD diets lead to decreased incidence of methylcholanthrene tumors in mice, further suggesting that the production of dienoic prostanoids such as PGE_2 suppresses tumor surveillance.[76] In addition, both corn and safflower oil diets lead to increased tumor incidence and volume in rats with aflatoxin-B liver tumors in rats[38] or in mice with B16 melanoma.[50] There is concern that as PUFA intake in the American diet has increased to replace saturated fat and decrease the risk of atherosclerotic disease, the incidence of neoplastic disease may rise.[82]

Not all PUFA facilitate tumor growth. Supplementation with fish oil on a daily basis decreases the weight and volume of mammary tumors in rats.[83] Supplementation with fish oils in this model is associated with both decreased PGE_2 in tumor microsomes and increased ω-3 PUFA in tumor cell phosphatidylcholine, thus confirming the association between PGE_2 and tumor surveillance. It is also known from specimens of human hepatocellular carcinoma that membrane ALA and DHA are lower than in reference specimens, and levels of thromboxane and prostacyclin are higher.[84] Permeability of tumor cells also increases after incubation with DHA,[85] suggesting yet another mechanism for the suppressive effects of fish oil on tumor growth. Fish oil may benefit the tumor-bearing host by decreasing prostanoid production and destabilizing tumor cell membranes.

Immune responses to tumors can either enhance the growth of a tumor or cause its eradication from the host. Several types of cytotoxic interactions occur between tumor cells and host effector mechanisms. The lytic interaction through which cytotoxic T-lymphocytes destroy appropriate target cells is a multi-step process in which the plasma membrane of the cytotoxic T cell is undoubtedly important because of direct contact with the target cell.[53] Fatty acids incorporated into cytotoxic T-lymphocytes may enhance or inhibit cell-mediated cytotoxicity, while unsaturated fatty acids enhance cytolysis, and saturated fatty acids inhibit it.[53] In addition, fatty acids alter the susceptibility of tumor cells to cytolysis mediated by antibody or complement.[61,62,86] Increases in membrane PUFA increase susceptibility of tumor target cells to lysis in some experiments,[61,62] although other investigators have found that in vivo feeding experiments did not show the same results as those with cultured cells.[86] Nonetheless, in experiments in which increased tumor growth is associated with either increased amount of dietary fat or increases in polyunsaturated fat, both suppression of cytotoxicity[50] and lymphoproliferation in response to mitogens[38] have been noted. To the degree that the host immune response is important in maintaining suppression of tumor growth, dietary lipids play a significant role in enhancement or suppression of tumor growth via modulation of lymphocyte activity and target cell membrane susceptibility.

Outcome from infection

Cyclooxygenase products play a role in the morbidity and mortality that accompany Gram-negative bacterial infections. EFAD rats, which produce prostaglandins in smaller quantities, are more resistant to endotoxin shock than controls,[87] and ibuprofen protects against mortality after cecal ligation and puncture (a model of bacterial peritonitis) in mice that have been subjected to hemorrhagic shock.[88]

As expected, diets rich in PUFA also modulate the response to bacterial infection, although the experimental results are not consistent. On the one hand, several investigators have found that fish oil, given either enterally or parenterally, improves survival from endotoxin or bacterial shock.[89–92] On the other hand, some investigators have found that the type and amount of fish oil have no effect on survival from bacterial infection.[93] In one of our own studies, we found that diets based on ω-6 PUFA-rich vegetable oils or fish oils have no difference in their effect on survival from bacterial peritonitis in guinea pigs fed enterally for 2 weeks during the infection.[36] Paradoxically, in another of our studies, we reported that fish oil dramatically increases mortality from *Pseudomonas* infection in burned mice.[43] Interestingly, our best survival rates in the guinea pig study were achieved with a diet composed of a mixture of vegetable and fish oils, with a resultant ω-6 to ω-3 ratio of approximately 2 to 1. Although the pure fish oil diets were associated with a decrease in splenocyte PGE_2 production and an increase in delayed-type hypersensitivity responses, these changes in prostanoid production and immune function appeared to be insufficient by themselves to improve survival.

Conclusion

In summary, lipids modulate the immune system by (1) altering membrane fluidity, (2) producing lipid peroxides, (3) regulating eicosanoid metabolites, and (4) interacting directly with cellular activation processes. Dietary lipids are absorbed and distributed to essentially every cell membrane in the body. There they perform important structural and functional roles. Membrane fluidity and eicosanoid synthesis are the two realms in which lipids have their most potent effects. Lipid peroxidation challenges the plasma membrane to protect the cell, and under conditions of stress or antioxidant depletion, significant free radical damage can occur.

These multiple effects result in marked differences in outcome in animal models in which activation of the immune system plays a significant role. Examples include transplantation, in which allograft survival is prolonged by PUFA; cancer, in which low fat or ω-3 PUFA diets decrease the incidence and growth of tumors; and autoimmune disease, in which survival is prolonged by dietary ω-3 PUFA. These studies and others suggest the following syllogism: (a) PGE_2 is immunosuppressive; (b) ω-3 PUFA inhibit PGE_2 synthesis; and (c) therefore, ω-3 PUFA are immunostimulators.

Not all results in animal models, however, are consistent with each other. For example, ω-3 PUFA may improve outcome from infection in one model, while increasing mortality in others. Also puzzling are the observations that both ω-6 and ω-3 PUFA prolong allograft survival in transplantation models. Although it may be predicted that increased produc-

tion of PGE_2 by LA would suppress the immune response (thus increasing mortality from infection and prolonging allograft survival), fish oil diets sometimes have a similar effect. Therefore, the syllogism suggested above may be too simplistic to describe the effects of PUFA on the immune response.

The immune modulating effects should thus be anticipated based on all the effects of PUFA, not just those secondary to eicosanoid metabolites. Models in which outcome correlates with the degree of unsaturation rather than the family classification can be explained by either the increased fluidity of leucocyte membranes or the increased burden of lipid peroxides. The area of direct effects of fatty acids on signal transduction is yet unexplored in animal models of disease or injury. Further research on lipids and the immune response should help to integrate measurements of eicosanoid activity, membrane fluidity, and peroxide formation, thus summing up the multiple effects of the lipid treatment. The direct effect of fatty acids on transmembrane signalling is also a fertile area for exploration.

References

1 Peck, M.D. (1994). Interactions of lipids with immune function I: Biochemical effects of dietary lipids on plasma membranes. *J. Nutr. Biochem.* **5,** 466–478

2 Lowry, S.F. (1993). Cytokine mediators of immunity and inflammation. *Arch. Surg.* **128,** 1235–1241

3 Rock, C.S. and Lowry, S.F. (1991). Tumor necrosis factor-α. *J. Surg. Res.* **51,** 434–445

4 Dinarello, C.A. (1984). Interleukin-1 and the pathogenesis of the acute-phase response. *N. Engl. J. Med.* **311,** 1413–1418

5 Fassbender, K., Pargger, H., Muller, W., and Zimmerli, W. (1993). Interleukin-6 and acute-phase protein concentrations in surgical intensive care unit patient: Diagnostic signs in nosocomial infection. *Crit. Care Med.* **21,** 1175–1180

6 Drost, A.C., Burleson, D.G., Cioffi, W.G., Mason, A.D., and Pruitt, B.A., Jr. (1993). Plasma cytokines after thermal injury and their relationship to infection. *Ann. Surg.* **218,** 74–78

7 Santoli, D. and Zurier, R.B. (1989). Prostaglandin E precursor fatty acids inhibit human IL-2 production by a prostaglandin E-independent mechanism. *J. Immunol.* **143 ,** 1303–1309

8 Venkatraman, J.T. and Fernandes, G. (1993). Differential effects of fatty acids on proliferative response and IL-2 production by EL-4 cells. *FASEB J.* **7,** A56

9 Billiar, T.R., Bankey, P.E., Svingen, B.A., Curran, R.D., West, M.A., Holman, R.T., Simmons, R.L., and Cerra, F.B. (1988). Fatty acid intake and Kupffer cell function: Fish oil alters eicosanoid and monokine production to endotoxin stimulation. *Surgery* **104,** 343–349

10 Ertel, W., Morrison, M.H., Ayala, A., and Chandry, I.H. (1993). Modulation of macrophage membrane phospholipids by n-3 polyunsaturated fatty acids increases interleukin 1 release and prevents suppression of cellular immunity following hemorrhagic shock. *Arch. Surg.* **128,** 15–21

11 Hardardottir, I. and Kinsella, J.E. (1991). Tumor necrosis factor production by murine resident peritoneal macrophages is enhanced by dietary n-3 polyunsaturated fatty acids. *Biochim. Biophys. Acta* **1095,** 187–195

12 Watanabe, S., Hayashi, H., Onozaki, K., and Okuyama, H. (1991). Effect of dietary α-linolenate/linoleate balance on lipopolysaccharide-induced tumor necrosis factor production in mouse macrophages. *Life Sci.* **48,** 2013–2020

13 Endres, S., Ghorbani, R., Kelley, V.E., Georgilis, K., Lonnemann, G., van der Meer, J.W.M., Cannon, J.G., Wolff, S.M., and Dinarello, C.A. (1989). The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N. Engl. J. Med.* **320,** 265–271

14 Meydani, S.N., Endres, S., Woods, M.M., Goldin, B.R., Soo, C., Morrill-Labrode, A., Dinarello, C.A., and Gorbach, S.L. (1991). Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: Comparison between young and older women. *J. Nutr.* **121,** 547–555

15 Karmiol, S., Curry, R., and Phan, S.H. (1993). Fatty acid modification of human lung fibroblasts (HLFS) results in differential IL-6 production due to IL-1β stimulation. *FASEB J.* **7,** A429

16 Yoshino, S. and Ellis, E.F. (1987). Effect of a fish-oil-supplemented diet on inflammation and immunological processes in rats. *Int. Archs. Allergy Appl. Immun.* **84,** 233–240

17 Hubbard, N.E., Somers, S.D., and Erickson, K.L. (1991). Effect of dietary fish oil on development and selected functions of murine inflammatory macrophages. *J. Leukoc. Biol.* **49,** 592–598

18 Costa Rosa, L.F., Guimaraes, A.R., Safi, D.A., and Williams, J.F. (1993). Effects of various dietary fatty acids on enzyme activities of carbohydrate and glutamine metabolism and the metabolic response of lymphocytes and macrophages during Walker-256 ascites cell tumour growth in rats. *Biochem. Mol. Biol. Int.* **29,** 33–45

19 Lefkowith, J.B. (1990). Essential fatty acid deficiency: probing the role of arachidonate in biology. *Adv. Prostagland. Thromb. Leukot. Res.* **20,** 224–231

20 Hawley, H.P. and Gordon, G.B. (1976). The effects of long chain free fatty acids on human neutrophil function and structure. *Lab. Invest.* **34,** 216–222

21 Morrow, W.J.W., Ohashi, Y., Hall, J., Pribnow, J., Hirose, S., Shirai, T., and Levy, J.A. (1985). Dietary fat and immune function I. Antibody responses, lymphocyte and accessory cell function in (NZB X NZW) F_1 mice *J. Immunol.* **135,** 3857–3863

22 Lokesh, B.R. and Kinsella, J.E. (1987). Modulation of prostaglandin synthesis in mouse peritoneal macrophages by enrichment of lipids with either eicosapentaenoic or docosahexaenoic acids in vitro. *Immunobiology* **175,** 406–419

23 Peck, M.D., Ogle, C.K., Alexander, J.W., and Babcock, G.F. (1991). Dietary fat and infection in burned animals. *J. Burn Care Rehab.* **12,** 43–45

24 Blonk, M.C., Bilo, H.J., Nauta, J.J.P., Popp-Snijders, C., Mulder, C., and Donker, A.J.M. (1990). Dose-response effects of fish-oil supplementation in healthy volunteers. *Am. J. Clin. Nutr.* **52,** 120–127

25 Naccache, P.H., Molski, T.F.P., Volpi, M., and Sha'afi, R.I. (1984). Modulation of rabbit neutrophil aggregation and degranulation by free fatty acids. *J. Leukoc. Biol* **36,** 333–340

26 Wong, K. and Chew, C. (1984). The respiratory burst of human neutrophils treated with various stimulators in vitro is dampened by exogenous unsaturated fatty acids. *J. Cell Physiol.* **119,** 89–95

27 Magrum, L.J. and Johnston, P.V. (1985). Effect of culture in vitro with eicosatetraenoic (20:4(n-6)) and eicosapentaenoic (20:5(n-3)) acids on fatty acid composition, prostaglandin synthesis and chemiluminescence of rat peritoneal macrophages. *Biochim. Biophys. Acta* **836,** 354–360

28 Badwey, J.A., Curnutte, J.T., Robinson, J.M., Berede, C.B., Karnovsky, M.J., and Karnovsky, M.L. (1984). Effects of free fatty acids on release of superoxide and on change of shape by human neutrophils. *J. Biol. Chem.* **259,** 7870–7877

29 Gadd, M.A. and Hansbrough, J.F. (1990). Postburn suppression of murine lymphocyte and neutrophil functions is not reversed by prostaglandin blockade. *J. Surg. Res.* **48,** 84–90

30 Kelly, J.P. and Parker, C.W. (1979). Effects of arachidonic acid and other unsaturated fatty acids on mitogenesis in human lymphocytes. *J. Immunol.* **122,** 1556–1562

31 Erickson, K.L., McNeill, C.J., Gershwin M.E., and Ossmann, J.B. (1980). Influence of dietary fat concentration and saturation on immune ontogeny in mice. *J. Nutr.* **110,** 1555–1572

32 Ossman, J.B., Erickson, K.L., and Canolty, N.L. (1980). Effects of saturation and concentration of dietary fats on lymphocyte transformation in mice. *Nutr. Rep. Int.* **22,** 279–285

33 Erickson, K.L., Adams, D.A., and McNeill, C.J. (1983). Dietary lipid modulation of immune responsiveness. *Lipids* **18,** 468–474

34 Adams, D.A., Freauff S.J., and Erickson, S.L. (1985). Biophysical characterization of dietary lipid influences on lymphocytes. In *Pharmacological Role of Lipids*, (J. Kabana, ed.), pp. 9–21, AOCS, Champaign, IL USA

35 Egami, M.I., Guimaraes, A.R.P., Nascimento Curi, C.M.P.O., and Curi, R. (1993). Effect of fatty acid-rich diets on thymocyte proliferation and thymus involution during growing. *Physiol. Behav.* **53,** 531–534

36 Peck, M.D., Ogle, C.K., Alexander, J.W., and Babcock, G.W. (1991). Composition of fat in enteral diets can influence outcome in experimental peritonitis. *Ann. Surg.* **213,** 74–82

37 Meydani, S.N., Nicolosi R.J., and Hayes, K.C. (1985). Effect of long-term feeding of corn oil or coconut oil diets on immune response and prostaglandin E_2 synthesis of squirrel and cebus monkeys. *Nutr. Res.* **5,** 993–1002

38 Newberne, P.M. (1981). Dietary fat, immunological response, and cancer in rats. *Cancer Res.* **41,** 3783–3785

39 Locniskar, M., Nauss, K.M., and Newberne, P.M. (1983). The effect of quality and quantity of dietary fat on the immune system. *J. Nutr.* **113,** 951–961

40 Kelley, D.S., Nelson, G.J., Serrato, C.L., Schmidt, P.C., and Branch, L.B. (1988). Effects of type of dietary fat on indices of immune status of rabbits. *J. Nutr.* **118,** 1376–1384

41 Fernandes, G. (1989). Effect of dietary fish oil supplement on autoimmune disease: Changes in lymphoid cell subsets, oncogene mRNA expression and neuroendocrine hormones. In *Health Effects of Fish and Fish Oil* (R.K. Chandra, ed.), ARTS Biomedical, St. John's, Newfoundland, Canada

42 Fowler, K.L., Chapkin, R.S., and McMurray, D.N. (1993). Effects of purified dietary N-3 ethyl esters on murine T-lymphocyte function. *FASEB J.* **7,** A72

43 Peck, M.D., Alexander, J.W., Ogle, C.K., and Babcock, G.F. (1990). The effect of dietary fatty acids on response to *Pseudomonas* infection in burned mice. *J. Trauma* **30,** 445–452

44 Shapiro, A.C., Wu, D., O'Donnell, E.K., Ansair, P., and Meydani, S.N. (1992). Effect of in-vitro addition PGE_2 and PGE_3 on proliferative response (PR) of splenocytes from mice fed high n-3 or n-6 polyunsaturated fatty acid (PUFA) diets. *FASEB J.* **6,** A1371

45 Trocki, O., Heyd, T.J., Waymack, J.P., and Alexander, J.W. (1987). Effects of fish oil on postburn metabolism and immunity. *JPEN* **11,** 521–528

46 Friend, J.V., Lock, S.O., Gurr, M.I., and Parish, W.E. (1980). Effect of different dietary lipids on the immune responses of Hartley strain guinea pigs. *Int. Arch. Allergy Appl. Immun.* **62,** 292–301

47 Bennett, M., Uauy, R., and Grundy, S.M., (1987). Dietary fatty acid effects on T-cell-mediated immunity in mice infected with *Mycoplasma Pulmonis* or given carcinogens by injection. *Am. J. Pathol.* **126,** 103–113

48 Alexander, J.W., Saito, H., Trocki, O., and Ogle, C.K. (1986). The importance of lipid type in the diet after burn injury. *Ann. Surg.* **204,** 1–8

49 DeWille, J.W., Fraker, P.J., and Romsos, D.R. (1979). Effects of essential fatty acid deficiency, and various levels of dietary polyunsaturated fatty acids, on humoral immunity in mice. *J. Nutr.* **109,** 1018–1027

50 Erickson, K.L. (1984). Dietary fat influences on murine melanoma growth and lymphocyte-mediated cytotoxicity. *J. Natl. Cancer Inst.* **72,** 115–120

51 Richieri, G.V., Mescher, M.F., and Kleinfeld, A.M. (1990). Short term exposure to *cis* unsaturated free fatty acids inhibits degranulation of cytotoxic T lymphocytes. *J. Immunol.* **144,** 671–677

52 Mandel, G. and Clark, W. (1978). Functional properties of EL-4 tumor cells with lipid-altered membranes. *J. Immunol.* **120,** 1637–1643

53 Bialick, R., Gill, R., Berke, G., and Clark, W.R. (1984). Modulation of cell-mediated cytotoxicity function after alteration of fatty acid composition in vitro. *J. Immunol.* **132,** 81–87

54 Meydani, S.N., Yogeeswaran, G., Liu, S., Baskar, S., and Meydani, M. (1988). Fish oil and tocopherol-induced changes in natural killer cell-mediated cytotoxicity and PGE_2 synthesis in young and old mice. *J. Nutr.* **118,** 1245–1252

55 Yamashita N., Maruyama, M., Yamazaki, K., Hamazaki, T., and Yano, S. (1991). Effect of eicosapentaenoic and decosahexaenoic acid on natural killer cell activity in human peripheral blood lymphocytes. *Clin. Immunol. Immunopathol.* **59,** 335–345

56 Dustin L.B., Shea, C.M., Soberman, R.J., and Lu, C.Y. (1990). Docosahexaenoic acid. A constituent of rodent fetal serum and fish oil diets, inhibits acquisition of macrophage tumoricidal function. *J. Immunol.* **144,** 4888–4897

57 Somers, S.D., Chapkin, R.S., and Erickson, K.L. (1989). Alteration of in vitro murine peritoneal macrophage function by dietary enrichment with eicosapentaenoic and docosanexaenoic acids in menhaden fish oil. *Cell Immunol.* **123,** 201–211

58 Schlager, S.L., Meltzer, M.S., and Madden, L.D. (1983). Role of membrane lipids in the immunological killing of tumor cells: II. Effector cell lipids. *Lipids* **18,** 483–488

59 Huang, S.C., Misfeldt, M.L., and Fritsche, K.L. (1992). Dietary fat influences Ia antigen expression and immune cell populations in the murine peritoneum and spleen. *J. Nutr.* **122,** 1–14

60 Gill, R. and Clark, W. (1980). Membrane structure-function relationships in cell-mediated cytolysis. *J. Immunol.* **125,** 689–695

61 Schlager, S.I. and Ohanian, S.H., (1979). A role for fatty acid composition of complex cellular lipids in the susceptibility of tumor cells to humoral immune killing. *J. Immunol.* **123,** 146–152

62 Yoo, T.J., Chiu, H.C., Spector, A.A., Whiteaker, R.S., Denning, G.M., and Lee, N.F. (1980). Effect of fatty acid modifications of cultured hepatoma cells on susceptibility to complement-mediated cytolysis. *Cancer Res.* **40,** 1084–1090

63 Schlager, S.I. and Ohanian, S.H. (1983). Role of membrane lipids in the immunological killing of tumor cells: I. Target cell lipids. *Lipids* **18,** 475–482

64 Cook, J.A., Wise, W.C., Knapp, D.R., and Halushka, P.V. (1981). Essential fatty acid deficient rats: A new model for evaluating arachidonate metabolism in shock. *Adv. Shock. Ther.* **6,** 93–105

65 Boissonneault, G.A. and Johnston, P.V. (1983). Essential fatty acid deficiency, prostaglandin synthesis and humoral immunity in Lewis rats. *J. Nutr.* **113,** 1187–1194

66 Lefkowith, J.B., Jakschik, B.A., Stahl, P., and Needleman, P. (1987). Metabolic and functional alterations in macrophages induced by essential fatty acid deficiency. *J. Biol. Chem.* **262,** 6668–6675

67 Lefkowith, J.B. (1988). Essential fatty acid deficiency inhibits the in vivo generation of leukotriene B_4 and suppresses levels of resident and elicited leukocytes in acute inflammation. *J. Immunol.* **140,** 228–233

68 Gyllenhammar, H., Palmblad, J., Ringertz, B., Hafstrom, I., and Borgeat, P. (1988). Rat neutrophil function, and leukotriene generation in essential fatty acid deficiency. *Lipids* **23,** 89–95

69 Lefkowith, J.B., Rogers, M., Lennartz, M.R., and Brown, E.J. (1991). Essential fatty acid deficiency impairs macrophage spreading and adherence. *J. Biol. Chem.* **266,** 1071–1076

70 Prickett, J.D., Robinson, D.R., and Steinberg, A.D. (1983). Effects of dietary enrichment with eicosapentaenoic acid upon autoimmune nephritis in female NZB X NZW/F_1 Mice. *Arthritis Rheum.* **26,** 133–139

71 Kelley, V.E., Ferretti, A., Izui, S., and Strom, T.B. (1985). A fish oil diet rich in eicosapentaenoic acid reduces cyclooxygenase metabolites, and suppresses lupus in MRL-lpr mice. *J. Immunol.* **134,** 1914–1919

72 Kremer, J.M., Jubiz, W., Michalek, A., Rynes, R.I., Bartholomew, L.E., Bigauette, J., Timchalk, M., Beeler, D., and Lininger, L. (1987). Fish oil fatty acid supplementation in active rheumatoid arthritis. *Ann. Intern. Med.* **106,** 497–503

73 Soyland, E., Funk, J., Rajka, G., Sandberg, M., Thune, P., Rustad, L., Helland, S., Middelfart, K., Odu, S., Falk, E.S., Solvoll, K., Bjorneboe, G.E.A., and Drevon, C.A. (1993). Effect of dietary supplementation with very-long chain n-3 fatty acids in patients with psoriasis. *N. Engl. J. Med.* **328,** 1812–1816

74 Clark, W.F., Parbtani, A., Naylor, C.D., Levinton, C.M., Muirhead, N., Spanner, E., Huff, M.W., Philbrick, D.J., and Holub, B.J. (1993). Fish oil in lupus nephritis: Clinical findings and methodological implications. *Kidney Int.* **44,** 75–86

75 Mertin, J. (1976). Effect of polyunsaturated fatty acids on skin allograft survival and primary and secondary cytotoxic response in mice. *Transplantation* **21,** 1–4

76 Mertin, J. and Hunt, R. (1976). Influence of polyunsaturated fatty acids on survival of skin allografts and tumor incidence in mice. *Proc. Nat. Acad. Sci. U.S.A.* **73,** 928–931

77 Mertin, J., Meade, C.J., Hunt, R., and Sheena, J. (1977). Importance of the spleen for the immuno-inhibitory action of linoleic acid in mice. *Int. Arch. Allergy Appl. Immun.* **53,** 469–473

78 Kelley, V.E., Kirkman, R.L., Bastos, M., Barrett, L.V., and Strom, T.B. (1989). Enhancement of immunosuppression by substitution of fish oil for olive oil as a vehicle for cyclosporine. *Transplantation* **48,** 98–102

79 Sweny, P. (1993). Use of dietary fish oils in renal allograft

recipients with chronic vascular rejection. *Transplant Proc.* **25,** 2089–2091

80 King, M.M., Bailey, D.M., Gibson, D.D., Pitha, J.V., and McCay, P.B. (1979). Incidence and growth of mammary tumors induced by 7,12-Dimethylbenz[a]anthracene as related to the dietary content of fat and antioxidant. *J. Natl. Cancer Inst.* **63,** 657–663

81 Kollmorgen, G.M., Sansing, W.A., Lehman, A.A., Fischer, G., Longley, R.E., Alexander, Jr., S.S., King, M.M., and McCay, P.B. (1979). Inhibition of lymphocyte function in rats fed high-fat diets. *Cancer Res.* **39,** 3458–3462

82 Henderson, M.M. (1991). Correlations between fatty acid intake and cancer incidence. In *Health Effects of Dietary Fatty Acids,* (G.J. Nelson, ed.), pp. 136–149, AOCS, Champaign, IL USA

83 Karmali, R.A., Marsh, J., and Fuchs, C. (1984). Effect of Omega-3 fatty acids on growth of a rat mammary tumor. *JNCI* **73,** 457–461

84 Hanai, T., Hashimoto, T., Nishiwaki, K., Ono, M., Akamo, Y., Tanaka, M., Mizuno, I., Isamu, M., and Yura, J. (1993). Comparison of prostanoids and their precursor fatty acids in human hepatocellular carcinoma and noncancerous reference tissues. *J. Surg. Res.* **54,** 57–60

85 Stillwell, W., Ehringer, W., and Jenski, L.J. (1993). Docosahexaenoic acid increases permeability of lipid vesicles and tumor cells. *Lipids* **28,** 103–108

86 Erickson, K.L. and Thomas, I.K. (1985). Susceptibility of mammary tumor cells to complement-mediated cytolysis after in vitro or in vivo fatty acid manipulation. *J. Natl. Cancer Inst.* **75,** 333–340

87 Ball, H.A., Cook, J.A., Wise, W.C., and Halushka, P.V. (1986). Role of thromboxane, prostaglandins and leukotrienes in endotoxic and septic shock. *Inten. Care Med.* **12,** 116–126

88 Ertel, W., Morrison, M.H., Meldrum, D.R., Ayala, A., and Chaudry, I.H. (1992). Ibuprofen restores cellular immunity and decreases susceptibility to sepsis following hemorrhage. *J. Surg. Res.* **53,** 55–61

89 Mascioli, E., Leader, L., Flores, E., Trimbo, S., Bistrian, B., and Blackburn, G. (1988). Enhanced survival to endotoxin in guinea pigs fed IV fish oil emulsion. *Lipids* **23,** 623–625

90 Mascioli, E.A., Iwasa, Y., Trimbo, S., Leader, L., Bistrian, B.R., and Blackburn, G.L., (1989). Endotoxin challenge after menhaden oil diet: Effects on survival of guinea pigs. *Am. J. Clin. Nutr.* **49,** 277–282

91 Barton, R.G., Wells, C.L., Carlson, A., Singh, R., Sullivan, J.J., and Cerra, F.B. (1991). Dietary Omega-3 fatty acids decrease mortality and Kupffer cell prostaglandin E_2 production in a rat model of chronic sepsis. *J. Trauma* **31,** 768–774

92 Murray, M.J., Svingen, B.A., Holman, R.T., and Yaksh, T.L. (1991). Effects of a fish oil diet on pigs' cardiopulmonary response to bacteremia. *JPEN* **15,** 152–158

93 Clouva-Molyvdas, P., Peck, M.D., and Alexander, J.W. (1992). Short-term dietary lipid manipulation does not affect survival in two models of murine sepsis. *JPEN* **16,** 343–347