The effects of fatty acids on lymphocyte functions in vitro

Effects of fatty acids on lymphocyte proliferation

Experimentally, the most widely used test of lymphocyte function is proliferation of the cells in response to a mitogenic signal. Commonly used mitogens are concanavalin A (Con A) and phytohaemagglutinin (PHA), which stimulate T lymphocyte mitogenesis only, and pokeweed mitogen (PWM), which stimulates both T and B lymphocyte mitogenesis only, bacterial lipopolysaccharide (LPS), which stimulates B lymphocyte mitogenesis, and pokeweed mitogen (PWM), which stimulates both T and B lymphocytes. The proliferative response of lymphocytes may be followed by measuring the increase in the number of cells or, more conveniently, by measuring the incorporation of $[^{3}H]$thymidine into the DNA of the cells.

A number of studies have investigated the effects of adding non-esterified fatty acids to lymphocytes in culture on mitogen-stimulated lymphocyte proliferation; fatty acids have been added to the culture medium either dissolved in ethanol [1-5] or, more physiologically, as preformed complexes with albumin [6-16]. The concentrations of fatty acids used in these studies have usually been between 30 and 300 μM. Early studies compared the effects of saturated (myristic, palmitic, stearic), n-9 monounsaturated (oleic) and n-6 polyunsaturated (linoleic, arachidonic) fatty acids (PUFAs) [1-4,6-9]. More recent studies have included other n-6 PUFAs ($\gamma$-linolenic, dihomo-$\gamma$-linolenic) and n-3 PUFAs ($\alpha$-linolenic, eicosapentaenoic, docosahexaenoic) in their comparisons [5,10-17]. These studies have clearly shown that oleic, linoleic, $\alpha$-linolenic, $\gamma$-linolenic, dihomo-$\gamma$-linolenic, arachidonic, eicosapentaenoic and docosahexaenoic acids inhibit Con A- and/or PHA-stimulated proliferation of lymphocytes isolated from rat lymph nodes [11-13], spleen [8,16], thymus [16] and thoracic duct [12], from mouse spleen [8,9] and thymus [5], from pig lymph nodes [8] and from human peripheral blood [1-4,6-8,14,15,17] and tonsils [16]. These fatty acids also inhibit the proliferation of rat lymph node lymphocytes in response to PWM [16], and eicosapentaenoic acid has been shown to inhibit anti-
CD3-stimulated proliferation of human peripheral blood lymphocytes (PBLs) [18]. The inhibition of lymphocyte proliferation by unsaturated fatty acids is dependent upon the concentration of fatty acid used, the time during culture that the fatty acid was added and the duration of exposure of the cells to the fatty acid [11,15]. Typically, eicosapentaenoic acid added as a complex with albumin and at a final concentration of 100 μM to a culture of rat or human lymphocytes will cause approximately 85% inhibition of proliferation over a 66-h culture period [11-14]. Most studies agree that the extent of inhibition is also partly dependent on the degree of unsaturation of the fatty acid, with chain length also being important; the early studies showed that arachidonic acid caused the greatest inhibition of proliferation, whereas the later studies, which included a greater range of fatty acids, indicate an approximate order of potencies as: oleic < linoleic = α-linolenic < γ-linolenic = dihomo-γ-linolenic = docosahexaenoic < arachidonic = eicosapentaenoic

A number of studies have also shown inhibition of lymphocyte proliferation by at least some saturated fatty acids. Most studies agree that saturated fatty acids cause less inhibition than unsaturated fatty acids [3,5,11-15]; there have been a number of reports of lack of inhibition by the medium-chain saturated fatty acids, lauric and myristic [6-8,11-15] and there appears to be general agreement that stearic acid is the most potent inhibitor of lymphocyte proliferation among the saturated fatty acids, causing a degree of inhibition similar to that of oleic acid [3,11-15]. Thus among the saturated fatty acids the order of potencies appears to be:

lauric = myristic < palmitic < stearic.

In contrast to the findings of these studies, Kelly and Parker [19] reported that low concentrations (less than 5 μM) of some unsaturated fatty acids enhanced PHA-stimulated proliferation of murine spleen lymphocytes; arachidonic acid was inhibitory even at very low concentrations. This may be because proliferating lymphocytes require an exogenous supply of unsaturated fatty acids; however, once a particular fatty acid concentration is exceeded (~10–15 μM), the fatty acids begin to exert inhibitory effects. This was borne out by the study of Kelly and Parker [19], in which higher concentrations of unsaturated fatty acids did cause inhibition of lymphocyte proliferation.

The specificity of PHA and Con A as T lymphocyte mitogens indicates that fatty acids inhibit proliferation of these cells. Indeed, the pattern of inhibition of Con A-stimulated proliferation of purified rat lymph node T-cells by fatty acids is similar to the pattern observed in complete lymphocyte preparations [12]. Two studies have investigated the effect of fatty acids on the proliferation of B-cells, by using LPS as a mitogen [9,10]; these studies showed that palmitic, stearic, oleic, linoleic, α-linolenic, arachidonic, eicosapentaenoic and docosahexaenoic acids inhibit B-cell proliferation, although the extent of inhibition caused by each fatty acid is less than that caused upon T-cell proliferation [9,10].

Mitogen-stimulated lymphocyte proliferation is a model in vitro for the stimulation of lymphocytes by presentation of processed antigen to the T-cell receptor by an antigen presenting cell. The response of lymphocytes to antigen in vitro can also be measured; most commonly the antigen used is tuberculin, the purified protein derivative of Mycobacterium tuberculosis also known as PPD. Early studies showed that oleic, linoleic and arachidonic acids inhibited the response of human PBLs to PPD [2,3]. More recently, it has been demonstrated that the n-3 PUFAs eicosapentaenoic and docosahexaenoic acids also inhibit PPD-stimulated human PBL proliferation [14]. Not only may fatty acids affect the response of lymphocytes to antigen, they may also affect the ability of antigen presenting cells to present antigen: in the only study that has investigated this possibility, Fujikawa et al. [20] showed that a triacylglycerol (TAG) containing eicosapentaenoic acid inhibited the ability of murine spleen cells to present antigen (keyhole limpet haemocyanin) to two helper T-cell lines.

The proliferation of lymphocytes, both in vivo and in vitro, is dependent on the presence of various cytokines, including interleukin-2 (IL-2); thus proliferation of lymphocytes can be measured in response to the provision of cytokines. Santoli et al. [21] found that palmitic, oleic, linoleic, α-linolenic, dihomo-γ-linolenic, arachidonic and eicosapentaenoic acids cause concentration-dependent inhibition of the proliferation of human PBLs in response to IL-2, with arachidonic acid causing the greatest inhibition, and palmitic and oleic acids the least. Recently, Rotondo et al. [5] showed that several unsaturated fatty acids inhibited the proliferation of murine thymic lymphocytes in response to either IL-2 or IL-1β; dihomo-γ-linolenic, arachidonic and eicosapentaenoic acids were the most inhibitory fatty acids. In accordance with these findings, Borofsky et al. [22] found that some unsaturated fatty acids (dihomo-γ-linolenic,
arachidonic) inhibited the proliferation of an IL-2-dependent cell line.

Because most fatty acids are carried in the bloodstream as TAGs rather than in non-esterified form, the effect of different TAGs upon lymphocyte proliferation must be addressed. The only study to have investigated the effect of pure TAGs on lymphocyte proliferation found that those containing linoleic, \(\alpha\)-linolenic or arachidonic acids caused concentration-dependent inhibition of Con A-stimulated proliferation of rat lymph node lymphocytes, whereas TAGs containing saturated fatty acids (myristic, palmitic, stearic) or oleic acid were without effect [23].

Many eicosanoids derived from arachidonic acid, particularly prostaglandin \(E_2\) (PGE\(_2\)), suppress lymphocyte proliferation (see [12] for references). It is therefore possible that fatty acids exert their inhibitory effects upon lymphocyte proliferation by modulation of eicosanoid synthesis. However, because eicosapentaenoic and arachidonic acids both inhibit lymphocyte proliferation to the same extent, this would require that eicosanoids derived from eicosapentaenoic acid (e.g. PGE\(_3\)) have the same effects as those derived from arachidonic acid (e.g. PGE\(_2\)). In fact it has been demonstrated that PGE\(_3\) does not inhibit Con A-stimulated lymphocyte proliferation [12,21]. This suggests that fatty acids inhibit lymphocyte proliferation by an eicosanoid (or at least a PGE)-independent mechanism. The strongest evidence for an eicosanoid-independent mechanism comes from experiments using inhibitors of eicosanoid synthesis such as quinacrine (inhibits phospholipase \(A_2\)), indomethacin (inhibits cyclo-oxygenase), caffeic acid and nordihydroguaiaretic acid (inhibit lipoxygenase). Addition of such inhibitors to lymphocyte culture medium enhances proliferation [12], but, importantly, fatty acids retain their inhibitory effect in the presence of these compounds [5,12,15,17]. The major source of eicosanoids in lymphocyte cultures is believed to be macrophages present in the cell preparation (see [12] for references). Nevertheless, fatty acids inhibit the proliferation of thoracic duct lymphocytes that are devoid of phagocytes [12], of lymphocyte preparations that have been depleted of macrophages [12] and of lymphocyte cell lines [22].

Owing to the presence of double bonds, PUFAs are sensitive to peroxidation, and the resulting lipid peroxides are toxic to certain cells. Therefore one explanation for the inhibitory effect of unsaturated fatty acids upon lymphocyte proliferation could be the formation of toxic peroxides. If antioxidants such as \(\alpha\)-tocopherol are included in the lymphocyte culture medium they prevent the formation of lipid peroxidation products [24]. However, such antioxidants do not prevent the inhibitory effects of fatty acids [15,24], leading to the conclusion that these effects are independent of lipid peroxidation.

Fatty acids, ketone bodies and short-chain fatty acids can all be used by lymphocytes as fuels [25–27]. It is possible that fatty acids exert their inhibitory effects upon lymphocyte proliferation by inhibiting the utilization of other, key, fuels, such as glucose and glutamine, by lymphocytes. However, the relative rates of utilization of fatty acids as fuels by lymphocytes [26] do not coincide with their inhibitory effects. Also, the inclusion of potent inhibitors of fatty acid oxidation in the lymphocyte culture medium does not prevent the inhibition of lymphocyte proliferation [16]. Furthermore, fatty acids do not appear to decrease the rates of utilization of glucose or glutamine by lymphocytes in culture or the rate of conversion of glucose to lactate [16,25]. In addition, lymphocyte proliferation is not inhibited by ketone bodies or by all short-chain fatty acids [16]. Thus it appears that the inhibition of lymphocyte proliferation by fatty acids is independent of their use as fuels.

When lymphocytes are stimulated in culture they undergo subtle changes in the fatty acid composition of their plasma membrane phospholipids [28,29]; in addition there is an increase in plasma membrane fluidity [28,29]. Addition of fatty acids to the culture medium results in their uptake by the cells and subsequent incorporation into phospholipids [29]. As a result the fatty acid composition of the phospholipids is markedly changed [29]. Such manipulation of the fatty acid composition of the cells also results in significant changes in plasma membrane fluidity [29]. It is possible that these changes in some way affect the ability of the cells to respond optimally to mitogenic stimulation. Changes in plasma membrane fatty acid composition and/or fluidity could affect ion or substrate transport into the cells, the activity of membrane-associated enzymes (adenylate cyclase, protein kinase C, phospholipases), the functioning of receptors and/or signal transduction processes.

**Effects of fatty acids on lymphocyte-derived cytokine production**

The proliferation of lymphocytes depends upon the production of IL-2. The possibility that fatty acids suppress proliferation by inhibiting IL-2 production has been investigated by measuring the concentra-
tion of IL-2 in the medium of mitogen-stimulated lymphocytes. Culture of Con A-stimulated rat lymph node lymphocytes with oleic, linoleic, α-linolenic, arachidonic, eicosapentaeanoic or docosahexaenoic acids resulted in a 40–50% lower concentration of IL-2 in the culture medium than when the cells were cultured in the absence of fatty acids or in the presence of saturated fatty acids [13]. Similarly, culture of human PBLs with these unsaturated fatty acids resulted in 70–90% lower IL-2 concentrations in the culture medium [14]. These findings suggest that unsaturated fatty acids may affect IL-2 production by mitogen-stimulated lymphocytes. However, lymphocyte proliferation could not be returned to control levels by the addition of exogenous IL-2 to the culture medium [13]. Thus unsaturated fatty acids may inhibit lymphocyte proliferation by exerting effects upon two or more key processes, including IL-2 production. It is worth noting that the response of lymphocytes to IL-2 is also diminished by culture with unsaturated fatty acids [5,21,22].

Effects of fatty acids on lymphocyte-mediated cytolyis

Lymphocyte-mediated cytolysis of target cells is performed by cytotoxic T lymphocytes (CTLs) and by natural killer (NK) cells. Experimentally the activity of these cell types is measured as the lysis in vitro of suitable target cell lines. Two studies investigated the effect of a range of fatty acids (pentadecanoic, heptadecanoic, stearic, nonadecanoic, palmitoleic, oleic, elaidic, linoleic) upon the activity of murine spleen CTLs [30,31]; these studies showed that the saturated fatty acids suppressed, whereas oleic, elaidic and linoleic acids enhanced, CTL activity. Several of the fatty acids used in these studies are physiologically rare. In another study, Richieri et al. [32] showed that oleic, linoleic, α-linolenic and arachidonic acids inhibit the extracellular release of the contents of the granules that are responsible for target cell killing by rat spleen CTLs; this observation suggests that these unsaturated fatty acids should inhibit CTL activity.

Rice et al. [33] reported that γ-linolenic acid significantly suppressed the NK activity of human peripheral blood lymphocytes, whereas oleic acid caused a marginal enhancement of this activity. More recently, Yamashita et al. [34,35] showed that TAGs containing either eicosapentaeanoic or docosahexaenoic acid cause concentration-dependent inhibition of human peripheral blood NK cell activity.

Dietary lipids and lymphocyte functions

Effects of dietary lipids on lymphocyte proliferation

Because mitogen-stimulated lymphocyte proliferation is relatively simple to measure in vitro and because it is believed to be a reliable measure of lymphocyte function in vivo, many studies have used this test to assess the effects of dietary lipids upon lymphocytes. However, it is difficult to make direct comparisons between these studies because they differ greatly in the amount of fat used, the composition of other dietary components, the amount and type of antioxidant present, the duration of feeding and the age, sex and species of animals used. Furthermore, it is now apparent that the result of lymphocyte proliferation measurements ex vivo is strongly influenced by the cell culture conditions, particularly the nature of the serum used [36].

Studies in laboratory animals

A number of studies have shown that feeding rats or mice on diets rich in n-6 PUFAs, such as corn, soybean, safflower or sunflower oils, results in suppressed Con-A- or PHA-stimulated proliferation of spleen lymphocytes ex vivo compared with feeding on diets rich in saturated fatty acids, such as lard, tallow or hydrogenated coconut oil, or low-fat diets [37–44]. In contrast, some studies have shown that feeding rodents on n-6 PUFA-rich diets does not affect [45] or enhances [46] T-cell proliferation ex vivo. The results of investigations on B lymphocyte proliferation are also contradictory: it has been reported that n-6 PUFA-rich diets suppress [46], do not affect [38,44,45] or enhance [46] LPS-stimulated proliferation of rodent spleen lymphocytes ex vivo. Friend et al. [47] reported that feeding guinea pigs on a corn oil-rich diet results in suppressed lymph node lymphocyte proliferation in response to PHA, whereas de Deckere et al. [48] reported that the Con A- or PHA-stimulated proliferation of rabbit PBLs was unaffected by feeding on palm or sunflower oils.

There is little doubt that many of the differences in the effects reported by these studies are due to differences in the level of fat fed, the duration of feeding and the cell culture conditions used when measuring proliferation. There also appear to be differences due to the anatomical site of origin of the lymphocytes and the species and strain of animal used. The latter is clearly illustrated by the study of Alexander and Smythe [49], who showed that feeding fish oil to BALB/c mice significantly
decreased Con A-stimulated spleen lymphocyte proliferation compared with feeding on lard or corn oil, whereas feeding NZB/NZW mice on fish oil had no effect on spleen lymphocyte proliferation. That the anatomical site of origin of the lymphocytes to be tested may affect the experimental result observed is shown by the report of Locniskar et al. [40] that feeding rats on corn oil suppressed the proliferation of spleen, but not lymph node lymphocytes in response to Con A or PHA.

In recent years there has been increased interest in the effects of \( \text{n}-3 \) PUFA-containing oils (linseed, canola, fish) on lymphocyte proliferation. Marshall and Johnston [42] showed that feeding rats on a linseed oil-rich diet suppressed spleen T lymphocyte proliferation compared with feeding on hydrogenated coconut oil. Kelley et al. [50] found that feeding fish oil to rabbits suppressed the proliferation of spleen T and peripheral blood T (and B) lymphocytes compared with feeding hydrogenated coconut, safflower or linseed oils. Fritsche et al. [51] showed that feeding fish oil to chickens significantly suppressed spleen lymphocyte proliferation in response to Con A or PWM compared with feeding linseed, canola or corn oils or lard; furthermore, feeding linseed oil suppressed proliferation compared with feeding canola or corn oil or lard. These effects were demonstrated when the cells were cultured in autologous serum, but were lost if the cells were cultured in foetal calf serum (FCS). Similarly, Meydani et al. [52] reported that feeding cebus or squirrel monkeys on a corn oil diet for several years resulted in suppression of PBL proliferation in response to either PHA or PWM, compared with feeding on coconut oil, but that these effects were observed only if the cells were cultured in autologous serum; FCS completely masked the differential effects of the diets. Culture of cells in FCS may explain the lack of effect upon spleen lymphocyte proliferation of feeding fish, linseed, safflower or olive oils to mice that was recently reported by Berger et al. [53].

Recently we reported the results of a direct comparison of feeding weanling rats for 10 weeks on diets containing 20% hydrogenated coconut oil, olive oil, safflower oil, evening primrose oil or fish (menhaden) oil; all other components of the diets, including the levels of antioxidants, were identical. Feeding each of these diets suppressed Con A- and PHA-stimulated spleen lymphocyte proliferation \( \text{ex vivo} \) compared with feeding a low-fat diet [36], and feeding the olive oil, evening primrose oil or menhaden oil diets suppressed Con A- and PHA-stimulated proliferation of lymph node lymphocytes compared with feeding the low-fat, hydrogenated coconut oil or safflower oil diets [36]. For both cell types the effects of the dietary manipulation were masked if the cells were cultured in FCS rather than autologous serum [36]. We have recently shown that the effects of feeding these diets to more mature rats upon spleen lymphocyte proliferation are very similar to the effects of feeding to weanling rats [54]. Furthermore we found that the diets that appear to be particularly immunosuppressive (i.e. olive, evening primrose and fish oils) decrease the level of expression of adhesion molecules (CD2, ICAM-1, LFA-1) on resting and/or mitogen-stimulated spleen lymphocytes [54]. Spleen lymphocytes from animals fed on these diets also showed a lower level of expression of the IL-2 receptor after mitogenic stimulation [54]. Thus we proposed that the immunomodulatory effects of dietary lipids may be due to the decreased level of expression of key surface molecules.

The use of a whole-blood culture system to test the effects of fatty acids on lymphocyte functions avoids the removal of erythrocytes and the loss of non-cellular physiological components normally in contact with the cells in the circulation. It also avoids the need for the addition of serum, either autologous or from a different species. Importantly, the technique has the advantage that the ratios between different cell types and the ratios between cells and components such as growth factors and nutrients are the same as those \textit{in vivo}. It was found that feeding rats for 10 weeks on diets containing hydrogenated coconut oil, olive oil or fish oil suppressed the proliferation of lymphocytes in response to low concentrations of Con A (or PHA) in whole-blood culture compared with feeding on a low-fat diet, or diets containing safflower or evening primrose oil [55].

\textbf{Studies in man}

Kelley et al. [56,57] reported that a reduction in total fat intake enhanced PBL proliferation in response to Con A or PHA, suggesting that high-fat diets might suppress human lymphocyte proliferation. The studies of the effects of dietary lipid manipulation on human lymphocyte proliferation have been restricted to investigating the effects of fish oils and fish oil-derived fatty acids. Meydani et al. [58] reported the results of supplementing the diets of healthy young (22-33 years of age) or older (51-68 years of age) women with encapsulated \( \text{n}-3 \) PUFAs (approximately 2.4 g/day) for 12 weeks; the mitogenic response of PBLs to PHA was lowered after 12 weeks of supplementation in
the older women. Virella et al. [59] found that feeding healthy volunteers on 8 g of fish oil/day did not affect the response of PBLs to Con A or PHA, although there was a trend towards a decrease in the response to anti-CD3. Recently, Meydani et al. [60] reported a decreased response of PBLs to Con A or PHA after supplementation of volunteers on a low-fat, low-cholesterol diet with encapsulated n-3 PUFAs, whereas Endres et al. [61] found that 18 g of fish oil/day for 6 weeks resulted in lowered PHA-stimulated proliferation of PBLs 10 weeks after supplementation had ended (but not at the end of the supplementation period).

**Effects of dietary lipids on lymphocyte-mediated cytolyis**

Olson et al. [44] showed that feeding weanling mice on 5 or 20% (by weight) soybean oil for 9 months resulted in suppression of spleen cytotoxic T lymphocyte (CTL) activity, with the cells from the 20% group having lower activity. This finding was supported by Erickson [62], who found that feeding mice on 8 or 20% safflower oil for 4 weeks decreased spleen CTL activity compared with feeding on a fat-free diet, a low-fat diet (containing corn oil) or diets containing 8 or 20% hydrogenated coconut oil. Fritsche and Johnston [63] reported that the CTL activity of mouse spleen lymphocytes was higher after feeding mice on 10% linseed oil for up to 10 weeks than after feeding on 10% corn oil.

Erickson and Schumacher [64] reported no effect of feeding mice for 4 weeks on diets containing 5% or 20% palm or safflower oil on spleen natural killer (NK) cell activity compared with feeding on a low-fat diet. Similarly, Leung and Ip [65] showed little effect of feeding 20% corn oil to rats for up to 87 days on spleen NK cell activity. In contrast to these studies, Morrow et al. [41] showed that feeding weanling mice on 9% lard or 9% corn oil decreased spleen NK cell activity compared with feeding on 1% corn oil; the 9% lard diet caused a greater decrease than the 9% corn oil diet. Meydani et al. [66] found that feeding young mice on a diet containing 10% fish oil for 6 weeks caused a decrease (not statistically significant) in spleen NK cell activity compared with feeding on chow or 10% corn oil; there were no differences in NK cell activity when these diets were fed to older mice. In the study of Berger et al. [53] female mice were fed for 5 months on diets containing 10% olive, safflower, linseed or fish oil and the spleen NK cell activity of the pups was determined before they were weaned; the activity was lower in the fish oil group than in the safflower or olive oil groups.

Recently, Yaqoob et al. [67] fed weanling rats for 10 weeks on a low-fat diet or on diets containing 20% hydrogenated coconut oil, olive oil, safflower oil, evening primrose oil or fish oil before measuring spleen NK cell activity. It was found that feeding each of the high-fat diets resulted in lower NK cell activity compared with feeding the low-fat diet; the fish oil diet was the most suppressive, followed by the olive oil and evening primrose oil diets [67]. Similar results have been found in more mature rats fed on these diets for 12 weeks [54].

No studies have investigated the effect of dietary lipids on human NK cell activity, although Barone et al. [68] and Hebert et al. [69] reported that a reduced fat intake is associated with a significant increase in NK activity of PBLs, suggesting that high fat consumption will suppress NK cell activity in man. Yamashita et al. [35] reported that intravenous injection of a TAG containing eicosapentaenoic acid into healthy human volunteers resulted in the suppression of peripheral blood NK cell activity 24 h later.

**Effects of dietary lipids on the production of lymphocyte-derived cytokines**

In contrast to the large number of studies of the effects of dietary lipids, especially fish oils, on the production of macrophage-derived cytokines ex vivo (see [70] for review), there have been only three studies on lymphocyte-derived cytokines. The only animal study that has been reported to date involved feeding weanling mice for 8 weeks on a low-fat diet or on diets containing 20% hydrogenated coconut oil, olive oil, safflower oil or fish oil; the spleen lymphocytes were subsequently stimulated with Con A [71]. This study found that the concentration of IL-2 was higher in the medium of spleen lymphocytes from mice fed on olive or safflower oil than in the medium of cells from mice fed on the low-fat diet or hydrogenated coconut oil; fish oil feeding had no effect on the IL-2 concentration in the medium. However, mitogen-stimulated spleen lymphocytes from mice fed on olive oil, safflower oil or fish oil produced less IL-4 and IL-10 than those from mice fed on the low-fat or hydrogenated coconut oil diets [71].

Two studies have investigated the effects of consumption of fish oil capsules by healthy human volunteers on subsequent IL-2 production by PBLs [58,61]. Both of these studies claimed that dietary supplementation with fish oil capsules lowers IL-2 production. However, close scrutiny of the results indicates that this may not be so. In the study of Endres et al. [61] there was, in fact, no decrease in
the ability of lymphocytes to produce IL-2 at the end of the supplementation period; there was, however, lowered IL-2 production 10 weeks after the supplementation had finished. In the earlier study of Meydani et al. [58], there was no significant effect of fish oil supplementation on the ability of lymphocytes from either young or older women to produce IL-2 after 1, 2 or 3 months of supplementation (there was a non-significant trend towards lowered IL-2 production by lymphocytes from the older women).

**Concluding remarks**

It is clear that unsaturated fatty acids inhibit a variety of lymphocyte functions in vitro, including the proliferation of T- and B-cells, cytokine production, NK cell activity and antigen presentation; it appears that the fish oil-derived n-3 PUFA eicosapentaenoic acid is the most potent inhibitor of these functions. Feeding studies confirm many of the observations in vitro: diets rich in unsaturated fatty acids, especially fish oil, result in suppression of lymphocyte functions, although it is clear that the exact result is affected by the conditions of the feeding regimen and by the subsequent cell culture conditions. These studies strongly suggest that dietary fish oil, and perhaps also olive and some n-6 PUFA-containing oils, are immunosuppressive.

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