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## Symposium on 'Lipids and the immune system'

### Dietary fatty acids and lymphocyte functions

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#### Lymphocytes

Lymphocytes are the cells that specifically recognize and respond to foreign antigens. They are present as circulating cells in blood and lymph, as anatomically-defined collections of cells in lymphoid organs (thymus, spleen, lymph nodes) or as scattered cells in other tissues. Lymphocytes exist as distinct subsets that have quite different functions and protein products, although they appear to be morphologically similar. The principal types of lymphocytes are T and B lymphocytes and natural killer (NK) cells; along with monocytes, lymphocytes are termed mononuclear cells.

#### T lymphocytes

The precursors of T lymphocytes arise in the bone marrow and mature in the thymus. T lymphocytes are further subdivided into functionally-distinct populations, the best defined of which are helper T (Th) lymphocytes and cytotoxic T lymphocytes (CTL); these classes of T lymphocytes are defined by the presence on their surface of CD4 or CD8 molecules respectively. T lymphocytes do not produce antibodies. They recognize peptide antigens attached to major histocompatibility complex proteins on the surface of so-called antigen-presenting cells. In response to antigenic stimulation T lymphocytes secrete cytokines, whose function is to promote the proliferation and differentiation of the T lymphocytes as well as other cell types, including B lymphocytes, NK cells and macrophages. CTL lyse cells that produce foreign antigens, such as cells infected by viruses or intracellular microbes.

The Th lymphocytes are further subdivided according to the pattern of cytokines they produce (Fig. 1). It is believed that naive Th cells produce mainly interleukin (IL)-2 on initial encounter with antigen. These cells may differentiate into a population sometimes referred to as Th0 cells, which differentiate further into either Th1 or Th2 cells (Fig. 1). This differentiation is regulated by cytokines; IL-12 and

interferon- $\gamma$  (IFN- $\gamma$ ) promote the development of Th1 cells, while IL-4 promotes the development of Th2 cells (Fig. 1). Th1 and Th2 themselves have relatively restricted profiles of cytokine production; Th1 cells produce IL-2 and IFN- $\gamma$  which activate macrophages, NK cells and CTL and are the principal effectors of cell-mediated immunity and delayed-type hypersensitivity. Interactions with intracellular microbes tend to induce Th1 activity. Th2 cells produce IL-4, which stimulates immunoglobulin (Ig)E production, IL-5, an eosinophil-activating factor, and IL-10, which together with IL-4 suppresses cell-mediated immunity (Fig. 1). Th2 cells are responsible for defence against helminthic parasites and for allergic reactions, which are due to IgE-mediated activation of mast cells and basophils.

#### B lymphocytes

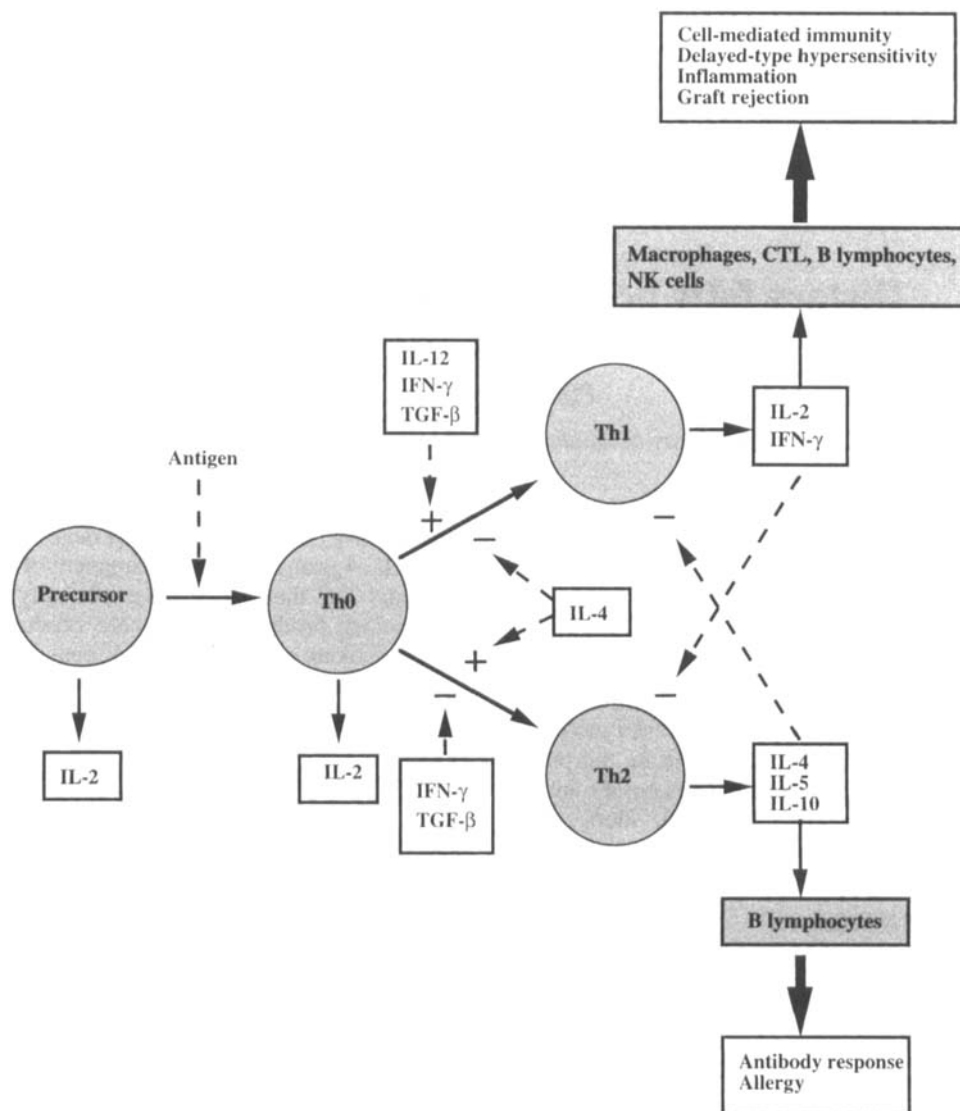
These are the cells responsible for producing antibodies. The role of these antibodies is to neutralize and eliminate the antigen that induced their formation. The antibodies produced belong to different Ig classes, depending on the type of stimulus and the anatomical site of the lymphocytes involved. Cytokines determine the types of antibodies produced by selectively promoting Ig heavy-chain class switching and by stimulating B lymphocyte proliferation. The most potent cytokines involved in B lymphocyte activation are those produced by Th cells (Fig. 2).

#### Natural killer cells

These are a class of lymphocytes which do not express surface markers identifying them as either T or B lymphocytes. They are capable of lysing tumour- or virus-infected cells and have a role in graft rejection. NK cells are activated by IL-2, IL-12, IFN- $\gamma$  or tumour necrosis factor- $\alpha$ . Treatment of NK cells with IL-2 causes them to differentiate into lymphokine-activated killer cells.

**Abbreviations:** Con A, concanavalin A; CTL, cytotoxic T lymphocytes; DTH, delayed-type hypersensitivity; G v. H, graft v. host; H v. G, host v. graft; IFN- $\gamma$ , interferon- $\gamma$ ; Ig, immunoglobulin; IL, interleukin; NK, natural killer; PBMNC, peripheral blood mononuclear cells; PG, prostaglandin; PHA, phytohaemagglutinin; PUFA, polyunsaturated fatty acids; Th, helper T lymphocytes.

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**Fig. 1.** Differentiation and activities of helper T (Th) lymphocytes. CTL, cytotoxic T lymphocytes; IFN- $\gamma$ , interferon- $\gamma$ ; IL, interleukin; NK, natural killer; TGF- $\beta$ , transforming growth factor- $\beta$ ; +, promote; -, suppress; --->, modulate; -->, produce.

### Dietary fatty acids and immune cell functions

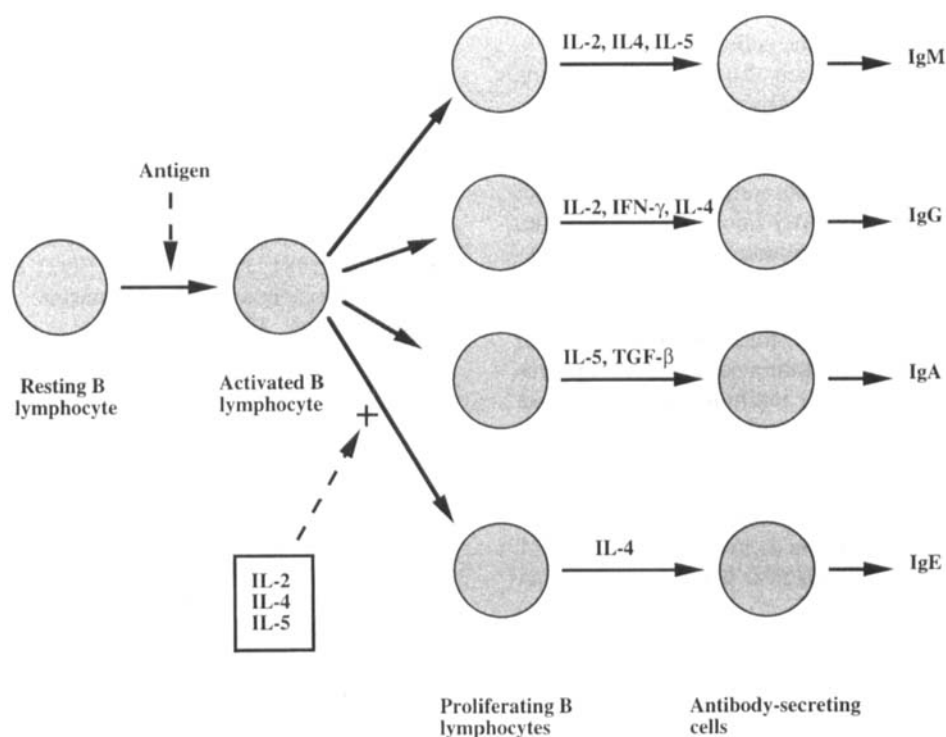
Fatty acid nomenclature and the key dietary sources of different fatty acids are outlined in Table 1. Interest in the effects of fatty acids and dietary lipids on the immune system dates back many years (for an early review, see Meade & Mertin, 1978); but this interest has intensified with the elucidation of the roles of eicosanoids derived from arachidonic acid in modulating inflammation and immunity (for reviews, see Goodwin & Cueppens, 1983; Hwang, 1989; Roper & Phipps, 1994), and with the knowledge that the metabolism of arachidonic acid to yield these mediators can be inhibited by the long-chain *n*-3 polyunsaturated fatty acids (PUFA) found in some marine fish oils (for reviews, see Hwang, 1989; Kinsella *et al.* 1990; Calder, 1996*a,b,c*). The present review will describe the effects of fatty acids on lymphocyte proliferation, lymphocyte-mediated cytotoxicity, lymphocyte-derived cytokine production, antibody production, and cell-mediated immunity. These and other aspects of lipids and immunity have been reviewed a number of times (Gurr,

1983; Peck, 1994; Calder, 1995, 1996*a,b,c*, 1997, 1998; Blok *et al.* 1996; Miles & Calder, 1998); thus, the present review will focus on more recent advances. Similarly, mechanisms by which fatty acids might influence the functions of lymphocytes will not be reviewed in depth; these have been discussed elsewhere (Calder, 1996*c*; Miles & Calder, 1998).

### *In vitro* effects of fatty acids on lymphocyte functions

#### *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 lymphocytes only, bacterial lipopolysaccharide which stimulates B lymphocytes only, and pokeweed (*Phytolacca americana*) mitogen 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,



**Fig. 2.** B lymphocyte differentiation. IFN- $\gamma$ , interferon- $\gamma$ ; Ig, immunoglobulin; IL, interleukin; TGF- $\beta$ , transforming growth factor- $\beta$ ; +, promote;  $-\rightarrow$ , modulate;  $\rightarrow$  produce.

**Table 1.** Fatty acid nomenclature and sources

Systematic name	Trivial name	Shorthand notation	Sources
Dodecanoic	Lauric	12 : 0	<i>De novo</i> synthesis Coconut oil
Tetradecanoic	Myristic	14 : 0	<i>De novo</i> synthesis Milk
Hexadecanoic	Palmitic	16 : 0	<i>De novo</i> synthesis Milk, eggs, animal fats, meat, cocoa butter, palm oil (other vegetable oils contain lesser amounts), fish oils
Octadecanoic	Stearic	18 : 0	<i>De novo</i> synthesis Milk, eggs, animal fats, meat, cocoa butter
9-Hexadecenoic	Palmitoleic	16 : 1 <i>n</i> -7	Desaturation of palmitic acid Fish oils
9-Octadecenoic	Oleic	18 : 1 <i>n</i> -9	Desaturation of stearic acid Milk, eggs, animal fats, meat, cocoa butter, most vegetable oils especially olive oil
9,12-Octadecadienoic	Linoleic	18 : 2 <i>n</i> -6	Cannot be synthesized in mammals Some milks, eggs, animal fats, meat, green leaves, most vegetable oils especially maize, sunflower, safflower and soyabean oils
9,12,15-Octadecatrienoic	$\alpha$ -Linolenic	18 : 3 <i>n</i> -3	Cannot be synthesized in mammals Green leaves, some vegetable oils especially rapeseed, soyabean and linseed oils
6,9,12-Octadecatrienoic	$\gamma$ -Linolenic	18 : 3 <i>n</i> -6	Synthesized from linoleic acid Borage* and evening primrose† oils
11,14,17-Eicosatrienoic	Mead	20 : 3 <i>n</i> -9	Synthesized from oleic acid Indicator of essential fatty acid deficiency
8,11,14-Eicosatrienoic	Dihomo- $\gamma$ -linolenic	20 : 3 <i>n</i> -6	Synthesized from $\gamma$ -linolenic
8,11,14,17-Eicosatetraenoic	Arachidonic	20 : 4 <i>n</i> -6	Synthesized from linoleic acid via $\gamma$ -linolenic and dihomo- $\gamma$ -linolenic acids
5,8,11,14,17-Eicosapentaenoic	Eicosapentaenoic	20 : 5 <i>n</i> -3	Synthesized from $\alpha$ -linolenic acid Fish oils
4,7,10,13,16,19-Docosahexaenoic	Docosahexaenoic	22 : 6 <i>n</i> -3	Synthesized from $\alpha$ -linolenic acid via eicosapentaenoic acid Fish oils

\* *Borago officinalis*.

† *Oenothera biennis*.

more conveniently, by measuring the incorporation of [<sup>3</sup>H]thymidine into the DNA of the cells.

Low concentrations (less than 5 μM) of fatty acids enhance mitogen-stimulated proliferation of lymphocytes. However, once a particular fatty acid concentration is exceeded (approximately 10–15 μM), many fatty acids begin to inhibit lymphocyte proliferation. Oleic, linoleic, α-linolenic, γ-linolenic, dihomo-γ-linolenic, arachidonic, eicosapentaenoic and docosahexaenoic acids inhibit Con A- and/or PHA-stimulated proliferation of lymphocytes isolated from rodent lymphoid tissues (lymph nodes, spleen, thymus) and from human peripheral blood (for references, see Gurr, 1983; Calder, 1995, 1996a,b,c); saturated fatty acids are less inhibitory or without effect. The inhibition of lymphocyte proliferation by unsaturated fatty acids is dependent on the concentration of fatty acid used, the time during culture of fatty acid addition, and the duration of exposure of the cells to the fatty acid. 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; an approximate order of potencies is:

lauric = myristic < palmitic < stearic = oleic < linoleic =  
α-linolenic < γ-linolenic = dihomo-γ-linolenic =  
docosahexaenoic < arachidonic ≤ eicosapentaenoic.

Recent studies have confirmed that γ-linolenic, eicosapentaenoic and docosahexaenoic acids inhibit mitogen-stimulated lymphocyte proliferation (Khalfoun *et al.* 1996b; Purasiri *et al.* 1997). The inhibition of lymphocyte proliferation by unsaturated fatty acids is not prevented by inhibitors of phospholipase A<sub>2</sub> (EC 3.1.1.4), cyclooxygenase (EC 1.14.99.1) or lipoxygenase (EC 1.13.11.12; Santoli *et al.* 1990; Calder *et al.* 1992; Kumar *et al.* 1992; Soyland *et al.* 1993; Rotondo *et al.* 1994; Khalfoun *et al.* 1996b), suggesting that the effects of the fatty acids are independent of eicosanoid synthesis. Furthermore, the inhibition of proliferation by unsaturated fatty acids is not prevented by α-tocopherol and other antioxidants (Calder & Newsholme, 1993; Soyland *et al.* 1993; Khalfoun *et al.* 1996b), suggesting that the effects of the fatty acids are independent of lipid peroxidation.

#### *Lymphocyte-derived cytokine production*

Culture of Con A-stimulated rat lymph node or human peripheral blood lymphocytes with oleic, linoleic, α-linolenic, arachidonic, eicosapentaenoic or docosahexaenoic acids results in lower IL-2 concentrations in the culture medium than if the cells are cultured in the absence of fatty acids or in the presence of saturated fatty acids (Calder & Newsholme, 1992a,b). It has been recently confirmed that eicosapentaenoic and docosahexaenoic acids inhibit IL-2 production by human peripheral blood mononuclear cells (PBMNC) *in vitro* (Purasiri *et al.* 1997); in contrast, low concentrations of γ-linolenic acid enhanced IL-2 production (Purasiri *et al.* 1997). This recent study also examined, for the first time, the effect of PUFA on IFN-γ production by cultured PBMNC; low levels of docosahexaenoic acid enhanced production, while γ-linolenic and eicosapentaenoic acids were without effect at the concentrations used (Purasiri *et al.* 1997).

#### *Lymphocyte-mediated cytotoxicity*

Oleic, linoleic, α-linolenic and arachidonic acids inhibit the extracellular release of the contents of the granules which are responsible for target cell killing by rat spleen CTL (Richieri & Kleinfeld, 1990); this observation suggests that these unsaturated fatty acids should inhibit CTL activity.

Human peripheral blood NK cell activity has been shown to be suppressed by culture of the cells with γ-linolenic acid (Rice *et al.* 1981; Purasiri *et al.* 1997), or eicosapentaenoic or docosahexaenoic acids (Yamashita *et al.* 1986, 1991; Purasiri *et al.* 1997). Lymphokine-activated killer cell activity is also suppressed by culture with γ-linolenic, eicosapentaenoic or docosahexaenoic acids (Purasiri *et al.* 1997).

#### *Lymphocyte surface molecule expression*

Adhesion molecules are involved in many cell-to-cell interactions. For example, interaction between T lymphocytes and antigen-presenting cells is, in part, mediated by the ligand-receptor pairs CD11a and CD18-CD54, CD11a and CD18-CD102 and CD2-CD58 (for a review, see Springer, 1990). Thus, an efficient cell-mediated immune response requires appropriate levels of expression of these molecules on T lymphocytes. In addition, lymphocyte adhesion to the endothelium involves a number of ligand-receptor pairs including CD11a and CD18-CD54, CD54-CD11a and CD18, CD49d and CD29-CD106, CD2-CD58, CD62L-MAcCAM-1 and CD44-hyaluronate (for reviews, see Stoolman, 1989; Springer, 1990; Hogg & Landis, 1993). Thus, movement of lymphocytes between body compartments, into and out of lymphoid organs, and into sites of immune or inflammatory reactivity requires adhesion molecule expression. Adhesion molecule expression appears to be involved in several acute and chronic inflammatory disease processes (for a review, see Faull, 1995), and antibodies against certain adhesion molecules can reduce chronic inflammatory disease (see Faull, 1995).

Khalfoun *et al.* (1996a) have recently shown that incubation of human peripheral blood lymphocytes with eicosapentaenoic or docosahexaenoic acids reduces the cell surface expression of the adhesion molecules L-selectin (CD62L) and leucocyte-function-associated antigen 1 (CD11a and CD18) without affecting expression of very late antigen 1 (CD49a and CD29); arachidonic acid did not influence surface expression of these molecules. In accordance with the effects on expression of some cell surface adhesion molecules, incubation of human lymphocytes with eicosapentaenoic or docosahexaenoic acids reduced adhesion between the lymphocytes and untreated or cytokine- or bacterial lipopolysaccharide-stimulated endothelial cells (Khalfoun *et al.* 1996a).

#### *Antibody production*

A recent study has examined in detail the effects of culturing rat mesenteric lymph node lymphocytes with different fatty acids on Ig production (Yamada *et al.* 1996). These workers measured total IgM, IgE, IgG and IgA production by the cells, which were not stimulated in culture. Saturated fatty acids (lauric, myristic, palmitic, stearic) at a concentration of



10  $\mu\text{M}$  did not alter IgM, IgE or IgG production, but they reduced IgA production by approximately 30 % (Yamada *et al.* 1996). Unsaturated fatty acids (oleic, linoleic, arachidonic) did not affect Ig production at concentrations below 100  $\mu\text{M}$ . However, at a concentration of 1 mM these fatty acids strongly inhibited production of IgM, IgG and IgA and enhanced the production of IgE;  $\alpha$ -linolenic,  $\gamma$ -linolenic, eicosapentaenoic and docosahexaenoic acids also enhanced IgE production. The pattern of these effects suggests that unsaturated fatty acids suppress the production of Ig which are promoted by Th1-type cytokines (see Fig. 2; this is in accordance with their reported inhibitory effects on IL-2 production), and enhance the production of IgE which is promoted by Th2-type cytokines (see Fig. 2). Thus, the effects of unsaturated fatty acids on Ig production might be mediated via their differential effects on the two classes of Th lymphocytes.

Ascorbic acid had only minimal influence on the effects of arachidonic acid on Ig production (Yamada *et al.* 1996). However,  $\alpha$ -tocopherol partly or totally abolished the IgE-enhancing effect of oleic,  $\alpha$ -linolenic,  $\gamma$ -linolenic, arachidonic, eicosapentaenoic and docosahexaenoic acids, although it did not affect the inhibition of production of the other Ig by arachidonic acid (Yamada *et al.* 1996).

### Effects of the amount and type of fat in the diet on lymphocyte functions

#### *Lymphocyte proliferation: studies in laboratory animals*

*Effect of the amount of fat in the diet.* Many studies have compared the effects of feeding laboratory animals on low- and high-fat diets on lymphocyte proliferation (for references, see Calder, 1995, 1996a,b,c, 1998). Such studies have often found that high-fat diets result in diminished *ex vivo* lymphocyte proliferation compared with low-fat diets, but the precise effect depends on the level of fat used in the high-fat diet and its source. These studies have been reviewed in detail elsewhere (Calder, 1995, 1996a,b,c, 1998) and will be summarized here before more recent studies are described.

*Effect of saturated fatty acids.* High-fat diets using lard, beef tallow, palm oil or hydrogenated coconut oil as the source of fat have been used in many studies of lymphocyte proliferation; in some studies the high-saturated-fat diet has been compared, along with other high-fat diets, with a low-fat control, while in other studies the high-saturated-fat diet appears to serve as a high-fat 'control' with which the effects of PUFA-rich diets were compared (for references, see Calder, 1995, 1996a,b,c, 1998). Some studies have revealed that high-saturated-fat diets do not affect lymphocyte proliferation compared with feeding low-fat diets, while others have shown that they are suppressive, but less so than PUFA-rich diets (for references, see Calder, 1995, 1996a,b,c, 1998).

*Effect of n-6 polyunsaturated fatty acids.* Several studies have reported lower Con A- or PHA-stimulated T lymphocyte proliferation following the feeding of diets rich in maize or safflower oils to laboratory rodents compared with feeding diets rich in saturated fatty acids (for references, see Calder, 1995, 1996a,b,c, 1998). In contrast, some studies

have reported no effect of feeding linoleic acid-rich diets on rodent T lymphocyte proliferation (for references, see Calder, 1995, 1996a,b,c, 1998). However, it is now apparent that the outcome of such measures of lymphocyte function is strongly influenced by the conditions used to culture the cells *ex vivo*, and this may account for the discrepancies in the literature.

One study has reported diminished rat lymph node and spleen lymphocyte proliferation following the feeding of a diet rich in evening primrose oil (*Oenothera biennis*; Yaqoob *et al.* 1994a).

*Effect of oleic acid.* Berger *et al.* (1993) reported that a 100 g olive oil/kg diet did not affect Con A-stimulated proliferation of spleen lymphocytes; this study cultured the lymphocytes in fetal calf serum. In contrast, feeding rats on a 200 g olive oil/kg diet resulted in diminished *ex vivo* lymphocyte proliferation if the cells were cultured in autologous serum (but not if they were cultured in fetal calf serum; Yaqoob *et al.* 1994a); this finding was confirmed recently using a 200 g oleic acid-rich sunflower oil/kg diet (Jeffery *et al.* 1996c).

*Effect of  $\alpha$ -linolenic acid.* Feeding rats on diets containing large amounts of linseed oil (rich in  $\alpha$ -linolenic acid) suppressed spleen T lymphocyte proliferation compared with feeding diets rich in hydrogenated coconut oil (Marshall & Johnston, 1985) or sunflower oil (Jeffery *et al.* 1996a). Similarly, feeding chickens on a linseed oil-rich diet suppressed spleen lymphocyte proliferation compared with feeding diets rich in rapeseed or maize oils or lard (Fritsche *et al.* 1991).

*Effect of eicosapentaenoic and docosahexaenoic acids.* Feeding diets rich in fish oil to rabbits, chickens, rats or mice results in suppressed proliferation of T (and in some studies B) lymphocytes compared with feeding hydrogenated coconut, safflower, maize or linseed oils, or lard (Alexander & Smythe, 1988; Kelley *et al.* 1988; Fritsche *et al.* 1991; Yaqoob *et al.* 1994a; Yaqoob & Calder, 1995; Sanderson *et al.* 1995a).

*Summary of animal studies and factors which might account for different outcomes.* Animal studies indicate that high-fat diets in general lower T lymphocyte proliferation compared with low-fat diets. Among high-fat diets the order of potency is:

saturated fat < n-6 PUFA-rich oils  $\leq$  olive  
oil  $\leq$  linseed oil  $\leq$  fish oil.

However, the extent of inhibition reported by different studies using the same type of oil is variable. Such discrepancies are most probably due to a variety of effects. These include: differences in the amount of fat in the diet and the duration of feeding; the exact comparison being made (i.e. with a low-fat diet or with another type of high-fat diet); the species and strain of animal used; the anatomical source of the lymphocytes used; the mitogen used and its concentration; the type of serum used for the *ex vivo* lymphocyte cultures. The last factor appears to be particularly important. For example, suppressive effects of dietary n-6 or n-3 PUFA were demonstrated when the cells were cultured in autologous serum, but were lost if the cells were cultured in fetal calf serum (Meydani *et al.* 1985; Fritsche *et al.* 1991; Yaqoob *et al.* 1994a). Culture of cells in fetal calf serum may

explain the lack of effect on spleen lymphocyte proliferation of feeding fish, linseed, safflower or olive oils to mice reported by Berger *et al.* (1993), and the earlier report of lack of effect of maize or fish oil feeding on mouse spleen lymphocyte proliferation in response to Con A or bacterial lipopolysaccharide (Cathcart *et al.* 1987). It has been shown that the changes in lymphocyte fatty acid composition induced by dietary manipulations are better maintained if the cells are cultured in autologous rather than fetal calf serum (Yaqoob *et al.* 1995). The effect of serum type in influencing the outcome of lymphocyte proliferation tests is likely to be a major factor in accounting for the discrepancies in the literature, particularly regarding the effects of *n*-6 and *n*-3 PUFA.

#### *Lymphocyte proliferation: studies in man*

A reduction in total dietary fat intake (from 40 to 25 % total energy) resulted in greatly enhanced human PBMNC proliferation in response to Con A or PHA (Kelley *et al.* 1989, 1992a), suggesting that high-fat diets suppress human lymphocyte proliferation. No difference in the responses to T-cell mitogens were observed for human PBMNC taken from volunteers consuming low-fat diets which were rich (12.9 % energy) or poor (3.5 % energy) in *n*-6 PUFA (Kelley *et al.* 1989, 1992a); the cells were cultured in fetal calf serum. Supplementation of the diets of healthy women (51–68 years of age) with encapsulated *n*-3 PUFA (approximately 2.4 g/d) resulted in a lowered mitogenic response of PBMNC to PHA (Meydani *et al.* 1991); the mitogenic response of PBMNC taken from young women (21–33 years of age) supplemented with this level of *n*-3 PUFA was unaffected. More recently, a decreased response of PBMNC to Con A and PHA was reported following supplementation of the diet of volunteers on a low-fat low-cholesterol diet with 1.23 g *n*-3 PUFA/d (Meydani *et al.* 1993), while 18 g fish oil/d (approximately 6 g *n*-3 PUFA/d) for 6 weeks resulted in lowered PHA-stimulated proliferation of peripheral blood lymphocytes 10 weeks after supplementation had ended (but not at the end of the supplementation period) (Endres *et al.* 1993). Soyland *et al.* (1994) reported no effect of 6 g *n*-3 PUFA/d on the proliferative response of PBMNC from patients with inflammatory skin disorders.

#### *Lymphocyte proliferation: recent studies*

Recent animal studies have endeavoured to establish the effects of particular discrete changes in dietary fatty acid composition on lymphocyte proliferation; as a result they represent a step forward from studies involving the feeding of diets containing one, or a mixture of two oils. This is because the oils used in such experiments (e.g. sunflower, safflower, linseed or fish oils) differ greatly in background fatty acid composition, in total PUFA content, and in *n*-6 : *n*-3 PUFA value. The recent studies are summarized in Table 2.

*Effects of saturated, monounsaturated and n-6 polyunsaturated fatty acids.* It appears that lymphocyte proliferation is affected by the nature of the principal saturated fatty acid in the rat diet (Jeffery *et al.* 1997c). In this study diets containing 178 g fat/kg were fed to rats; the diets differed according to the principal saturated fatty acids they contained

(medium-chain, lauric, palmitic or stearic) and according to the position of the palmitic acid on the dietary triacylglycerols (*sn*-1(3) or *sn*-2), but the levels of total saturated, oleic, polyunsaturated, linoleic and  $\alpha$ -linolenic acids were identical. It was found that spleen lymphocyte proliferation in response to Con A was enhanced if the animals were fed on the diet with palmitic acid at the *sn*-2 position of dietary triacylglycerols compared with feeding the other diets (Jeffery *et al.* 1997c).

More recently, the effects of exchanging palmitic, oleic and linoleic acids in the rat diet have been investigated (Jeffery *et al.* 1997a): the diets contained 178 g fat/kg, and apart from the levels of the three fatty acids under study and  $\alpha$ -linolenic acid they were identical (*n*-6 : *n*-3 PUFA value was maintained at 7, and thus if the level of linoleic acid was changed, that of  $\alpha$ -linolenic acid also changed). This study found a significant inverse relationship between lymphocyte proliferation and oleic acid : linoleic acid in the diet (Jeffery *et al.* 1997a), and suggested complex interactions between dietary palmitic, oleic and linoleic acids.

One study of the effect of dietary intervention with oleic acid on human lymphocyte proliferation has been performed (Yaqoob *et al.* 1998). In this study subjects increased their oleic acid intake at the expense of saturated fatty acids. After 2 months there was a trend towards reduced proliferative responses to Con A of whole-blood cultures and of isolated PBMNC, but the effect of diet was not statistically significant (Yaqoob *et al.* 1998).

*Effects of polyunsaturated fatty acid content,  $\alpha$ -linolenic acid and n-6 : n-3 polyunsaturated fatty acids.* Wu *et al.* (1996) fed monkeys for 14 weeks on diets containing 3.5 or 5.3 % energy as  $\alpha$ -linolenic acid (PUFA comprised 28 g/100 g total fatty acids and *n*-6 : *n*-3 PUFA values for the two diets were 1.0 and 0.5 respectively). PBMNC proliferation in response to Con A or PHA was unaffected compared with the basal diet which had an *n*-6 : *n*-3 PUFA value of 36.

In a recent study rats were fed on diets containing 178 g fat/kg but differing in PUFA content (17.5 or 35 g/100 g fatty acids) and *n*-6 : *n*-3 PUFA value (100, 20, 10, 5, 1); the PUFA content was altered by replacing a proportion of palmitic acid with linoleic and  $\alpha$ -linolenic acids (Jeffery *et al.* 1997b). It was found that lymphocyte proliferation decreased as the *n*-6 : *n*-3 PUFA value of the 'low'-PUFA diet decreased; the *n*-6 : *n*-3 PUFA value of the 'high'-PUFA diet did not significantly affect lymphocyte proliferation (Jeffery *et al.* 1997b). At *n*-6 : *n*-3 PUFA values of 100 and 20, the proliferation of lymphocytes from rats fed on the high-PUFA diets was lower than that of rats fed on the low-PUFA diets. This study indicates that dietary  $\alpha$ -linolenic acid reduces lymphocyte proliferation, but that its effect is dependent on the total PUFA content of the diet and its level relative to that of linoleic acid.

The observations of Wu *et al.* (1996) and Jeffery *et al.* (1997b) are in agreement; both studies indicate that replacing a proportion of linoleic acid with  $\alpha$ -linolenic acid in a 'high'-PUFA diet has minimal effect on lymphocyte proliferation.

*Effect of arachidonic acid.* Feeding mice on a diet containing 20 g safflower oil plus 10 g arachidonic acid/kg did not affect Con A-stimulated spleen lymphocyte proliferation compared with feeding a diet containing safflower oil (30 g/kg; Jolly *et al.* 1997). Peterson *et al.* (1998) also observed

**Table 2.** Recent studies of the effects of dietary lipids on lymphocyte proliferation\*

Cell source	Details of diets	Stimulus	Effect	Reference
Rat spleen	200 g/kg; SO v. OO v. HOSO; 6 weeks	Con A	Proliferation decreased in OO and HOSO groups compared with low-fat or SO groups	Jeffery <i>et al.</i> (1996b)
Rat spleen	200 g/kg; sunflower oil v. LO v. mixtures of sunflower oil and LO; 6 weeks	Con A	Proliferation decreased when LO added to diet	Jeffery <i>et al.</i> (1996a)
Monkey blood	30 % energy as fat; total saturated fatty acids, MUFA and PUFA constant; proportion of linoleic acid replaced by either EPA + DHA (1.3 or 3.3 % energy) or $\alpha$ -linolenic acid (3.5 or 5.3 % energy); 14 weeks	Con A, PHA	No effect of $\alpha$ -linolenic acid Increased proliferation as level of EPA + DHA increased	Wu <i>et al.</i> (1996)
Rat spleen	77 or 178 g/kg; fat containing different saturated fatty acids and different positional isomers of palmitic acid (total saturated, MUFA, <i>n</i> -6 PUFA, <i>n</i> -3 PUFA constant); 6 weeks	Con A	Proliferation increased by diet containing palmitic acid at <i>sn</i> -2 position	Jeffery <i>et al.</i> (1997c)
Rat spleen	178 g/kg; fat varying in the proportions of palmitic, oleic, linoleic and $\alpha$ -linolenic acids ( <i>n</i> -6 : <i>n</i> -3 PUFA constant); 6 weeks	Con A	Proliferation decreased as oleic acid : linoleic acid increased	Jeffery <i>et al.</i> (1997a)
Rat spleen	178 g/kg; fat containing low (17.8 g/100 g fat) or high (35 g/100 g fat) proportions of total PUFA and varying in <i>n</i> -6 : <i>n</i> -3 PUFA (total saturated fatty acids and MUFA constant); 6 weeks	Con A	Proliferation decreased as <i>n</i> -6 : <i>n</i> -3 PUFA value of low-PUFA diet decreased Little effect of <i>n</i> -6 : <i>n</i> -3 PUFA value of high-PUFA diet At <i>n</i> -6 : <i>n</i> -3 PUFA value of 100 or 20, proliferation lower for high-PUFA than low-PUFA-fed animals	Jeffery <i>et al.</i> (1997b)
Mouse spleen	30 g SO/kg v. 20 g SO/kg plus 10 g arachidonic acid or EPA or DHA/kg; 10 d	Con A	No effect of arachidonic acid Proliferation decreased (by approximately 80 %) by replacement of $\alpha$ -linolenic acid with either EPA or DHA	Jolly <i>et al.</i> (1997)
Human blood	Arachidonic acid-enriched diet providing 1.5 g arachidonic acid/d (at expense of MUFA); 50 d	Con A, PHA, pokeweed† mitogen, influenza virus	No effect	Kelley <i>et al.</i> (1997)
Rat spleen	178 g/kg; fat with saturated fatty acid, MUFA, total PUFA and <i>n</i> -6 : <i>n</i> -3 PUFA constant; $\alpha$ -linolenic acid (4.4 g/100 g fatty acids) replaced with either EPA or DHA; a proportion (4.4 g/100 g fatty acids) of linoleic acid replaced with either arachidonic acid or GLA; 6 weeks	Con A	No effect of partial replacement of linoleic acid with arachidonic acid or GLA Proliferation decreased (by 20–25 %) by inclusion of either EPA or DHA in diet	Peterson <i>et al.</i> (1998)
Human blood	Enrichment of diet with MUFA at expense of saturated fatty acids; 2 months	Con A	Non-significant decrease in proliferation	Yaqoob <i>et al.</i> (1998)

Con A, concanavalin A; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA,  $\gamma$ -linolenic acid; HOSO, high-oleic sunflower oil; LO, linseed oil; MUFA, monounsaturated fatty acid; OO, olive oil; PHA, phytohaemagglutinin; PUFA, polyunsaturated fatty acid; SO, safflower oil.

\* This table is designed to complement Table 2 of Calder (1996c); it is restricted to results published since 1996.

† *Phytolacca americana*.

that inclusion of 4.4 g arachidonic acid/100 g fatty acids in the rat diet did not affect Con A-stimulated spleen lymphocyte proliferation. These observations agree with those of Kelley *et al.* (1997), who reported the outcome of the first study of dietary arachidonic acid and human lymphocyte function; 1.5 g arachidonic acid/d for 50 d did not affect the proliferative response of PBMNC to Con A, PHA or pokeweed mitogen.

*Effects of eicosapentaenoic and docosahexaenoic acids.* Previous studies (see pp. 491–492) do not indicate whether the suppressive effects of fish oil feeding on lymphocyte proliferation are due to eicosapentaenoic or docosahexaenoic acids or both. Furthermore, there is no indication of the level

of long-chain *n*-3 PUFA required to affect lymphocyte proliferation. These questions have recently been addressed (Jolly *et al.* 1997; Peterson *et al.* 1998). Jolly *et al.* (1997) reported that feeding mice diets containing 20 g safflower oil plus 10 g eicosapentaenoic or docosahexaenoic acid/kg reduced Con A-stimulated spleen lymphocyte proliferation compared with feeding a diet containing 30 g safflower oil/kg; both *n*-3 PUFA were equipotent and reduced proliferation by approximately 80 %. Peterson *et al.* (1998) fed rats on diets containing 178 g fat/kg, and replaced  $\alpha$ -linolenic acid (4.4 g/100 g fatty acids) with either eicosapentaenoic or docosahexaenoic acid while keeping the total PUFA content and *n*-6 : *n*-3 PUFA value of the diet constant. Both

eicosapentaenoic and docosahexaenoic acids reduced lymphocyte proliferation to the same extent (approximately 30–35 %) compared with the diet containing  $\alpha$ -linolenic acid. Thus, these studies suggest that both eicosapentaenoic and docosahexaenoic acids result in inhibition of lymphocyte proliferation, and that relatively low levels are required to exert this effect, compared with the levels present in fish oil.

Wu *et al.* (1996) fed monkeys for 14 weeks on diets containing 1.3 or 3.3 % energy as eicosapentaenoic plus docosahexaenoic acids (PUFA comprised 30 g/100 g total fatty acids and the  $n-6 : n-3$  PUFA values of the two diets were 4.4 and 1.1 respectively); the proliferative response of PBMNC to Con A or PHA was enhanced. This observation is contradictory to previous observations in experimental animals (Alexander & Smythe, 1988; Kelley *et al.* 1988; Fritsche *et al.* 1991; Yaqoob *et al.* 1994a; Yaqoob & Calder, 1995; Sanderson *et al.* 1995a) and human subjects (Meydani *et al.* 1991, 1993; Endres *et al.* 1993). The authors provide some evidence that this discrepancy is due to better maintained levels of vitamin E in their study compared with previous studies, thereby suggesting that long-chain  $n-3$  PUFA inhibit lymphocyte proliferation via a process against which vitamin E protects.

#### Cytotoxic T lymphocyte activity

CTL activity is reduced following feeding of laboratory animals on high-fat diets rich in safflower oil, soyabean oil, linseed oil or fish oil (for references, see Calder, 1995, 1996a,b,c, 1998). Thus, it appears that high-fat diets lower

CTL cell activity compared with low-fat diets. Animal studies indicate that among high-fat diets the order of potency is:

saturated fat <  $n-6$  PUFA-rich oils < linseed oil < fish oil.

#### Natural killer cell activity

Early studies suggest little effect of high-saturated-fat or  $n-6$  PUFA-rich-oil diets on rodent NK cell activity (for references, see Calder, 1995, 1996a,b,c, 1998). In contrast, feeding a linseed oil-rich diet decreased rat spleen lymphocyte NK cell activity compared with feeding a sunflower oil-rich diet (Jeffery *et al.* 1996a). A number of studies have shown that feeding rats or mice on fish oil-rich diets results in suppressed spleen lymphocyte NK cell activity compared with feeding low-fat diets or high-fat diets rich in saturated fat or  $n-6$  PUFA (Meydani *et al.* 1988; Berger *et al.* 1993; Lumpkin *et al.* 1993; Yaqoob *et al.* 1994b; Sanderson *et al.* 1995a); fish oil appears to be more suppressive than linseed oil (Fritsche & Johnstone, 1979). Although one study reports no effect of an olive oil-rich diet on *ex vivo* rat spleen NK cell activity (Berger *et al.* 1993), diets containing 200 g olive oil or oleic acid-rich sunflower oil/kg were found to significantly reduce this activity (Yaqoob *et al.* 1994b; Sanderson *et al.* 1995a; Jeffery *et al.* 1996b).

Thus, it appears that high-fat diets lower NK cell activity compared with low-fat diets. Animal studies indicate that among high-fat diets the order of potency is:

saturated fat  $\leq$   $n-6$  PUFA-rich oils < olive oil < linseed oil < fish oil.

**Table 3.** Recent studies of the effects of dietary lipids on natural killer (NK) cell activity\*

Cell source	Details of diets	Effect	Reference
Rat spleen	200 g/kg; SO v. OO v. HOSO; 6 weeks	NK cell activity decreased in OO and HOSO groups compared with low-fat or SO groups	Jeffery <i>et al.</i> (1996b)
Rat spleen	200 g/kg; sunflower oil v. LO v. mixtures of sunflower oil and LO; 6 weeks	NK cell activity decreased as proportion of LO in the diet increased	Jeffery <i>et al.</i> (1996a)
Rat spleen	77 or 178 g/kg; fat containing different saturated fatty acids and different positional isomers of palmitic acid (total saturated, MUFA, $n-6$ PUFA, $n-3$ PUFA constant); 6 weeks	NK cell activity increased by diets containing palmitic acid NK cell activity decreased by high-fat diet containing stearic acid	Jeffery <i>et al.</i> (1997c)
Rat spleen	178 g/kg; fat varying in the proportions of palmitic, oleic, linoleic and $\alpha$ -linolenic acids ( $n-6 : n-3$ PUFA constant); 6 weeks	NK cell activity decreased as the proportion of oleic acid in diet increased	Jeffery <i>et al.</i> (1997a)
Rat spleen	178 g/kg; fat containing low (17.8 g/100 g fat) or high (35 g/100 g fat) proportions of total PUFA and varying in $n-6 : n-3$ PUFA (total saturated fatty acids and MUFA constant); 6 weeks	NK cell activity decreased as $n-6 : n-3$ PUFA value of diet decreased Greater effect of $n-6 : n-3$ PUFA value of low-PUFA diet than high-PUFA diet At $n-6 : n-3$ PUFA value of 100, 20 or 10, NK cell activity lower for high-PUFA than low-PUFA-fed animals	Jeffery <i>et al.</i> (1997b)
Human blood	Arachidonic acid-enriched diet providing 1.5 g arachidonic acid/d (at expense of MUFA); 50 d	No effect	Kelley <i>et al.</i> (1997)
Rat spleen	178 g/kg; fat with saturated fatty acid, MUFA, total PUFA and $n-6 : n-3$ PUFA constant; $\alpha$ -linolenic acid (4.4 g/100 g fatty acids) replaced with either EPA or DHA; a proportion (4.4 g/100 g fatty acids) of linoleic acid replaced with either arachidonic acid or GLA; 6 weeks	No effect of partial replacement of linoleic acid with arachidonic acid or GLA or of replacement of $\alpha$ -linolenic acid with DHA NK cell activity decreased by replacement of $\alpha$ -linolenic acid with EPA	Peterson <i>et al.</i> (1998)
Human blood	Enrichment of diet with MUFA at expense of saturated fatty acids; 2 months	Non-significant decrease	Yaqoob <i>et al.</i> (1998)

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA,  $\gamma$ -linolenic acid; HOSO, high-oleic sunflower oil; LO, linseed oil; MUFA, monounsaturated fatty acid; OO, olive oil; PUFA, polyunsaturated fatty acid; SO, safflower oil.

\* This table is designed to complement Table 2 of Calder (1996c); it is restricted to results published since 1996.



### Natural killer cell activity: recent studies

Recent animal studies have endeavoured to establish the effects of particular discrete changes in dietary fatty acid composition on NK cell activity and are summarized in Table 3.

*Effects of saturated, monounsaturated and n-6 polyunsaturated fatty acids.* The type of saturated fatty acid in the rat diet has been reported to influence spleen NK cell activity, which was higher if the animals had been fed on a diet containing palmitic acid as the principal saturated fatty acid than if they had consumed diets rich in medium-chain, lauric or stearic acids (Jeffery *et al.* 1997c). In another study, a significant inverse linear correlation between the level of oleic acid or the oleic acid : linoleic acid in the rat diet on spleen lymphocyte NK cell activity was reported (Jeffery *et al.* 1997a).

One study of the effect of dietary intervention with oleic acid on human NK cell activity has been performed (Yaqoob *et al.* 1998). In this study, subjects increased their oleic acid intake at the expense of saturated fatty acids. After 2 months there was a trend towards reduced NK cell activity, but the effect of diet was not statistically significant (Yaqoob *et al.* 1998).

*Effects of polyunsaturated fatty acid content and n-6 : n-3 polyunsaturated fatty acids.* In a recent study rats were fed on diets containing 178 g fat/kg but differing in PUFA content (17.5 or 35 g/100 g fatty acids) and n-6 : n-3 PUFA values (100, 20, 10, 5, 1); the PUFA content was altered by replacing a proportion of palmitic acid with linoleic and  $\alpha$ -linolenic acids (Jeffery *et al.* 1997b). It was found that NK cell activity decreased as the n-6 : n-3 PUFA of the 'low'-PUFA diet decreased; the n-6 : n-3 PUFA of the 'high'-PUFA diet had less impact on NK cell activity (Jeffery *et al.* 1997b). At n-6 : n-3 PUFA values of 100, 20 and 10, the NK cell activity of lymphocytes from rats fed on the high-PUFA diets was lower than that of rats fed on the low-PUFA diets. This study indicates that dietary  $\alpha$ -linolenic acid reduces NK cell activity, but that its effect is dependent on the total PUFA content of the diet and its level relative to that of linoleic acid.

*Effects of arachidonic acid.* Peterson *et al.* (1998) observed that inclusion of 4.4 g arachidonic acid/100 g fatty acids in the rat diet did not affect NK cell activity of spleen lymphocytes. Similarly, Kelley *et al.* (1997) reported that 1.5 g arachidonic acid/d for 50 d did not affect human NK cell activity.

*Effects of eicosapentaenoic and docosahexaenoic acids.* Peterson *et al.* (1998) report that replacing  $\alpha$ -linolenic acid (4.4 g/100 g fatty acids) with eicosapentaenoic in a high-fat diet (with the total PUFA content and n-6 : n-3 PUFA value of the diet kept constant) reduced rat spleen NK cell activity. In contrast, replacing  $\alpha$ -linolenic acid with docosahexaenoic acid did not affect NK cell activity (Peterson *et al.* 1998).

### Lymphocyte-derived cytokine production

In contrast to the large number of studies of the effects of dietary lipids, especially fish oils, on the *ex vivo* production of macrophage-derived cytokines (for reviews, see Calder,

1996c, 1997), there have been relatively few studies on lymphocyte-derived cytokines. Fish and linseed oils have been shown to reduce IL-2 production by pig alveolar lymphocytes (Turek *et al.* 1994). Recently, Jolly *et al.* (1997) reported that feeding mice on diets rich in either eicosapentaenoic or docosahexaenoic acid diminished *ex vivo* IL-2 production by Con A-stimulated spleen lymphocytes. Supplementation of the diet of healthy volunteers with n-3 PUFA (1.23–approximately 6 g/d for up to 24 weeks) resulted in lower *ex vivo* IL-2 production (Meydani *et al.* 1991, 1993; Virella *et al.* 1991; Endres *et al.* 1993; Gallai *et al.* 1993). Gallai *et al.* (1993) also reported decreased *ex vivo* production of IFN- $\gamma$  following supplementation of the diet of healthy subjects or patients with multiple sclerosis with 6 g fish oil/d for 6 months. These findings suggest that fish oil-derived n-3 PUFA inhibit the response of Th1 lymphocytes. The only study to have compared the effects of dietary lipids on the production of both Th1- and Th2-derived cytokines (IL-2, IL-4, IL-10 and IFN- $\gamma$ ) revealed little effect of diet in mice (Yaqoob & Calder, 1995).

In the study of Wu *et al.* (1996) described earlier, the  $\alpha$ -linolenic acid-rich diets did not affect IL-2 production by PBMNC stimulated with either Con A or PHA. In contrast, IL-2 production was elevated in the groups fed on diets enriched with eicosapentaenoic plus docosahexaenoic acids. Again, this observation is contradictory to other studies in animals (Turek *et al.* 1994; Jolly *et al.* 1997) and human subjects (Meydani *et al.* 1991, 1993; Virella *et al.* 1991; Endres *et al.* 1993; Gallai *et al.* 1993); however, the authors suggest that this difference is due to the level of vitamin E included in the monkey diets.

### Lymphocyte surface molecule expression

Stimulation of lymphocytes increases the cell surface expression of a number proteins, including some cytokine receptors (e.g. the IL-2 receptor) and the transferrin receptor. Feeding rats on high-fat diets rich in olive oil, evening primrose oil or fish oil lowered the proportion of spleen lymphocytes bearing the IL-2 receptor and the proportion of thymic lymphocytes bearing the IL-2 receptor and transferrin receptor following Con A stimulation (Yaqoob *et al.* 1994a); feeding the fish oil diet also lowered the proportion of spleen and lymph node lymphocytes bearing the transferrin receptor following Con A stimulation. Spleen lymphocytes from animals fed on the olive oil, evening primrose oil and fish oil diets also showed a lower level of expression of the IL-2 receptor following mitogenic stimulation (Sanderson *et al.* 1995a). Supplementation of the diet of patients with psoriasis or atopic dermatitis with 6 g n-3 PUFA ethyl esters/d caused a significant reduction in the percentage of IL-2 receptor-positive blood lymphocytes following PHA stimulation (Soyland *et al.* 1994); the level of expression of the IL-2 receptor on the positive cells was also significantly reduced (Soyland *et al.* 1994).

Recent studies also indicate effects of dietary lipids on adhesion molecule expression on lymphocytes and other mononuclear cells. Supplementation of the human diet with 3 g fish oil/d resulted in significantly lower levels of expression of CD11a and CD54 (intercellular adhesion molecule-1) on peripheral blood monocytes (Hughes *et al.* 1996).

Furthermore, it has been reported that feeding rats on a diet rich in fish oil results in significantly reduced levels of expression of CD2 and CD11a on freshly-prepared lymphocytes (Sanderson *et al.* 1995a), of CD2, CD11a and intercellular adhesion molecule-1 on Con A-stimulated lymphocytes (Sanderson *et al.* 1995a), and of CD2 and CD11a on popliteal lymph node lymphocytes following localized graft *v.* host (G *v.* H) or host *v.* graft (H *v.* G) responses (Sanderson *et al.* 1995b). These studies have been recently extended; feeding the fish oil diet to rats also decreased the level of expression of CD18 and CD44 on the surface of freshly-prepared lymphocytes and of CD18 and CD62L on the surface of Con A-stimulated lymphocytes (Sanderson & Calder, 1998). An olive oil-rich diet also resulted in decreased expression of some adhesion molecules (Sanderson *et al.* 1995a,b; Sanderson & Calder, 1998). The fish oil or olive oil diets decreased the adhesion of both freshly-prepared and Con A-stimulated lymphocytes to macrophage monolayers or to untreated endothelial cells (Sanderson & Calder, 1998). Furthermore, the fish oil diet resulted in a 50 % reduction in Con A-stimulated lymphocyte adhesion to tumour necrosis factor- $\alpha$ -stimulated endothelial cells (Sanderson & Calder, 1998). These studies demonstrate that dietary lipids affect the expression of functionally-important adhesion molecules on the surface of lymphocytes. Furthermore, the study of Sanderson & Calder (1998) suggests that such diet-induced effects on adhesion molecule expression might alter the ability of lymphocytes to bind to macrophages and to endothelial cells.

In accordance with the effect of feeding olive oil to rats, Yaqoob *et al.* (1998) have demonstrated that increasing the proportion of oleic acid in the human diet, at the expense of saturated fatty acids, results in a significantly decreased number of PBMNC expressing intercellular adhesion molecule-1; the proportion of PBMNC expressing CD11b also declined, but this was not statistically significant.

#### Antibody production

**Studies in experimental animals.** Dietary *n*-6 PUFA reduced the production of antibodies, including IgG and IgM, following antigenic challenges, as compared with feeding low-fat or high-saturated-fat diets (Friend *et al.* 1980; Erickson *et al.* 1986). Enhanced production of IgE to ovalbumin was reported in rats fed on a high-fish oil diet compared with those fed on a saturated-fat diet (Prickett *et al.* 1982). These observations are consistent with effects of *n*-6 and *n*-3 PUFA on the Th1 lymphocyte response, which, if suppressed would result in reduced IgM and IgG production and, perhaps, enhanced IgE production.

**Studies in human subjects and other primates.** Feeding cebus (*Cebus apella*) or squirrel (*Saimiri sciureus*) monkeys on diets containing 143 g coconut or maize oil/kg for several years did not result in different antibody responses to measles vaccine (Meydani *et al.* 1985). Kelley *et al.* (1989, 1992a) reported no effect on circulating IgM, IgG, IgE or IgA levels of reducing total fat intake (from 40 to 25–30 % total energy) or of varying the amount of PUFA (3.5 or 12.9 % energy) in the human diet. They also found that including linseed oil or salmon in the diet did not alter circulating antibody levels (Kelley *et al.* 1991, 1992b).

**Recent studies.** Wander *et al.* (1997) report that the production of antibodies in response to keyhole limpet (*Megathura crenulata*) haemocyanin is unaffected by feeding beagle dogs on diets with differing *n*-6 : *n*-3 PUFA values (31, 5.4 or 1.4).

Matsuo *et al.* (1996) fed Brown Norway rats on diets containing 100 g safflower, evening primrose or Korean pine (*Pinus orientalis*) seed oils (rich in pinoleic acid; 5,9, 12-18:3) and sensitized the animals by injection of ovalbumin. Subsequently, circulating total and ovalbumin-specific IgG and IgE, and the *ex vivo* production of total and specific IgG and IgE by spleen lymphocytes were examined. Compared with safflower oil, evening primrose and pine seed oils increased total and ovalbumin-specific IgE production by spleen lymphocytes and increased circulating total and ovalbumin-specific IgE levels 1 week after ovalbumin injection. In contrast, evening primrose oil suppressed ovalbumin-specific IgG production by lymphocytes without affecting total IgG production, while pine seed oil enhanced total IgG production. These effects were not seen *in vivo*, however, where ovalbumin-specific IgG levels were unaffected by diet, and total IgG levels were elevated by evening primrose and safflower oils compared with pine seed oil.

#### Dietary fatty acids and *in vivo* measures of cell-mediated immunity

The studies outlined previously have investigated the effects of dietary manipulations on *ex vivo* functions of isolated cell populations. Although a number of consistent patterns have emerged from these studies, there are also contradictory reports, and it is evident that the outcome of such *ex vivo* measures is strongly influenced by the experimental conditions used. Furthermore, *in vivo* cells exist as part of a network being influenced by other cell types; often such interactions are disturbed by the purification of the particular cell types to be studied. Thus, it is important to investigate the effect of dietary fats on the intact fully-functioning system in which all normal cellular interactions are in place. The ability to make *in vivo* measures of inflammation and of cell-mediated immunity offers the prospect of investigating the effects of dietary manipulations on the overall responses of these systems.

#### Delayed-type hypersensitivity

The delayed-type hypersensitivity (DTH) reaction is the result of a cell-mediated response to challenge with an antigen to which the individual has already been primed.

**Animal studies.** The DTH response in rats or guinea-pigs is reduced by feeding high-fat diets compared with feeding low-fat diets (Friend *et al.* 1980; Crevel *et al.* 1992); *n*-6 PUFA-rich diets are more suppressive than high-saturated-fat diets (Friend *et al.* 1980; Crevel *et al.* 1992). Dietary fish oil reduces the DTH response in mice compared with *n*-6 PUFA-rich or olive oil-rich diets (Yoshino & Ellis, 1987), while addition of ethyl esters of either eicosapentaenoic or docosahexaenoic acids to the diet of mice consuming a safflower oil diet reduced the DTH response (Fowler *et al.* 1993); both *n*-3 PUFA were equally effective. The DTH

response to sheep erythrocytes in mice was diminished following tail-vein injections of emulsions of triacylglycerols rich in eicosapentaenoic or docosahexaenoic acids (Taki *et al.* 1992). Recently, Wander *et al.* (1997) showed that feeding beagle dogs on a diet with an *n*-6 : *n*-3 PUFA value of 1.4 resulted in a reduced DTH response to intradermal keyhole limpet haemocyanin compared with diets with *n*-6 : *n*-3 PUFA values of 3.1 or 5.4; the increased *n*-3 PUFA content was brought about by replacing linoleic acid with eicosapentaenoic plus docosahexaenoic acids. These observations are consistent with the effects of different types of fatty acids on *ex vivo* lymphocyte responses (e.g. proliferation).

Thus, the animal studies indicate that high-fat diets reduce the DTH response compared with low-fat diets. Among high-fat diets the order of potency is:

saturated fat < *n*-6 PUFA-rich oils < fish oil.

*Studies in human subjects.* A 40 d reduction in fat intake (from 40 to 25–30 % energy) by healthy human volunteers did not alter the DTH responses to seven recall antigens (Kelley *et al.* 1992a); these responses were also unaffected by differences in the PUFA level of the diet (3.2 or 9.1 % energy; Kelley *et al.* 1992a) or by consuming a salmon-rich diet (500 g/d for 40 d; Kelley *et al.* 1992b). Feeding a linseed oil-rich diet to healthy human volunteers for 8 weeks lowered the DTH response to seven recall antigens, although this reduction was not statistically significant (Kelley *et al.* 1991). Supplementation of the diet of volunteers consuming a low-fat low-cholesterol diet with 1.25 g *n*-3 PUFA/d diminished the DTH responses to seven recall antigens (Meydani *et al.* 1993). Kelley *et al.* (1997) reported no effect of 1.5 g arachidonic acid/d on the DTH response to seven recall antigens applied intradermally.

#### *Graft v. host and host v. graft responses*

The so-called popliteal lymph node assay provides a useful experimental model in rodents for measuring graft *v.* host (G *v.* H) and host *v.* graft (H *v.* G) responses elicited by injection of allogeneic cells into the footpad of the host. The G *v.* H response primarily involves the polyclonal activation, and subsequent proliferation, of host B-cells, although NK cells may also be involved in the host defence. In contrast, the H *v.* G reaction is a T-cell mediated response, in which CTL of the host recognize major histocompatibility complex antigens on the injected cells. In both cases, the enlargement in popliteal lymph node size is due largely to proliferation of activated host cells; most of these originate within the popliteal lymph node, although there is also some recruitment of cells from the bloodstream. Using this assay, Mertin *et al.* (1985) reported that both the G *v.* H and H *v.* G responses were suppressed following a single administration of fish oil concentrate (750 mg/kg body weight) by oesophageal catheter to mice before, or immediately after, the inoculation with allogeneic cells. A suppressed H *v.* G response was observed in mice fed on a 160 g fish oil/kg diet compared with those fed on a standard chow diet (Hinds & Sanders, 1993); lower levels of fish oil (25, 50, 100 g/kg) did not significantly affect the response. Significantly

diminished G *v.* H and H *v.* G responses (by 34 and 20 % respectively) were observed in rats fed on 200 g fish oil/kg compared with those fed on a low-fat diet or diets containing 200 g coconut, olive, safflower or evening primrose oils/kg (Sanderson *et al.* 1995b). Such observations accord with the demonstrations of significantly diminished *ex vivo* T lymphocyte proliferation, NK cell activity and CTL activity following fish oil feeding. The fish oil diet resulted in less IL-2 receptor-positive cells and CD16<sup>+</sup> and CD3<sup>+</sup> cells in the popliteal lymph node following the G *v.* H response (Sanderson *et al.* 1995b), indicating an inhibition of lymphocyte activation and a decrease in the proportion of NK cells respectively. Recently, Jeffrey *et al.* (1996a) reported a dose-dependent effect of linseed oil compared with sunflower oil on the G *v.* H response in rats; the level of fat in the diet was 200 g/kg. More recently, replacing  $\alpha$ -linolenic acid (4.4 g/100 g fatty acids) with eicosapentaenoic acid was observed to significantly decrease (by 15–20 %) the G *v.* H in rats (Peterson *et al.* 1998); replacement of  $\alpha$ -linolenic acid with docosahexaenoic acid was without effect.

Thus, animal studies indicate that high-fat diets reduce the G *v.* H and H *v.* G responses compared with low-fat diets. The order of potency is:

low fat = high saturated fat < high *n*-6 PUFA-rich oils < high linseed oil  $\leq$  high fish oil.

#### *Animal models of organ transplantation*

Graft rejection in transplantation surgery is caused by an immune reaction to the foreign material introduced into the body; T-cells have been implicated in accelerated graft rejection, but antibodies with specificity for the graft donor have also been observed following rejection, implying that both cell-mediated and humoral immunity play a part in the rejection process.

Linoleic acid administered subcutaneously, intraperitoneally or orally prolongs the survival of skin allografts in mice or rats (Ring *et al.* 1974). Renal or cardiac transplants have been shown to survive for longer if recipient rats are fed on oleic, linoleic, or eicosapentaenoic acids or fish oil (for references, see Calder, 1996c, 1998). Greater prolongation of cardiac survival has been reported in rats receiving an infusion of fish oil post-transplantation compared with those receiving soyabean oil infusion (Grimm *et al.* 1995; Grimminger *et al.* 1996); in turn, soyabean oil enhanced survival compared with saline (9 g NaCl/l) infusion. Oral fish oil (4.5 g/d) has also been shown to prolong the survival of Islets of Langerhans grafts in mice (Linn *et al.* 1990). These observations are in accordance with the reduced lymphocyte responses observed following fish oil feeding in particular, and they indicate that intervention with *n*-3 PUFA may be useful before and following organ transplantation in man. Human renal transplant survival and/or function is improved if the patients receive fish oil (6–9 g/d for 1 year post-transplant) in addition to traditional immunosuppressive therapy (Berthoux *et al.* 1992; Homan van der Heide *et al.* 1993; Bennett *et al.* 1995; Maachi *et al.* 1995). Cyclosporin nephrotoxicity is also reduced by *n*-3 PUFA consumption in these patients (Berthoux *et al.* 1992; Bennett *et al.* 1995).



### Do dietary lipids diminish host defence?

The diminished lymphocyte responses observed, particularly after feeding experimental animals on diets rich in *n*-3 PUFA, result in suppressed cell-mediated immune responses, suggesting that these fatty acids could affect the host response to infection. Some animal studies support this suggestion. For example, diets rich in fish oil reduce the survival of mice to orally-administered *Salmonella typhimurium* (Chang *et al.* 1992), decrease the clearance of inspired *Staphylococcus aureus* in neonatal rabbits (D'Ambola *et al.* 1991), and decrease the survival of mice to an intraperitoneal injection of *Listeria monocytogenes* (Fritsche *et al.* 1997). Since the response to microbial infections is predominantly a Th1-mediated response, the reduced survival of rodents fed on large amounts of fish oil to bacterial challenges suggests that fish oil suppresses the Th1 response; this is consistent with the observed effects of fish oil on cytokine and antibody production.

There have been no reports of compromised immunity in human subjects supplementing their diet with *n*-3 PUFA.

### Mechanisms by which fatty acids might affect lymphocyte functions

Mechanisms by which different fatty acids might affect lymphocyte functions are discussed in detail elsewhere (Calder, 1996c; Miles & Calder, 1998); these mechanisms include: changes in the amount and type of eicosanoids produced; depletion of antioxidants, especially vitamin E; alterations in plasma-membrane phospholipid-fatty acid composition and/or membrane fluidity; alterations in intracellular signalling mechanisms; alterations in expression of key genes, such as those for cytokines, cytokine receptors and adhesion molecules. These mechanisms may not be mutually exclusive and might be interrelated (for example see, Fig. 3 of Miles & Calder, 1998). Some recent studies have sought to examine further the mechanisms by which fatty acids might influence lymphocyte function.

*The effects of n-3 polyunsaturated fatty acids appear not to be mediated by changes in eicosanoid production.* In the past it has often been assumed that the effects of dietary *n*-3 PUFA on immune cell functions, such as lymphocyte proliferation, must relate to changes in production of eicosanoids, particularly prostaglandin (PG)E<sub>2</sub>. However, PGE<sub>2</sub> inhibits lymphocyte proliferation (for reviews see Goodwin & Cueppens, 1983; Hwang, 1989); thus, reducing the ability of cells to produce PGE<sub>2</sub>, as observed after feeding diets rich in *n*-3 PUFA, should enhance lymphocyte proliferation. This is not observed (see pp. 491–493). A number of *in vitro* studies have concluded that the inhibitory effects of *n*-3 PUFA on lymphocyte proliferation are independent of their effects on PGE<sub>2</sub> production (Santoli *et al.* 1990; Calder *et al.* 1992; Kumar *et al.* 1992; Soyland *et al.* 1993; Rotondo *et al.* 1994; Khalfoun *et al.* 1996b). Furthermore, supplementation of the human diet with fish oil has been shown to reduce both lymphocyte proliferation and PGE<sub>2</sub> production (Meydani *et al.* 1991, 1993); these studies concluded that the effect of *n*-3 fatty acid supplementation on lymphocyte proliferation seems independent of, and is unlikely to be

due to, decreases in PGE<sub>2</sub> production. The recent study of Wu *et al.* (1996) in part also supports this conclusion; these workers found that feeding monkeys on diets containing increased amounts of  $\alpha$ -linolenic acid resulted in significantly reduced PGE<sub>2</sub> production but did not affect lymphocyte proliferation.

*Effects mediated by diminished antioxidant status.* Wu *et al.* (1996) showed that enrichment of the diet of monkeys with eicosapentaenoic plus docosahexaenoic acids significantly reduced PGE<sub>2</sub> production, but increased the response of lymphocytes to mitogens. The authors' explanation for the latter observation was that the monkeys had been fed on variable levels of  $\alpha$ -tocopherol, and so were better able to maintain antioxidant defences in the face of differing dietary PUFA levels. This suggests that *n*-3 PUFA may exert at least some of their inhibitory effects by inducing some sort of oxidative damage to the cells. This is supported by another recent study which showed that increasing the *n*-3 PUFA content of the diet of beagle dogs decreases plasma  $\alpha$ -tocopherol levels and increases the levels of the products of lipid peroxidation in the plasma (Wander *et al.* 1997).

*Effects on cell signalling within lymphocytes.* Secretion of IL-2 by Jurkat T cells is preceded by a fall in mRNA for c-myc and a rise in mRNA for c-fos; c-myc and c-fos encode nuclear proteins that play a role in transcriptional regulation. Williams *et al.* (1996) found that dihomo- $\gamma$ -linolenic acid prevented the reduction in c-myc mRNA levels, arachidonic and eicosapentaenoic acids had more modest effects, and oleic acid was inactive (Williams *et al.* 1996). Furthermore, dihomo- $\gamma$ -linolenic and arachidonic acids reduced (by 75 %) the level of c-fos mRNA; eicosapentaenoic and oleic acids were also active, but less so (Williams *et al.* 1996). These studies suggest that some *n*-6 and *n*-3 PUFA may act to suppress IL-2 production by altering the early steps in the signal transduction pathway which ultimately leads to activation of expression of the IL-2 gene.

Vassilopoulos *et al.* (1997) have recently shown that incubation of human PBMNC with  $\gamma$ -linolenic or dihomo- $\gamma$ -linolenic acids (approximately 30  $\mu$ M) reduced the anti-CD3-induced rise in intracellular free Ca levels; both fatty acids also reduced anti-CD3-mediated generation of the second messenger inositol-1,4,5-trisphosphate. The findings of this study agree with earlier studies which have shown that several unsaturated fatty acids, including  $\alpha$ -linolenic, eicosapentaenoic and docosahexaenoic acids, inhibit the anti-CD3-induced increase in intracellular free Ca concentration in the Jurkat cell line (Chow *et al.* 1990; Breitmayer *et al.* 1993); the fatty acids appear to act by blocking Ca entry into the cells and it has been concluded that they act directly on receptor-operated Ca channels.

The study of Jolly *et al.* (1997) provides some insight into how long-chain *n*-3 PUFA might act to suppress lymphocyte proliferation. These workers observed a marked reduction in generation of the intracellular second messengers diacylglycerol and ceramide in Con A-stimulated cells from eicosapentaenoic or docosahexaenoic acid-fed mice. Both diacylglycerol and ceramide are key second messengers in lymphocytes, ultimately giving rise to transcription factor activation, and so regulating gene expression. The findings are suggestive of effects of fish oil-derived *n*-3 PUFA on



intracellular signalling pathways which control the functional activities of the cells.

### Concluding statement

The amount and type of eicosanoids made can be affected by the type of fat consumed in the diet (for a review see Kinsella *et al.* 1990). It is now apparent that both eicosanoids and PUFA are potent modulators of lymphocyte functions *in vitro*. Inclusion in the diet of high levels of certain lipids, particularly those containing *n*-3 PUFA, markedly affects the functions of lymphocytes subsequently tested *ex vivo*. Components of both natural and acquired immunity are affected. Recent studies have sought to identify the effects of lower levels of particular fatty acids in the diet; these studies reveal complex interactions between fatty acids. *Ex vivo* studies do reveal some contradictory observations with respect to the effects of both *n*-6 and *n*-3 PUFA. It is likely that these discrepancies result from the different experimental protocols used; in particular, the type of serum used for *ex vivo* culture of cells has a marked influence on functional tests. *In vivo* tests are perhaps the most appropriate approach for determining the effect of different dietary fatty acids on immune function. Several studies indicate that diets rich in *n*-3 PUFA are anti-inflammatory and immunosuppressive *in vivo*, although there have been relatively few studies in man. Although some of the effects of *n*-3 PUFA may be brought about by modulation of the amount and type of eicosanoids made, it is possible that these fatty acids might elicit some of their effects by eicosanoid-independent mechanisms, including actions on intracellular signalling pathways and transcription factor activity (for reviews see Calder, 1996c; Miles & Calder, 1998). Such *n*-3 PUFA-induced effects may be of use as a therapy for acute and chronic inflammation, for disorders which involve an inappropriately-activated immune response and for the enhancement of graft survival (for reviews, see Calder, 1996c, 1998).

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