Fatty acids and lymphocyte functions

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Fatty acids and lymphocyte functions

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The immune system acts to protect the host against pathogenic invaders. However, components of the immune system can become dysregulated such that their activities are directed against host tissues, so causing damage. Lymphocytes are involved in both the beneficial and detrimental effects of the immune system. Both the level of fat and the types of fatty acid present in the diet can affect lymphocyte functions. The fatty acid composition of lymphocytes, and other immune cells, is altered according to the fatty acid composition of the diet and this alters the capacity of those cells to produce eicosanoids, such as prostaglandin E_2, which are involved in immunoregulation. A high fat diet can impair lymphocyte function. Cell culture and animal feeding studies indicate that oleic, linoleic, conjugated linoleic, γ-linolenic, dihomo-γ-linolenic, arachidonic, α-linolenic, eicosapentaenoic and docosahexaenoic acids can all influence lymphocyte proliferation, the production of cytokines by lymphocytes, and natural killer cell activity. High intakes of some of these fatty acids are necessary to induce these effects. Among these fatty acids the long chain *n*-3 fatty acids, especially eicosapentaenoic acid, appear to be the most potent when included in the human diet. Although not all studies agree, it appears that fish oil, which contains eicosapentaenoic acid, down regulates the T-helper 1-type response which is associated with chronic inflammatory disease. There is evidence for beneficial effects of fish oil in such diseases; this evidence is strongest for rheumatoid arthritis. Since *n*-3 fatty acids also antagonise the production of inflammatory eicosanoid mediators from arachidonic acid, there is potential for benefit in asthma and related diseases. Recent evidence indicates that fish oil may be of benefit in some asthmatics but not others.

Fatty acid: Fish oil: Lymphocyte: Monocyte: Cytokine: Eicosanoid: Inflammation: Immunity

Introduction

Interest in the effects of fatty acids upon the immune system dates back many years, but this interest intensified with the elucidation of the roles of eicosanoids derived from arachidonic acid (ARA; 20:4*n*-6) in modulating inflammation and immunity and with the knowledge that the metabolism of ARA to yield these mediators can be inhibited by the long chain *n*-3 polyunsaturated fatty acids (PUFA) found in fish oils. This article will review the effects of fatty acids upon lymphocyte proliferation, lymphocyte-mediated cytotoxicity, lymphocyte-derived cytokine production, antibody production, and cell-mediated immunity. Since lymphocytes are activated *in vivo* by presentation of antigen by antigen-presenting cells and by cytokines derived from them, this review will also describe the effects of fatty acids on these processes. These, and other, aspects of fatty acids and immunity have been reviewed a number of times (Calder, 1995, 1996*a*,*b*, 1997, 1998*a*,*b*,*c*, 1999; Blok *et al.* 1996; Alexander, 1998; Grimble, 1998; Harbige, 1998; Hughes, 1998; Miles & Calder, 1998; Sperling, 1998; Wu & Meydani, 1998; Yaqoob, 1998*a*,*b*) and the reader is referred to these articles for a more complete overview of the field. Mechanisms by which fatty acids might influence the functions of lymphocytes, apart from effects upon eicosanoid production, will not be reviewed here; these have been discussed elsewhere (Calder 1996*b*; Miles & Calder 1998; Yaqoob, 1998*b*).

Lymphocytes

Lymphocytes are the cells that specifically recognise and respond to foreign antigens and, as such, they form the core of the acquired (or specific) immune system. They are

Abbreviations: EPA, eicosapentaenoic acid; ARA, arachidonic acid; PUFA, polyunsaturated fatty acid; GLA, γ-linolenic acid; DGLA, di-homo-γ-linolenic acid; ALNA, α-linolenic acid; DHA, docosahexaenoic acid; NK, natural killer; Th-lymphocyte, helper T-lymphocyte; CTL, cytotoxic T-lymphocyte; MHC, major histocompatibility complex; DTH, delayed type hypersensitivity; MNC, mononuclear cell.

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present as circulating cells in blood and lymph, as anatomically defined collections of cells in lymphoid organs (thymus, spleen, lymph nodes, gut-associated lymphoid tissue) or as scattered cells in other tissues. Lymphocytes exist as distinct subsets that are quite different in their functions and protein products, although they are morphologically fairly similar. The principal types of lymphocytes are T- and B-lymphocytes and natural killer (NK) 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-lymphocytes (Th-lymphocytes) and cytotoxic T-lymphocytes (CTL); these classes of T-lymphocyte are characterised by the presence of CD4 or CD8 molecules, respectively, on their surface. T-lymphocytes recognise peptide antigens attached to major histocompatibility complex (MHC) proteins on the surface of so-called antigen presenting cells. MHC II is expressed on dendritic cells, monocytes and macrophages, and B-lymphocytes. The role of MHC II is the presentation of antigenic peptides derived from intracellular proteins of exogenous origin to the T-cell receptor-CD3 complex on $CD4^+$ (Th) lymphocytes (Fig. 1). MHC I is expressed on most cells, but the level of expression varies among different cell types. The role of MHC I is the presentation of antigenic peptides derived from intracellular proteins of endogenous origin to the T-cell receptor-CD3 complex on $CD8^+$ lymphocytes (CTL). Thus, the MHC I-CTL interaction is involved in the destruction of cells infected with viruses. The MHC-T-cell receptor interaction on its own is insufficient to promote T-lymphocyte activation even in the presence of CD4 or CD8; other interactions

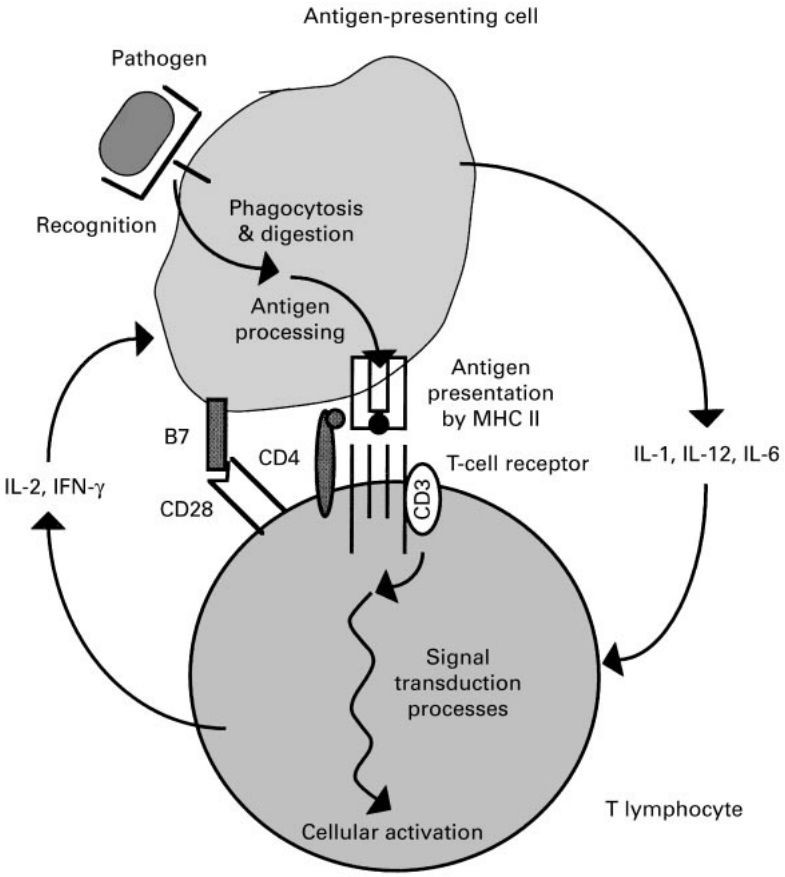

Fig. 1. The interaction between antigen presenting cells and T-lymphocytes. In the example shown, an extracellular pathogen is recognised and phagocytosed by an antigen presenting cell; this process is enhanced by coating of the pathogen with complement or antibodies (not shown). The phagocytic vesicle containing the pathogen fuses with a lysosome and the pathogen is subsequently digested. Peptides from the pathogen associate with MHC II and are translocated to the cell surface. The peptide is presented to the T-cell receptor on the T-lymphocyte surface. The interaction between MHC II and the T-cell receptor is stabilised by CD4 (CD8 stabilises the interaction between MHC I and the T-cell receptor), while accessory molecules on the surface of each cell (e.g. B7 and CD28) interact to provide further stability. CD3 tranduces signals from the T-cell receptor into the T-lymphocyte. Both the antigen presenting cell and the T-lymphocyte secrete cytokines to influence the activities of one another.

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between molecules on the surface of the antigen presenting cell and the T-cell are required (e.g. B7 and CD28). These molecules are termed accessory molecules.

In response to stimulation T-lymphocytes secrete cytokines whose function is to promote an increase in T-lymphocyte number (termed lymphocyte proliferation), the differentiation of T-lymphocytes and the activation of other cell types including B-lymphocytes, NK cells and macrophages.

The Th-lymphocytes are further sub-divided according to the pattern of cytokines they produce (Fig. 2). Upon initial encounter with antigen naive Th-cells produce mainly interleukin-2 (IL-2). These cells may differentiate into a population sometimes referred to as Th0 cells which differentiate further into either Th1 or Th2 cells (Fig. 2); this is a simplistic view and it is now recognised that there are most likely other classes of Th cell (e.g. Th3) (Mossmann & Sad, 1996). The differentiation into Th1 or Th2 is regulated by cytokines: IL-12 and IFN-γ promote the development of Th1 cells, while IL-4 promotes the development of Th2 cells (Fig. 2). Th1 and Th2 themselves have relatively restricted profiles of cytokine production: Th1 cells produce IL-2 and IFN-γ which activate macrophages, NK cells and CTL and are the principal effectors of cell-mediated immunity and delayed type hypersensitivity (DTH). Intracellular pathogens tend to induce Th1 activity. This is because antigen presenting cells are a major source of IL-12. Thus, infection with intracellular pathogens will promote differentiation along the Th1 pathway, and this will in turn produce cytokines which promote the activity of cells able to destroy such pathogens (e.g. macrophages, CTL, NK cells) and the immunoglobulin (Ig) G-type of antibody response (Fig. 2). Th2 cells produce IL-4, which stimulates IgE production, IL-5, an eosinophil activating factor, and IL-10, which together with IL-4 suppresses cell-mediated immunity and inflammation (Fig. 2). Infection with extracellular pathogens, such as helminthic worms, tends to induce differentiation along the Th2 pathway, and this will in turn induce responses which are able to destroy such pathogens, e.g. IgE production (Fig. 2). One important aspect of the Th1–Th2 paradigm is that Th1-type cytokines, especially IFN-γ, inhibit the differentiation of Th2 cells, while Th2-type cytokines, especially IL-4, inhibit the differentiation of Th1 cells, Fig. 2 (Mossmann & Sad, 1996).

B-lymphocytes

B-lymphocytes mature in the bone marrow. They are the cells responsible for producing antibodies. The role of these antibodies is to neutralise and promote elimination of the antigen that induced their formation (Fig. 2). The antibodies produced belong to different Ig classes depending upon 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).

NK cells

These are a class of lymphocyte 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-γ and tumour necrosis factor (TNF)-α.

Lymphocytes in health and disease

Clearly a well functioning immune system is essential to health. It serves to protect the host from the effects of ever present pathogenic organisms. Cells of the immune system also have a role in identifying and eliminating cancer cells. There are however some detrimental effects of the immune system and lymphocytes are central to these effects. First, in the course of its activity to recognise and eliminate foreign antigens, the immune system is responsible for the rejection of transplanted tissues. Second, in some individuals the immune system appears to recognise host antigens as 'non-self' rather than as 'self'. As a result, an immune response to host tissues is generated and this leads to tissue damage. This is the characteristic of so-called chronic inflammatory or autoimmune diseases. Such diseases are linked to genes coding for proteins involved in antigen presentation or recognition such as the MHC II proteins and the T-cell receptor; thus there is a genetic predisposition to these diseases. These diseases are typified by an ongoing chronic inflammation involving the pro-inflammatory cytokines produced by monocytes and macrophages and by a dysregulated Th1 lymphocyte response. Examples of this type of disease include rheumatoid arthritis, type-1 diabetes, Crohn's disease, psoriasis, and multiple sclerosis. Third, the immune system of some individuals can become sensitised to usually benign antigens from the environment and can respond inappropriately to them by the release of IgE. Such antigens can include components of foods or of so-called allergens (e.g. cat or dog fur, house dust mite, some pollens), such that this response can lead to allergies, asthma and related atopic diseases. Although these diseases are also termed chronic inflammatory diseases they have a different immune basis from the diseases described above, although again they are typified by inappropriate recognition of and/or responses to antigens. However, atopic diseases are characterised by a dysregulated Th2 lymphocyte response such that excessive amounts of IL-4, IL-5 and IL-10 are found. IL-10 suppresses the Th1 response, IL-4 stimulates IgE production by B-lymphocytes (IgE promotes histamine release from mast cells), and IL-4 and IL-5 activate eosinophils which are involved in the persistent inflammation which is a characteristic of these diseases.

Assessment of the effect of fatty acids on lymphocyte function

General considerations

There is a wide range of methodologies available by which to assess the impact of fatty acids on specific lymphocyte functions. *In vitro* studies adding pure fatty acids, fatty acid mixtures or other lipids (e.g. triacylglycerols, phospholipids,

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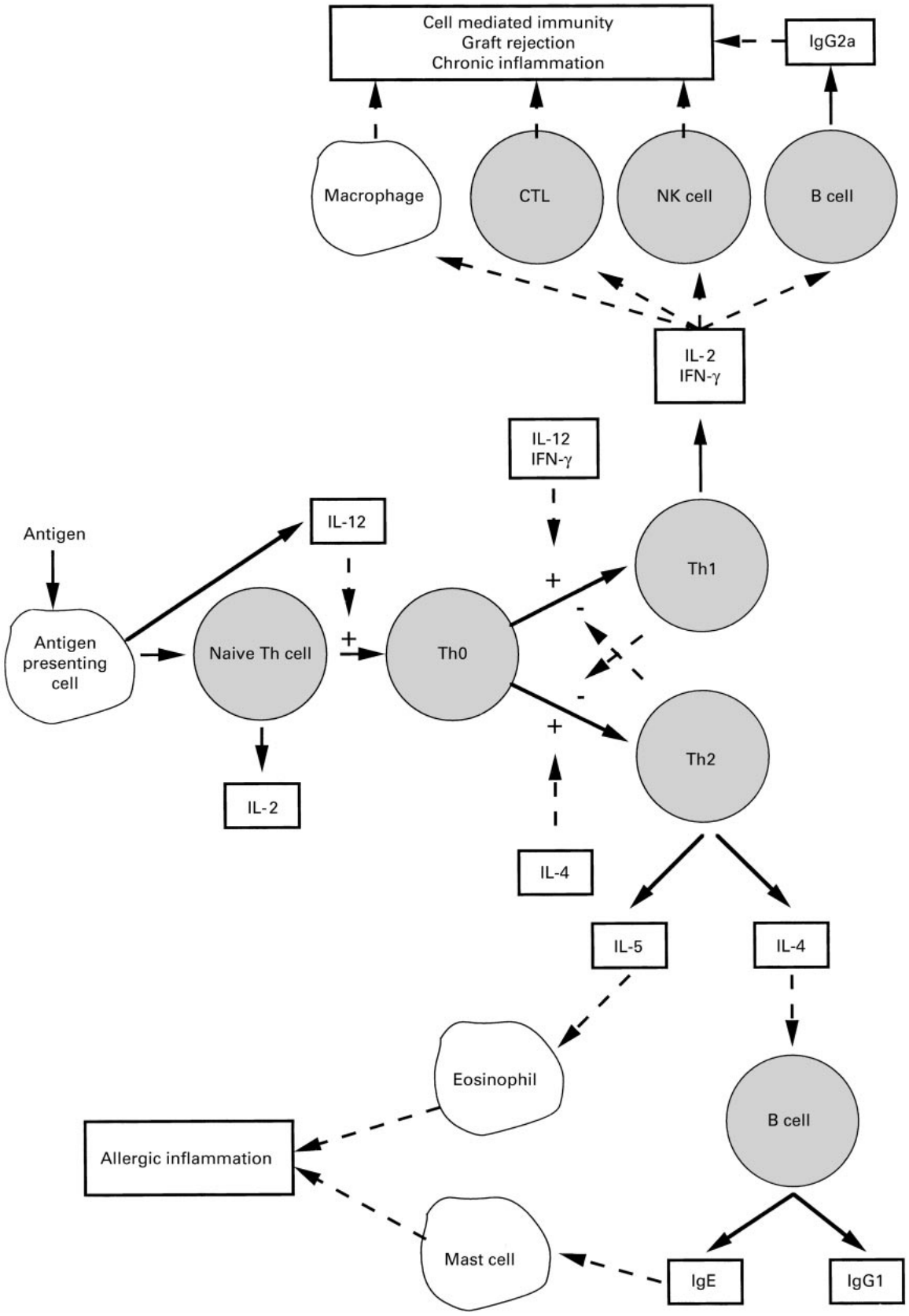

Fig. 2. The differentiation and roles of Th1 and Th2 lymphocytes. → indicates produces; ⇢ indicates regulates.

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lipoproteins) to lymphocytes in culture can be used; these studies use conditions which are highly controlled, although they are often rather unphysiological in nature. For example, the cells are cultured in isolation from the other types of cells with which they would come into contact in the body and the concentrations of fatty acids added to the cultures are often greatly in excess of those which can be attained *in vivo*. The effects of changing the amounts or types of fat in the diet on lymphocyte functions can be studied by isolating lymphocytes from animals or humans which have undergone dietary changes and culturing them *ex vivo*. While this has some of the disadvantages of the pure *in vitro* approach, its controlled nature allows effects on specific cell types and functions to be assessed and allows the exploration of the mechanisms underlying functional changes. One solution to the problem of using isolated lymphocytes in culture has been the development of whole blood culture systems (e.g. Yaqoob *et al.* 1999), which have been used in some studies investigating the influence of dietary fatty acids in animals (Yaqoob *et al.* 1995) and humans (Yaqoob *et al.* 2000). Clearly one limitation to the isolation and subsequent culture of lymphocytes is having a suitable source. For animal studies this does not represent a problem and lymphocytes from the bloodstream, spleen, thymus, lymph nodes, lungs and gut-associated lymphoid tissue are accessible and have been studied. However, most human studies have been restricted to using blood lymphocytes, often as a mixture with monocytes. Preparations containing such mixtures of lymphocytes and monocytes are often described as mononuclear cell (MNC) preparations; typically a MNC preparation from human peripheral blood would comprise 85–90 % lymphocytes and 10–15 % monocytes. One other important difference between dietary experiments in animals and humans is that animal experiments have often used very high amounts of the fatty acids under investigation and these represent intakes that could never be achieved in humans. While the use of such dietary regimens means that effects can be established and mechanisms studied, it is important that the effects of fatty acids at levels in the animal diet relevant to human consumption be demonstrated and that effects be determined in humans under appropriate conditions.

Measures using isolated cells

Probably the most widely used indicator of lymphocyte function is proliferation, i.e. the ability of the cells to divide when stimulated. Most often this is measured as the incorporation of radioactively-labelled thymidine into the DNA of the dividing lymphocytes, although a number of other measures are available. Agents used to stimulate lymphocyte proliferation include mitogens like concanavalin A (Con A) and phytohaemagglutinin (PHA), which stimulate T-lymphocytes, pokeweed mitogen, which stimulates a mixture of T- and B-lymphocytes, and bacterial lipopolysaccharide (LPS), which stimulates B-lymphocytes (and also monocytes). T-lymphocyte proliferation can also be triggered by using antibodies to CD3 or to CD3 in combination with antibodies to other surface proteins (e.g. CD4) or by a combination of agents which stimulate protein kinase C and a rise in the intracellular free calcium concentration. If the animal or individual has been sensitised to an antigen (or allergen) then the antigen (or allergen) can be used to stimulate lymphocyte proliferation. The proliferative response to the antigen or allergen will be much smaller than the response to mitogens or antibodies, since the antigenic response is specific, targeting only those T-lymphocytes which recognise the antigen; in contrast mitogenic stimulation is non-specific and will target a large proportion, perhaps all, of T- or B-lymphocytes in the cell preparation. In response to stimulation with mitogen, antibodies, antigen or allergen, lymphocytes will produce cytokines (e.g. IL-2, IFN-γ, IL-4, IL-10, IL-6) and the concentrations of these can be measured. LPS stimulation of monocytes, macrophages or MNC leads to production of TNF-α, IL-1 and IL-6. B-lymphocytes produce Ig in culture and the total concentration of particular Ig classes (e.g. IgG) can be measured, as can the concentration of Ig for a specific antigenic (or allergenic) stimulus. The cell surface expression of molecules involved in cell–cell interaction (e.g. adhesion molecules), in antigen presentation (e.g. MHC II) and in cellular activation (e.g. cytokine receptors) can be measured, as can the ability of antigen presenting cells to present antigen to sensitised T-lymphocytes. NK cell and CTL activity can be measured as the killing of tumour cells or virally-infected cells known to be specific targets for these cells.

In vivo measures

The effects of changing the amounts or types of fat in the diet can also be studied by assessing certain indicators of lymphocyte function *in vivo*, particularly in response to an immunological challenge. Clearly, animals offer greater access to the immune system, but it is important that observations made in animal studies be confirmed in humans. The number, types and percentages of lymphocytes circulating in the bloodstream and the cell surface expression of molecules involved in cell–cell interaction, antigen presentation, and cellular activation can be measured using staining procedures. The size and cellularity (the numbers and types of cells present) of lymphoid organs can be determined. In animal studies the thymus, spleen, lymph nodes and some components of the gut-associated lymphoid tissue can be removed and weighed. In human studies thymus size can be estimated by imaging techniques. The concentrations of total Ig and of the Ig subclasses in the bloodstream can be measured, as can the circulating concentrations of Ig specific for antigens after an antigen challenge or vaccination. Secretory IgA (sIgA) is found in extracellular fluids and its concentration in saliva, tears and intestinal washings can be measured. Circulating concentrations of cytokines and soluble cytokine receptors can be measured. The overall cell-mediated immune response can be determined by assessing responses to challenges known to involve T-lymphocytes. For example, the DTH response to intradermal application of an antigen to which the individual has already been exposed has been used in some human studies; the response is measured as the size of the swelling (termed induration) around the area of application at a period (usually 48 h) after the application. In animal studies the ability to reject grafted tissues, especially skin, has been used.

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Variation in lymphocyte responses

Variation in cellular responses among individuals is not a great problem in animal studies since these most often use inbred strains. However, it is important to note that any particular response will differ among animal species and even among different strains within a species (Table 1). It is highly likely that immune cells from different species and strains will exhibit different sensitivities to the amount of fat in the diet and to different types of dietary fatty acids. Thus, extrapolations from animal studies to humans should be made cautiously.

There is wide variation in lymphocyte responses among healthy human subjects (Table 2). This is in part related to genetic polymorphisms which regulate the expression of cytokines, cytokine receptors, MHC II (in humans this is termed human leucocyte antigen or HLA), adhesion molecules and so on. However, other factors such as age, gender, smoking habits, habitual levels of exercise, alcohol consumption, diet, stage in the female menstrual cycle, and history of infections and vaccinations are likely to be important contributors to the observed variation. Consideration of this variation is important when designing and interpreting human studies. It is not clear whether there is variation in responsiveness of human lymphocytes to the effects of dietary fatty acids, although one study suggests that among women this varies with age (Meydani *et al.* 1991).

Eicosanoids: a link between fatty acids and lymphocyte functions

Eicosanoids

Eicosanoids are a group of chemical messengers synthesised from the 20-carbon PUFA dihomo-γ-linolenic acid (DGLA; 20:3*n*-6), ARA and eicosapentaenoic acid (EPA; 20:5*n*-3). Eicosanoids include prostaglandins (PG), thromboxanes (TX), leukotrienes (LT), lipoxins, hydroperoxy-eicosatetraenoic acids and hydroxyeicosatetraenoic acids (HETE). The fatty acid precursor for eicosanoid synthesis is released from cell membrane phospholipids, usually by the action of phospholipase A_2 activated in response to a cellular stimulus. Because the membranes of most cells contain large amounts of ARA, compared with DGLA and EPA, ARA is usually the principal precursor for eicosanoid synthesis and gives rise to the 2-series PG and TX and the 4-series LT (Fig. 3). PG and TX are produced by the cyclooxygenase enzymes (COX 1 and COX 2), while LT and related compounds are produced by lipoxygenase enzymes (LOX) of which there are three types (5-, 12- and 15-LOX). Lymphocytes are poor sources of eicosanoids (Goldyne, 1988), although they are subject to regulation by them. PGE_2 inhibits lymphocyte proliferation, NK cell activity and the production of Th1 cytokines (IL-2, IFN-γ) (see Kinsella *et al.* 1990; Roper & Phipps, 1994 for reviews). PGE_2 also inhibits MHC II expression and the production of TNF-α and IL-1β by monocytes and macrophages (see Kinsella *et al.* 1990; Roper & Phipps, 1994). In contrast, PGE_2 does not appear to directly influence the production of the Th2 cytokine IL-4 and promotes IgE production by B-lymphocytes (see Roper & Phipps, 1994). LTB_4 enhances production of TNF-α, IL-1 and IL-6 by monocytes and macrophages, enhances Th1 cytokine production and enhances NK cell activity (see Kinsella *et al.* 1990; Lewis *et al.* 1990 for reviews). Thus, ARA gives rise to mediators with opposing actions. Therefore, the overall effect will depend upon the timing of production of the different eicosanoids and upon the sensitivity of target cells to the concentrations of the different eicosanoids produced.

Table 1. Cytokine production by spleen lymphocytes from two different stains of mice

	Concentration (pg/ml)			
Mouse strain	IL-2	IFN-γ	IL-4	IL-10
C57Bl/6	660 (60)	510 (15)	92 (10)	72 (10)
Balb/c	1100 (50)*	35 (5)*	275 (25)*	170 (15)*

Spleen lymphocytes from male C57Bl/6 and Balb/c mice (weighing approximately 24 g) were prepared by standard techniques and were cultured for 48 h in the presence of 2·5 μg/ml Con A. Cytokine concentrations in the culture supernatants were measured by specific ELISA. Data are mean (SEM) for four separate cell preparations and are previously unpublished. *Indicates significantly different from C57B1/6.

γ-Linolenic acid and eicosanoids

γ-Linolenic acid (GLA; 18:3*n*-6) is found in borage, blackcurrant seed and evening primrose oils where it contributes about 20–25 %, 15–20 % and 5–10 % of fatty acids, respectively. Apart from the presence of GLA, these oils tend to resemble typical *n*-6 PUFA-rich vegetable oils with a high content of linoleic acid (18:2*n*-6), although blackcurrant seed oil also contains α-linolenic acid (ALNA; 18:3*n*-3). GLA does not accumulate in cell membranes, even when it is provided in the diet (Rossetti *et al.* 1997; Yaqoob *et al.* 2000). Instead, the content of its derivative, DGLA, is increased (Rossetti *et al.* 1997; Johnson *et al.* 1997; Yaqoob *et al.* 2000) (Table 3). The significance of this is that DGLA is a substrate for COX, giving rise to the 1-series PG (e.g. PGE_1), for 5-LOX giving rise to 3-series LT and for 15-LOX, giving rise to 15-hydroxy-DGLA (Fig. 3). Like PGE_2, PGE_1 inhibits IL-2 and IFN-γ production by lymphocytes (Gold *et al.* 1994), inhibits lymphocyte proliferation (Santoli *et al.* 1990) and inhibits TNF, IL-1 and IL-6 production by macrophages (Haynes *et al.* 1992). 15-hydroxy-DGLA is an inhibitor of 5-LOX (Iverson *et al.* 1991, 1992), and so can decrease the synthesis of some ARA-derived mediators (Iverson *et al.* 1991, 1992). Supplementing the diet of healthy humans with GLA results in increased PGE_1 production and decreased production of PGE_2, LTB_4 and LTC_4 (Pullman-Moar *et al.* 1990; Johnson *et al.* 1997; Wu *et al.* 1999).

n-3 Polyunsaturated fatty acids and eicosanoids

Fish oil contains the long chain *n*-3 PUFA EPA, docosapentaenoic acid (22:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3). Feeding humans increased amounts of fish oil results in an increase in the amount of EPA and DHA in the membranes of cells involved in inflammation such as

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Table 2. Variation in lymphocyte responses among healthy adult humans

Subjects	Thymidine incorporation (cpm/well)†	IL-2 production (pg/ml)¶	NK cell activity (% Cytolysis)*
Males aged 18 to 40 y; $n = 48$	48722 (38742, 57277)	453·6 (344·4, 848·4)	ND
Males aged 18 to 40 y; $n = 72$	30071 (25056, 37260)	260·4 (176·4, 428·4)	ND
Males aged 21 to 44 y; $n = 15$	25622 (16398, 29760)	219·2 (94·1, 484·0)	29·5 (19·9, 56·1)
Males aged 46 to 71 y; $n = 20$	18458 (13837, 21362)	297·3 (136·0, 384·1)	38·8 (28·6, 53·9)
Males aged 55 to 75 y; $n = 24$	16729 (11802, 20681)	579·6 (361·2, 1075·2)	32·9 (27·0, 45·0)
Females aged 56 to 75 y; $n = 23$	18089 (11532, 20304)	453·6 (226·8, 680·4)	27·4 (18·6, 38·0)

Data are the median values observed in various studies carried out by the authors; values in parentheses indicate the 25th and 75th centiles. †Determined over the final 18 h of a 66 h period of culture of MNC in the presence of 15 μg/ml Con A. ¶Determined (by enzyme-linked immunosorbant assay) in the culture medium after 24 h of culture of MNC in the presence of 15 μg/ml Con A. *Determined as lactate dehydrogenase release from K562 target cells over 4 h at a MNC to K562 cell ratio of 100:1. ND indicates not determined. Data are previously unpublished in this form.

monocytes, macrophages, neutrophils and lymphocytes (Lee *et al.* 1985; Endres *et al.* 1989; Sperling *et al.* 1993; Gibney and Hunter, 1993; Caughey *et al.* 1996; Kelley *et al.* 1999; Yaqoob *et al.* 2000; Healy *et al.* 2000) (Table 3). In parallel with this there is a decrease in the ARA content of cell membranes (Table 3). This means that there is less substrate available for synthesis of eicosanoids from ARA. Furthermore, EPA competitively inhibits the metabolism of ARA. Thus, fish oil feeding results in a decreased capacity (by 40–75 %) of immune cells to synthesise eicosanoids from ARA (Lee *et al.* 1985; Endres *et al.* 1989; Meydani *et al.* 1993; Sperling *et al.* 1993;

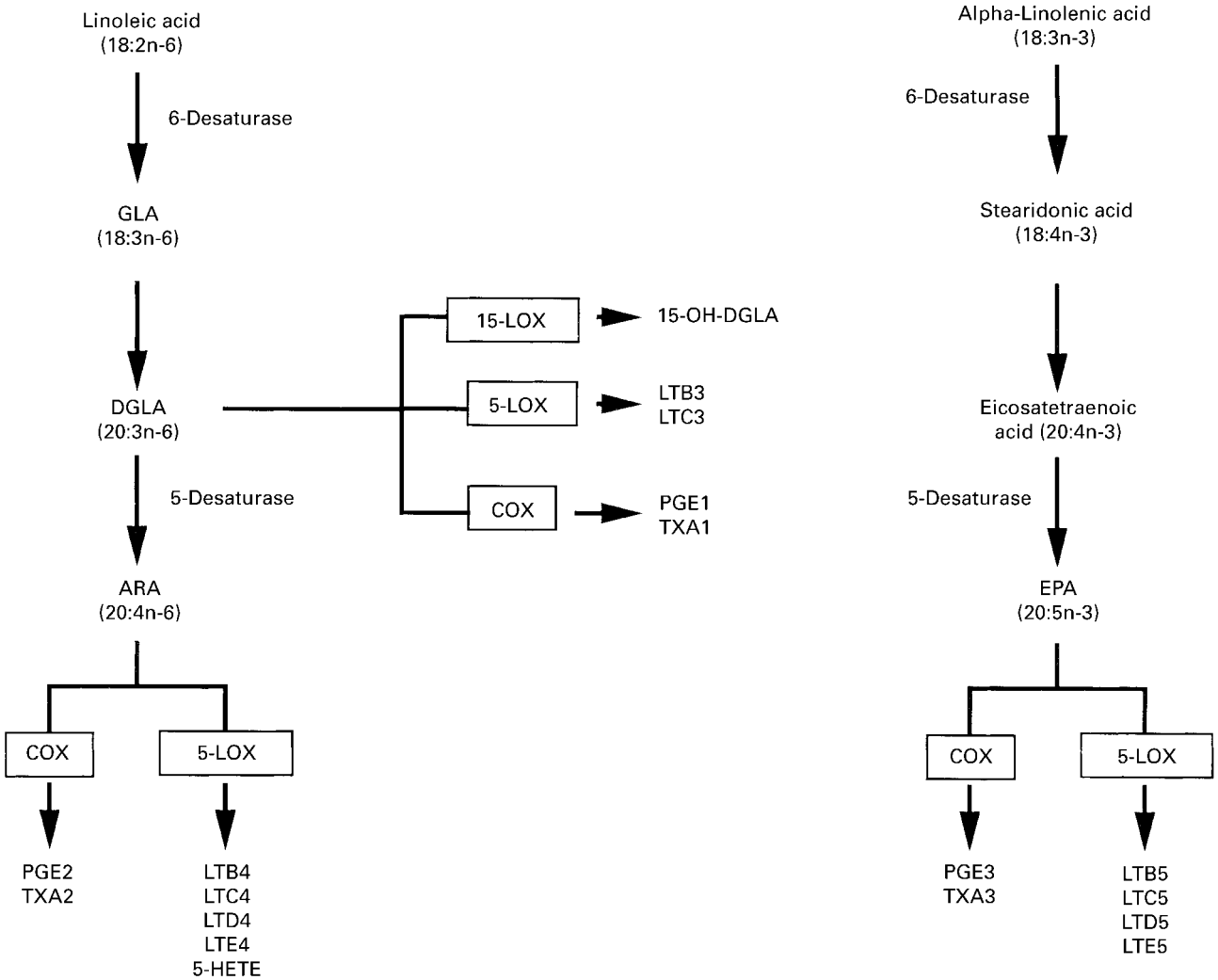

Fig. 3. Synthesis of eicosanoids from different precursor fatty acids.

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Table 3. Fatty acid composition of human mononuclear cells after supplementation of the diet with evening primrose oil or fish oil

	Fatty acid (g/100 g of total fatty acids)			
	Evening primrose oil		Fish oil	
Fatty acid	Before	After	Before	After
DGLA	1·2 (0·4)	2·2 (0·4)*	1·3 (0·5)	1·9 (0·5)
ARA	20·6 (2·1)	21·2 (0·5)	22·3 (0·8)	18·8 (1·3)*
EPA	1·0 (0·3)	1·1 (0·4)	0·8 (0·4)	2·8 (0·2)*
DHA	2·7 (0·4)	2·6 (0·4)	1·9 (0·5)	3·3 (0·3)

Healthy volunteers supplemented their diet with 9 g evening primrose oil (providing 1 g GLA) per day or with 9 g fish oil (providing 3·2 g EPA plus DHA) per day for 8 weeks. Mononuclear cells were isolated by standard techniques and the fatty acid composition determined. Data are mean (SEM) for six subjects per group and are taken from Yaqoob *et al.* (2000). *Indicates significantly different from before supplementation.

Caughey *et al.* 1996). A recent study reported that 6 g DHA per day resulted in a 60 % decrease in PGE_2 production and a 75 % decrease in LTB_4 production (Kelley *et al.* 1999); whether this is due to DHA itself or due to retroconversion of DHA to EPA is not clear from the data provided. ALNA is the precursor of EPA. Caughey *et al.* (1996) reported that 13·7 g ALNA per day resulted in a 33 % decrease in *ex vivo* PGE_2 production by human MNC; this is most likely due to the conversion of ALNA to EPA. In addition to competitively inhibiting the metabolism of ARA to eicosanoids, EPA is able to act as a substrate for both COX and 5-LOX (Fig. 3), giving rise to derivatives which have a different structure from those produced from ARA (i.e. 3-series PG and 5-series LT). Thus, the EPA-induced suppression in the production of ARA-derived eicosanoids is believed to be mirrored by an elevation in the production of EPA-derived eicosanoids. Although this has been clearly demonstrated by Lee *et al.* (1985) and Sperling *et al.* (1993), who showed that dietary fish oil results in significantly increased generation of the 5-LOX products of EPA by stimulated neutrophils and monocytes, the production of 3-series PG by human cells after fish oil feeding has not been demonstrated. The eicosanoids produced from EPA are considered to be less biologically potent than the analogues synthesised from ARA, although the full range of biological activities of these compounds has not been investigated.

Influence of the amount of fat in the diet on lymphocyte functions

Animal feeding studies

Many studies have compared the effects of feeding laboratory animals low and high fat diets upon lymphocyte proliferation and NK cell activity (see Calder, 1998*a* for references). Such studies have often found that high fat diets result in diminished lymphocyte functions compared with low fat diets, but the precise effect depends upon the exact level of fat used in the high fat diet and its source. The DTH response was reduced after feeding high fat, compared with low fat, diets (Friend *et al.* 1980; Crevel *et al.* 1992). These data indicate that a high fat diet diminishes lymphocyte activities and the cell-mediated immune response.

Human studies

A reduction in total dietary fat intake (from 41 % to 25 % to 31 % of total energy) enhanced blood lymphocyte proliferation in response to Con A or PHA by about 200 % (Kelley *et al.* 1989, 1992). NK cell activity was increased by 50 % by a reduction in fat intake from 30 % to an average of 22 % of total energy (Barone *et al.* 1989; Hebert *et al.* 1990). In these latter studies the reduction in fat intake was achieved without altering the fatty acid composition of the diet. These studies suggest that human lymphocyte responses can be enhanced by decreasing the amount of fat in the diet. In contrast, reducing fat intake of elderly Danish subjects from 40 to 29 % energy did not affect NK cell activity (Rasmussen *et al.* 1994), although in this study the reduction in total fat intake was accompanied by an increased contribution of linoleic acid and of *n*-3 PUFA to dietary fat.

Influence of the type of fatty acid in the diet on lymphocyte functions

The influence of saturated fatty acids, oleic acid (18:1*n*-9), linoleic acid, ALNA, ARA and the long chain *n*-3 PUFA, EPA and DHA, on the functions of lymphocytes and antigen presenting cells, on cytokine production by these cells and on adhesion molecule expression and cell–cell interactions has been reviewed elsewhere in detail (Calder, 1995, 1996*a*,*b*, 1997, 1998*a*,*b*,*c*). Thus, these effects will be summarised here; data from new studies and for fatty acids whose effects were not described previously will be described in detail.

Saturated fatty acids

In vitro and animal feeding studies suggest that saturated fatty acids have limited impact on lymphocyte proliferation, Th1-type and Th2-type cytokine production and NK cell activity (see Calder, 1995, 1996*b*, 1998*a*,*c*). The lack of effect of varying the intake of saturated fatty acids on NK cell activity is supported by human studies (Hebert *et al.* 1990; Rasmussen *et al.* 1994).

Oleic acid

In vitro and animal feeding studies show that oleic acid in sufficient amounts can partially inhibit lymphocyte proliferation, IL-2 production, IL-2 receptor and adhesion molecule expression and NK cell activity (see Calder, 1995, 1996*a*, 1998*a*,*c*). These observations suggest that dietary oleic acid has the potential to modulate lymphocyte functions, although the contribution of oleic acid to the animal diets in these studies was greatly in excess of the contribution to the diet of free living humans. Yaqoob *et al.* (2000) included a group who supplemented their habitual diet with an extra 9 g of olive oil/day for 12 weeks. This had no effect on lymphocyte proliferation, NK cell activity, or production of TNF-α, IL-1α, IL-1β, IL-2, or IFN-γ by

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MNC. One study of the effect of dietary intervention with oleic acid upon human lymphocyte functions has been performed (Yaqoob *et al.* 1998). In this study healthy free living subjects increased their oleic acid intake from 11 % to 18 % of energy at the expense of saturated fatty acids. After two months there was a non-significant reduction in NK cell activity and lymphocyte proliferation, and the number of MNC expressing CD54 was significantly decreased (Yaqoob *et al.* 1998).

Linoleic acid

In vitro and animal feeding studies have shown that linoleic acid in sufficient amounts can partially inhibit lymphocyte proliferation, production of IL-2, CTL activity, NK cell activity and the production of IgG and IgM (see Calder, 1995, 1996*a*, 1998*a*,*c*). The DTH response was lower after feeding *n*-6 PUFA-rich diets compared with saturated fat-rich or low fat diets. These observations suggest that high levels of linoleic acid in the diet impair the cell-mediated and antibody responses. However, more modest changes in the amount of linoleic acid in the rat diet did not markedly affect lymphocyte proliferation or NK cell activity (Jeffery *et al.* 1997).

Surprisingly few human studies have investigated the immunological impact of linoleic acid. The most detailed of the studies which have been performed are those of Kelley *et al.* (1989, 1992), which involved providing volunteers with low fat diets (25 % energy as fat) which were rich in linoleic acid (12·9 % of energy) or poor in linoleic acid (3·5 % of energy). No differences were observed in the responses of lymphocytes to various T-cell mitogens, in circulating IgM, IgG, IgE or IgA levels, or in the DTH response to seven recall antigens. Yaqoob *et al.* (2000) included a group who supplemented their habitual diet with an extra 9 g of sunflower oil/day for 12 weeks. This had no effect on lymphocyte proliferation, NK cell activity, or production of TNF-α, IL-1α, IL-1β, IL-2, or IFN-γ by MNC. These studies suggest limited impact of linoleic acid (at levels between 3·5 and 12·9 % of dietary energy) upon human immune function. However, in another study, the low fat diet induced increase in human NK cell activity (Barone *et al.* 1989; Hebert *et al.* 1990) was reversed by adding 15 g safflower oil/day to the diet for 2 months (Hebert *et al.* 1990); in this study linoleic acid contributed about 8·2 % of dietary energy when the subjects were consuming the safflower oil supplement. Furthermore, the NK cell activity of blood lymphocytes from elderly Danish subjects correlated negatively with linoleic acid intake and with serum levels of linoleic acid (Rasmussen *et al.* 1994); unfortunately this paper does not provide the data for linoleic acid intakes among the subjects.

Conjugated linoleic acid

Cook *et al.* (1993) reported that including conjugated linoleic acid (CLA) in the diet of chickens (0·5 g/100 g diet) increased lymphocyte proliferation in response to T-cell mitogens. More recent studies have reported some immunological effects of feeding a mixture of isomers of CLA to laboratory rodents. These studies have employed a fairly low fat diet (5 % fat by weight) in which CLA comprised 20 % of fatty acids present. Feeding this diet to mice increased lymphocyte proliferation and IL-2 production but did not affect NK cell activity (Hayek *et al.* 1999), while feeding it to rats decreased TNF-α and IL-6 production by macrophages (Turek *et al.* 1998). It is unclear which CLA isomers are responsible for these effects. At the time of writing no studies of the influence of CLA on human immune function have been published.

γ-Linolenic and di-homo-γ-linolenic acids

Both GLA and DGLA inhibited proliferation of cultured human lymphocytes (Kelley & Parker, 1979; Santoli *et al.* 1990; deMarco *et al.* 1994; Purasiri *et al.* 1997; Zurier *et al.* 1999), and DGLA decreased the production of IL-2 by human lymphocytes (Santoli *et al.* 1990); GLA probably also decreases IL-2 production but this has not been studied. GLA and DGLA inhibited production of IL-1β and of TNF-α by human monocytes in culture (DeLuca *et al.* 1999). Human peripheral blood NK cell activity was decreased after culture of the cells with GLA (Purasiri *et al.* 1997).

Feeding rats oils rich in GLA (e.g. evening primrose oil) decreased lymphocyte proliferation and NK cell activity, and decreased the expression of the IL-2 receptor and of some adhesion molecules (CD2, CD11a, CD18, CD54, CD62L) on the surface of lymphocytes (Yaqoob *et al.* 1994*a*,*b*; Sanderson *et al.* 1995*a*,*b*; Sanderson & Calder, 1998; Peterson *et al.* 1999). These observations suggest that GLA in sufficient quantities will diminish the Th1-type response. This is confirmed by the work of Matsuo *et al.* (1996) who showed that evening primrose oil increased total and ovalbumin-specific IgE production by spleen lymphocytes and increased circulating total and ovalbumin-specific IgE levels following ovalbumin challenge to ovalbumin-sensitised Brown Norway rats. In contrast, evening primrose oil suppressed ovalbumin-specific IgG production by lymphocytes, although circulating total and ovalbumin-specific IgG levels were unaffected by diet. Thus, it appears that GLA acts to diminish the Th1-type lymphocyte response.

Supplementation studies using GLA-rich oils to provide ≥2·4 g GLA per day in healthy human volunteers report decreased production of TNF-α, IL-1 and IL-6 by monocytes (Watson *et al.* 1993; DeLuca *et al.* 1999), and decreased lymphocyte proliferation (Rossetti *et al.* 1997). Recent studies providing ≤1 g GLA report no effect on lymphocyte proliferation (Yaqoob *et al.* 2000), NK cell activity (Yaqoob *et al.* 2000; Thies *et al.* 2001*a*) or production of TNF-α, IL-1β, IL-2 or IFN-γ (Wu *et al.* 1999; Yaqoob *et al.* 2000; Thies *et al.* 2001*b*,*c*). These observations suggest that a GLA intake of somewhere between 1 and 2·4 g per day is required to exert immunological effects in healthy humans.

Arachidonic acid

ARA increased IL-1β production by cultured human monocytes (Baldie *et al.* 1993). In contrast, ARA inhibited the proliferation of cultured lymphocytes, decreased the

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production of IL-2, and inhibited CTL degranulation (see Calder, 1995, 1996*a*, 1998*c*). Feeding rats or mice on diets containing significant amounts of ARA did not affect spleen lymphocyte proliferation, IL-2 production, NK cell activity or the graft versus host response, despite the increased capacity of the cells to produce PGE_2 (Jolly *et al.* 1997; Peterson *et al.* 1998*a*). These studies suggest that even significant amounts of ARA in the rodent diet do not influence cell-mediated immunity.

Two studies of the influence of dietary ARA upon human immune function have been performed. Including ARA (1·5 g per day) as part of a low fat diet (27 % energy as fat) for 8 weeks did not alter the proliferation of lymphocytes, NK cell activity, the DTH response to seven recall antigens, TNF-α, IL-1β, IL-6 or IL-2 production by MNC or the *in vivo* antibody responses to immunisation with three strains of influenza virus (Kelley *et al.* 1997, 1998*a*). ARA did however increase production of PGE_2 and LTB_4 by LPS-stimulated MNC. Supplementing the diet of healthy subjects aged 55 to 75 years with encapsulated ARA (approximately 700 mg/day) for 12 weeks did not effect the NK cell activity, lymphocyte proliferation or the production of TNF-α, IL-1β, IL-6, IL-2 or IFN-γ by MNC (Thies *et al.* 2001*a*,*b*,*c*). Although these studies suggest that increasing ARA consumption in healthy adults does not have adverse immunological effects, the length of ARA administration in these studies was no more than 12 weeks and the immunological effects of ARA over a longer term are not known.

α-Linolenic acid

In vitro and animal feeding studies indicated that ALNA inhibits lymphocyte proliferation, the production of IL-2, CTL activity, NK cell activity and the graft versus host response (see Calder 1995, 1996*a*,*b*, 1998*a*,*c*). The precise effect of ALNA on lymphocyte functions appears to depend on the level of linoleic acid and the total PUFA content of the diet (Jeffery *et al.* 1997).

A high dose of ALNA in the human diet (approximately 13 g per day for 4 weeks) decreased IL-1 and TNF production by LPS-stimulated monocytes (Caughey *et al.* 1996). Adding linseed oil (providing about 15 g ALNA per day) to a low fat diet (29 % energy from fat) resulted in a significant decrease in human blood lymphocyte proliferation and in the DTH response to seven recall antigens after 6 weeks, but circulating antibody levels were unaffected (Kelley *et al.* 1991). Supplementing the diet of healthy subjects aged 55 to 75 years with linseed oil providing 2 g ALNA per day did not significantly affect NK cell activity, lymphocyte proliferation or production of TNF-α, IL-1β, IL-6, IL-2 or IFN-γ by MNC (Thies *et al.* 2001*a*,*b*,*c*). These studies suggest that a moderate increase in ALNA intake by healthy adults does not affect immunity, but that a marked increase in ALNA intake can induce immunomodulatory effects. It is not clear whether these are exerted by ALNA itself or by EPA, a product of ALNA metabolism.

Eicosapentaenoic and docosahexaenoic acids

Since dietary fish oil leads to decreased PGE_2 production, it is often stated that it should reverse the effects of PGE_2, simply acting as a PGE_2 antagonist. If this were so then fish oil should increase the production of the classic pro-inflammatory cytokines (TNF, IL-1 and IL-6), increase the production of Th1-type cytokines, enhance MHC II expression, lymphocyte proliferation and NK cell activity and decrease IgE production by B-lymphocytes. Many studies, especially those conducted in laboratory animals, have demonstrated that, while in some situations fish oil does act as a 'PGE_2 antagonist', it often induces effects which are the opposite to those predicted on this basis. Thus, the situation is more complex than fish oil simply being a PGE_2 antagonist. This is because PGE_2 is not the sole mediator produced from ARA, and the range of mediators produced has varying, sometimes opposite, actions (see earlier). Thus, if fish oil was to act as a '4-series LT antagonist' it would be expected to decrease production of pro-inflammatory and Th1-type cytokines, and NK cell activity. EPA itself will give rise to eicosanoids with varying actions, some augmenting the actions of ARA-dereived mediators and others antagonising those actions. In addition long chain *n*-3 PUFA may exert a range of eicosanoid-independent effects, especially upon intracellular signalling mechanisms. Thus, the overall effect of fish oil feeding cannot be predicted solely on the basis of an abrogation of PGE_2-mediated effects.

In vitro studies have revealed a marked influence of long chain *n*-3 PUFA on inflammatory and immune cell function. EPA and DHA inhibited cytokine-induced cell surface expression of MHC II on macrophages and monocytes (Khair-el-Din *et al.* 1995; Hughes *et al.* 1996*a*), and decreased the ability of human monocytes to present antigen (tetanus toxoid) to autologous lymphocytes (Hughes & Pinder, 1997). In culture, EPA and DHA inhibit the production of IL-1β, TNF-α, IL-6 and IL-2, the proliferation of lymphocytes, and NK cell activity (see Calder 1995, 1996*a*,*b*, 1997, 1998*a*,*b*,*c*).

Feeding fish oil to laboratory rodents decreased MHC II expression and diminished presentation of antigen *ex vivo* (see Calder, 1996*a*,*b*, 1998*a*,*b*,*c*). A recent study reported that dietary fish oil decreased expression of the IFN-γ receptor on murine macrophages and splenocytes (Feng *et al.* 1999). Fish oil feeding has been reported to decrease *ex vivo* production of TNF-α, IL-1β and IL-6 by rodent inflammatory macrophages and monocytes (see Calder, 1996*a*,*b*, 1997, 1998*b*). These studies suggest that dietary fish oil might diminish the cell-mediated immune response by decreasing the activity of antigen presenting cells and by decreasing the sensitivity of macrophages to T-lymphocyte-derived cytokines. High levels of dietary fish oil decrease NK cell activity, CTL activity, lymphocyte proliferation, expression of the IL-2 receptor and adhesion molecules on lymphocytes (see Calder, 1995, 1996*a*,*b*, 1998*a*,*c*) and the production of IL-2 (Jolly *et al.* 1997; Wallace *et al.* 2001) and IFN-γ (Byleveld *et al.* 1999; Wallace *et al.* 2001). Dietary fish oil decreased the DTH and graft versus host responses compared with low fat or *n*-6 PUFA-rich or olive oil-rich diets (see Calder, 1995, 1996*a*,*b*, 1998*a*,*c*). Dietary fish oil also prolonged the survival of skin, kidney, heart or Islets of Langerhans transplants in rodents (see Calder, 1996*a*,*b*, 1998*a*,*c*). Taken together, these studies suggest

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that fish oil induces a shift in the T-lymphocyte response away from the Th1-type response, which is involved in cell-mediated immunity, chronic inflammation and graft rejection. In accordance with this, fish oil enhanced production of IgE to ovalbumin in rats (Prickett *et al.* 1982). Animal studies have often used very large amounts of fish oil in the diet: a diet in which fish oil contributes 20 % by weight will mean that EPA plus DHA comprise up to 30 % of dietary fatty acids and up to 12 % of dietary energy. Recent studies in rats and mice have indicated that relatively low levels of the long chain *n*-3 fatty acids (EPA or DHA at a level of 4·4 % of total fatty acids or 1·7 % of dietary energy) are sufficient to bring about some of the effects of fish oil, that dietary EPA and DHA both inhibit lymphocyte proliferation and IL-2 production and that dietary EPA, but not DHA, inhibits NK cell activity (Jolly *et al.* 1997; Peterson *et al.* 1998*a*,*b*).

Supplementation of the diet of human volunteers with 1·6 g EPA plus DHA per day for 3 weeks resulted in decreased expression of MHC II (HLA-DP, -DQ and -DR) and of CD54 on the surface of blood monocytes (Hughes *et al.* 1996*b*). Fish oil providing more than 2·4 g EPA plus DHA per day decreased production of TNF, IL-1 and IL-6 by MNC (Endres *et al.* 1989; Meydani *et al.* 1991; Gallai *et al.* 1993; Caughey *et al.* 1996). One other study in which subjects consumed a low fat diet including oily fish daily (providing 1·2 g EPA plus DHA per day) showed decreased production of TNF, IL-1 and IL-6 (Meydani *et al.* 1993). In contrast to these observations, a number of studies which provided from 0·55 g to 3·4 g EPA plus DHA per day have failed to demonstrate an effect of fish oil on production of TNF, IL-1 and IL-6 (Molvig *et al.* 1991; Cooper *et al.* 1993; Cannon *et al.* 1995; Schmidt *et al.* 1996; Blok *et al.* 1997; Yaqoob *et al.* 2000; Thies *et al.* 2001*c*). Data from studies investigating the influence of fish oil on human lymphocyte functions are also conflicting. Supplementation of the diet of healthy human volunteers with fish oil providing 2·4 g EPA plus DHA per day resulted in decreased proliferation of lymphocytes from older (aged 51–68 years) but not young (aged 21–33 years) women and decreased IL-2 production (Meydani *et al.* 1991). Molvig *et al.* (1991) reported decreased lymphocyte proliferation after providing 1·7 or 3·4 g EPA plus DHA per day to men, while Gallai *et al.* (1993) reported that 5·2 g EPA plus DHA per day decreased IL-2 and IFN-γ production. Providing 1·2 g EPA plus DHA to healthy subjects aged 55–75 years resulted in decreased NK cell activity (Thies *et al.* 2001*a*) and lymphocyte proliferation (Thies *et al.* 2001*b*), but did not affect IL-2 or IFN-γ production (Thies *et al.* 2001*b*). Finally, inclusion of oily fish providing 1·2 g EPA plus DHA per day in the diet of volunteers consuming a low fat diet decreased lymphocyte proliferation, IL-2 production and the DTH response to seven recall antigens (Meydani *et al.* 1993). In contrast to these observations, there are reports of no effect of 3·2 g EPA plus DHA per day on NK cell activity, lymphocyte proliferation and IL-2 and IFN-γ production (Yaqoob *et al.* 2000) and of no effect of 4·6 g EPA plus DHA per day on lymphocyte proliferation and IL-2 production (Endres *et al.* 1993). Thus, several studies indicate that a high level of dietary fish oil can inhibit monocyte and lymphocyte responses. Other studies indicate that more modest addition of fish oil to the diet does not affect these responses. However, there are a large number of studies which fall between the extremes of 'modest addition' and 'high level' and these studies provide conflicting results. It is unclear what the reasons for these discrepancies are, but they might be related to different experimental protocols used, particularly those involving cell preparation, cell culture and cytokine assays and/or to different subject characteristics (e.g. gender, age, habitual diet).

Some recent studies in humans have examined whether the effects of fish oil are due to EPA or DHA. Kelley *et al.* (1998*b*, 1999) reported the effects in males aged 20–40 years of including 6 g DHA per day in a low fat diet (30 % energy as fat) for 90 days. There was no effect of DHA on lymphocyte proliferation, serum IgG concentration or the DTH response to seven recall antigens or on the serum antibody response to immunisation with three strains of influenza virus. NK cell activity was unaffected at day 55 but was decreased at day 90. The production of TNF-α and IL-1β tended to decrease at day 55 but was significantly decreased at day 80. More recently, 750 mg DHA per day was shown not to affect NK cell activity, lymphocyte proliferation or the production of TNF-α, IL-1β, IL-6, IL-2 or IFN-γ in healthy subjects aged 55–75 years (Thies *et al.* 2001*a*,*b*,*c*). Taken together these data indicate that high levels of DHA (e.g. 6 g per day) can mimic some of the effects of fish oil but that lower levels (e.g. <1 g per day) do not exert any immunological effects in healthy adults.

Summary of the effects of fatty acids on lymphocyte functions

It is apparent that lowering the amount of fat consumed in the diet will enhance lymphocyte responses, although a supply of essential fatty acids is necessary to support the development of lymphoid organs and the responsiveness of lymphocytes. It is also apparent that at any particular level of fat in the diet, varying the fatty acid composition may impact on lymphocytes. *In vitro* and animal feeding studies suggest that saturated fatty acids have a limited effect on immune function, but that unsaturated fatty acids have the capacity to modulate lymphocyte functions and the cell-mediated and antibody responses to challenge. Among the unsaturated fatty acids, PUFA appear particularly potent with GLA, DGLA, ARA, EPA and DHA all exerting fairly similar effects *in vitro* upon lymphocyte proliferation, IL-2 production, and NK and CTL activities. Animal feeding studies indicate that a high intake of oleic acid, linoleic acid, GLA, ALNA, EPA or DHA can influence lymphocyte function and cell-mediated immunity. The intakes of these fatty acids used in animal studies are often greatly in excess of those which could be achieved in free living humans, and it appears from human studies that reasonable variation in intakes of oleic acid, linoleic acid and ALNA probably has limited impact on immune function. However, large long-term differences in habitual intakes of these fatty acids might influence immune function, and affect the incidence of diseases that have an immunological component (e.g. Linos *et al.* 1991). It is the PUFA which are habitually consumed in small amounts (GLA, EPA, DHA) which appear to have the more potent immunological effects. The studies performed to date have not clearly defined the dose–response effects for these fatty acids. However, it appears

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that the levels at which these fatty acids will influence lymphocyte functions can be achieved only through consumption of the pure oils. The immunological effects of GLA and of the long chain *n*-3 PUFA are generally termed as anti-inflammatory and the applications of these effects have been described in terms of chronic inflammatory diseases, allergic inflammation, acute systemic inflammation in response to trauma and graft rejection.

Applications

Chronic inflammatory diseases

Chronic inflammatory diseases are characterised by a dysregulated, overactive Th1-type response and often by an inappropriate production of ARA-derived eicosanoids, especially PGE_2 and LTB_4. The effects of fish oil outlined above suggest that it might have a role in prevention and therapy of these diseases (Fig. 4). Dietary fish oil has been shown to have beneficial clinical, immunological and biochemical effects in various animal models of human chronic inflammatory diseases, including decreased incidence and severity of inflammation in mice with type II collagen-induced arthritis (Leslie *et al.* 1985) and less inflammation in rat models of colitis (Wallace *et al.* 1989; Vilaseca *et al.* 1990). It was recently reported that both EPA and DHA suppress streptococcal cell wall-induced arthritis in rats, but that EPA was more effective (Volker *et al.*

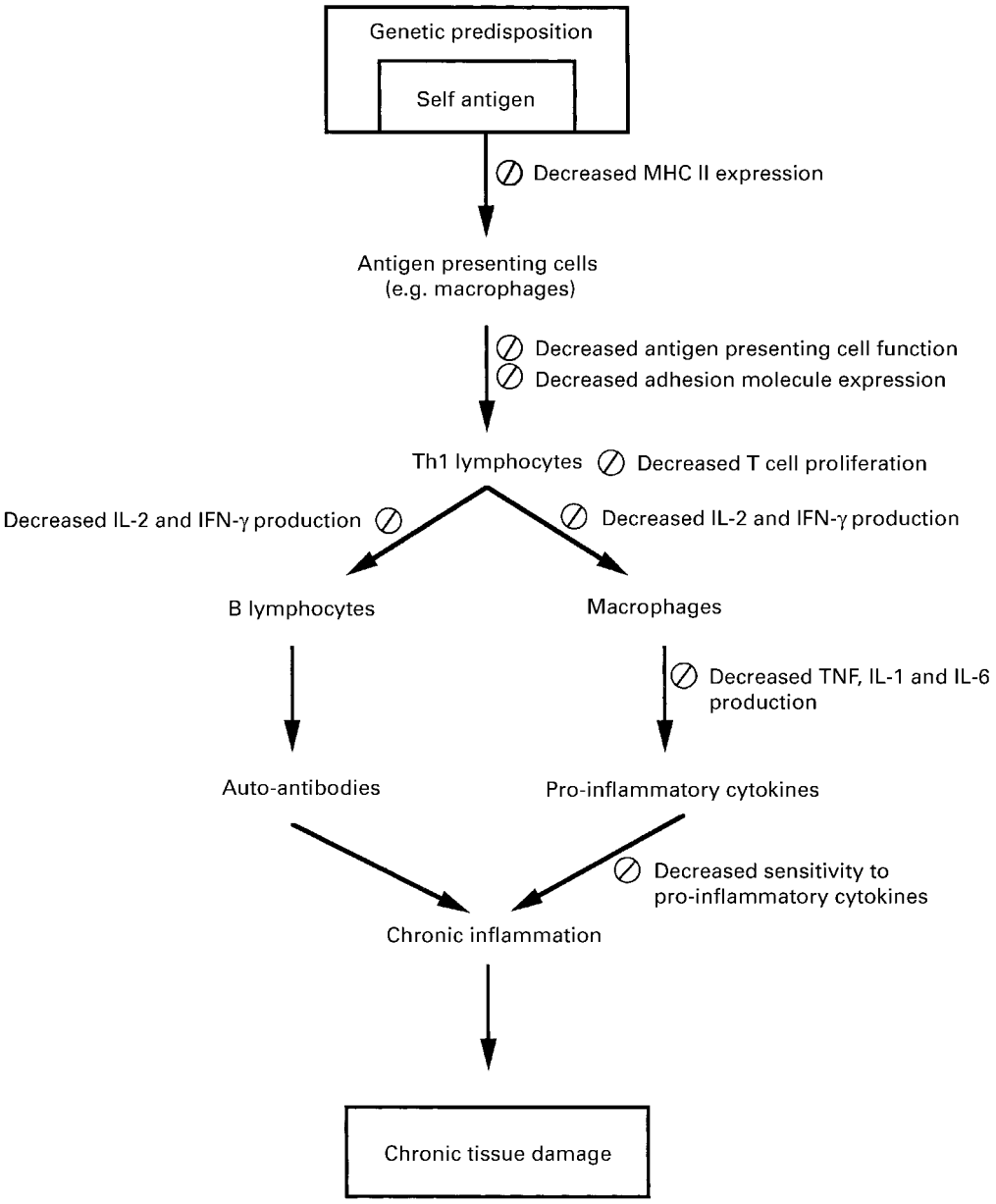

Fig. 4. Potential sites of action of *n*-3 PUFA in ameliorating chronic inflammatory diseases. Chronic inflammatory diseases are characterised by a dysregulated Th1 response resulting from inappropriate recognition of self-antigen in genetically predisposed individuals. *n*-3 PUFA can exert anti-inflammatory effects at several points (Ø) to induce clinical benefit.

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Table 4. Summary of clinical trials of fish oil in chronic inflammatory diseases

Disease	Number of double-blind, placebo-controlled studies	Doses of EPA + DHA used (g/day)	Duration (weeks)	Key findings	Reviews
Rheumatoid arthritis	13	1–6·4	12–52	All studies reported improvements, including reduced duration of morning stiffness, reduced number of tender or swollen joints, reduced joint pain, reduced time to fatigue, and increased grip strength. Twelve studies reported improvement in at least two clinical measures, and five studies reported improvement in at least four clinical measures. Ten studies reported decreased joint tenderness. Three studies reported significant decrease in the use of non-steroidal anti-inflammatory drugs.	James & Cleland, 1997; Geusens, 1998; Calder, 2001
Crohn's Disease	3	2·7–5·1	12–52	Two studies reported no benefit. One study reported a significiant decrease in relapses. One other study which used oily fish (100–250 g/day for 2 years) reported a significant decrease in relapses.	Belluzzi & Miglio, 1998
Ulcerative colitis	4	1·8–5·4	12–52	One study reported no benefit (this study used the lowest dose of EPA plus DHA) One study reported a non-significant decrease in disease activity and a significant decrease in use of corticosteroids. Two studies reported benefit including improved histological appearance of the colon, decreased disease activity, weight gain and decreased use of prednisolone. Two other open studies reported improved symptoms, improved histological appearance of the rectal mucosa and decreased use of prednisolone.	Rodgers, 1998
Psoriasis	2	1·8	8–12	One study reported significant improvement in itching, scaling and erythema. One study reported no benefit. Three open studies (providing 10 to 18 g EPA plus DHA per day for 6–8 weeks) reported mild to moderate (two studies) or moderate to excellent (one study) improvements in scaling, itching, lesion thickness and erythema in the majority of patients.	Ziboh, 1998

2000); this fits with the more potent effects of EPA than DHA on inflammation and immunity.

There have been a number of clinical trials assessing the benefits of dietary supplementation with fish oil in several inflammatory diseases in humans including rheumatoid arthritis, Crohn's Disease, ulcerative colitis, and psoriasis (see Table 4 for a summary). Many of the placebo-controlled, double-blind trials of fish oil in chronic inflammatory diseases reveal significant benefit including decreased disease activity and a lowered use of anti-inflammatory drugs; the evidence for a beneficial effect of fish oil is strongest in rheumatoid arthritis (Table 4).

Asthma

ARA-derived eicosanoids such as PGD_2, LTC_4, D_4 and E_4 are among the major mediators of allergic inflammation (Fig. 5). Although its action as a precursor to LT has highlighted the significance of ARA in the aetiology of allergic inflammation, a second link with this fatty acid has been made. This is because PGE_2 regulates the differentiation and functions of lymphocytes (see earlier). Of particular relevance in the context of asthma and related IgE-mediated allergic diseases is the ability of PGE_2 to inhibit the production of the Th1-type cytokine IFN-γ without affecting the production of the Th2-type cytokines, and to stimulate B-cells to produce IgE. These observations suggest that PGE_2 regulates the development of these diseases (Fig. 5). As a result of this there has been speculation that the increased intake of linoleic acid, the precursor of ARA, which has occurred since the mid-1960s, is causally linked to the increased incidence of asthma and allergic diseases over this period (Hodge *et al.* 1994; Black & Sharpe, 1997). Thus, a case has been made for increasing the consumption of *n*-3 fatty acids by patients with IgE-mediated allergic diseases (Fig. 5). There is some epidemiological evidence to support a protective role of long chain *n*-3 PUFA in these diseases (see Calder & Miles, 2000 for references). However, several studies of fish oil supplementation in asthma reveal limited clinical impact,

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despite significant biochemical changes (e.g. reduced 4-series LT production) (see Calder & Miles, 2000 for references). In contrast, some studies have shown significant clinical improvements, at least in some patient groups, and suggest that this type of approach may be useful in conjunction with other drug- and diet-based therapies (see Calder & Miles, 2000 for references). A very careful study by Broughton *et al.* (1997) found that low *n*-3 PUFA ingestion resulted in increased methacholine-induced respiratory distress in adult asthmatics (Table 5). In contrast, high *n*-3 PUFA ingestion resulted in an improved response in more than 40 % of the subjects (Table 5); all measures of respiratory function were markedly improved in this group of patients who also showed elevated appearance of the EPA-derived 5-series LT in their urine. However, some patients did not respond to the high *n*-3 PUFA intake (Table 5). This study indicates that there are patients who respond positively to fish oil intervention and patients who are non-responders. This suggests that such therapies should be approached cautiously until more is understood about the interaction between fatty acid consumption and disease activity.

Concluding remarks

Amongst the fatty acids it is the *n*-3 PUFA which possess the most potent immunomodulatory activities, and amongst the *n*-3 PUFA those from fish oil (EPA and DHA) are more biologically potent than ALNA. Components of both natural and acquired immunity, including the production of key inflammatory mediators, can be affected by *n*-3 PUFA. Animal studies indicate that diets rich in EPA plus DHA are anti-inflammatory and immunomodulatory *in vivo*, although there have been relatively few good studies in humans. Although some of the effects of *n*-3 PUFA may be brought about by modulation of the amount and types of eicosanoids made, it is possible that these fatty acids might elicit some of their effects by eicosanoid-independent mechanisms,

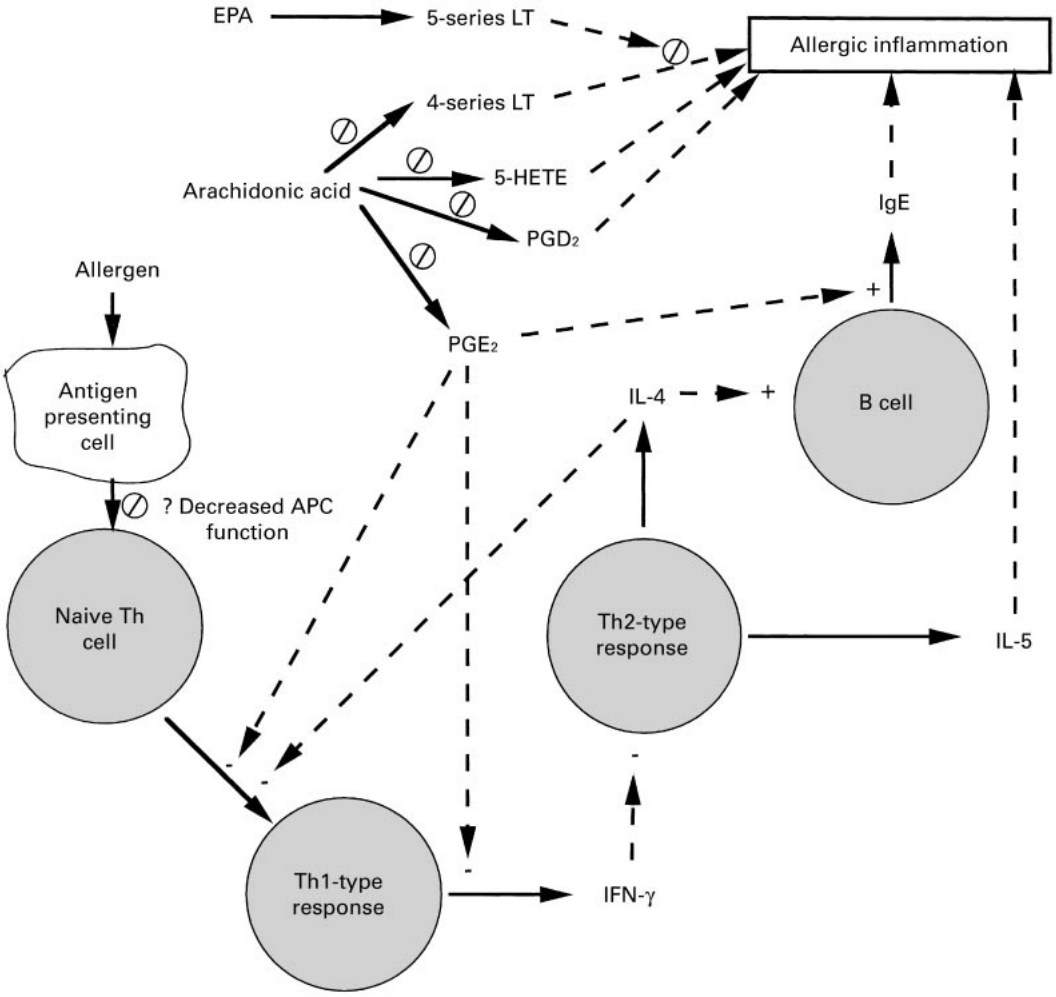

Fig. 5. Potential sites of action of *n*-3 PUFA in ameliorating IgE-mediated allergic diseases. ARA gives rise to mediators of allergic inflammation and PGE_2 inhibits the development of the Th1 phenotype and the production of IFN-γ. This allows the development of the Th2 phenotype, with production of IL-4. IL-4 acts to reinforce the inhibition of the Th1-type response and also promotes IgE production by B cells. PGE_2 acts directly on B-cells to promote IgE production. IL-5 is a mediator of allergic inflammation, while IL-10 (produced by Th2-type lymphocytes) inhibits development of the Th1-type response (not shown). *n*-3 PUFA can exert anti-inflammatory effects at several points (Ø) to induce clinical benefit. ⟶ indicates produces; ⇢ indicates regulates.

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Table 5. Influence of fish oil intervention in adult asthma

	Cumalitive dose of methacholine to cause a 20 % decline (units)			
	Baseline	Low dose fish oil	High dose fish oil	
	($n = 19$)	($n = 19$)	Responders ($n = 9$)	Non-responders ($n = 10$)
Forced vital capacity	24·1	11·8	>100	3·7
Peak expiratory flow	17·1	5·9	>100	3·5
Forced expiratory volume/sec	16·9	1·9	>100	4·9
Forced expiratory flow 25–75 %	9·0	0·7	>100	9·9

Adult asthmatics consumed fish oil capsules for 4 weeks such that the *n*-6 to *n*-3 PUFA ratio of their diet was 10 (low dose fish oil) or 2 (high dose fish oil). At baseline, after consuming the low dose fish oil and after consuming the high dose fish oil subjects underwent challenge with increasing amounts of methacholine to give cumulative doses up to 68 units. Data are the cumulative dose of methacholine required to cause a 20 % reduction in each indicator of lung function. Data are from Broughton *et al.* (1997).

including actions upon intracellular signalling pathways and transcription factor activity (see Miles & Calder, 1998; Yaqoob, 1998*b*). Such *n*-3 PUFA-induced effects may be of use as a therapy for chronic inflammatory conditions characterised by an overactive Th1 response, and for disorders like asthma characterised by an overactive Th2 response. Moderate levels of ARA and DHA do not appear to have any significant effects on human immune function, but the effects of these fatty acids have been studied in the short term and only in healthy adults. All studies of fatty acids and the human immune system have used adults as subjects and most studies have used men only or a mixture of men and women. The only study which has used women exclusively as subjects was that of Meydani *et al.* (1991); in that study it was found that the immune system of older women is more sensitive to fish oil than is that of young women. This age-related difference in sensitivity to dietary intervention may explain some of the contradictory observations in the literature (e.g. between Yaqoob *et al.* 2000 and Thies *et al.* 2001*a*,*b*). It is clear that more needs to be understood about the impact of *n*-6 and *n*-3 PUFA on the human immune system and on how variations in age, gender, ethnicity, hormone status, antioxidant status and genetics influence sensitivity to dietary PUFA.

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