Dietary *n*-6 and *n*-3 fatty acids in immunity and autoimmune disease

Laurence S. Harbige

School of Chemical and Life Sciences, University of Greenwich, Wellington Street, London SE18 6PF, UK

Lipid biology plays a significant role in the normal and pathological functioning of cells of the immune system (Hopkins *et al.* 1981; Dvorak *et al.* 1983; Schlager *et al.* 1983; Shipman *et al.* 1988; Roper *et al.* 1990; Goetzl *et al.* 1995; Pushkareva *et al.* 1995; Hennig *et al.* 1996). In addition to the physiological requirements of cells of the immune system for essential fatty acids, dietary fatty acid modulation of the membrane composition and functions of immune cells can affect both normal and pathological processes (Calder, 1996; Harbige, 1996; Hennig *et al.* 1996). Among the first immunologists to recognize the importance of dietary fatty acids in immunity and autoimmune disease were Peter Medawar and Jürgen Mertin (Medawar *et al.* 1979) in the UK and Gabriel Fernandes and Robert Good (Fernandes *et al.* 1972) in the USA. The importance of dietary fatty acids in the prevention or control of autoimmune disease in animal models is now well documented (Mertin, 1981; Levy *et al.* 1982; Kelley *et al.* 1985; Morrow *et al.* 1985; Robinson *et al.* 1986; Alexander *et al.* 1987; Harbige *et al.* 1995; Lin *et al.* 1996). However, the biochemical and immunological mechanisms by which dietary fatty acids affect the immune system and autoimmunity have remained largely obscure. In recent years important new concepts in immunoregulation have emerged which are likely to be of significance to effects of fatty acid nutrition on immunity. Mosman & Coffman (1989) have characterized two different patterns of cytokine secretion by T-cells which lead to different functional responses. T helper$_1$ (Th_1) cells produce interleukin (IL)-2, interferon-γ and tumour necrosis factor-β which are not synthesized by Th_2 cells. In contrast, Th_2 cells (but not Th_1) produce IL-4, IL-5 and IL-10. Th_1 cells enhance cell-mediated inflammatory activity, whereas Th_2 cells synthesize cytokines that help B-cells develop into antibody-producing cells. There are also T-cells able to produce both Th_1 and Th_2 cytokines, referred to as Th_0 cells (Male *et al.* 1996). Weiner (1997) has further characterized a Th_3 T-cell subset that primarily produces transforming growth factor-β (TGFβ), provides help for immunoglobulin (Ig) A production, and has suppressive properties. Importantly, the overall balance of cytokine production by Th_1, Th_2, and Th_3 cells affects the type of immune response generated, e.g. Th_2 cytokines can down-regulate production of Th_1 cytokines and *vice versa* (Male *et al.* 1996). Eicosanoids are known to affect these immunoregulatory mechanisms (Phipps *et al.* 1991), which may, in part, explain the complex relationship between the immune system, eicosanoids and the eicosanoid precursor polyunsaturated fatty acids derived from the diet. The balance of membrane fatty acids and eicosanoids is also known to influence inflammatory reactions (Terano *et al.* 1984; Lefkowith, 1988; Tate *et al.* 1989; Ross, 1993; Calder, 1996; Hennig *et al.* 1996).

Essential fatty acid-deficient diets and high- and low-fat diets in autoimmune disease

Diets deficient in essential fatty acids and diets low in fat markedly increase the survival and reduce spontaneous autoimmune disease in NZB × NZW F_1 mice (primarily autoantibody mediated), a model of the human disease systemic lupus erythematosus (Hurd *et al.* 1981; Levy *et al.* 1982). Essential fatty acid-deficient diets also protect against autoimmune diabetes in the BB rat and in a low-dose-streptozotocin-treated mouse model of autoimmune diabetes (Lefkowith *et al.* 1990). High-fat (both lard and maize oil) diets increase the levels of natural thymocytotoxic and anti-DNA antibodies along with increased immune complex deposition, decrease T-cell mitogenic responses to concanavalin A (Con A), and accelerate the disease course in NZB × NZW F_1 mice (Levy *et al.* 1982; Yumura *et al.* 1985). In addition, the same group (Morrow *et al.* 1985) reported that high-saturated-fat (lard) diets have deleterious effects on both macrophage phagocytosis and natural killer cell activity, the latter correlating with *in vitro* interferon production. In the NZB × NZW F_1 autoimmune model a high-fat diet consisting of equal amounts of lard and soyabean oil (rich in linoleic acid) causes animals to develop more severe disease and have a shortened lifespan associated with increased IgG anti-cardiolipin antibodies (Lin *et al.* 1996, 1997). In contrast to some of the previously described findings, in rats clinical manifestations of a T-cell-mediated disease experimental autoimmune encephalomyelitis (EAE), an animal model for the human disease multiple sclerosis, appear potentiated by fat deficiency (Clausen & Moller, 1967; Selivonchick & Johnston, 1975). However, there does appear to be disparity in the effects of fat deficiency on EAE (Levine & Sowinski, 1980), and the effects of high-fat diets have not been investigated.

Abbreviations: Con A, concanavalin A; EAE, experimental autoimmune encephalomyelitis; Ig, immunoglobulin; IL, interleukin; LT, leukotriene; ova, ovalbumin; PG, prostaglandin; PHA, phytohaemagglutinin; Th, T helper; TGFβ, transforming growth factor-β.

Corresponding author: Dr L. S. Harbige, fax +44 (0)181 331 8305, email L. Harbige@greenwich.ac.uk

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Autoimmunity, immune functions and *n*-3 fatty acids

Animal and human studies

The effects of dietary *n*-3 fatty acids on *ex vivo* lymphocyte functions, as judged by mitogen stimulation, have been consistent in both human and animal studies showing suppressed responses (Calder, 1996). Kelley *et al.* (1988) found that an *n*-3 fatty acid-rich diet (76 g fish oil/kg) decreased rabbit peripheral-blood lymphocyte mitogenic responses to Con A, phytohaemagglutinin (PHA) and pokeweed (*Phytolacca americana*) mitogen *ex vivo*. Similarly, in rats fed on a high-fish-oil diet Yaqoob *et al.* (1994) reported decreased proliferative responses to Con A for spleen and lymph node lymphocytes. In mice fed on a high-fish-oil diet spleen lymphocytes showed decreased responses to Con A (Yaqoob & Calder, 1995). In both fish oil-fed and fish oil plus high- or low-dose oral ovalbumin (ova) tolerance induction, ova immunized mice exhibited markedly reduced spleen lymphocyte responses to ova *ex vivo* and, in ova-fed animals only, reduced ova-specific levels of serum IgG_1, IgG_{2a} and IgG_{2b} (LS Harbige and BAC Fisher, unpublished results). In contrast, Morrissey *et al.* (1990) observed increased Con A -induced proliferation with a low-fish-oil diet (50 g/kg) plus additional vitamin E (150 mg/kg). This suggests that low-dose-fish-oil feeding may have different effects on proliferative responses, or that vitamin E may have an effect on mitogen-induced lymphoproliferation independent of fish oil *n*-3 fatty acids. Interestingly, Kelley *et al.* (1988) observed enhanced lymphocyte responses in rabbits fed on α-linolenic acid-rich linseed oil. However, this response may reflect evolutionary adaptation of rabbits to an α-linolenic acid-rich herbivorous diet, and highlights the importance of species differences. All the previously described *ex vivo* findings may not reflect fully those *in vivo*. For example, there appear to be site-specific differences in lymphocyte responses to dietary fatty acids (Yaqoob *et al.* 1994), which appear to be dependent on the local adipose-tissue lipid composition (Pond, 1996). It is interesting to note that adipose tissue around lymph nodes preferentially incorporates or selectively retains polyunsaturated fatty acids (Pond, 1996). This suggests that lymphoid cells inside lymph nodes can be locally supplied with the appropriate fatty acids for membrane and eicosanoid synthesis.

Several investigators have found reduced major histocompatibility complex class II molecule expression in rodents fed on fish oil (Huang *et al.* 1992; Sherrington *et al.* 1995), indicating a decreased ability of antigen-presenting cells to present antigen to T lymphocytes. Consistent with the previous findings, Fujikawa *et al.* (1992) found splenocytes acting as antigen-presenting cells from mice fed on the *n*-3 fatty acid eicosapentaenoic acid had a reduced ability to present antigen to T-cell clones. Experimental data on the effects of feeding *n*-3 fatty acids on macrophage phagocytic functions and the production of H_2O_2, superoxide and NO have been inconsistent (Calder, 1996). There are also inconsistent reports of the effects of feeding fish oil to rodents on tumour necrosis factor and IL-1 production by peritoneal macrophages (Calder, 1996). In rats fed on fish oil (Leitch *et al.* 1984) or pure ethyl ester of eicosapentaenoic acid (Terano *et al.* 1984), *ex vivo* peritoneal leucocytes produced less pro-inflammatory leukotriene (LT) B_4 and more of the less-potent LTB_5.

Hughes *et al.* (1995) found that fish oil supplementation in human subjects reduced expression of major histocompatibility complex class II DR molecules on peripheral-blood monocytes which suggests antigen presentation may be affected. Fish oil supplementation for 24 weeks reduced the relative percentage of peripheral blood $CD4^+$ and increased the percentage of $CD8^+$ cells in man (Meydani *et al.* 1993). In non-human primates fed on diets containing 1·3 and 3·3 % energy as eicosapentaenoic acid and docosahexaenoic acid for 14 weeks increased peripheral-blood lymphocyte responses to Con A and PHA have been reported (Wu *et al.* 1996). These investigators also found that the plasma vitamin E level was maintained, and suggested that in the presence of adequate vitamin E concentrations lymphocyte mitogenic proliferative responses are enhanced. Again this indicates vitamin E may have independent effects on immune function and that the balance of *n*-3 fatty acids and vitamin E is important. The effects of fish oil supplementation have been extensively studied on human peripheral-blood mononuclear cell cytokine production *ex vivo*, particularly IL-1α, IL-1β, IL-2, IL-6, and tumour necrosis factor-α, showing decreased production of these cytokines (Calder, 1996). Administration of 12-O-tetradecanoylphorbol-13-acetate induces tumour necrosis factor-α gene expression in human HL-60 cells through arachidonic acid metabolites acting as regulating messengers (Horiguchi *et al.* 1989). It is well known that on feeding fish oil the *n*-3 fatty acid, eicosapentaenoic acid, displaces arachidonic acid in membrane phospholipids. It is possible, therefore, that the effects of *n*-3 fatty acids on some pro-inflammatory cytokines may be indirect, and may indicate the direct importance of arachidonic acid metabolism in their regulation. The delayed-type hypersensitivity response, which is an *in vivo* measure of cell-mediated immunity, was reported by Meydani *et al.* (1993) to be reduced in response to several recall antigens, including tetanus toxoid and *Mycobacterium tuberculosis*, in subjects supplemented daily with 1·23 g eicosapentaenoic acid and docosahexaenoic acid. Investigators have shown that feeding fish oil induces a decrease in pro-inflammatory neutrophil LTB_4 production and an increase in the less potent pro-inflammatory lipid-mediator LTB_5 in human subjects with inflammatory diseases (Schmidt & Dyerberg, 1989).

Autoimmune disease

In mouse models of the human disease, systemic lupus erythematosus, fish oils rich in *n*-3 fatty acids increase the longevity and delay the onset of clinical manifestations of spontaneous autoimmunity (autoantibody mediated) in NZB × NZW F_1 and MRL/lpr mice (Kelley *et al.* 1985; Godfrey *et al.* 1986; Robinson *et al.* 1986). Feeding fish oil compared with maize oil delayed the onset of autoimmune disease in NZB × NZW F_1 mice (Fernandes *et al.* 1994). This observation was associated with elevated IL-2, IL-4 and $TGFβ_1$ and lower c-myc and c-ras mRNA in the spleen, with protein products following the same pattern (Fernandes *et al.* 1994). The same group reported high kidney $TGFβ_1$

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mRNA for NZB × NZW F_1 mice fed on maize oil and low kidney TGFβ_1 mRNA expression for NZB × NZW F_1 mice fed on fish oil, the reverse of their findings in the spleen (Fernandes, 1994). In contrast, antioxidant enzyme mRNA expression in the liver was higher in fish oil-fed mice than in maize oil-fed mice (Fernandes, 1994). These workers suggested that these findings were important to the renal disease normally observed in the NZB × NZW F_1 mouse, and also that dietary fatty acids regulate TGFβ_1 and antioxidant enzyme expression in an organ-specific manner (Chandrasekar *et al.* 1995). Interestingly, Kubo *et al.* (1997) reported organ-specific susceptibility to lipid peroxidation and organ-specific differences for antioxidants, and suggested that the kidney, in terms of its fatty acid composition, is more resistant to dietary *n*-3 fatty acid influences. Fernandes *et al.* (1996) have also shown that feeding fish oil to the NZB × NZW F_1 mouse increases programmed cell death of lymphocytes, which may prevent the accumulation of self-reactive immune cells in lymphoid organs.

Evidence for beneficial effects of *n*-3 fish oil fatty acids in experimentally-induced T-cell-mediated models of autoimmune arthritis appear conflicting. In the mouse, Leslie *et al.* (1985) found fish oil to protect against experimental collagen-induced autoimmune arthritis, whilst in the rat fish oil was reported to augment the disease (Prickett *et al.* 1984). We found that feeding fish oil augmented EAE in the rat (Harbige, 1993), although suppression of EAE using lower doses of fish oil has been described by Mertin (1983), but the animals developed more severe clinical disease after discontinuation of treatment. The mechanism(s) involved in fish oil-induced augmentation of experimental autoimmune disease in rats is not well understood.

Autoimmunity, immune functions and *n*-6 fatty acids

Animal studies

Erickson *et al.* (1983) concluded from data obtained using a mixed lymphocyte reaction (alloantigens) system as a measure of lymphocyte function in mice that high dietary levels of safflower oil (rich in linoleic acid) suppressed the response whereas lower levels intensified the response. Similarly Yaqoob *et al.* (1994) reported that high levels of dietary linoleic acid-rich oil suppressed Con A-stimulated lymphocyte proliferation in rats. In contrast, De Deckere *et al.* (1988) found no effect on spleen lymphocyte proliferation in response to Con A in rabbits and rats fed on diets containing a high amount of linoleic acid (35 % digestible energy as sunflower oil) compared with palm oil. Studies by Kelley *et al.* (1988) in rabbits suggest that a low intake of linoleic acid-rich safflower oil does not affect spleen-derived lymphocyte responses or peripheral-blood-derived lymphocyte responses to PHA and Con A. Cantillon *et al.* (1990) also reported no effect of feeding linoleic acid-rich oil on rat lymphocyte proliferation *ex vivo*. Spleen lymphocytes from rats orally administered safflower-seed oil showed enhanced Con A responses *ex vivo* compared with lymphocytes from rats fed on the basal diet (Harbige *et al.* 1995). Kollmorgen *et al.* (1979) reported that feeding rats on maize oil reduced the *ex vivo* lymphocyte proliferative response to Con A; an important consideration in the Kollmorgen *et al.* (1979) study is the use of maize oil stripped of vitamin E. Young *et al.* (1987) showed reduced spleen lymphocyte proliferation in response to Con A in mice treated with a pure linoleic acid preparation at an estimated ten times higher dose than those used by other workers; in addition, linoleic acid was administered by subcutaneous injection. Cinader *et al.* (1983) found loss of 'suppressor activity' to the tolerance inducibility of rabbit γ-globulin in mice fed on a linoleic acid-rich diet. In mice fed a γ-linolenic acid-rich diet high- and low-dose oral ova tolerance induction reduced ova-specific proliferative responses (but not in animals fed on borage oil only) in ova immunized mice and decreased (high dose oral ova only) ova-specific serum IgG_{2a} and IgG_{2b} (LS Harbige and BAC Fisher, unpublished results). In summary, the effects of dietary linoleic acid on immune functions in animals appears inconsistent, and more information is required on other fatty acids of the *n*-6 family.

Human studies

The effects of *n*-6 fatty acids on immune functions, particularly the desaturated and elongated metabolites of linoleic acid, have not been studied as much as the *n*-3 fatty acids in human subjects. Supplementation with γ-linolenic acid-rich borage oil (*Borago officinalis*) did not affect the lymphoproliferative response to PHA and anti-CD3, but did increase the production of mitogen-stimulated peripheral-blood mononuclear cell TGFβ_1 and decreased the production of IL-4 and IL-10 (Harbige & Fisher, 1997). These findings are consistent with the effects of prostaglandin (PG) E_1 and PGE_2 on IL-4 and IL-10 in human T-cell clones (Harbige *et al.* 1997*a*). However, the effects of PGE on T-cell clones reported by Harbige *et al.* (1997*a*) are not consistent with previous findings obtained using murine and human T-cell clones (Betz & Fox, 1991; Watanabe *et al.* 1994), possibly due to differences in clones, mode of activation and eicosanoid concentrations used. Membrane lipid-oxidation products have been shown to increase TGFβ_1 expression and production (Leonarduzzi *et al.* 1997). It is possible, therefore, that an increase in membrane unsaturation, leading to an increased peroxidizability index, by feeding γ-linolenic acid-rich borage oil might lead to increased production of TGFβ_1. However, we found no change in plasma malondialdehyde concentration, a marker of peroxidation, in a γ-linolenic acid-rich borage oil supplementation study in human subjects (LS Harbige, F Kelley, C Dunster and K Ghebremeskel, unpublished results).

Autoimmune disease

In the NZB × NZW F_1 autoimmune mouse, linoleic acid-rich oil appears to exacerbate the disease (Levy *et al.* 1982) or to have no effect (Hurd et al. 1981). Similarly, in the MLR/Ipr autoimmune-prone mouse, linoleic acid-rich oil also appears to enhance the disease or to have no effect (Kelley *et al.* 1985; Godfrey *et al.* 1986). In addition, in the MLR/Ipr autoimmune-prone mouse, Godfrey *et al.* (1986) observed a greater survival rate for mice supplemented with 50 g evening primrose (*Oenothera biennis*) oil/kg diet (72 g linoleic acid and 9 g γ-linolenic acid/100 g total fatty acids). These findings suggest that linoleic acid, the parent *n*-6 fatty

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acid, is unable to ameliorate antibody-mediated spontaneous autoimmune disease, whereas the desaturated *n*-6 metabolites can. Studies in the guinea-pig have shown linoleic acid to partially suppress the incidence and severity of EAE (Meade *et al.* 1978). Similarly, high levels of a linoleic-rich oil containing low levels of γ-linolenic acid (linoleic acid : γ-linolenic acid 7 : 1, based on total fatty acid content) partially suppressed the incidence and severity of EAE in the rat (Mertin & Stackpoole, 1978). Using γ-linolenic acid-rich oils from fungal or plant sources we demonstrated complete protection against EAE in both rats and mice (Harbige *et al.* 1995, 1997*b*). This series of investigations demonstrated important disease-modifying effects of linoleic and γ-linolenic acid on clinical and histopathological manifestations of EAE. Depending on dose, γ-linolenic acid is completely protective in acute rat EAE, whereas linoleic acid has a dose-dependent action on the clinical severity of EAE, although not abolishing it. Analysis of spleen-cell-membrane fatty acid composition suggested that the previously described clinical response in EAE is due to conversion of γ-linolenic acid to the longer-chain *n*-6 eicosanoid precursor fatty acids, dihomo-γ-linolenic and arachidonic acids.

In chronic relapsing EAE induced in SJL mice with an encephalitogenic peptide (92–106) of myelin oligodendrocyte glycoprotein, feeding γ-linolenic acid markedly inhibited both the acute and relapse phases of the disease (Harbige *et al.* 1997*b*). Spleen-cell lymphoproliferative responses to Con A, myelin oligodendrocyte glycoprotein peptide (92–106) and protein purified derivative were unaltered when comparing γ-linolenic acid-fed and control EAE mice, indicating no gross immunosuppression by the fatty acid treatment (Harbige *et al.* 1997*b*). However, on activation with myelin oligodendrocyte glycoprotein peptide, Con A, and protein purified derivative there was a non-specific increase in the production of $TGF\beta_1$ and PGE_2 in γ-linolenic acid-fed mice *ex vivo*, with no effect on the production of IL-2 and interferon-γ (Harbige *et al.* 1997*b*). Furthermore, there was higher $TGF\beta_1$ mRNA expression and increased membrane eicosanoid precursor fatty acids (PGE_1 and PGE_2) in the spleens of γ-linolenic acid-fed mice. These findings are consistent with reports that administration of TGFβ protects in acute and relapsing EAE (Racke *et al.* 1993; Santambrogio *et al.* 1993), and that PG inhibitors, such as indomethacin, augment EAE (Ovadia & Paterson, 1982). In addition, during the natural recovery phase from EAE, TGFβ-secreting T-cells can inhibit EAE effector cells, TGFβ is expressed in the central nervous system and, in oral- tolerance-induced protection in EAE, TGFβ and PGE_2 are expressed in the brain (Karpus & Swanborg, 1991; Khoury *et al.* 1992). Feeding γ-linolenic acid can increase PGE_1 and PGE_2 production (Fan & Chapkin, 1992; Harbige *et al.* 1997*b*), and PGE_2 has been shown to inhibit the production of Th_1 but not Th_2 cytokines *in vitro* (Phipps *et al.* 1991). Importantly, adoptive transfer experiments have shown EAE to be a T-cell-mediated autoimmune disease in which Th_1-type cytokine-producing cells cause pathology (Liblau *et al.* 1995). On this basis we could propose that dietary γ-linolenic acid prevents T-cell-mediated autoimmune disease by altering the balance of cytokines from a Th_1- to a Th_2-type response. However, our findings suggest that the effects of dietary γ-linolenic acid on EAE are mediated through Th_3-like mechanisms involving $TGF\beta_1$ (Harbige *et al.* 1997*b*). It is interesting to note that Vidard *et al.* (1995) suggested from studies of T-cell tolerance and site-specific lymphokine profiles that the type of Th subset cytokine pattern produced depends on the environment in which they are found. In relation to the observations made by Pond (1996) mentioned earlier, this could be one mechanism by which local fatty acids affect Th subset cytokines.

The antioxidant enzymes superoxide dismutase (*EC* 1.15.1.1) and catalase (*EC* 1.11.1.6) have been shown to beneficially modify the clinical course of EAE (Guy *et al.* 1989). Previously we have shown that feeding a γ-linolenic acid-rich oil to rats increases the activity of superoxide dismutase in rat tissues (Phylactos *et al.* 1994). Increased antioxidant activity may therefore be an important mechanism by which linoleic acid metabolites control EAE. Similarly, Fernandes (1994) has suggested this as a mechanism in fish oil protective effects in NZB × NZW F_1 mice.

The nature of the autoimmune model appears to be important. The Th_2 cytokines IL-4, IL-6 and IL-10 are pro-inflammmatory in the NZB × NZW F_1 model, which is antibody-mediated (Fernandes, 1994). In contrast, in EAE, which is T-cell-mediated, IL-4 and IL-10 are associated with remission (Kennedy *et al.* 1992; Khoury *et al.* 1992; Liblau *et al.* 1995). Consistent with the previous findings, in the antibody-mediated NZB × NZW F_1 autoimmune model, animals fed on a high-fat diet consisting of equal amounts of lard and soyabean oil (linoleic acid-rich) develop a more severe disease and have a shortened lifespan, which is associated with increased autoantibody, and a decreased Th_1 and increased Th_2 cytokine profile (Lin *et al.* 1996).

Supplementation with *n*-6 and *n*-3 fatty acids in human autoimmune inflammatory disease

In clinical studies significant benefits have been reported in patients with systemic lupus erythematosus following a low-fat diet plus an *n*-3 fatty acid-rich fish oil supplement (Walton *et al.* 1991). Controlled trials in patients with rheumatoid arthritis on diets high in *n*-3 fatty acids without background saturated fat manipulation have also shown clinically beneficial effects (Kremer *et al.* 1985; Cleland *et al.* 1988). Interestingly, Kremer *et al.* (1987) found a significant correlation between decreases in neutrophil LTB_4 production and decreases in the number of tender joints in individual patients supplemented with fish oil. In patients with rheumatoid arthritis, treatment with evening primrose oil enabled 73 % of patients to stop or reduce non-steroidal anti-inflammatory drug treatment (Belch *et al.* 1988). Using γ-linolenic acid-rich borage oil (23 g γ-linolenic acid and 62 g linolenic acid/100 g total fatty acids), Leventhal *et al.* (1993) managed to significantly reduce clinically-important signs and symptoms of disease activity in patients with active rheumatoid arthritis. Belluzzi *et al.* (1996), feeding a novel fish oil preparation in a double-blind placebo-controlled trial with patients with Crohn's disease have reported a 60 % reduction in relapse rate. Double-blind trials to determine the therapeutic efficacy of diets supplemented with sunflower

oil, a source of linoleic acid, have been carried out in patients with multiple sclerosis by Miller *et al.* (1973), Bates *et al.* (1978) and Paty *et al.* (1978). In the first two studies, relapse rate and severity of disease were reduced in the treated groups, but Paty *et al.* (1978) showed no such effect. In a meta-analysis of the previously described trials, Dworkin *et al.* (1984) showed reduced relapse rate and severity, and a decrease in the long-term progression of the disease in patients with mild multiple sclerosis. Furthermore, open studies with patients with multiple sclerosis suggest that a low-fat diet and/or manipulation of dietary *n*-6 and *n*-3 fatty acids may be beneficial (Swank & Grimsgaard, 1988; Harbige *et al.* 1990). Importantly, many trial designs, including those for multiple sclerosis, have utilized olive oil as the placebo control and have not taken into account the total fat and saturated fat intake. It is apparent that olive oil (65–85 g oleic acid/100 g total fatty acids) increases the survival rate of MLR/Ipr mice and reduces the incidence of EAE in the guinea-pig (Meade *et al.* 1978; Godfrey *et al.* 1986). Patients with rheumatoid arthritis in the olive oil comparative control group had improvement in some clinical indices in the fish oil study of Cleland *et al.* (1988). Olive oil has been observed also to be of some clinical benefit in rheumatoid arthritis, with corresponding improvements in several laboratory variables, including C-reactive protein (G Darlington, personal communication). There is a clear need, therefore, for more carefully designed and controlled trials in the therapeutic application of fatty acids to human autoimmune inflammatory conditions. Since side effects are a problem with current treatment of human autoimmune disease, safe and more effective treatments would greatly improve their management. Nutritional approaches using fatty acid supplementation in human autoimmune disease, whether as an alternative or adjunct therapy, is therefore potentially very important.

Summary and conclusions

Clearly there is much evidence to show that under well-controlled laboratory and dietary conditions fatty acid intake can have profound effects on animal models of autoimmune disease. Studies in human autoimmune disease have been less dramatic; however, human trials have been subject to uncontrolled dietary and genetic backgrounds, infection and other environmental influences, and basic trial designs have been inadequate. The impact of dietary fatty acids on animal autoimmune disease models appears to depend on the animal model and the type and amount of fatty acids fed. Diets low in fat, essential fatty acid-deficient, or high in *n*-3 fatty acids from fish oils increase the survival and reduce disease severity in spontaneous autoantibody-mediated disease, whilst linoleic acid-rich diets appear to increase disease severity. In experimentally-induced T-cell-mediated autoimmune disease, essential fatty acid-deficient diets or diets supplemented with *n*-3 fatty acids appear to augment disease, whereas *n*-6 fatty acids prevent or reduce the severity. In contrast, in both T-cell and antibody-mediated auto- immune disease the desaturated and elongated metabolites of linoleic acid are protective. Suppression of autoantibody and T lymphocyte proliferation, apoptosis of autoreactive lymphocytes, and reduced pro-inflammatory cytokine production by high-dose fish oils are all likely mechanisms by which *n*-3 fatty acids ameliorate autoimmune disease. However, these could be undesirable long-term effects of high-dose fish oil which may compromise host immunity. The protective mechanism(s) of *n*-6 fatty acids in T-cell- mediated autoimmune disease are less clear, but may include dihomo-γ-linolenic acid- and arachidonic acid-sensitive immunoregulatory circuits such as Th_1 responses, $TGF\beta_1$-mediated effects and Th_3-like responses. It is often claimed that *n*-6 fatty acids promote autoimmune and inflammatory disease based on results obtained with linoleic acid only. It should be appreciated that linoleic acid does not reflect the functions of dihomo-γ-linolenic and arachidonic acid, and that the endogenous rate of conversion of linoleic to arachidonic acid is slow (Hassam *et al.* 1975, 1977; Phylactos *et al.* 1994; Harbige *et al.* 1995). In addition to effects of dietary fatty acids on immunoregulation, inflammation as a consequence of immune activation in autoimmune disease may also be an important mechanism of action whereby dietary fatty acids modulate disease activity.

In conclusion, regulation of gene expression, signal transduction pathways, production of eicosanoids and cytokines, and the action of antioxidant enzymes are all mechanisms by which dietary *n*-6 and *n*-3 fatty acids may exert effects on the immune system and autoimmune disease. Probably the most significant of these mechanisms in relation to our current understanding of immunoregulation and inflammation would appear to be via fatty acid effects on cytokines. The amount, type and balance of dietary fatty acids and associated antioxidant nutrients appear to impact on the immune system to produce immune-deviation or immunosuppressive effects, and to reduce immune-mediated inflammation which will in turn affect the susceptibility to, or severity of, autoimmune disease.

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