

Dietary fatty acid modulation of mucosally-induced tolerogenic immune responses

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Immunological unresponsiveness or hyporesponsiveness (tolerance) can be induced by feeding protein antigens to naïve animals. Using a classical oral ovalbumin gut-induced tolerance protocol in BALB/c mice we investigated the effects of dietary *n*-6 and *n*-3 polyunsaturated fatty acids (PUFA) on high- and low-dose oral tolerance (and in non-tolerised animals, i.e. effects of antigen challenge alone) in relation to lymphoproliferative, cytokine and antibody responses. Fish oil rich in long-chain *n*-3 fatty acids decreased both T-helper (Th) 1- and Th2-like responses. In contrast, borage (*Borago officinalis*) oil rich in *n*-6 PUFA, of which γ -linolenic acid is rapidly metabolised to longer-chain *n*-6 PUFA, increased Th1-like responses and decreased Th2-like responses, and possibly enhanced suppressor cell or Th3-like activity. These findings are in general agreement with other studies on the effects of long chain *n*-3 PUFA on immune system functions, and characterise important differences between long-chain *n*-3 and *n*-6 PUFA, defining more precisely and broadly the immunological regulatory mechanisms involved. They are also discussed in relation to autoimmune disease.

Dietary *n*-3 and *n*-6 fatty acids: Cytokine and antibody responses: Tolerogenic and antigenic immune responses

The normal gut immune system can discriminate between harmless food antigens (tolerance) and those associated with pathogenic and non-pathogenic microbes. How this discrimination is achieved, however, is not fully understood, but fundamentally involves regulation of the balance between tolerance (suppression) and sensitisation (priming). Factors that influence this balance include: genetic, e.g. major histocompatibility complex (Lamont *et al.* 1988); environmental, e.g. microbial flora (Chin *et al.* 2000; Matsuzaki & Chin, 2000); developmental or physiological, e.g. gut closure, hormonal, lactation (Kolb & Pozzilli, 1999; Heyman, 2001). Increased understanding of the underlying regulatory mechanisms in tolerance have demonstrated the importance (often in relation to antigen dose) of CD4⁺ T-cells, clonal deletion, clonal anergy, T-cell cytokine profiles (i.e. T-helper (Th) 1 *v.* Th2), active suppression (e.g. Th3 cytokines), apoptosis, lack of co-stimulatory molecules on antigen-presenting cells (e.g. B7.1/7.2 (CD80)), and the local environment (Mowat *et al.* 1982; Melamed & Friedman, 1993; Friedman & Weiner, 1994; Garside *et al.* 1995a; Vidard *et al.* 1995; Bailey *et al.* 2001; Strobel, 2001). The role of T-cell subpopulations secreting different cytokines has received considerable, and often controversial, attention

in relation to the mechanisms underlying high- and low-dose tolerance induction, and to tolerance induction during the neonatal period, which is said to be a critical window for tolerance induction (Burstein & Abbas, 1993; Garside *et al.* 1995b; Forsthuber *et al.* 1996; Ridge *et al.* 1996; Sarzotti *et al.* 1996; Weiner, 1997).

Lymphocyte subsets, cytokines and immunoregulation

Two different patterns of cytokine secretion by T-cells have been identified, which lead to different functional responses and are referred to as Th1 and Th2 T-cells (Mosman & Coffman, 1989). Th1 cells produce interleukin (IL) 2, interferon- γ (IFN- γ) and tumour necrosis factor- β , which are not synthesised by Th2 cells. In contrast, Th2 cells (but not Th1 cells) produce IL-4, IL-5, IL-10 and others. Th1 cells enhance cell-mediated inflammatory activity (such as delayed hypersensitivity) and induce B-cell antibody-class switching to immunoglobulin (Ig) G2a, whereas Th2 cells synthesise cytokines that help B-cells develop into IgE (mediates immediate hypersensitivity) and IgG1 antibody-producing cells. There are also T-cells able to produce both Th1 and Th2 cytokines, referred to as Th0 cells (Mosman &

Abbreviations: EAE, experimental autoimmune encephalomyelitis; IFN- γ , interferon- γ ; Ig, immunoglobulin; IL, interleukin; OVA, ovalbumin; PG, prostaglandins; PUFA, polyunsaturated fatty acids; TGF β , transforming growth factor β ; Th, T-helper.

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Sad, 1996). Weiner (1997) has further characterised a Th3 T-cell subset that primarily produces transforming growth-factor β (TGF β), provides help for B-cell IgA production, has potent suppressive properties, and is involved in oral tolerance. Importantly, the overall balance of cytokine production by Th1, Th2, and Th3 cells affects the type of immune response generated (Mosman & Sad, 1996).

Metabolism and immunobiology of polyunsaturated fatty acids

Little is known about the regulatory effects, impact and/or requirements of the gut mucosal immune system in relation to the essential macro- and micronutrient components of the diet. The macronutrient component of the diet includes the polyunsaturated fatty acids (PUFA). There are several families of PUFA, the major ones are the *n*-9, *n*-6 and *n*-3 (Sinclair, 1990). Both plants and animals can synthesise the *n*-9 parent PUFA oleic acid (18:1) from stearic acid (18:0) by the action of the enzyme delta-9-desaturase. Importantly, plants are able to make the *n*-3 and *n*-6 parent PUFA α -linolenic acid (18:3*n*-3) and linoleic acid (18:2*n*-6) respectively from oleic acid (18:1) due to the possession of appropriate desaturase enzymes. Animals, however, cannot synthesise the parent *n*-3 and *n*-6 PUFA (Sinclair, 1990). Nevertheless, they can elongate and desaturate the parent *n*-3 and *n*-6 PUFA which occur in the diet (Fig. 1), and which are classically referred to as essential fatty acids (Mead, 1981; Rivers & Frankel, 1981; Crawford, 1983; Sinclair, 1990). The *n*-6 parent PUFA linoleic acid (e.g. found in leafy vegetables, sunflower- and safflower-seed oils) and the *n*-3 parent PUFA α -linolenic acid (e.g. found in leafy vegetables and linseed oil) require delta-6-desaturase enzyme activity for conversion to γ -linolenic acid (18:3*n*-6, e.g. found in blackcurrant and borage- (*Borago officinalis*) seed oils) and stearidonic acid (18:4*n*-3) respectively (Fig. 1). Further chain elongation through elongase enzymes and desaturation by delta-5 and delta-4 desaturation enzymes produce the longer-chain *n*-6 metabolites such as dihomo- γ -linolenic (20:3*n*-6), arachidonic (20:4*n*-6), docosapentaenoic (22:5*n*-6), arachidonic (20:4*n*-6), docosatetraenoic (22:4*n*-6)

and docosapentaenoic (22:5*n*-6) acids, and the *n*-3 longer-chain PUFA eicosapentaenoic (20:5*n*-3, e.g. found in seafoods, fish and fish oils), docosapentaenoic (22:5*n*-3) and docosahexaenoic (22:6*n*-3, e.g. found in seafoods, fish and fish oils) acids (Crawford, 1983; Sinclair, 1990). Although the same enzyme systems are responsible for the alternative desaturation and elongation of the PUFA families, they have differing affinities, *n*-3 > *n*-6 > *n*-9 (Sinclair, 1990). Some of the PUFA containing three, four and five C=C are precursors for the bioactive eicosanoids (prostaglandins (PG), thromboxanes, leukotrienes and lipoxins). The precursor PUFA 20:3*n*-6, 20:4*n*-6 and 20:5*n*-3 are cyclised and incorporate molecular oxygen, at a specific site in the molecule, to produce unstable peroxidised intermediates such as PG. Depending on whether the C₂₀ PUFA precursor is 20:3*n*-6, 20:4*n*-6 or 20:5*n*-3, PGE is referred to as E₁, E₂, or E₃ respectively, and thromboxane A as A₁, A₂ or A₃ respectively; although there is no PGI₁, there is a PGI₂ (prostacyclin) and PGI₃. Metabolism by other enzymic routes produces the leukotrienes such as B₄ and B₅ by lipoxygenase activity and the 5-, 12- and 15-hydroxy acids by hydroperoxidase activity (Sinclair, 1990). Dietary-derived *n*-6 PUFA and their metabolites are essential for the normal development and structural and functional integrity of the immune system (Clausen & Moller, 1967; Selivonchick & Johnston, 1975; Scott *et al.* 1980; Goldyne & Stobo, 1981; Goodwin & Webb, 1981; Hopkins *et al.* 1981; Tripp *et al.* 1986; Shipman *et al.* 1988; Roper *et al.* 1990; Betz & Fox, 1991; Dvorak & Stepankova, 1991; Phipps *et al.* 1991; Ushikubi *et al.* 1993; Goetzl *et al.* 1995; Harbige, 1998). Dietary, PUFA (*n*-3, *n*-6 and *n*-9) are known to affect the membrane fatty acid composition of cells of the immune system, and through dietary PUFA manipulation systemic immune system functions as well as autoimmune disease can be modulated (Calder, 1998; Harbige, 1998; de Pablo & Alvarez de Cienfuegos, 2000). In view of the importance of PUFA in immune system functions we undertook studies to investigate the effects of different dietary PUFA on tolerogenic immune responses of the gut mucosal immune system generated via oral dosing with ovalbumin (OVA).

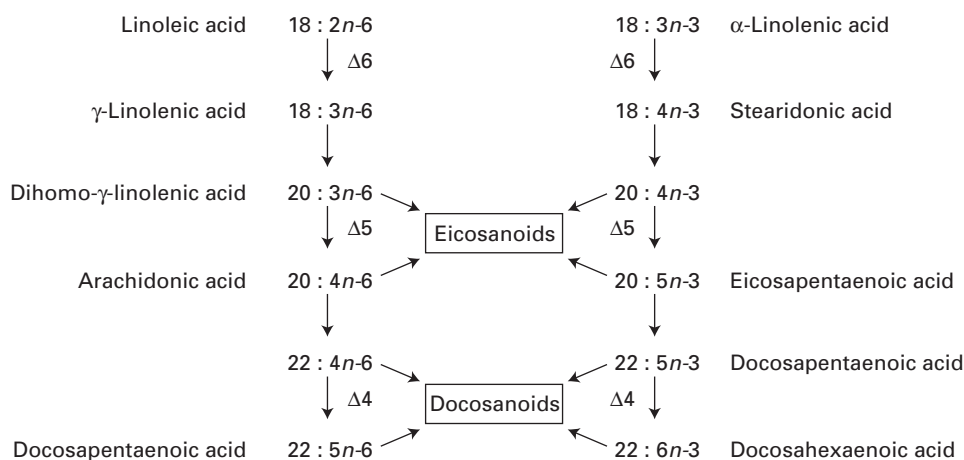


Fig. 1. An outline of essential fatty acid metabolism. Dietary precursor fatty acids are alternatively desaturated and elongated to produce longer-chain fatty acids with more double bonds. Eicosanoids are produced by oxygenation via either cyclooxygenase or lipoxygenase enzymic pathways and include: prostaglandins, e.g. prostaglandin E₂; thromboxanes, e.g. thromboxane A₂; leukotrienes, e.g. leukotriene B₄; the lipoxins, e.g. lipoxin A₄. Docosanoids are less well characterised.

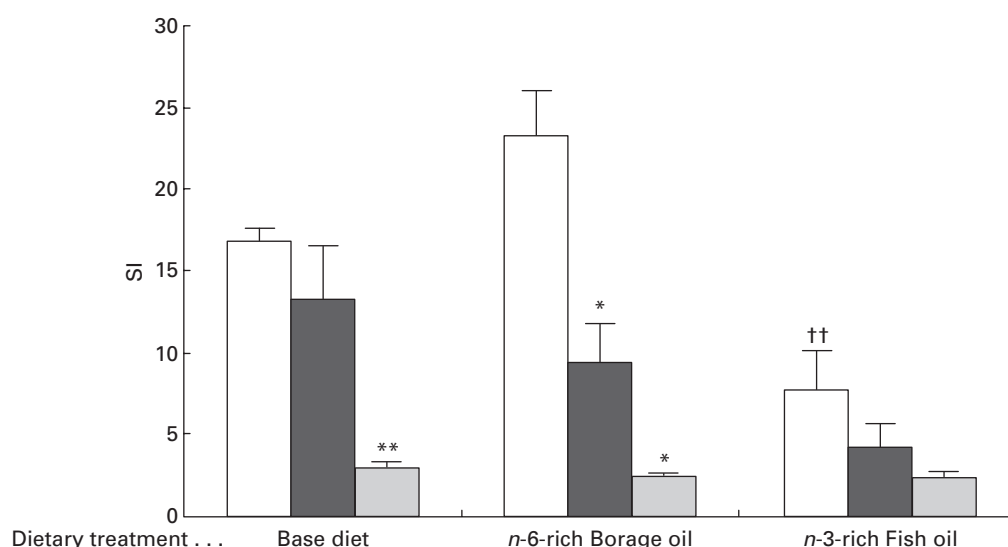


Fig. 2. Effect of dietary *n*-6 and *n*-3 fatty acids on spleen lymphocyte proliferative responses to ovalbumin (OVA) in both tolerised and non-tolerised animals. BALB/c mice were fed one of three diets; normal chow (low fat, 2.5% (w/w)), normal chow plus 10% (w/w) *n*-3 fatty acid-rich fish oil or normal chow plus 10% (w/w) *n*-6 fatty acid-rich borage oil. After 14 d animals were either tolerised (orally by gavage) with 2 (■) or 25 (▨) mg OVA respectively or sham-operated (□). After 7 d post-tolerisation animals were immunised with OVA in complete Freund's adjuvant (100 µg in 50 µl). Spleen cells were obtained 7 d post-immunisation and cultured in the presence of 1 mg OVA/ml and the lymphoproliferative responses measured as described previously (Harbige *et al.* 2000). Results are expressed as a stimulation index (SI), i.e. the mean count/min for OVA-stimulated cultures divided by the mean count/min for cultures without OVA. Values are means and standard deviations represented by vertical bars for four animals per group. Mean values within dietary groups were significantly different: * $P < 0.05$, ** $P < 0.01$. Mean value was significantly different from the corresponding value for base diet: †† $P < 0.01$.

n-6 and *n*-3 Fatty acids, lymphoproliferative, cytokine and tolerogenic responses

BALB/c male mice were fed one of three diets: normal chow (which is low in fat), normal chow plus 10% (w/w) *n*-3 PUFA-rich fish oil or normal chow plus 10% (w/w) *n*-6 PUFA-rich borage oil. After 14 d the mice in each group were either tolerised orally by gavage with 2 or 25 mg OVA to induce low- and high-dose tolerance respectively, or sham-operated. After 7 d post-tolerisation all mice were immunised with OVA in complete Freund's adjuvant. Spleen cells were harvested from some of the animals at day 7 post immunisation to investigate OVA-specific lymphoproliferative responses and cytokine production, and at day 14 serum was collected from the remaining animals for estimation of OVA-specific antibodies.

In all dietary groups high-dose oral tolerance decreased lymphocyte proliferation to a greater extent than low-dose tolerance (Fig. 2). In the fish oil group, however, OVA-specific proliferation was significantly reduced ($P < 0.01$) in the control group. This finding contrasts with the borage oil control where there was increased proliferation (Fig. 2). The decreased proliferation seen in the fish oil group was associated with decreased IL-2 production (Fig. 3). These findings are in accordance with previous work showing that fish oils decrease lymphoproliferation and IL-2 production in animal and human studies (Calder, 1996, 1998). In the present study low-dose tolerance, and to a greater extent high-dose tolerance, both decreased IL-2 production in all dietary groups (Fig. 3). However, the percentage reduction

compared with the corresponding control was greatest in the borage oil group and lowest in the fish oil group (Fig. 3). The reason for this difference is unclear, but it may be due to changes in the generation of suppressor cells, even though in this study there was no discrimination between active suppression and anergy. Initial proliferation induced by feeding OVA occurs in the mesenteric lymph nodes, and is similar in magnitude to that seen in the popliteal lymph nodes of mice immunised with OVA and complete Freund's adjuvant (Williamson *et al.* 1999; Nagler-Anderson, 2000). Furthermore, in experimental autoimmune encephalomyelitis (EAE) using myelin basic protein-specific T-cell receptor-transgenic mice, a significant increase in cells secreting IL-2, IFN- γ , IL-4 and IL-5 is seen in the mesenteric lymph nodes on day 1 after feeding myelin basic protein (Benson & Whitacre, 1997). If feeding fish oil reduces this initial proliferation, then it could be postulated that the number of Th3 or Tr1 (T-regulatory 1 e.g. produce IL-10, TGF β) cells generated would also be fewer. Different mechanisms have been shown for suppression of proliferation by fish oil, including lower major histocompatibility complex class II expression on dendritic cells and other antigen-presenting cells, and therefore decreased antigen presentation (Fujikawa *et al.* 1992; Hughes *et al.* 1998; Sanderson *et al.* 1997). It is also possible that there may be increased apoptosis rather than simply decreased proliferation (Fernandes *et al.* 1996). The increased apoptosis of lymphocytes observed by Fernandes *et al.* (1996) in fish oil-fed mice is likely to be the result of increased oxidative stress (Buttke & Sandstrom, 1994) induced by the highly-

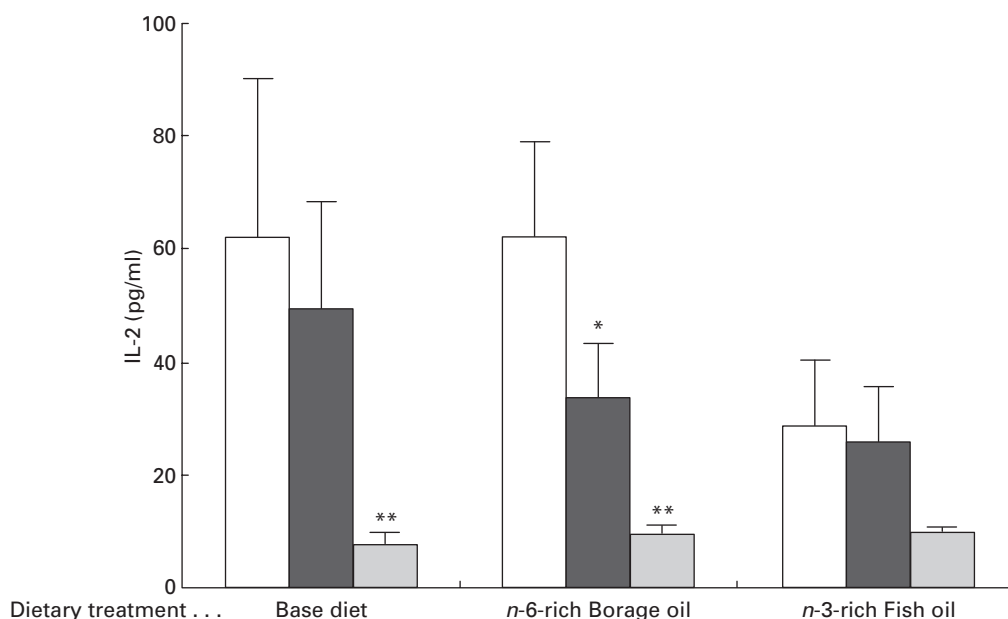


Fig. 3. Effect of dietary *n*-6 and *n*-3 fatty acids on ovalbumin (OVA)-specific interleukin (IL) 2 production by spleen lymphocytes in response to OVA in both tolerised and non-tolerised animals. BALB/c mice were fed one of three diets; normal chow (low fat, 2.5 % (w/w)), normal chow plus 10 % (w/w) *n*-3 fatty acid-rich fish oil or normal chow plus 10 % (w/w) *n*-6 fatty acid-rich borage oil. After 14 d animals were either tolerised (orally by gavage) with 2 (■) or 25 (▒) mg OVA respectively or sham-operated (□). After 7 d post-tolerisation animals were immunised with OVA in complete Freund's adjuvant (100 µg in 50 µl). Spleen cells were obtained 7 d post-immunisation and cultured in the presence of 1 mg OVA/ml for 24 h and the supernatant fractions removed and stored at -70°C. Mouse IL-2 was measured in supernatant fractions using a commercially available ELISA system (Harbige *et al.* 2000). Values are means and standard deviations represented by vertical bars for four animals per group. Mean values within dietary groups were significantly different: **P*<0.05, ***P*<0.02.

peroxidisable long-chain *n*-3 PUFA (Harbige *et al.* 1990; Avula *et al.* 1999).

Immunosuppression following fish oil feeding has been shown in several animal studies. For example, diets rich in fish oil increase the mortality of mice to whom *Salmonella typhimurium* has been administered orally (Chang *et al.* 1992), and prolong graft survival (Grimm *et al.* 1998). In contrast, the effects of *n*-6 fatty acids are less clear. The lack of lymphoproliferative suppression in the borage oil control (non-tolerised) group suggests a maintenance of response to novel antigens, whereas there are enhanced effects of oral tolerance on lymphoproliferation and IL-2, i.e. with low dose. It is therefore interesting to note that in the borage oil control group the production of IFN-γ was significantly (*P*<0.05) enhanced (LS Harbige and BAC Fisher, unpublished results). This finding is also consistent with those of previous studies in which feeding borage oil was observed to be associated with a trend of increasing IFN-γ production, despite suppressing EAE, a CD4⁺ Th1-mediated disease (Harbige *et al.* 2000; LS Harbige, L Layward, MM Morris-Downes, DC Dumonde and S Amor, unpublished results). These findings could be further tested by deriving lymphocytes (including Peyer's patch and mesenteric lymphocytes) from orally-tolerised mice fed *n*-3 or *n*-6 PUFA, and determining the concentration needed to suppress the antigen-specific proliferative response of cells from animals immunised with OVA. If fewer suppressor cells are generated through feeding fish oil and more with feeding

borage oil, then there are important implications for the study of oral tolerance in autoimmune disease. Since a wide variety of autoantigens are implicated in many autoimmune diseases through epitope spreading, the concept of bystander suppression is pivotal, whereby tolerised cells are able to suppress responses to other antigens in their vicinity, through the elaboration of cytokines such as IL-10 and TGFβ. Similar mechanisms could explain our observations in EAE where *n*-6 PUFA-rich seed oils are protective and *n*-3 PUFA-rich fish oils increase severity and delay recovery (Harbige, 1993, 1998; Harbige *et al.* 1995, 2000). Natural recovery in EAE is mediated by expansion of suppressor lymphoid cells (Adda *et al.* 1977), some of which have been characterised as TGFβ-producing CD4⁺ T cells by Karpus & Swanborg (1991). The protective effect of borage oil in EAE is linked to increased TGFβ transcription and production by T-cells and/or monocytes and/or PGE₂-producing monocytes (Harbige *et al.* 2000). The increased severity and delayed recovery in EAE induced by feeding fish oil may be the consequence of an inhibitory effect on the proliferative expansion of the suppressor cells (or decrease in PGE₂ from suppressor monocytes?) involved in the natural recovery from EAE. Given the immunosuppressive effects of fish oils and their suppressive effects in spontaneous autoantibody-mediated disease (Harbige, 1998), why there should be lack of suppression in EAE in the first place is difficult to explain. One possibility is that the potent immunogenic nature of the central nervous

system antigen mixture with adjuvant used to induce EAE may be sufficient to overcome any mild immunosuppressive effect of fish oil, as milder EAE disease induction can be suppressed by fish oil (Mertin, 1993).

In the PUFA and oral-tolerance studies with OVA our preliminary findings indicate that we are unable to detect OVA-specific TGF β production. However, it is important to note that we have only used spleen-derived lymphoid cells, as other researchers have been able to detect TGF β -secreting cells from Peyer's patches but not from popliteal lymph nodes in BALB/c mice (Shi *et al.* 1999). This finding may be due to there being low numbers of antigen-specific TGF β -secreting cells in the periphery. It is also possible to induce oral tolerance in TGF β 1-null mice with both low- and high-OVA doses (Barone *et al.* 1998), but these animals are abnormal, requiring injections of LFA-1 (leucocyte function-associated antigen-1) for survival, and may not therefore reflect normal immune system physiology.

There are further conceptual reasons why active suppression:apparent anergy might differ between the dietary *n*-6 and *n*-3 PUFA groups. PG have important roles in mucus secretion and in maintaining small intestine epithelial tight junctions (for review, see Mohajer & Ma, 2000). Alteration of the eicosanoid milieu by alteration of the proportions of precursor PUFA might therefore affect the amount of tolerogen reaching the peripheral circulation where anergy is induced. In addition, PGE₂ is important in lymph node 'shutdown', allowing an adequate proliferative response to antigen within the lymph node (Hopkins *et al.* 1981). Thus, the known decrease in PGE₂ production by *n*-3

PUFA feeding (Calder, 1998) and the increase in PGE₂ production by *n*-6 PUFA (Harbige *et al.* 2000) could give differential immunological responses. Interestingly, it has been shown recently that the maternal dietary *n*-6:*n*-3 balance can influence the intestinal functions/ontogeny of the offspring (Jarocka-Cyrta *et al.* 1998). In the context of 'tolerance' the Jarocka-Cyrta *et al.* (1998) findings not only indicate that modification of intestinal function by dietary PUFA might be important, but that there may also be important effects on the fetal and neonatal periods of development. Dietary PUFA effects on tolerance and thymic education during the fetal and neonatal period could, therefore, have implications for the development of immunological disorders, and would be in keeping with current views on nutrition and 'fetal programming' (Barker, 1998). Furthermore, it is interesting to note that human breast milk contains both preformed 20 : 4*n*-6 and 22 : 6*n*-3, whereas formula milks do not (Drury & Crawford, 1990; Crawford *et al.* 1997).

n-6 and *n*-3 Fatty acids, antibody and tolerogenic responses

The effects of *n*-3 PUFA-rich fish oil and *n*-6 PUFA-rich borage oil on OVA-specific antibody production was investigated as described earlier. In all dietary groups IgG1 production was decreased by high-dose tolerance to a greater extent than by low-dose tolerance (Fig. 4). The most interesting finding, however, was that OVA-specific IgG1 was significantly reduced ($P < 0.01$) in the borage oil control

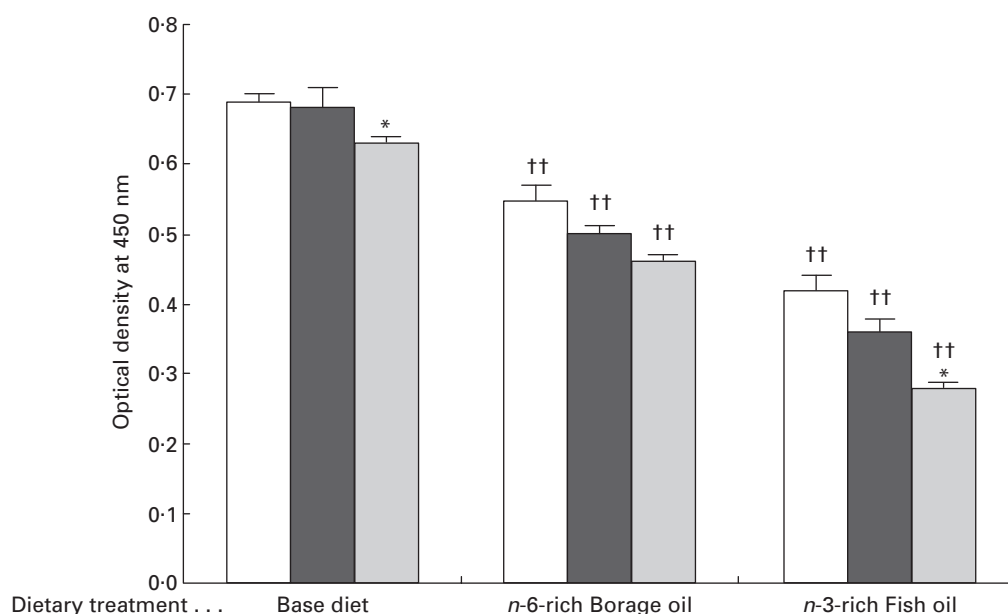


Fig. 4. Effect of dietary *n*-6 and *n*-3 fatty acids on ovalbumin (OVA)-specific serum (1 : 100 dilution) immunoglobulin G1 in both OVA-tolerised and non-tolerised animals. BALB/c mice were fed one of three diets; normal chow (low fat, 2.5% (w/w)), normal chow plus 10% (w/w) *n*-3 fatty acid-rich fish oil or normal chow plus 10% (w/w) *n*-6 fatty acid-rich borage oil. After 14 d animals were either tolerised (orally by gavage) with 2 (■) or 25 (▒) mg OVA respectively or sham-operated (□). After 7 d post-tolerisation animals were immunised with OVA in complete Freund's adjuvant (100 µg in 50 µl). Serum was collected 14 d post-immunisation and OVA-specific subclass antibodies detected using an ELISA format. Values are means and standard deviations represented by vertical bars for four animals per group. Mean values within groups were significantly different: * $P < 0.05$. Mean values were significantly different from the corresponding value for the base group diet: †† $P < 0.01$.

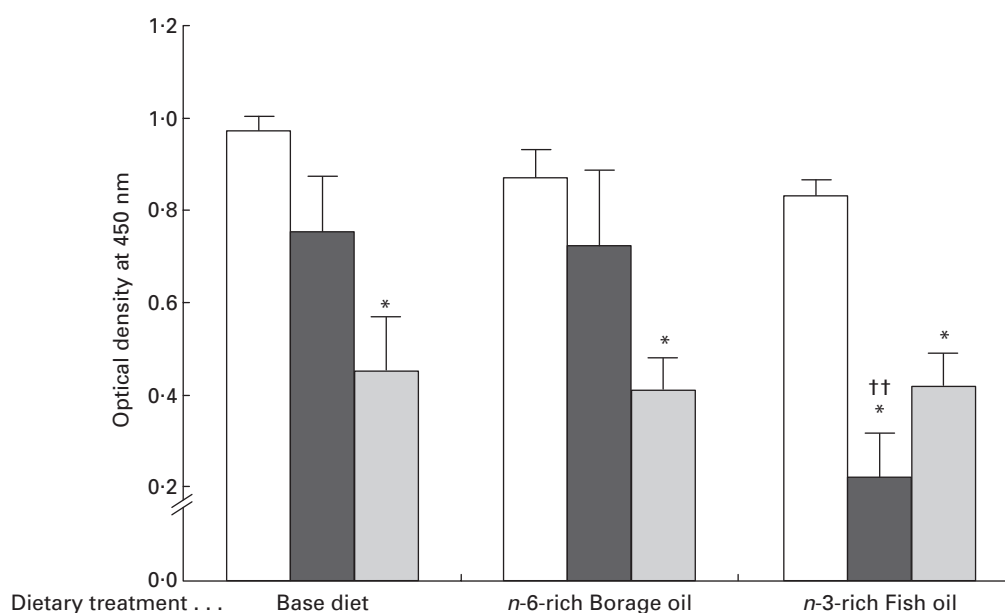


Fig. 5. Effect of dietary *n*-6 and *n*-3 fatty acids on ovalbumin (OVA)-specific serum (1 : 100 dilution) immunoglobulin G2a in both OVA-tolerised and non-tolerised animals. BALB/c mice were fed one of three diets; normal chow (low fat, 2.5% (w/w)), normal chow plus 10% (w/w) *n*-3 fatty acid-rich fish oil or normal chow plus 10% (w/w) *n*-6 fatty acid-rich borage oil. After 14 d animals were either tolerised (orally by gavage) with 2 (■) or 25(▨)mg OVA respectively or sham-operated (□). After 7 d post-tolerisation animals were immunised with OVA in complete Freund's adjuvant (100 µg in 50 µl). Serum was collected 14 d post-immunisation and OVA-specific subclass antibodies detected using an ELISA format. Values are means and standard deviations represented by vertical bars for four animals per group. Mean values within groups were significantly different: **P*<0.05. Mean value was significantly different from the corresponding value for the base group diet: ††*P*<0.01.

group, and to a greater extent in the fish oil control (Fig. 4). IgG1 class switching is mediated by IL-4, and is part of the Th2 response. The implication is that fish oil, and to a lesser extent borage oil, inhibit Th2 responses. This finding is in contrast to some studies that have shown increased production of IgE to OVA in rats fed on a high fish oil diet (Calder, 1998). This inconsistency may be due to the amount of PUFA fed or species differences, or indicate that there is a differential regulation of IgG1 and IgE in the model used. The decrease in IgG1 could result from B-cell apoptosis (see Fig. 2) or from decreased production. The effect on IgG2a production is less marked, although low-dose tolerance appears to be enhanced in the fish oil group (Fig. 5). Taking into account the decreased IL-2 with fish oil feeding (all groups), our data support the view that fish oil feeding results in suppression of both Th1 and Th2 responses. In contrast to our findings with *n*-6 PUFA-rich borage oil, Cinader *et al.* (1983) found loss of 'suppressor activity' to the tolerance inducibility of rabbit γ -globulin in mice fed on an *n*-6 linoleic acid-rich diet. The difference between the Cinader *et al.* (1983) observations and our findings is likely to be due to the use of the parent *n*-6 PUFA linoleic acid, rather than its desaturated product which is rapidly metabolised to longer-chain *n*-6 PUFA, i.e. 20 : 3*n*-6 and 20 : 4*n*-6 (Harbige *et al.* 1995; Harbige, 1998).

Conclusion

The intestinal immune system is a complex interaction between factors such as the diet, the local microflora, the

physical intestinal barrier and the immune cells themselves. We have shown that dietary lipids can modulate gut mucosal-induced immune responses. Long-chain *n*-3 PUFA-rich fish oil decreases both Th1- and Th2-like responses with and without oral tolerogen. Fish oils rich in long-chain *n*-3 PUFA can be viewed as being generally immunosuppressive at high doses, which is in accordance with most published studies (Calder, 1998; Harbige, 1998). One of the mechanisms behind some of the effects of fish oil appears to be peroxide-induced apoptosis of lymphocytes. It would be interesting, therefore, to know if vitamin E administration could reverse fish oil-induced lymphocyte apoptosis and the fish oil-induced increase in disease severity and delayed recovery in EAE. In contrast, *n*-6 PUFA-rich borage oil has a more complex role. There is an increase in proliferative response (in control OVA-immunised but not tolerised animals) to antigen with a concomitant increase in IFN- γ production (Th1 response). In addition, borage oil decreases the level of IgG1 (in all groups; a Th2 response) and enhances the tolerogenic response to OVA, i.e. decreased proliferative and IL-2 responses, which suggests increased suppressor cell or Th3-like activity. It appears, therefore, that *n*-6 PUFA-rich borage oil can increase Th1-like responses, and possibly Th3-like responses, and decrease Th2-like responses, and may therefore be considered immunoregulatory (immune-deviation?) rather than immunosuppressive in our experimental system. Other mechanisms may also be important in PUFA and eicosanoid effects on gut-induced tolerance; e.g. intestinal functions such as barrier, absorptive and

processing functions and mesenteric lymph node shutdown. Dietary PUFA could also have important effects during the fetal and neonatal periods in relation to tolerance induction and thymic education.

The disappointing results of oral tolerance trials in established human autoimmune disease may require more than manipulation of antigen dose. Indeed, whey proteins from cow's milk, when given in single doses equivalent to a tolerogenic dose of OVA, are able to induce active immunity and tolerance in the same animal (Strobel & Mowet, 1998). Furthermore, the gut microflora and their products have been shown to influence the induction and maintenance of oral tolerance (Wardrop & Whitacre, 1999; Isolauri *et al.* 2001), and the gut microflora is known to vary between individuals. The impact of diet on the human gut microflora is also largely unknown. Any therapeutic approach might therefore consider using multiple targets and exploring, for example, the use of oral tolerance with probiotic and/or PUFA manipulation.

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