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N-3 polyunsaturated fatty acids modulate the expression of functionally associated molecules on human monocytes and inhibit antigen presentation *in vitro*

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SUMMARY

N-3 polyunsaturated fatty acid (PUFA)-rich diets are associated with suppression of cell-mediated immune responses, but the mechanisms are unclear. Specific immune responses are initiated by antigen-presenting cells (APC). We have previously shown *in vitro* that the n-3 PUFA, eicosapentaenoic acid (EPA), inhibits the expression of HLA-DR, an MHC class II molecule required for normal APC function on human blood monocytes. In contrast, docosahexaenoic acid (DHA) enhanced the expression of this molecule on unstimulated monocytes, but both n-3 PUFA suppressed its expression on interferon-gamma (IFN-γ)-activated monocytes. In the present study we show that when EPA and DHA were combined at the same ratio as is commonly found in fish oil supplement capsules (3:2) there was no significant effect *in vitro* on the expression of HLA-DR on unstimulated monocytes, but the expression on IFN-γ-activated monocytes remained significantly inhibited. In the same *in vitro* system a significant reduction in the ability of IFN-γ-activated monocytes to present tetanus toxoid antigen to autologous lymphocytes was observed following culture with the combined n-3 PUFA. These findings support previous animal studies which suggest that n-3 PUFA can inhibit the antigen-presenting function of mononuclear phagocytes.

Keywords n-3 PUFA MHC class II monocytes humans antigen presentation

INTRODUCTION

N-3 polyunsaturated fatty acid (PUFA)-rich diets are associated with suppression of the immune system [1], and the results of several well controlled dietary supplementation studies have shown that fish oils (rich in n-3 PUFA) can improve the condition of patients suffering from a number of disorders involving over-reactive immune responses, such as rheumatoid arthritis (RA) [2].

Mononuclear phagocyte cell types, which include monocytes and macrophages, initiate cell-mediated immune responses by processing and subsequently expressing antigens on their surface membranes for recognition by appropriate T cells [3]. A prerequisite for this antigen-presenting cell (APC) function is the expression of MHC (which is known as the human leucocyte antigen (HLA) system in man) class II antigens, such as HLA-DR, HLA-DP and HLA-DQ [4]. It has been shown that the T cell proliferative response to antigen is proportional to the number of MHC class II molecules on the surface of APC [5], and that the percentage of MHC class II-positive cells and the density of these molecules on the cell surface can alter the degree of immune responsiveness of an individual [6].

In addition to requiring the expression of MHC class II molecules, cell–cell adhesion appears to be critical for the initiation of a primary immune response. Several adhesion receptor–ligand pairs can facilitate an immune response not only by enhancing adhesion, but by providing an additional distinct co-stimulatory signal. The binding of the adhesion molecule, leucocyte function-associated antigen-1 (LFA-1) to its ligand, intercellular adhesion molecule-1 (ICAM-1), has been shown to be capable of co-stimulating an immune response [7].

We have previously reported that the n-3 PUFA, eicosapentaenoic acid (EPA), can inhibit the expression of HLA-DR and ICAM-1 on normal human monocytes *in vitro* in a dose-dependent manner. In contrast, a significant increase in the expression of HLA-DR and -DP was observed on monocytes following incubation with the other major n-3 PUFA found in fish oil, docosahexaenoic acid (DHA) [8]. Since it has been reported that synovial fluid monocytes obtained from patients with RA express elevated levels of MHC class II molecules [9], we also examined the effect of n-3 PUFA on activated monocytes, cultured in the presence of interferon-gamma (IFN-γ), to up-regulate the expression of MHC

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class II molecules on the monocytes. Both EPA and DHA significantly inhibited the expression of HLA-DR, -DP and ICAM-1 on the activated monocytes [8]. We have also shown that dietary fish oil supplementation (containing a mixture of EPA and DHA) can inhibit the expression of these molecules on human peripheral blood monocytes [10]. Since EPA and DHA had exhibited opposing effects *in vitro* on unstimulated monocytes, the aim of this study was to investigate the combined effect of EPA and DHA *in vitro*, when provided at the same ratio as is commonly found in fish oil supplement capsules (3:2), on the expression of functionally associated surface molecules on human monocytes. In addition, an *in vitro* assay of antigen presentation was used to investigate whether changes in the expression of monocyte surface molecules was associated with an alteration in antigen-presenting function.

MATERIALS AND METHODS

N-3 polyunsaturated fatty acids

EPA and DHA were obtained from Sigma (Poole, UK) and were solubilized in 95% ethanol. A concentrated stock mixture of EPA and DHA, at a ratio of 3:2, was stored under nitrogen until immediately before use.

Antibodies for immunostaining

The following MoAbs were used to investigate the modulatory effects of the fatty acids on cell surface antigen expression: anti-HLA-DR, -DP and -DQ (Becton Dickinson, Oxford, UK), anti-CD54 (ICAM-1; Serotec, Oxford, UK), anti-CD11a (LFA-1) and anti-CD58 (LFA-3; Serotec). FITC-labelled rabbit anti-mouse immunoglobulin $F(ab')_2$ fragment (Dako, High Wycombe, UK) was used as a second layer antibody to detect MoAb binding. This FITC-labelled antibody was also used alone to control for non-specific binding. Anti-CD45 (common to all leucocytes; Serotec) was used as a positive control.

Antigen for assay of antigen presentation

Soluble tetanus toxoid (TT), with a limited flocculation (Lf) value of 2050 Lf/ml and a purity of 1073 Lf/mg, was kindly provided by Medeva PLC (Leatherhead, UK).

Subjects

The monocytes used in this study were obtained by venepuncture from healthy, non-smoking, adult volunteers. Although several volunteers were recruited into both studies, the surface molecule and antigen presentation experiments were performed on separate occasions. For the immunofluorescence study the volunteers comprised three males and six females (mean age 34 years, range 23–44 years). Monocytes from two males and four females (mean age 35 years, range 22–53 years) were used in the functional study, all of whom had been inoculated with TT within the previous 10 years. No volunteers were receiving medication, and none had been consuming fish oil supplements or were regular consumers of oily fish. The study was approved by the Institute of Food Research Ethical Committee.

Purification of monocytes

Monocytes were acquired by density gradient centrifugation, by the method previously described in detail [11]. Briefly, peripheral blood from each volunteer (usually 60 ml) was collected into syringes containing EDTA. Leucocyte-rich plasma was obtained by Dextran 500 sedimentation and subjected to a period of hyperosmolarity. The plasma was then layered onto NycoPrep 1.068 (Nycomed Ltd, Birmingham, UK) in 15-ml tubes (diameter 13 mm; Falcon 2097; Becton Dickinson), which were centrifuged for 15 min at 600 ***g*** at 22°C. The monocyte-containing fraction was aspirated and washed twice in 0·15 mol/*l* NaCl containing 0·04 mol/*l* EDTA and 10 g/*l* albumin, by centrifuging at 600 ***g*** for 7 min. The recovered cells were resuspended in 1 ml of 'culture medium' (HEPES-buffered RPMI containing 5% heat-inactivated fetal calf serum (FCS), 2 mmol/*l* L-glutamine, 1×10^5 U/*l* penicillin, 0·07 mmol/*l* streptomycin; GIBCO BRL, Paisley, UK), counted and adjusted to 5×10^9 cells/*l*. The monocytes were >95% viable, as assessed by trypan blue exclusion.

Purification of autologous lymphocytes

For use in the assays of antigen-presenting function, autologous lymphocytes were obtained from a further 50 ml blood sample from the same individual, by the method we have previously described in detail [12]. Briefly, mononuclear cells were obtained by centrifugation of defibrinated blood over lymphocyte separation medium (ICN Biomedicals, Thame, UK) at 600 ***g*** for 30 min. The cells at the interface were aspirated, washed twice in minimal essential medium (MEM; GIBCO BRL), counted and adjusted to 5×10^9 cells/*l* in culture medium. To remove contaminating monocytes, 5-ml aliquots of the cell suspension were placed into polystyrene tissue culture flasks (ICN Biomedicals; 25 ml capacity) which had been pre-coated with heat-inactivated FCS, and incubated for 4 h at 37°C. The non-adherent lymphocytes were recovered by gently washing the flasks using pre-warmed RPMI (37°C). They were then panned to remove any residual cells expressing HLA-D region products by labelling the cells with WR18 (Serotec; a mouse IgG2a directed against a common determinant of each MHC β-chain type) before incubation in Petri dishes which had been pre-coated with affinity-purified $F(ab')_2$ rabbit anti-mouse IgG (Serotec). Non-adherent lymphocytes were harvested by gentle washing with RPMI and resuspended at $1{\cdot}1\times10^9$ cells/*l* in culture medium.

Cell culture with n-3 PUFA

Monocytes were cultured in the presence or absence of the combined EPA and DHA in 15-ml polypropylene tubes (Falcon 2097). The stock solution of EPA and DHA (3:2) was diluted in culture medium immediately before use (to minimize oxidation) and added to the cultures at a final concentration of 39 μM EPA and 26 μM DHA. Control cells were given equivalent amounts of 95% ethanol (final concentration = 0·1% ethanol). In addition, cultures in the presence or absence of the n-3 PUFA were performed with the further addition of interferon-gamma (IFN-γ; Genzyme, West Malling, UK) to up-regulate surface molecule expression, using a previously determined optimal concentration of 4×10^5 U/*l*. After a 48-h incubation at 37°C, cells were placed on ice for 40 min to loosen any adherent monocytes. The cells were washed twice in MEM, counted using trypan blue exclusion to assess viability (which was always >93% after culture with or without n-3 PUFA), and adjusted to a concentration of 5×10^9 cells/*l* in MEM for staining before flow cytometry.

Immunofluorescence

The cells were stained with the various MoAbs by the technique described by Parker & Haslam [13]. Briefly, 5×10^5 cells in 100 μl of MEM were incubated with an optimal concentration of each MoAb for 30 min at 4°C. The cells were washed in MEM containing

10% Haemoccel (Hoechst, Hownslow, UK) to reduce cell clumping, followed by the addition of the FITC rabbit anti-mouse immunoglobulin, for 30 min at 4°C. After a further washing the cells were fixed in MEM containing 5% Haemoccel and 50% methanol, and washed in PBS. The cell nuclei were then stained with propidium iodide (0·025 g/*l*) in the presence of ribonuclease (0·25 g/*l*) for 20 min at 37°C immediately before flow cytometric analysis.

Analysis of surface marker expression by flow cytometry

A 488 nm laser line was used to simultaneously excite FITC and propidium iodide. The instrument was calibrated daily using fluorescent 1-μm latex beads (Fluoresbrite; Polysciences, Warrington, PA). Log FITC fluorescence was detected via a 530 nm band-pass filter and linear propidium iodide fluorescence emission via a 610 nm band-pass filter. A threshold intensity of propidium iodide fluorescence was set to selectively acquire data on nucleated cells only. Forward angle and 90° light scatter characteristics were also recorded for each cell, to give an indication of size and granularity, respectively, to aid in distinguishing the different populations. Monocytes were identified on this basis and gated appropriately. The percentages of FITC-positive cells (exhibiting a higher fluorescence intensity than the upper limit of the FITC-only controls without MoAbs) in the gated populations were determined. Cells within the gated population were >90% anti-CD14$^+$ (as assessed using the anti-CD14 clone, B-A8 (Serotec), which reacts primarily with monocytes and, unlike some other anti-CD14 antibodies, does not react with B lymphocytes).

The intensity of expression of the cell surface markers on monocytes was determined using a previously reported method [8]. The anti-log of the green (530 nm) fluorescence emission was used to calculate the linear median intensity value for the monocytes stained with MoAb and for the controls without MoAb. The intensity of FITC fluorescence relating to bound MoAb was obtained by subtracting the linear median intensity value for the control monocytes. The results were thus expressed as 'relative median intensity' values.

Assay of antigen-presenting function

To determine the effect of n-3 PUFA pre-treatment on monocyte function, an *in vitro* assay of antigen presentation was performed, as we have previously described [11], but using a colourimetric quantification technique in the place of ^{3}H-thymidine uptake. Briefly, the monocytes recovered from culture with or without the fatty acids were 'antigen pulsed' by incubating them with TT for 3 h at 37°C. An optimal dose of 25 Lf/ml was employed (determined from dose–response studies). The monocytes were then washed three times in RPMI, then re-suspended at 1×10^9/*l* in culture medium.

For each assay, 100-μl aliquots of antigen-pulsed monocytes, containing 1×10^5 cells, were added to 900-μl aliquots of purified autologous lymphocytes (containing 1×10^6 cells) in culture medium in 5-ml polypropylene tubes (Falcon 2063). The ratio of monocytes to lymphocytes used (1:10) in this assay was the one that we had previously determined as being optimal for these culture conditions [11]. Cultures were performed in triplicate. Control cultures consisted of tubes containing medium alone and tubes containing 10^6 lymphocytes alone to determine background levels. Tubes containing 10^5 monocytes alone were also included to ensure that these induced no detectable additional colour change. The cells were cultured for 5 days at 37°C, and lymphoproliferation was assessed by adding bromodeoxyuridine (BrdU) to the cultures 18 h before quantification of BrdU uptake using an ELISA kit (Boehringer Mannheim, Lewes, UK). The results were expressed as mean absorbance ± s.e.m. of the triplicate cultures.

Statistical analysis

Differences between pairs of n-3 PUFA-treated and untreated samples were analysed using the paired *t*-test [14]. Significance level was set at $P<0\cdot05$. Results are expressed as means and s.e.m. unless otherwise stated.

RESULTS

Effect of n-3 PUFA on surface molecule expression by monocytes

Unstimulated monocytes. Table 1 shows the percentages of monocytes expressing the various surface molecules after incubation for 48 h in the absence (control) or combined presence of 39 μM EPA and 26 μM DHA. No significant changes were seen in the percentage of the MHC class II molecules, but significant decreases in the percentage of ICAM-1$^+$ cells ($P<0\cdot05$) and LFA-3$^+$ cells ($P<0\cdot05$) were observed following incubation in the presence of the n-3 PUFA. There was also a significant decrease in the median intensity of expression of both ICAM-1 and LFA-3 on the monocytes cultured in the presence of the n-3 PUFA ($P<0\cdot05$). Representative staining profiles for the monocyte surface molecules are shown in Fig. 1.

No significant differences in cell viability, as assessed by trypan blue exclusion, or in the expression of any of the surface molecules studied were observed between monocytes cultured in culture medium alone and monocytes cultured with the addition of 0·1% ethanol (data not shown).

IFN-γ-stimulated monocytes. Table 2 shows the percentages of monocytes expressing the various surface molecules after incubation for 48 h in the additional presence of IFN-γ (using aliquots of monocytes from the same individuals used in Table 1) . There was a significant decrease in the percentage of monocytes expressing the MHC class II molecules, HLA-DR ($P<0\cdot05$) and -DP ($P<0\cdot01$), and the adhesion molecules, ICAM-1 ($P<0\cdot05$) and LFA-3 ($P<0\cdot01$), following incubation in the presence of the n-3 PUFA. There was also a significant decrease in the intensity of expression of HLA-DR ($P<0\cdot05$), -DP ($P<0\cdot001$), ICAM-1 ($P<0\cdot01$) and LFA-3 ($P<0\cdot01$) (Table 2 and Fig. 1). No significant differences in the expression of HLA-DQ and LFA-1 were observed.

Effect of varying the concentration of the combined n-3 PUFA

Figure 2 illustrates the appearance of a concentration-dependent effect of the combined n-3 fatty acids on the percentage of both unstimulated (Fig. 2a) and IFN-γ-stimulated (Fig. 2b) monocytes expressing ICAM-1 and LFA-3. In contrast, a dose-dependent effect of n-3 PUFA on HLA-DR expression was only observed on the IFN-γ-stimulated monocytes. Cell viability, as assessed by trypan blue exclusion, was monitored at each concentration of fatty acids, and always exceeded 93%.

Figure 3 shows that incubation with the combined fatty acids resulted in a dose-dependent decrease in the median intensity of expression of ICAM-1 and LFA-3 on both unstimulated (Fig. 3a) and IFN-γ-stimulated (Fig. 3b) monocytes, which was optimal at the concentration used in the main series of experiments (39 μM EPA, 26 μM DHA). Again, a dose-dependent effect of the combined n-3 PUFA on HLA-DR expression was only observed on the IFN-γ-stimulated monocytes.

Table 1. Effect of n-3 polyunsaturated fatty acids (PUFA) on the expression of surface molecules on unstimulated human monocytes

	Positive monocytes (%)						Relative median intensity of expression					
	Controls ($n=9$)			n-3 PUFA ($n=9$)			Controls ($n=9$)			n-3 PUFA ($n=9$)		
Molecule	Mean		s.e.m.	Mean		s.e.m.	Mean		s.e.m.	Mean		s.e.m.
HLA-DR	75·2	±	6·8	69·0	±	7·5	74·8	±	10·4	66·1	±	10·2
HLA-DP	50·8	±	9·0	45·3	±	9·7	44·3	±	9·1	37·1	±	9·4
HLA-DQ	36·0	±	10·1	34·4	±	10·5	30·4	±	9·1	27·4	±	8·8
ICAM-1	81·6	±	4·0	63·1*	±	8·2	53·1	±	5·0	39·3*	±	5·7
LFA-1	86·2	±	6·1	78·0	±	5·1	59·0	±	7·3	52·0	±	5·9
LFA-3	78·7	±	7·3	60·8*	±	9·1	49·6	±	4·5	39·4*	±	5·9

* $P<0{\cdot}05$ (paired t-test) compared with controls.

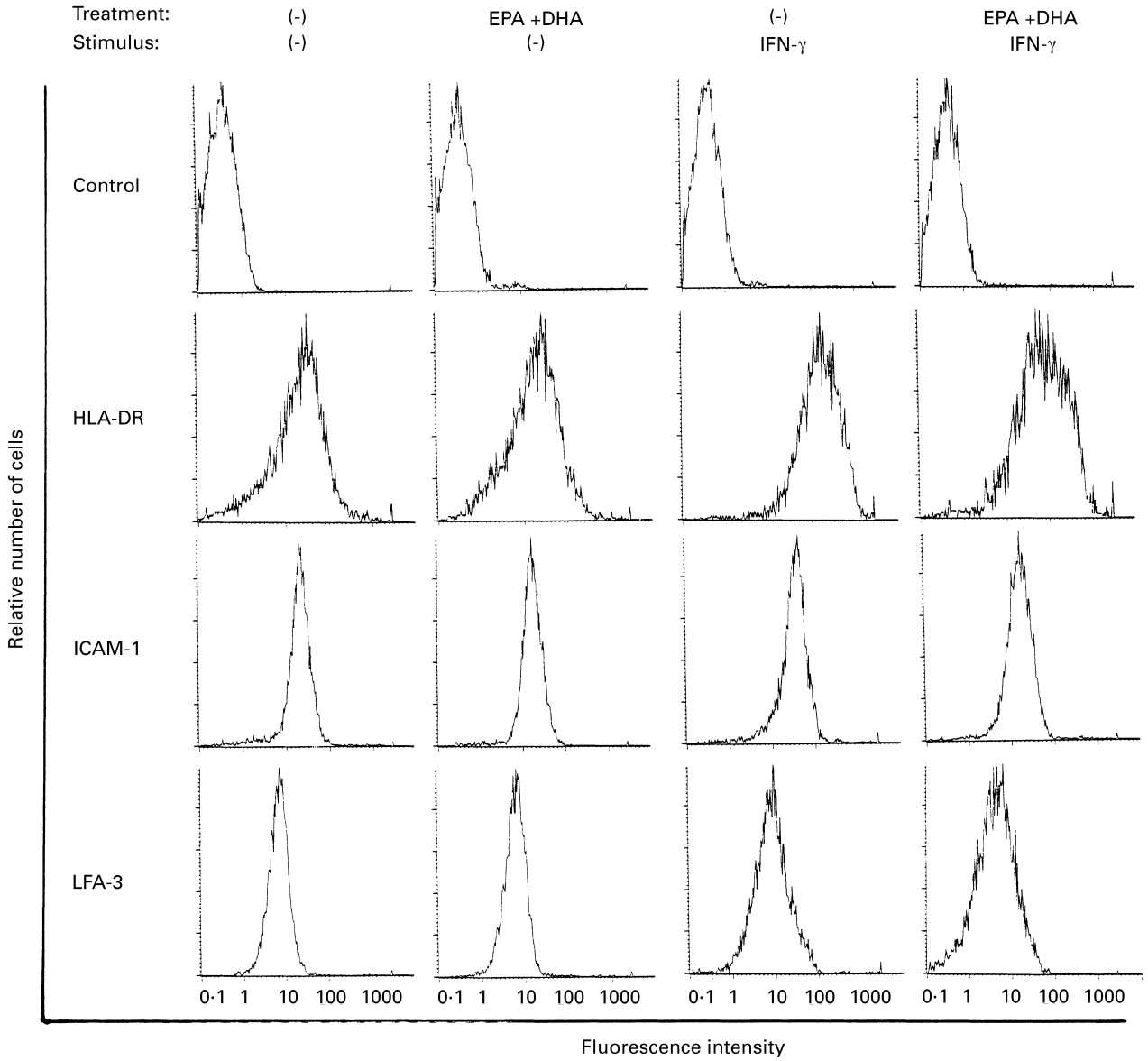

Fig. 1. Surface molecule expression on monocytes. Monocytes cultured without (−) or with eicosapentaenoic acid (EPA) (39 μM) + docasahexaenoic acid (DHA) (26 μM) and without (−) or with IFN-γ (4×10^5 U/l) for 48 h were stained with MoAbs as described in the text. Abscissa, fluorescence intensity in log scale; ordinate, cell numbers. Data from representative experiments from the nine performed.

Table 2. Effect of n-3 polyunsaturated fatty acids (PUFA) on the expression of surface molecules on IFN-γ-stimulated human monocytes

	Positive monocytes (%)						Relative median intensity of expression					
	Controls ($n=9$)			n-3 PUFA ($n=9$)			Controls ($n=9$)			n-3 PUFA ($n=9$)		
Molecule	Mean		s.e.m.	Mean		s.e.m.	Mean		s.e.m.	Mean		s.e.m.
HLA-DR	87·3	±	3·4	78·3*	±	4·4	107·2	±	6·4	91·9*	±	9·4
HLA-DP	82·3	±	4·8	70·0**	±	6·8	84·0	±	7·8	68·0***	±	9·2
HLA-DQ	62·8	±	11·5	59·4	±	10·9	57·7	±	12·9	52·8	±	11·7
ICAM-1	84·6	±	4·9	76·3*	±	5·9	72·9	±	6·4	60·3**	±	6·3
LFA-1	78·6	±	6·5	76·3	±	3·7	61·8	±	7·3	56·3	±	5·8
LFA-3	80·9	±	2·8	58·2**	±	7·4	56·4	±	7·5	38·0**	±	5·3

*$P<0·05$; **$P<0·01$; ***$P<0·001$ (paired t-test) compared with controls.

Effect of n-3 PUFA on the antigen-presenting function of monocytes

Figure 4b shows that the pre-incubation of monocytes from six individuals, in the presence of both n-3 PUFA (39 μM EPA and 26 μM DHA) and IFN-γ, resulted in a reduction in their ability to present TT to autologous lymphocytes, as assessed by lymphocyte proliferation, which was significant for the group ($P<0·05$). No significant effect was observed on unstimulated (no IFN-γ) monocytes (Fig. 4a). When sufficient monocytes were available, the suppressive effect appeared to be dose-dependent over the range of 16·25–97·50μM total PUFA, on IFN-γ-stimulated monocytes (mean absorbance (± s.e.m.) 0 μmol/*l*, 1·16 ± 0·13; 16·25μmol/*l*, 1·14 ± 0·23; 32·5μmol/*l*, 1·07 ± 0·13; 65 μmol/*l*, 0·9 ± 0·27; 97·5 μmol/*l*, 0·87 ± 0·29; $n=3$).

DISCUSSION

In a previous study we observed that EPA and DHA can inhibit the expression of the adhesion molecule ICAM-1 on both resting and IFN-γ-activated monocytes [8]. In contrast, these n-3 fatty acids exhibited opposing effects on the expression of the MHC class II molecules, HLA-DR and HLA-DP; EPA inhibited their expression whilst DHA enhanced. We now show that when the fatty acids are added to cell culture in combination, at the same ratio as is commonly found in commercially available preparations of fish oil (3:2), there is no significant effect on the expression of these molecules on resting monocytes but, as when the fatty acids are added individually, the expression of the MHC class II molecules on IFN-γ-stimulated monocytes is significantly inhibited. Variability in the expression of these functionally associated molecules is known to be capable of altering the degree of immune responsiveness of an individual to antigenic stimulation [6], and in this study we observed an associated reduction in the ability of IFN-γ-activated monocytes to present antigen to autologous lymphocytes following pre-incubation with the combined n-3 fatty acids. The inhibitory effects *in vitro* were obtained using levels of EPA and DHA which are achievable in blood plasma with relatively low supplementation with fish oil capsules (3 g/day) [15]. As Janeway and colleagues emphasized [6], functional assays are of central importance in indicating the potential *in vivo* situation. However, it

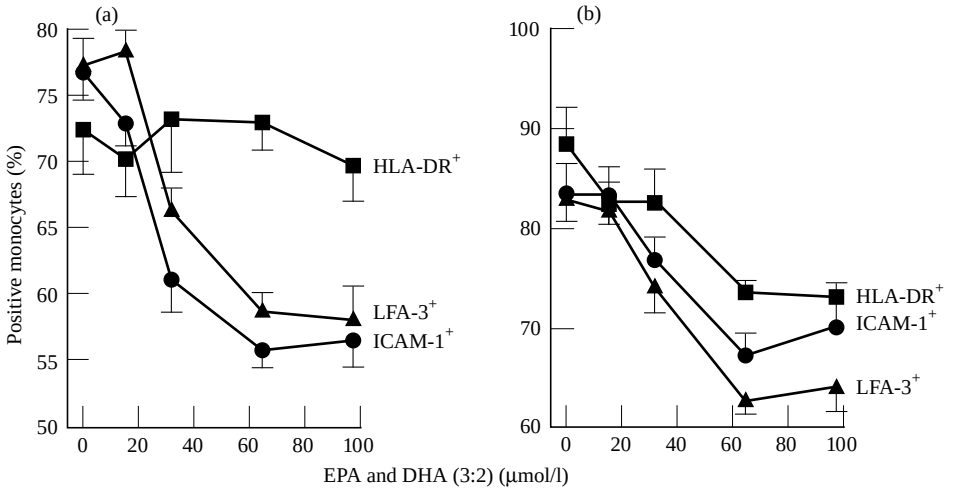

Fig. 2. Effect of increasing doses of the combined eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on the percentage of monocytes expressing HLA-DR, intercellular adhesion molecule-1 (ICAM-1) and LFA-3 in (a) the absence and (b) the presence of IFN-γ, following incubation at 37°C for 48 h. Values are the means ± s.e.m. of cell surface expression on monocytes from three individuals.

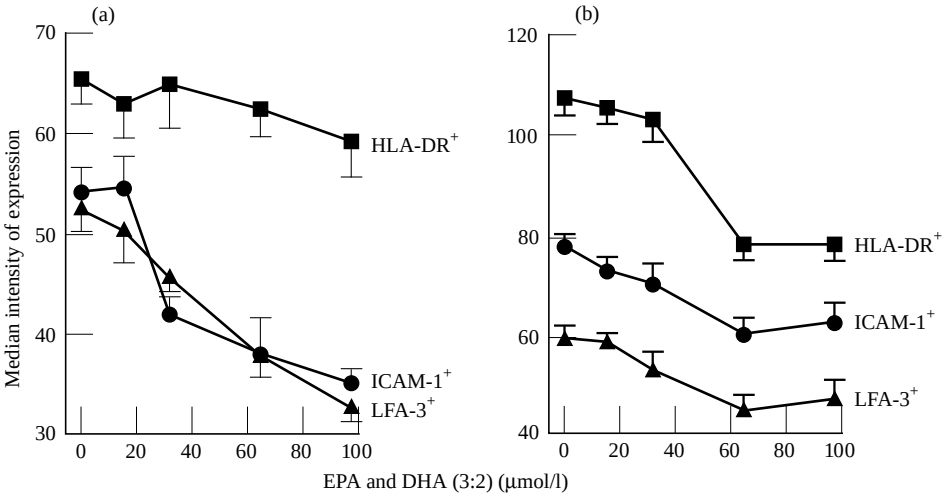

Fig. 3. Effect of increasing doses of the combined eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on the median intensity of expression of HLA-DR, intercellular adhesion molecule-1 (ICAM-1) and LFA-3 in (a) the absence and (b) the presence of IFN-γ, following incubation at 37°C for 48 h. Values are the means ± s.e.m. of cell surface expression on monocytes from three individuals.

remains unclear whether the reduced ability to present antigen is due to a moderate lack of MHC class II up-regulation on all cells, or a major lack on a few cells.

In a human supplementation study we examined the effect of 3 g fish oil/day on the expression of MHC class II molecules and adhesion molecules on peripheral blood monocytes [10]. The supplements used in the study contained the same EPA:DHA ratio as was used in the present *in vitro* experiments. Several animal studies have shown that n-3 PUFA can inhibit the expression of Ia molecules, the murine equivalent of the human MHC class II. Kelley and colleagues [16] reported that dietary supplementation with fish oil suppresses autoimmune lupus in MRL-*lpr* mice and prevents an increase in macrophage surface Ia expression. Mosquera *et al*. [17] demonstrated that fish oil administration to mice and rats by oesophageal gavage reduced the percentage of peritoneal macrophages that expressed Ia in comparison with saline gavaged controls, and Huang *et al*. [18] showed that fish oil-fed mice infected with *Listeria monocytogenes* had a reduced expression of Ia on the surface of peritoneal macrophages compared with mice fed other fat sources. Dietary enrichment with EPA has also been shown to inhibit the ability of spleen cells to present antigens to murine helper T cell clones, and *in vitro* pretreatment of splenocytes with EPA also resulted in inhibition of APC function [19]. Recently, it has been shown that dietary fish oil can diminish the ability of rat dendritic cells (another class of APC) to present antigen to autologous spleen lymphocytes [20]. Taken together, the results of these studies and those of the current study support the hypothesis that n-3 PUFA suppress cell-mediated immune responses, at least in part, by inhibiting APC function.

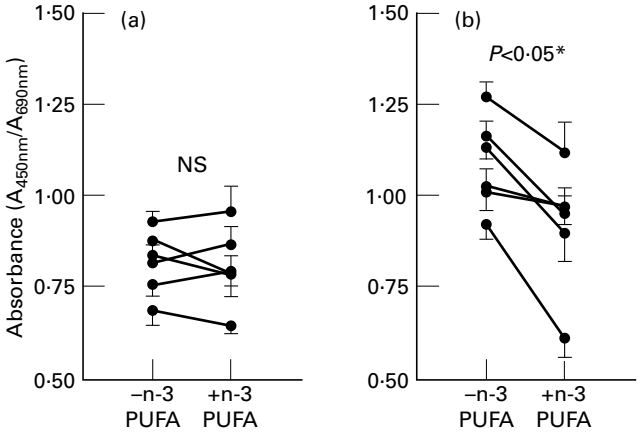

Fig. 4. Effect of the combined n-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), on the *in vitro* antigen-presenting function of (a) unstimulated and (b) IFN-γ-stimulated human monocytes. Purified lymphocytes (10^6) from six individuals were co-cultured with 10^5 autologous monocytes which had been pre-incubated with or without IFN in the absence (−) or presence (+) of n-3 PUFA and then with tetanus toxoid. Lymphocyte proliferation was assessed by bromodeoxyuridine uptake, quantified by ELISA, and the results are expressed as mean absorbance ± s.e.m. The mean (± s.e.m) absorbance of lymphocytes cultured alone was 0·55 ± 0·07. *Group comparisons were made using the paired *t*-test.

The ability of n-3 PUFA to inhibit the antigen-presenting function of activated monocytes supports the possibility that fish oil may be beneficial in the treatment of autoimmune disorders. A number of double-blind studies have reported that consumption of fish oil by patients with RA is associated with mild to moderate symptomatic improvement, including a reduction in morning stiffness and in the number of tender joints [2]. The striking inhibition of MHC class II molecules and ICAM-1 expression by EPA and DHA on IFN-γ-stimulated monocytes seen in this study may be particularly relevant to RA, since patients with this disorder have been shown to have abnormally elevated expression of both MHC class II molecules [9] and ICAM-1 [21] in chronically inflamed joints. A corresponding reduction in antigen-presenting function might lead to reduced helper T cell activation, thus decreasing both the production of inflammatory cytokines and the production of antibodies by B cells at these localized sites of disease.

The results of the present study may also be of relevance to the suggested inverse relation between long-term fish oil intake and atherogenesis. There is increasing evidence of a chronic immune and inflammatory involvement in the formation of atherosclerotic

lesions [22], and the presence of chronically stimulated T cells within lesions, and the expression of MHC class II molecules on lesional monocytes-macrophages indicates that these cells are actively participating in the local immune response occurring during atherogenesis [23]. Supplementation studies have shown that EPA and DHA are incorporated into the lipids of advanced atherosclerotic plaques in man [24], and it is possible that a reduced expression of MHC class II molecules might inhibit the antigen-presenting function of the local macrophages, thereby prolonging, if not preventing, lesion development.

There are several mechanisms which may be involved in the modulatory effect of n-3 PUFA on surface molecule expression. It is possible that the incorporation of these fatty acids into the cell membrane can increase its fluidity and thus alter the expression of membrane proteins [25], possibly by influencing the vertical displacement of the proteins within the membrane. It has been shown that different proteins exhibit disparate changes in cell surface expression following alterations in membrane fluidity [26]. This might explain why, in contrast to HLA-DR, ICAM-1 and LFA-3, no significant changes in HLA-DQ and LFA-1 expression were observed on unstimulated monocytes. Interestingly, ICAM-1 and LFA-3 belong to the same family of structurally related adhesion molecules, the immunoglobulin superfamily, whereas LFA-1 is a member of the integrin family. Therefore, it is possible that the structural form of the surface molecule is important in determining its expression relative to the fluidity of the membrane.

Since PUFA are more susceptible to lipid peroxidation than are monounsaturated and saturated fatty acids, it is possible that an increase in monocyte cell membrane lipid peroxidation may affect the expression of cell surface molecules. It has already been demonstrated that free radicals can suppress the expression of HLA-DR [27], and we have recently reported that dietary supplementation with the anti-oxidant carotenoid, beta-carotene, can enhance the expression of HLA-DR, ICAM-1 and LFA-3 on human peripheral blood monocytes [28].

A further possibility is that EPA and DHA are directly or indirectly influencing the expression of messenger RNA (mRNA) for the various cell surface molecules. It has recently been shown that DHA can inhibit the expression of vascular cell adhesion molecule-1 (VCAM-1), induced by IL-1 on human endothelial cells [29], in parallel with a decrease in VCAM-1 mRNA levels. The observed effect was concluded to be pretranslational, but whether it resulted from a decrease in mRNA stability or a decrease in transcription, possibly mediated by altering the activity of the transcription factor, NF-κB, remains undetermined.

In conclusion, we have shown that the n-3 PUFA, EPA and DHA, when combined at the same ratio as commonly found in fish oil supplements, can inhibit the antigen-presenting function of IFN-γ-stimulated human peripheral blood monocytes. This inhibitory effect appears to be associated with a reduced expression on monocytes of surface molecules that are involved in their function as APC. The inhibition of MHC class II molecule and ICAM-1 expression on IFN-γ-stimulated monocytes supports the possibility that fish oil may be beneficial in the treatment of disorders, such as autoimmune disease, which are associated with abnormally elevated expression of these molecules.

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