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Dietary fish oil diminishes lymphocyte adhesion to macrophage and endothelial cell monolayers

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SUMMARY

To further investigate the immunomodulatory effects of dietary lipids, rats were fed on a low-fat diet or on high-fat diets that contained hydrogenated coconut, olive, safflower, evening primrose or fish oil as the principal fat source. The fish oil diet decreased the level of expression of CD2, CD11a, CD18 and CD44 on the surface of freshly prepared lymphocytes and of CD2, CD11a, CD18, CD54 (intercellular adhesion molecule-1; ICAM-1) and CD62L (L-selectin) on the surface of concanavalin A (Con A)-stimulated lymphocytes. The olive oil diet also resulted in decreased expression of some adhesion molecules. The fish or olive oil diets, and to a lesser extent the safflower or evening primrose oil diets, decreased the adhesion of both freshly prepared and Con A-stimulated lymphocytes to macrophage monolayers. The fish oil diet, and to a lesser extent the olive or evening primrose oil diets, reduced the ability of Con A-stimulated lymphocytes to adhere to untreated endothelial cells. Furthermore, the fish oil diet resulted in a 50% reduction in Con A-stimulated lymphocyte adhesion to tumour necrosis factor-α (TNF-α)-stimulated endothelial cells. This study demonstrates that dietary lipids affect the expression of functionally important adhesion molecules on the surface of lymphocytes. Furthermore, this study suggests that such diet-induced effects on adhesion molecule expression might alter the ability of lymphocytes to bind to macrophages and to endothelial cells. Of the diets studied fish oil causes the most significant effects. The results of this study suggest that a reduction in cellular infiltration may partly explain the protective effect of a fish-oil-rich diet against the development of inflammatory and cardiovascular diseases.

INTRODUCTION

Adhesion molecules are involved in many cell-to-cell interactions. For example, interaction between T lymphocytes and antigen-presenting cells is in part mediated by the ligand–receptor pairs CD11a/CD18:CD54 (i.e. leucocyte function-associated antigen (LFA)-1:intercellular adhesion molecule-1 (ICAM-1)), LFA-1:CD102 and CD2:CD58.[1] Thus, an efficient cell-mediated immune response requires appropriate levels of expression of these molecules on T lymphocytes. In addition, lymphocyte adhesion to the endothelium involves a number of ligand–receptor pairs including LFA-1:ICAM-1, ICAM-1:LFA-1, CD49d/CD29:CD106 (i.e. very late antigen-4 (VLA-4):vascular cell adhesion molecule-1 (VCAM-1)), CD2:CD58, CD62L (L-selectin):MAdCAM-1 and CD44: hyaluronate.[1–3] Thus, movement of lymphocytes between body compartments, into and out of lymphoid organs and into sites of immune or inflammatory reactivity requires adhesion molecule expression. Adhesion molecule expression appears to be involved in several acute and chronic inflammatory disease processes,[4] and antibodies against certain adhesion molecules can reduce chronic inflammatory disease.[4]

In recent years there has been significant interest in the anti-inflammatory and immunomodulatory effects of dietary fish oils.[5,6] These oils are rich in the long chain *n*-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (20:5*n*-3) and docosahexaenoic acid (22:6*n*-3). In contrast, most vegetable oils, such as corn, sunflower and safflower oils, are rich in linoleic acid (18:2*n*-6) the precursor of arachidonic acid (20:4*n*-6) That fish oils might influence the inflammatory process was suggested by epidemiological studies which revealed that the traditional Eskimo diet, which is characterized by a high intake of *n*-3 PUFA, resulted in a significantly

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Abbreviations: CO, hydrogenated coconut oil; Con A, concanavalin A; EPO, evening primrose oil; FCS, fetal calf serum; HECs, high endothelial cells; ICAM, intercellular adhesion molecule; LF, low fat; LFA, leucocyte function associated molecule; MO, menhaden oil; OO, olive oil; PBS, phosphate-buffered saline; PUFA, polyunsaturated fatty acids; RAM-FITC, fluorescein-isothiocyanate-labelled rabbit anti-mouse IgG; SO, safflower oil; TNF, tumour necrosis factor; VCAM, vascular cellular adhesion molecule; VLA, very late antigen.

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lower incidence of chronic inflammatory diseases such as psoriasis, rheumatoid arthritis, asthma, type-1 diabetes and multiple sclerosis.[7] This idea is supported by clinical trials indicating beneficial effects of fish oil supplementation in patients with rheumatoid arthritis,[8–13] psoriasis,[14–18] atopic dermatitis and ezcema,[19] ulcerative colitis,[20–23] lupus[24] and multiple sclerosis.[25] The beneficial effects of fish oil in these diseases relate at least partly to the decreased production of arachidonic acid-derived pro-inflammatory mediators such as prostaglandin E_2 and the 4-series leukotrienes which accompanies fish oil feeding.[5,6] As well as influencing eicosanoid production, dietary fish oil has been shown to influence neutrophil and monocyte chemotaxis, the production of monocyte- and macrophage-derived cytokines, lymphocyte proliferation and natural killer cell activity.[5,6] Many of these effects appear to be exerted via eicosanoid-independent mechanisms.

It has also become apparent that *n*-3 PUFA can affect adhesion molecule expression by some cell types, at least *in vitro*. Calder *et al.*[26] observed that murine thoiglycollate-elicited peritoneal macrophages cultured in the presence of eicosapentaenoic or docosahexaenoic acid were less adherent to artificial surfaces (the adhesion to one of these surfaces is mediated by LFA-1) than those cultured with some other fatty acids. More recently, it has been shown that culture of endothelial cells of different origins (pig aorta, human umbilical vein, adult human saphenous vein) with eicosapentaenoic or docosahexaenoic acids reduces lipopolysaccharide- or cytokine-induced expression of VCAM-1, ICAM-1 and CD62E (E-selectin).[27–29] Such changes result in diminished binding of monocytes or lymphocytes to the endothelial cells,[27–29] suggesting that the reduced adhesion molecule expression has a functional effect. Khalfoun *et al.*[29] also reported, for the first time, the *in vitro* effect of *n*-3 PUFA on adhesion molecule expression on lymphocytes: incubation of lymphocytes with either eicosapentaenoic or docosahexaenoic acid reduced the level of expression of CD11a and L-selectin but did not affect CD49d expression.[29] In parallel with this reduction, the binding of lymphocytes to untreated or cytokine-stimulated endothelial cells was diminished.[29] In another recent study, incubation with eicosapentaenoic acid was shown to reduce the level of expression of ICAM-1 on the surface of resting or interferon-γ-stimulated human monocytes.[30] Thus, it appears that culture of macrophages, monocytes, lymphocytes or endothelial cells with *n*-3 PUFA can decrease adhesion molecule expression, resulting in diminished ability to bind to other cell types.

There are few studies of the effects of inclusion of *n*-3 PUFA in the diet upon adhesion molecule expression, although it was recently shown that supplementation of the human diet with *n*-3 PUFA results in significantly lower levels of expression of CD11a and ICAM-1 on peripheral blood monocytes.[31] We have previously reported that feeding rats a diet rich in fish oil results in significantly reduced levels of expression of CD2 and CD11a on freshly prepared lymphocytes,[32] of CD2, CD11a and ICAM-1 on concanavalin A (Con A)-stimulated lymphocytes,[32] and of CD2 and CD11a on popliteal lymph node lymphocytes following localized graft-versus-host or host-versus-graft responses.[33] Reduced adhesion molecule expression suggests that cells will be less able to interact with receptor-bearing cells. However, there have been no reports of the effects of dietary lipid manipulation upon cell-to-cell adhesion. Thus, this study set out to examine the effect of different dietary lipids upon the expression of a range of important adhesion molecules on the surface of lymphocytes and to examine the biological effect of any changes in such expression upon the ability of lymphocytes to bind to other cell types. To our knowledge this is the first study to investigate the effect of different dietary lipids upon cell-to-cell adhesion.

MATERIALS AND METHODS

Animals and diets

Lymphocytes were from male Lewis rats (obtained from Harlan-Olac, Bicester, UK) which had been fed for 8 weeks from weaning on a low fat (LF; 25 g/kg corn oil) diet or on one of five high fat (210 g/kg) diets (purchased from ICN Biomedicals, High Wycombe, UK). The high fat diets contained 200 g/kg of hydrogenated coconut oil (CO), olive oil (OO), safflower oil (SO), evening primrose oil (EPO) or fish (menhaden) oil (MO) plus 10 g/kg corn oil to prevent essential fatty acid deficiency. All diets contained identical amounts of protein, starch, sucrose and vitamin E. The fatty acid composition of these diets has been described elsewhere.[34] The LF and SO diets are rich in linoleic acid while EPO, which is also rich in linoleic acid, contains a small proportion of γ-linolenic acid (18:3*n*-6). OO is very rich in oleic acid (18:1*n*-9) while CO contains high proportions of medium-chain saturated fatty acids. Macrophages were from mature male Lewis rats which had been fed on standard laboratory chow (purchased from Special Diets Services, Witham, UK). Animals were killed in the fed state by an overdose of CO_2.

Chemicals

Chemicals, culture medium and medium supplements were obtained from the sources described elsewhere.[32–34] [4,5-^{3}H]Leucine was purchased from Amersham International, Amersham, UK. Monoclonal antibodies to CD11a (clone WT.1) and to ICAM-1 (clone IA.29) were gifts from Professor M. Miyasaka, Department of Immunology, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan. Monoclonal antibodies to CD2 (clone MRC OX34), CD18 (clone WT.3), CD44 (clone MRC OX50), CD49d (clone TA-2), L-selectin (clone MRC OX85) and VCAM-1 (clone TA-2) were generously supplied by the MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford. Fluoroscein isothiocyanate (FITC)-labelled rabbit anti-mouse immunoglobulin G (IgG) (RAM-FITC) was purchased from Serotec, Kidlington, UK. Recombinant tumour necrosis factor (rTNF)-α was purchased from R & D Systems Europe, Abingdon, UK.

Lymphocyte preparation and culture

Cervical and mesenteric lymph nodes were removed from the animals, freed of any attached adipose tissue and gently ground into phosphate-buffered saline (PBS). The cell suspension was filtered through lens tissue and lymphocytes were collected by centrifugation (1000 *g*, 10 min), resuspended in PBS and purified by centrifugation on Histopaque (1500 *g*, 20 min). The purified lymphocytes were washed once in PBS. Where Con A-stimulated lymphocytes were used, they were cultured for 24 hr at a density of 5×10^6 cells/well and a total

culture volume of 2 ml in RPMI medium containing 2·5% (v/v) autologous serum, 2 mM glutamine, 5 μg/ml Con A and antibiotics. Following culture, the cells were collected by centrifugation and washed twice in PBS.

Macrophage preparation

Rats were injected i.p. with 3 ml of Brewer's thioglycollate broth to elicit macrophage migration to the peritoneal cavity. After death the peritoneal exudate cells were collected by washing out the peritoneal cavity with a large volume of PBS. The cells were collected by centrifugation. Any contaminating erythrocytes were removed by a brief incubation in Tris-buffered 0·14 mM ammonium chloride, pH 7·2. The remaining cells were washed with PBS and collected by centrifugation. Finally, the cells were resuspended at a density of 2×10^6 cells/ml in RPMI medium supplemented with 10% (v/v) fetal calf serum (FCS), 2 mM glutamine and antibiotics. The cell suspension (100 μl) was plated into wells of flat-bottomed 96-well tissue culture plates. After 1 hr in a 19 : 1 air/CO_2 atmosphere at 37° the medium was aspirated and the adherent cells (macrophages) were washed three times with PBS containing 10% (v/v) FCS. Adherent macrophages (2×10^5 cells/well) were used in adhesion assays.

Endothelial cell culture

Cultures of high endothelial cells (HECs) from cervical lymph nodes of (AO × DA)F1 hybrid rats were a gift from Dr A. Ager, National Institute for Medical Research, London, UK. HECs were grown in RPMI medium containing 10% (v/v) FCS, supplemental non-essential amino acids, 2 mM glutamine and antibiotics. To subculture the cells at confluence, they were removed from the flasks using 0·1% (v/v) trypsin/0·025% (w/v) ethylenediamine tetra-acetic acid (EDTA) in PBS and re-cultured at 50% of confluent density. Adherent HECs (10^4 cells/well) were used in adhesion assays; in some cases the cells were pre-treated with rTNF-α (10^{-7} M) for 72 hr.

Flow cytometry

Flow cytometry was used to assess the expression of adhesion molecules on the surface of freshly prepared and Con A-stimulated lymphocytes and on the surface of untreated or TNF-α-treated HECs. Approximately 10^6 cells (resuspended in PBS containing 0·1% (w/v) bovine serum albumin (BSA) and 10 mM sodium azide (modified PBS)) were incubated for 20 min at 4° with monoclonal antibodies to CD11a, ICAM-1, CD2, CD18, CD44, CD49d, L-selectin or VCAM-1. Incubation with a monoclonal antibody to the human C3b inactivator protein (clone MRC OX-21) was used as a negative control. Following staining with monoclonal antibodies, the cells were washed twice with modified PBS and incubated with RAM-FITC for 20 min at 4°. They were washed twice with modified PBS and then suspended in 2% (v/v) formaldehyde in PBS and examined for fluorescence using a Becton Dickinson FACScan fluorescence-activated cell sorter. Fluorescence data were collected on 10^4 viable cells and analysed using Lysis II software.

Lymphocyte–macrophage and lymphocyte–endothelial cell adhesion assays

Total lymphocyte adhesion to monolayers of either elicited peritoneal macrophages or untreated or TNF-α-treated HECs was studied using [^{3}H]leucine-labelled lymphocytes. Lymphocytes ($2{\cdot}5 \times 10^7$/ml) were incubated for 60 min at 37° in leucine-free modified Eagle's medium (MEM) containing 10 μCi/ml [4,5-^{3}H]leucine. They were then washed three times in PBS and resuspended at a density of 10^7/ml in RPMI containing 1% (v/v) FCS. The resuspended lymphocytes (10^6/well) were added to wells containing macrophage (2×10^5/well) or HEC (10^4/well) monolayers and incubated at 37° in an air/CO_2 (19 : 1) atmosphere. After 1 hr, the medium containing unbound lymphocytes was aspirated from the wells and the adherent cells were washed five times with PBS. The adherent cells were solubilized with 100 μl 1 M ammonium hydroxide and the entire cell lysate was transferred to scintillation vials. Lymphocyte binding was assessed by scintillation counting and is expressed as a percentage of the total radioactivity added to each well. All incubations were conducted in quadruplicate.

For some assays, lymphocytes were pre-incubated with antibodies to adhesion molecules for 30 min at 0° prior to their use in adhesion assays.

Statistical analysis

Data are shown as mean ± SEM of the indicated number of separate observations. Statistical significance was determined using one-way analysis of variance, followed by a least significant difference test; a value of $P < 0{\cdot}05$ was taken to indicate statistical significance.

RESULTS

Effect of dietary lipid manipulation on expression of adhesion molecules on lymphocytes

Diet did not affect the proportion of lymphocytes staining positively for CD11a (100%), CD2 (65–75%), CD18 (100%), ICAM-1 (60–70%), CD44 (almost 100%), CD49d (≈98%) or L-selectin (65–70%). Nor did diet affect the level of expression of ICAM-1, CD49d or L-selectin (Table 1). However, there were significant effects of diet on the level of expression of CD11a, CD2, CD18 and CD44 (Table 1). Expression of each of these molecules was lowest on cells taken from rats fed the MO diet and was highest on those from LF-fed rats (Table 1). The expression of CD44 on lymphocytes from MO-fed rats was lower than on lymphocytes from rats fed each of the other diets, while the expression of CD11a and CD2 on lymphocytes from rats fed MO was lower than on cells from rats fed the LF, HCO, SO or EPO diets (Table 1). OO feeding also reduced the expression of CD11a, CD2 and CD18 compared with feeding some of the other diets (Table 1).

Culture with Con A for 24 hr did not alter the proportion of cells expressing CD11a, CD18, ICAM-1, CD44 or CD49d (not shown). However, the proportion of cells expressing CD2 was increased to 75–90% by Con A stimulation, while the proportion of L-selectin-positive cells was markedly decreased to 10–15% (Fig. 1). This reduction appears to be caused by a protein kinase C-dependent shedding of L-selectin from the lymphocyte surface.[35] The proportion of L-selectin-positive cells was lower ($P = 0{\cdot}043$) for cells from MO-fed rats (10·4 ± 1·5%) than for those from rats fed each of the other diets (all ≈15%).

There were significant effects of diet on the level of expression of CD11a, CD2, CD18, ICAM-1 and L-selectin on

Table 1. Effect of dietary lipid manipulation on the level of expression of adhesion molecules on the surface of freshly prepared lymphocytes

	Mean fluorescence intensity						
Diet	CD11a	CD2	CD18	ICAM-1	CD44	CD49d	L-selectin
LF	399[b]	409[b]	301[b]	160	456[c]	124	118
HCO	396[b]	384[bc]	291[bc]	149	444[bc]	131	114
OO	329[ac]	362[ac]	254[ac]	148	419[b]	124	109
SO	371[bc]	392[bc]	277[ab]	148	423[b]	122	107
EPO	377[bc]	374[bc]	307[b]	162	447[bc]	126	118
MO	313[a]	336[a]	243[a]	154	390[a]	116	111
Pooled SD	35·2	26·6	33·9	10·4	19·8	9·0	14·0
P	0·004	0·006	0·043	0·101	<0·001	0·134	0·732

Data are mean values for six animals fed on each diet. Values which do not share a common letter are significantly different.

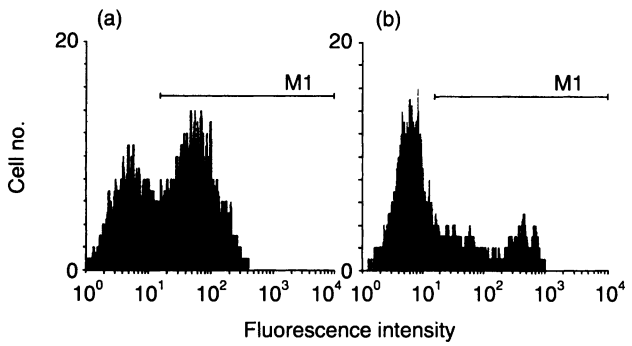

Figure 1. FACS profile of CD62L on (a) unstimulated and (b) Con A-stimulated lymphocytes. Lymphocytes were from animals fed on the LF diet.

the surface of Con A-stimulated lymphocytes (Table 2). Expression of each of these molecules was lowest on cells taken from rats fed the MO diet and was highest on those from LF-fed rats (Table 2). The expression of L-selectin on lymphocytes from MO-fed rats was lower than on lymphocytes from rats fed each of the other diets, while the expression of CD18 and ICAM-1 on lymphocytes from rats fed MO was lower than on cells from rats fed the LF, HCO, OO or SO diets (Table 2). The level of expression of CD11a and CD2 on lymphocytes from MO-fed rats was lower than on lymphocytes from rats fed the LF or HCO diets (Table 2).

Effect of dietary lipid manipulation on lymphocyte adhesion to macrophages

Lymphocytes were incubated with monoclonal antibodies prior to measuring their binding to macrophage monolayers. An anti-CD11a antibody reduced the adhesion of freshly prepared lymphocytes to macrophages by ≈45% while an anti-CD18 antibody reduced adhesion by ≈40% (Fig. 2). In contrast, antibodies to CD2, ICAM-1, CD49d or L-selectin did not inhibit binding (data not shown). Thus, CD11a and CD18 (the α and β2 subunits, respectively, of LFA-1) appear to be the principal adhesion molecules involved in binding of resting lymphocytes to macrophages. If the lymphocytes were stimulated with Con A for 24 hr adhesion to macrophages increased (Fig. 2). This adhesion could be reduced by ≈40% by an antibody to CD18 (Fig. 2). An antibody to CD11b did not influence the binding of stimulated lymphocytes to macrophages (Fig. 2). This suggests that binding in this situation is mediated by a β2 integrin other than LFA-1 (CD11b/CD18). We did not investigate the nature of this integrin further.

Feeding the MO or OO diets, and to a lesser extent the SO or EPO diets, decreased the adhesion of both freshly

Table 2 Effect of dietary lipid manipulation on the level of expression of adhesion molecules on the surface of Con A-stimulated lymphocytes

	Mean fluorescence intensity						
Diet	CD11a	CD2	CD18	ICAM-1	CD44	CD49d	L-selectin
LF	276[b]	404[b]	98[bc]	269[c]	309	61	448[b]
HCO	272[bc]	357[bc]	100[b]	248[bc]	319	62	422[b]
OO	245[ac]	334[ac]	102[b]	221[bcd]	320	60	419[b]
SO	246[ac]	335[ac]	96[bc]	241[bd]	313	60	427[b]
EPO	240[a]	315[ac]	92[ac]	203[ad]	329	61	428[b]
MO	225[a]	297[a]	89[a]	179[a]	318	61	361[a]
Pooled SD	22·0	31·0	4·6	27·4	23·1	2·9	25·8
P	0·003	0·001	0·048	0·001	0·811	0·818	0·001

Data are mean values for six animals fed on each diet. Values which do not share a common letter are significantly different.

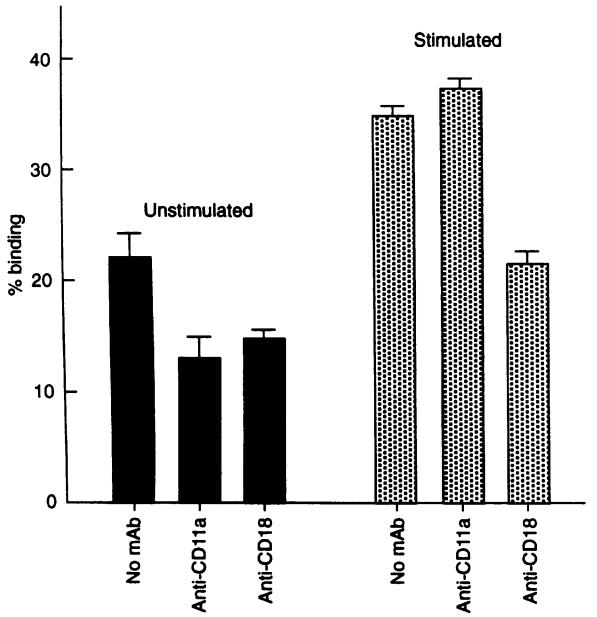

Figure 2. Effect of monoclonal antibodies against CD11a or CD18 upon the adhesion of lymphocytes to macrophage monolayers. Data are mean ± SEM for four separate lymphocyte preparations; lymphocytes were from animals fed on the LF diet.

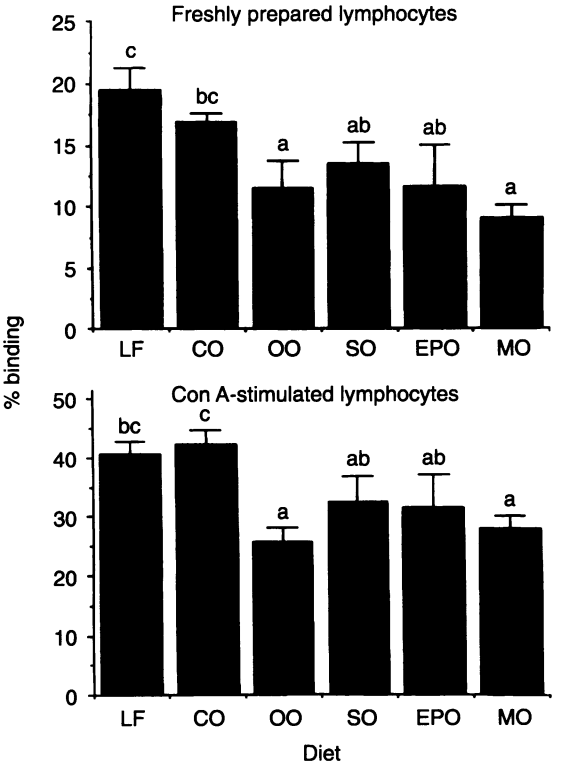

Figure 3. Effect of dietary lipid manipulation upon the adhesion of lymphocytes to macrophage monolayers. Data are mean ± SEM for six animals fed on each diet. Values which do not share a common letter are significantly different.

prepared and Con A-stimulated lymphocytes to macrophage monolayers (Fig. 3).

Effect of dietary lipid manipulation on lymphocyte adhesion to endothelial cells

Lymphocytes were incubated with monoclonal antibodies prior to measuring their binding to HEC monolayers. The HECs were used in either the resting or TNF-α-stimulated state. TNF-α significantly increased the level of ICAM-1 expression on the HEC surface (mean fluoresecence intensity increased from ≈15 to ≈80). In contrast, the expression of VCAM-1, which was weak (mean fluoresecence intensity ≈10), was unaffected by TNF-α stimulation. Lymphocyte adhesion was increased by stimulating the HECs with TNF-α and by stimulating the lymphocytes with Con A (Fig. 4). Binding of freshly prepared lymphocytes to unstimulated or TNF-α-stimulated HECs was reduced by more than 50% by an antibody to CD49d (Fig. 4a,b). Antibodies to CD11a, CD18 or ICAM-1 decreased adhesion of freshly prepared lymphocytes to HECs by 10–20%; an antibody to L-selectin was without effect (Fig. 4a,b). An antibody to CD44 appeared to enhance adhesion between freshly prepared lymphocytes and HECs (Fig. 4a,b). Antibodies to CD11a, CD18, ICAM-1 or CD49d decreased adhesion of Con A-stimulated lymphocytes to HECs by 40–50%; antibodies to CD44 or L-selectin did not greatly affect adhesion of stimulated lymphocytes to HECs (Fig. 4c,d). Thus, CD11a, CD18, ICAM-1 and CD49d all play a role in lymphocyte adhesion to HEC monolayers in this assay; CD49d appears to be the most important molecule for binding of unstimulated lymphocytes while all four of these molecules have an equal role in mediating adhesion of Con A-stimulated lymphocytes. These findings are in accordance with the observations of Pankonin *et al.*[36] who reported that anti-CD11a, anti-CD18 and anti-ICAM-1 antibodies inhibited the binding of interleukin-2 (IL-2)-stimulated lymphocytes to HEC monolayers.

Dietary lipids did not affect the binding of freshly prepared lymphocytes to either untreated HECs or HECs stimulated with TNF-α for 24 hr (data not shown). Feeding the MO diet, and to a lesser extent the OO and EPO diets, significantly reduced the ability of Con A-stimulated lymphocytes to adhere to untreated HECs (Fig. 5). Furthermore, feeding the MO diet resulted in a 50% reduction in Con A-stimulated lymphocyte adhesion to TNF-α-stimulated HECs compared with feeding each of the other diets (Fig. 5).

DISCUSSION

Previous studies have shown that culture of cells such as lymphocytes, monocytes or endothelial cells with long chain *n*-3 PUFA, such as those found in fish oil, results in a significant reduction in surface expression of some adhesion molecules.[27–30] As a result, adhesion between cells cultured in this way and cells bearing the adhesion molecule receptors is reduced.[27–29] The current study shows that dietary fish oil results in reduced expression of adhesion molecules on the surface of lymphocytes: in particular the levels of CD11a, CD2, CD18 and CD44 were lower on freshly prepared lymphocytes and the levels of CD11a, CD2, ICAM-1, CD18 and L-selectin were lower on Con A-stimulated lymphocytes. These observations are in accordance with those of Hughes *et al.*[31] who found that supplementation of the diet of healthy

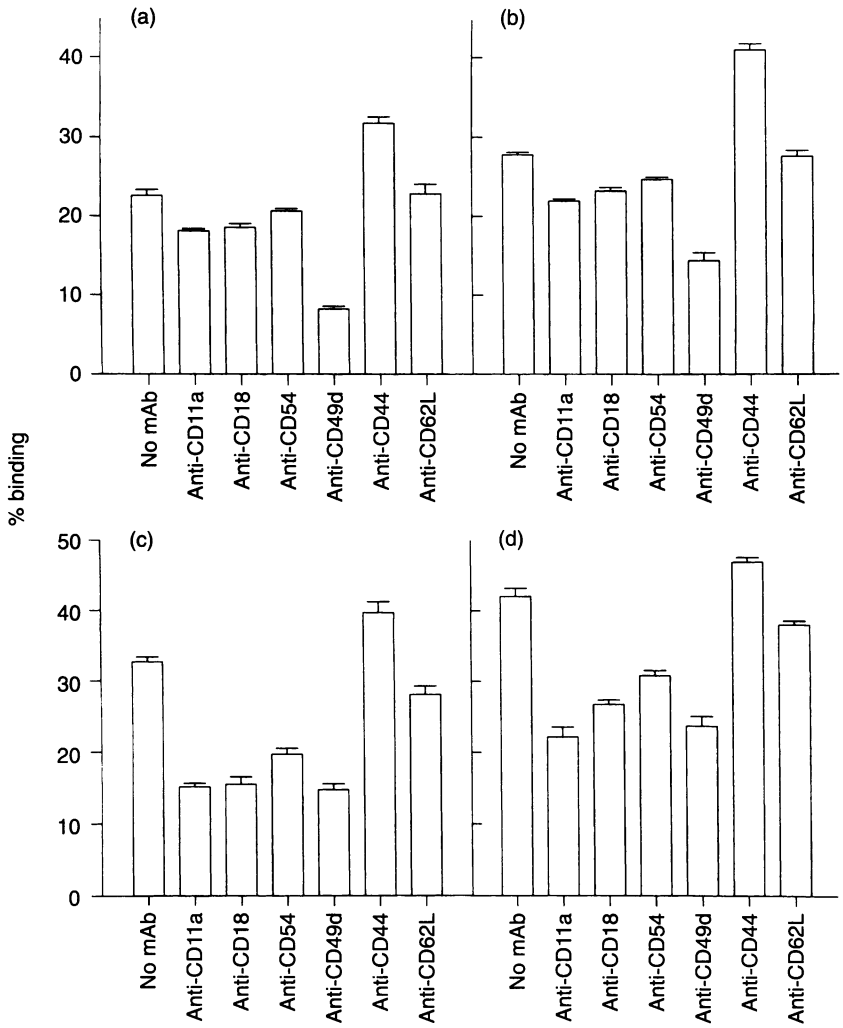

Figure 4. Effect of monoclonal antibodies against CD11a, CD18, CD54 (ICAM-1), CD49d, CD44 or CD62L (L-selectin) on the adhesion of lymphocytes to endothelial cell monolayers. (a) and (b) are unstimulated lymphocytes; (c) and (d) are Con A-stimulated lymphocytes; (a) and (c) are unstimulated HECs; (b) and (d) are TNF-α-stimulated HECs. Data are mean ± SEM for four separate lymphocyte preparations; lymphocytes were from animals fed on the LF diet.

volunteers with 3 g fish oil per day for 21 days resulted in a significant reduction in the levels of expression of ICAM-1 and CD11a on both freshly prepared and interferon-γ-stimulated peripheral blood monocytes. Furthermore, the observations of the current study are supported in part by those of Khalfoun *et al.*[29] who showed that culture of lymphocytes with *n*-3 PUFA results in reduced CD11a expression. In addition, the observation that dietary fish oil does not affect CD49d expression agrees with the lack of effect of culture of lymphocytes with *n*-3 PUFA upon expression of this molecule.[29] Although the most dramatic effects on adhesion molecule expression are exerted by dietary fish oil, the current study also shows that feeding diets rich in other unsaturated fatty acids can also influence expression of some adhesion molecules.

Diminished adhesion molecule expression might be expected to result in reduced adhesion of lymphocytes to cells bearing the adhesion molecule receptors. The current study identified lymphocyte-borne CD11a and CD18 as being important in the binding of unstimulated lymphocytes to macrophages, while CD18 was involved in the binding of Con A-stmulated lymphocytes to macrophages. Thus, the MO-induced reduction in the expression of CD11a and CD18 provides an explanation for the reduced ability of lymphocytes from MO-fed rats, whether fresh or Con A-stimulated, to bind to macrophage monolayers. The level of CD11a and CD18 was also low on freshly prepared lymphocytes from OO-fed rats; these cells also showed reduced binding to macrophages.

Among the molecules investigated, lymphocyte-borne CD49d was identified as the most important in the binding of freshly prepared lymphocytes to HECs whatever the activation state of the HECs. The lack of effect of diet on the expression of CD49d on freshly prepared lymphocytes accords with the lack of effect of diet on the binding of these cells to either unstimulated or TNF-α-stimulated HECs. In contrast, the binding of Con A-stimulated lymphocytes to HECs was affected by diet. It appears that lymphocyte-borne CD11a, CD18, ICAM-1 and Cd49d are involved to approximately equal extents in the binding of Con A-stimulated lymphocytes to HECs, whatever the activation state of the latter. Con A

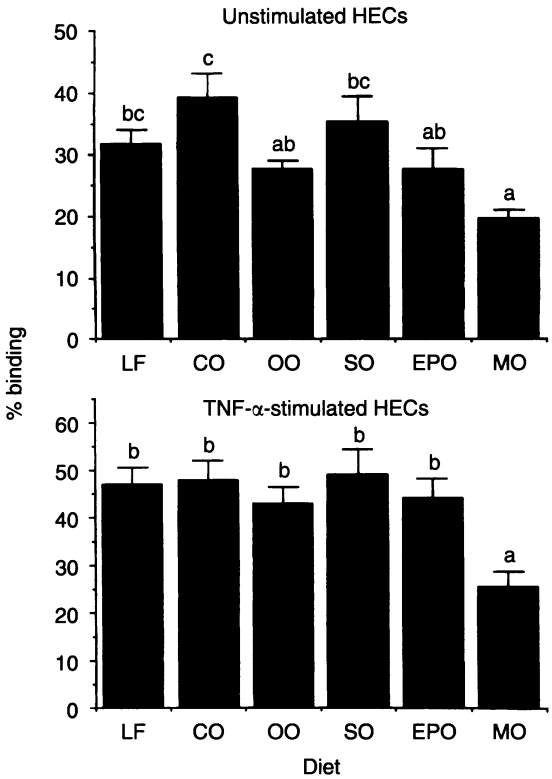

Figure 5. Effect of dietary lipid manipulation upon the adhesion of Con A-stimulated lymphocytes to HECs. Data are mean ± SEM for six animals fed on each diet. Values which do not share a common letter are significantly different.

stimulated lymphocytes from MO-fed rats exhibited reduced levels of expression of ICAM-1, CD18 and CD11a and showed decreased binding to both unstimulated and TNF-α-stimulated HECs. Thus, the reduced adhesion of these cells might be explained by the reduced levels of expression of some of the molecules involved.

Thus, this study demonstrates that dietary lipids affect the expression of functionally important adhesion molecules on the surface of lymphocytes; fish oil causes the most significant effects lowering the levels of expression of a range of adhesion molecules. Furthermore, this study demonstrates, for the first time, that dietary lipid-induced effects on adhesion molecule expression might alter the ability of lymphocytes to bind to macrophages and to endothelial cells. It is worth noting, however, that some adhesion molecules can undergo changes in activity which are independent of their cell surface expression. In the current study we have measured surface expression rather than the functional state of different adhesion molecules. It will be important to investigate the functional state of selected adhesion molecules in future studies so that the effects of fish oil upon the relationship between surface expression, functional state and cell-to-cell binding can be fully elucidated.

Adhesion of lymphocytes and other leucocytes to the endothelium is necessary for their movement into sites of inflammatory or autoimmune activity. Dietary modulation of adhesion molecule expression resulting in altered in cell-to-cell adhesion could play a role modifying the progression of such diseases. Fish oil has been shown to be of some benefit in rheumatoid arthritis[8–13] and other chronic inflammatory diseases.[14–25] It is tempting to speculate that the beneficial effects of fish oil in such diseases are at least partly caused by a reduction in the movement of leucocytes to sites of inflammation and tissue destruction. Likewise, the protective effects of fish oils towards atherosclerosis[7,37,38] may include a reduction in movement of cells from the bloodstream into the sub-endothelial space, where they cause tissue damage and contribute to the growth of the atherosclerotic plaque.

Lymphocyte-borne adhesion molecules such as CD11a, CD18 and CD2 are involved in interaction between T cells and antigen-presenting cells.[1] Thus, a diet-induced reduction in expression of these molecules, such as that which accompanies fish oil feeding, might result in diminished cell-mediated immune responses. Reduced adhesion molecule-mediated interaction between lymphocytes and accessory cells might contribute to the lower proliferation of lymphocytes observed following fish oil feeding.[39] Similarly, if fish oil feeding induces a similar decrease in expression of adhesion molecules on the surface of natural killer cells, which would result in lowered interaction between the cells and their targets, this might contribute to the reduced natural killer-cell activity which accompanies fish oil feeding.[40] Such effects would be expected to play a role in reducing the response to grafts. Indeed, fish oil has been shown to enhance the survival of transplanted kidneys and hearts in animal models.[41,42] Furthermore, Homan van der Heide *et al.*[43] reported enhanced survival of renal transplants in patients receiving fish oil plus cyclosporin A compared with those receiving coconut oil plus cyclosporin A. In addition to potential beneficial effects resulting from a reduction in adhesion molecule expression on lymphocytes, such an effect could result in an impairment in host defence. This is often overlooked when the effects of increasing the consumption of *n*-3 PUFA are discussed. However, it is clearly an important consideration. Inclusion of high levels of fish oil in the diet of experimental animals results in increased mortality following a challenge with *Salmonella typhimurium*,[44] *Staphylococcus aureus*,[45] or *Listeria monocytogenes*.[46]

Dietary lipids might affect the level of expression of cell surface molecules in at least three ways. Firstly, fish oil feeding significantly alters the the fatty acid composition of plasma membrane phospholipids of most cell types investigated including lymphocytes.[47] The changed composition could affect the displacement of proteins within the membrane and so their expression, as suggested by Muller *et al.*[48] Adhesion molecule expression is regulated by eicosanoids, cytokines and reactive species such as nitric oxide; dietary fish oil alters the levels of each of these types of regulatory molecules.[5,6] Thus, a second mechanism by which fish oil could exert its effects is by altering the production of molecules which, in turn, modulate adhesion molecule expression. A third possible mechanism by which dietary *n*-3 PUFA could affect the level of expression of adhesion molecules is via regulation of the expression of the genes coding for those proteins. There is some evidence for the regulation of adhesion molecule gene expression by *n*-3 PUFA: De Caterina *et al.*[27] reported decreased IL-1-induced VCAM-1 mRNA levels following the culture of endothelial cells with docosahexaenoic acid. This suggests that *n*-3 PUFA interact with transcription factors to modulate the expression

of genes for adhesion molecules. The precise nature of the interaction is at present not known but warrants further study.

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