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The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation

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SUMMARY

Polyunsaturated fatty acids (PUFA) have been shown to suppress immune cell functions *in vitro*. Dietary studies investigating the effects of PUFA-containing oils on lymphocyte functions have yielded contradictory findings: such studies are difficult to compare as there are many variations in protocols. The present study investigated the effects of diets containing oils rich in saturated fatty acids, monounsaturated fatty acids, *n*–6 PUFA or *n*–3 PUFA on rat lymphocyte proliferation and on receptor and surface marker expression. Rats were fed for 10 weeks on a low-fat (LF) diet (approximately 2% fat by weight) or on one of five high-fat diets, which contained 20% (by weight) hydrogenated coconut oil (HCO), olive oil (OO), safflower oil (SO), evening primrose oil (EPO) or menhaden (fish) oil (MO). Compared with feeding the LF diet, all of the high-fat diets suppressed the proliferation of lymphocytes from the spleen: although there was no significant effect of diet on the proliferation of lymphocytes from the thymus, there was a trend towards decreased proliferation with high-fat feeding. Feeding the OO, EPO or MO diets significantly suppressed proliferation of mesenteric lymph node lymphocytes compared with feeding the LF, HCO or SO diets. Dietary lipid manipulation had no effect on the proportion of T cells, B cells or monocytes/macrophages in the spleen, thymus or lymph nodes. Dietary lipid manipulation also had no significant effect on the proportions of $CD4^+$ or $CD8^+$ lymphocytes in spleen, thymus or lymph nodes, either in freshly prepared cells or in cells cultured in the presence of mitogen. There were no significant effects of dietary lipid manipulation on the expression of IL-2 receptors or transferrin receptors by concanavalin A (Con A)-stimulated lymphocytes. However, there was a trend towards a decrease in transferrin receptor expression by Con A-stimulated lymphocytes from the thymus and lymph nodes of the MO-fed rats and towards a decrease in the expression of IL-2 receptors by lymphocytes from the spleens and thymi of the MO-fed rats. These observations provide evidence that some dietary oils, particularly OO, EPO and MO, possess immunosuppressive properties and so may be useful in the therapy of diseases involving inappropriate lymphocyte activation.

INTRODUCTION

Lymphocytes play an important role in the development and progression of a number of inflammatory and autoimmune disorders, including rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus and psoriasis, all of which are characterized by the presence of activated T cells and cytokines at the site of tissue injury[1–4] and in the circulation.[5–8] There has been considerable interest in using dietary polyunsaturated fatty acids (PUFA), particularly the *n*–3 PUFA found in fish oils, in the therapy of these conditions;[9–11] such therapies aim, primarily, to inhibit T-lymphocyte functions.

Systematic investigation of a wide range of fatty acids *in vitro* has shown that, in general, fatty acids suppress mitogen-stimulated proliferation of lymphocytes isolated from rat lymph nodes,[12] spleen[13] and lymphatic duct,[14] from mouse spleen,[13,15] from pig lymph nodes[13] or human peripheral blood.[13,16–18] The effects of fatty acids on lymphocyte proliferation are concentration and time dependent.[12,16,18] Although both saturated and unsaturated fatty acids inhibit lymphocyte proliferation, unsaturated fatty acids tend to be more inhibitory and the greatest inhibition of proliferation is observed with the *n*–3 PUFA eicosapentaenoic acid.[12,18]

Activation of T lymphocytes results in the production of interleukin-2 (IL-2) and appearance on the cell surface of receptors for IL-2[19,20] and transferrin.[21,22] The expression of IL-2 receptors precedes that of transferrin receptors:[21,22] however, the appearance of both types of receptor is crucial for the subsequent proliferation of the activated T cell.[22,23] Proliferation of such cells requires continued production of IL-2:[19,20] the

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Abbreviations: Con A, concanavalin A; EPO, evening primrose oil; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; HCO, hydrogenated coconut oil; IL-2, interleukin-2; LF, low fat; 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.

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concentration of IL-2 produced, the period during which it is available and the expression of the IL-2 and transferrin receptors are the primary determinants of the magnitude and extent of T-cell proliferation.

The effects of fatty acids on the production of IL-2 and on expression of receptors for IL-2 and transferrin by lymphocytes have been examined *in vitro*.[17,24,25] It was found that unsaturated fatty acids, but not saturated fatty acids, decrease the concentration of IL-2 in the culture medium of mitogen-stimulated lymphocytes from rat lymph nodes[24] or human peripheral blood.[17] The greatest inhibition of IL-2 production was caused by the *n*–3 PUFA eicosapentaenoic acid.[17,24] These observations imply that the mechanism by which unsaturated fatty acids suppress lymphocyte proliferation is via inhibition of IL-2 synthesis and/or secretion. However, lymphocyte proliferation was only partially restored by addition of exogenous IL-2 to the culture medium in the presence of unsaturated fatty acids, indicating that this is not the sole mechanism for inhibition of proliferation.[24] Neither saturated nor unsaturated fatty acids affected the expression of the α subunit of the IL-2 receptor by concanavalin A (Con A)-stimulated rat lymph node lymphocytes.[24] However, a conflicting report by Virella *et al.*[25] has shown that eicosapentaenoic acid depresses the expression of IL-2 receptors by human peripheral blood lymphocytes in response to stimulation by pokeweed mitogen. Calder and Newsholme[24] also showed that unsaturated fatty acids, but not saturated fatty acids, decrease the expression of the transferrin receptor by Con A-stimulated rat lymph node lymphocytes *in vitro*.

These *in vitro* studies suggest that diets containing PUFA may have immunosuppressive properties. However, dietary studies in which animals have been fed PUFA-containing oils prior to *in vitro* assessment of T-lymphocyte functions have yielded contradictory findings; for example, diets rich in linoleic acid have been shown to have no effect,[26,27] to inhibit[28–30] or to enhance[31] lymphocyte proliferation. Results from such studies are difficult to compare, as they differ greatly in amount and source of fat, the composition of other dietary components, the amount and type of antioxidant present in diets, the duration of the diet, the species and sex of animal studied, and the conditions under which tests of immune function are made.

Because there is a lack of clear information regarding the effect of diets containing different types of fatty acids on lymphocyte functions, we have investigated the effects of dietary lipid manipulation on rat lymphocyte proliferation and on receptor and surface marker expression in both resting and Con A-stimulated lymphocytes. This is the first study to compare directly the effects of dietary oils containing saturated fatty acids, monounsaturated fatty acids, *n*–6 PUFA or *n*–3 PUFA on lymphocyte proliferation and the first to investigate the effects of such dietary manipulation on the expression of the IL-2 and transferrin receptors. This information may ultimately be important in terms of dietary advice for patients with conditions which involve inappropriate and prolonged activation of the immune system.

MATERIALS AND METHODS

Materials

The sources of chemicals were as described previously.[12,17,24] Monoclonal antibodies were a gift from the MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford.

Animals and diets

Weanling male Lewis rats (aged 3 weeks, weighing between 65 and 85 g) were obtained from Harlan-Olac (Bicester, U.K.). The animals were housed in the Department of Biochemistry for a period of 10 weeks prior to death, during which time they were allowed access, *ad libitum*, to water and to one of six experimental diets. Animals were fed either a low-fat (LF) diet (rat and mouse no. 1 diet, provided by SDS, Witham, U.K.) or one of five high-fat diets (custom diets, provided by ICN Biomedicals, High Wycombe, U.K.), all of which were in pelleted form. The LF diet contained approximately 2·4% lipid by weight and the high-fat diets contained 20% by weight of the lipid under study, plus 1% corn oil to prevent essential fatty acid deficiency. The high-fat diets contained either 20% hydrogenated coconut oil (HCO), 20% olive oil (OO), 20% safflower oil (SO), 20% evening primrose oil (EPO) or 20% menhaden (fish) oil (MO). The fatty acid composition of the diets was analysed by gas chromatography performed as described elsewhere.[32] The fatty acid composition of the diets is shown in Table 1.

Table 1. Fatty acid composition of the diets. Lipid was extracted from diet (0·05 g) using chloroform/methanol (2 : 1 v/v). Fatty acids were prepared by saponification in methanolic KOH and extracted into ethyl acetate. They were methylated with diazomethane and analysed by gas chromatography, as described elsewhere. Data are the mean of two determinations

	Fatty acid (mol %)											
Diet	8:0	10:0	12:0	14:0	16:0	16:1 *n*–7	18:0	18:1 *n*–9	18:2 *n*–6	18:3	20:5 *n*–3	22:6 *n*–3
LF	—	—	2·2	2·4	31·6	—	7·4	21·1	31·5	—	—	—
HCO	10·0	5·6	52·6	20·2	11·6	2·5	12·2	2·7	3·9	—	—	—
OO	—	—	3·0	1·5	11·1	—	3·9	69·9	15·3	—	—	—
SO	—	—	2·2	0·4	11·8	—	4·4	20·8	60·4	—	—	—
EPO	—	—	—	—	10·3	—	3·2	15·8	68·4	5·0[a]	—	—
MO	—	—	—	10·9	24·7	16·9	4·4	13·8	5·5	2·3[b]	11·7	5·2

[a] γ-linolenic acid (18 : 3 *n*–6); [b] α-linolenic acid (18 : 3 *n*–3).

Lymphocyte preparation

Spleen, thymus and mesenteric lymph nodes were dissected, freed of adipose tissue and gently ground into phosphate-buffered saline (PBS). The suspension was filtered through lens tissue and lymphocytes were collected by centrifugation (1000 *g*, 10 min), resuspended in PBS and purified by centrifugation on Lymphoprep (1500 *g*, 20 min). The purified lymphocytes were washed once in PBS.

Lymphocyte proliferation

Lymphocytes were cultured at 37° in an air/CO_2 (19:1) atmosphere in 96-well microtitre culture plates at a density of 5×10^5 cells/well and a total culture volume of 200 μl in HEPES-buffered RPMI supplemented with 2 mM glutamine, antibiotics and, for stimulated lymphocytes, 5 μg/ml Con A. The culture medium was also supplemented with either 10% (v/v) fetal calf serum (FCS) or 2·5% (v/v) autologous serum. After 48 hr of culture, [6-^{3}H]thymidine was added to each well (0·2 μCi/well) and the cells were incubated for a further 18 hr. The cells were then harvested onto glass fibre filters and washed and dried using a Skatron Cell Harvester. Radioactive thymidine incorporation was determined by liquid scintillation counting.

Thymidine incorporation data are expressed as stimulation index (SI) values where:

$$SI = \frac{\text{incorporation of } [^3H]\text{thymidine in the presence of mitogen}}{\text{incorporation of } [^3H]\text{thymidine in the absence of mitogen}}$$

Data are mean ± SEM of three, four or five separate experiments. Statistical significance was determined using one-way analysis of variance.

Analysis of lymphocyte subpopulations and receptor expression

FACS analysis was used to measure the presence of markers on the surface of freshly prepared and Con A-stimulated lymphocytes. Where Con A-stimulated cells were used, lymphocytes were cultured for 24 hr in the conditions described above, except at a density of 5×10^6 cells/well and a total culture volume of 2 ml. Following culture, the cells were collected by centrifugation and washed three times in PBS supplemented with 0·1% (w/v) bovine serum albumin and 10 mM sodium azide (modified PBS).

For FACS analysis, approximately 10^6 cells (resuspended in modified PBS) were incubated for 20 min at 4° with monoclonal antibodies to the T-cell receptor (R73), CD4 (W3/25), CD8 (MRC OX8), a B-cell surface structure (MRC OX12), a monocyte/macrophage surface structure (MRC OX42), the IL-2 receptor (MRC OX39) or the transferrin receptor (MRC OX26). Incubation with a monoclonal antibody to the human C3b activator protein (MRC OX21) was used as a negative control. Following staining with monoclonal antibodies, the cells were washed twice with modified PBS and incubated with fluorescein isothiocyanate-labelled rabbit anti-mouse IgG (RAM-FITC) for 20 min at 4°. They were washed twice with modified PBS and then suspended in FACS-Fix [2% (v/v) formaldehyde in PBS] and examined for fluorescence using a Becton Dickinson FACScan (Mountain View, CA). Fluorescence data were collected on 5×10^3 viable cells. Results are expressed in terms of the percentage of marker positive cells. Data are mean ± SEM of three, four or five separate cell preparations. Statistical significance was determined using one-way analysis of variance.

RESULTS

The effect of dietary lipid manipulation on lymphocyte subpopulations

FACS analysis using monoclonal antibodies directed against the T-cell receptor, CD4, CD8, a B-cell marker or a monocyte/macrophage marker showed that lymphocyte preparations from spleens consisted of approximately 65% T cells, 25% B cells and 10% monocytes/macrophages (Table 2). Between 50 and 55% of cells were $CD4^+$ and 20–25% were $CD8^+$ (Table 2). There was no significant effect of dietary lipid manipulation on lymphocyte subsets in the spleen.

Between 60 and 70% of the cells from the thymic preparations were positive for the T-cell receptor (Table 3); this probably represents the proportion of relatively mature T cells. As might be expected, these cell preparations contained only very small proportions of B cells and monocytes/macrophages. Between 80 and 90% of cells stained positive for both $CD4^+$ and $CD8^+$

Table 2. The effect of dietary lipid manipulation on receptor and surface marker expression by spleen lymphocytes. Rats were fed for 10 weeks on one of the diets described in the Materials and Methods. Spleen lymphocytes were prepared: Con A-stimulated cells were obtained by culture for 24 hr in the presence of 5 μg/ml Con A and 2·5% (v/v) autologous serum. Receptor and surface marker expression were determined using FACS analysis and the appropriate monoclonal antibodies (see the Materials and Methods). Data are mean ± SEM of three, four or five separate cell preparations. There were no statistical differences among the groups using one-way analysis of variance

	% marker positive cells								
	Freshly prepared cells					Con A-stimulated cells			
Diet	CD4	CD8	TcR	B cell	Mono/ macro	IL-2 receptor	Transferrin receptor	CD4	CD8
LF	53·9 ± 2·5	24·0 ± 1·6	63·0 ± 3·5	19·0 ± 1·8	10·2 ± 0·4	45·2 ± 6·8	35·0 ± 7·4	52·2 ± 2·1	37·5 ± 3·1
HCO	51·2 ± 2·7	25·2 ± 1·1	62·7 ± 3·1	21·0 ± 2·4	7·5 ± 0·7	32·4 ± 4·2	29·1 ± 3·9	48·3 ± 3·1	36·2 ± 3·0
OO	53·9 ± 4·0	22·2 ± 1·3	64·5 ± 5·2	22·1 ± 3·7	8·1 ± 1·3	37·9 ± 6·2	34·9 ± 3·3	56·0 ± 2·8	36·1 ± 4·4
SO	54·0 ± 4·4	21·7 ± 0·8	63·1 ± 4·8	21·4 ± 4·2	9·0 ± 0·7	47·7 ± 4·3	43·1 ± 4·0	54·2 ± 2·1	36·1 ± 3·0
EPO	53·7 ± 3·8	22·1 ± 1·0	62·3 ± 4·0	25·2 ± 4·0	8·2 ± 0·9	37·8 ± 3·4	34·9 ± 3·3	54·3 ± 2·4	35·6 ± 2·3
MO	51·6 ± 3·9	21·0 ± 0·7	60·0 ± 4·4	24·5 ± 4·1	8·8 ± 0·8	37·4 ± 7·0	37·6 ± 5·2	54·5 ± 5·0	34·2 ± 3·8

Table 3. The effect of dietary lipid manipulation on receptor and surface marker expression by thymic lymphocytes. Rats were fed for 10 weeks on one of the diets described in the Materials and Methods. Thymic lymphocytes were prepared: Con A-stimulated cells were obtained by culture for 24 hr in the presence of 5 μg/ml Con A and 2·5% (v/v) autologous serum. Receptor and surface marker expression were determined using FACS analysis and the appropriate monoclonal antibodies (see the Materials and Methods). Data are mean ± SEM of three, four or five separate cell preparations. There were no statistical differences among the groups using one-way analysis of variance

	% marker positive cells								
	Freshly prepared cells					Con A-stimulated cells			
Diet	CD4	CD8	TcR	B cell	Mono/ macro	IL-2 receptor	Transferrin receptor	CD4	CD8
LF	88·0 ± 0·7	85·8 ± 2·3	67·4 ± 2·9	1·3 ± 0·3	1·1 ± 0·1	59·6 ± 3·4	52·6 ± 5·5	54·6 ± 3·0	70·5 ± 2·1
HCO	87·0 ± 1·3	86·0 ± 2·6	64·2 ± 2·6	1·8 ± 0·7	0·8 ± 0·2	58·8 ± 4·6	50·3 ± 5·1	56·2 ± 2·0	75·4 ± 0·8
OO	88·2 ± 0·9	86·6 ± 1·4	67·1 ± 1·9	1·1 ± 0·2	0·8 ± 0·1	48·3 ± 2·7	39·0 ± 3·0	53·7 ± 2·2	72·4 ± 2·1
SO	88·7 ± 1·7	85·5 ± 3·1	68·1 ± 2·9	1·8 ± 0·4	0·9 ± 0·3	51·7 ± 5·1	48·1 ± 2·2	52·5 ± 4·3	70·1 ± 4·4
EPO	89·5 ± 1·5	87·9 ± 1·8	68·3 ± 2·6	1·4 ± 0·5	0·5 ± 0·2	49·8 ± 5·8	42·6 ± 4·0	58·1 ± 3·7	75·1 ± 1·7
MO	90·0 ± 1·5	90·9 ± 1·1	64·1 ± 2·5	0·6 ± 0·2	0·5 ± 0·2	40·4 ± 7·6	35·3 ± 6·1	54·8 ± 3·0	74·0 ± 1·4

(Table 3); this double-positive staining is typical of immature lympho-cytes. There was no significant effect of dietary lipid manipulation on lymphocyte subsets in the thymic preparations (Table 3).

In the lymph node preparations approximately 80% of cells were T lymphocytes, 15–20% were B lymphocytes and there were small proportions of monocytes/macrophages (Table 4). Up to 65% of lymph node lymphocytes were $CD4^+$ and approximately 20–25% were $CD8^+$ (Table 4). There were no significant effects of dietary lipid manipulation on lymphocyte subsets in lymph node preparations, apart from some minor differences in the monocyte/macrophage populations (Table 4).

The effect of dietary lipid manipulation on receptor and surface marker expression by Con A-stimulated lymphocytes

The effect of dietary lipid manipulation on the expression of receptors for IL-2 and transferrin and on the expression of CD4 and CD8 surface markers on lymphocytes from spleen, thymus and lymph nodes is shown in Tables 2 to 4.

In animals fed the LF diet approximately 45% of lymphocytes from the spleen expressed receptors for IL-2 and 35% expressed the transferrin receptor when stimulated by Con A (Table 2). Feeding the high-fat diets affected the proportion of IL-2 or transferrin receptor-positive spleen lymphocytes compared with feeding the LF diet. Although feeding the high-fat diets did not significantly affect the proportion of IL-2 receptor-positive spleen lymphocytes compared with feeding the LF diet, there was a trend towards a reduction in the proportion of IL-2 receptor-positive cells in animals fed the HCO, OO, EPO or MO diets (Table 2). There was no effect of dietary lipid manipulation on the proportions of $CD4^+$, $CD8^+$ or transferrin receptor-positive cells following Con A stimulation of lymphocytes from the spleen (Table 2).

In animals fed the LF diet, approximately 55% of lymphocytes isolated from thymi expressed receptors for IL-2 and

Table 4. The effect of dietary lipid manipulation on receptor and surface marker expression by mesenteric lymph node lymphocytes. Rats were fed for 10 weeks on one of the diets described in the Materials and Methods. Mesenteric lymph node lymphocytes were prepared: Con A-stimulated cells were obtained by culture for 24 hr in the presence of 5 μg/ml Con A and 2·5% (v/v) autologous serum. Receptor and surface marker expression was determined using FACS analysis and the appropriate monoclonal antibodies (see the Materials and Methods). Data are mean ± SEM of three, four or five separate cell preparations

	% marker positive cells								
	Freshly prepared cells					Con A-stimulated cells			
Diet	CD4	CD8	TcR	B cells	Mono/ macro	IL-2 receptor	Transferrin receptor	CD4	CD8
LF	60·8 ± 4·0	19·5 ± 1·6	77·7 ± 4·8	19·9 ± 4·1	0·9 ± 0·3	94·2 ± 3·2	94·0 ± 1·4	68·0 ± 2·1	44·1 ± 0·9
HCO	61·5 ± 3·0	21·6 ± 1·2	79·7 ± 3·7	17·1 ± 2·1	0·7 ± 0·2	94·2 ± 1·9	90·6 ± 2·0	68·5 ± 1·0	42·5 ± 3·7
OO	67·5 ± 3·6	19·1 ± 0·9	83·7 ± 4·0	13·5 ± 3·2	0·3 ± 0·1^{cd}	97·1 ± 1·0	94·9 ± 1·0	73·4 ± 2·8	41·6 ± 2·8
SO	65·4 ± 3·6	19·2 ± 0·8	82·0 ± 5·3	13·6 ± 3·4	0·4 ± 0·1^{cd}	96·3 ± 1·1	91·7 ± 2·3	76·6 ± 1·4	38·8 ± 3·2
EPO	63·3 ± 4·7	19·4 ± 0·7	80·0 ± 4·7	17·0 ± 3·9	1·2 ± 0·1^{ab}	96·7 ± 0·6	93·2 ± 2·0	74·4 ± 1·4	37·0 ± 2·2
MO	64·5 ± 3·8	18·9 ± 1·3	80·5 ± 3·8	15·4 ± 5·2	1·1 ± 0·4^{ab}	93·9 ± 2·2	84·9 ± 6·0	65·7 ± 2·3	38·0 ± 2·9

Statistical significance (one-way analysis of variance) for $P < 0·05$ at least is indicated as follows: [a] versus OO, [b] versus SO, [c] versus EPO, [d] versus MO.

transferrin following stimulation with Con A; approximately 55% of thymic lymphocytes were $CD4^+$ and approximately 70% were $CD8^+$ (Table 3). There were no statistically significant effects of dietary lipid manipulation on the proportion of thymic cells positive for CD4, CD8, the transferrin receptor or the IL-2 receptor. However, compared with feeding the LF diet, feeding the OO, EPO or MO diets reduced (by up to 30%) the proportion of IL-2 receptor-positive and transferrin receptor-positive thymus cells (Table 3).

In animals fed the LF diet more than 90% of lymph node lymphocytes expressed receptors for IL-2 and transferrin following stimulation with Con A (Table 4). Approximately 65% of lymphocytes were $CD4^+$ and 45% were $CD8^+$ (Table 4). There was no statistically significant effect of dietary lipid manipulation on the expression of the transferrin receptor, the IL-2 receptor, CD4 or CD8 by lymph node lymphocytes, although there was a trend towards decreased transferrin receptor expression by lymph node lymphocytes from animals fed the MO diet (Table 4).

The effect of dietary lipid manipulation on the proliferation of lymphocytes cultured in autologous serum or FCS

A FCS concentration of 10% (v/v) was used in lymphocyte culture, as this is the standard concentration used in cell culture and is the concentration most commonly used in previous studies which have investigated the effects of dietary lipid manipulation on lymphocyte proliferation, although 2·5% (v/v) is, in fact, the optimal FCS concentration to support lymphocyte proliferation (data not shown). Optimal proliferation of lymphocytes was supported by 2·5% (v/v) autologous serum (data not shown); this optimal autologous serum concentration was employed in these experiments. The incorporation of [^{3}H]thymidine by spleen lymphocytes cultured in autologous serum or FCS, in the presence of 5 μg/ml Con A, is shown in Fig. 1, that for thymic lymphocytes is shown in Fig. 2 and that for mesenteric lymph node lymphocytes is shown in Fig. 3. The SI values for all cell types were lower when cells were cultured in FCS than when the cells were cultured in autologous serum (Figs. 1–3). This is probably a reflection of the fact that 10% FCS is not the optimal concentration for proliferation of these cells *in vitro* and that autologous serum may contain growth factors which are absent in FCS.

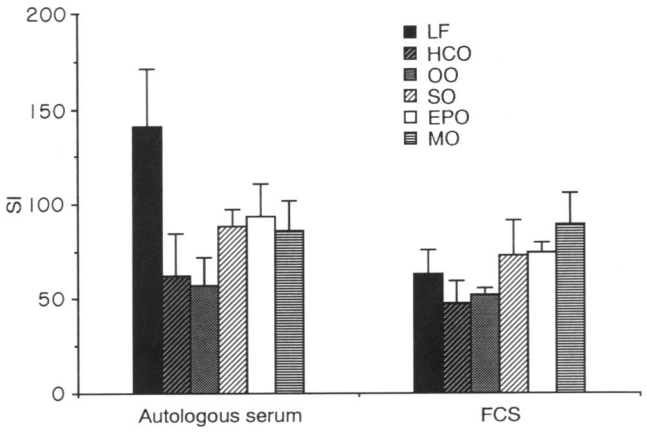

Figure 2. The effect of dietary lipid manipulation on the proliferation of Con A-stimulated thymic lymphocytes. Rats were fed for 10 weeks on one of the diets described in the Materials and Methods. Lymphocytes were prepared as described in the Materials and Methods and were cultured for 66 hr in either 2·5% (v/v) autologous serum or 10% (v/v) FCS, in the presence of 5 μg/ml Con A. Proliferation was measured by [^{3}H]thymidine incorporation over the final 18 hr of culture. Data are mean ± SEM of three, four or five separate experiments. *Significant difference (for $P < 0.05$ using one-way analysis of variance) versus the LF diet.

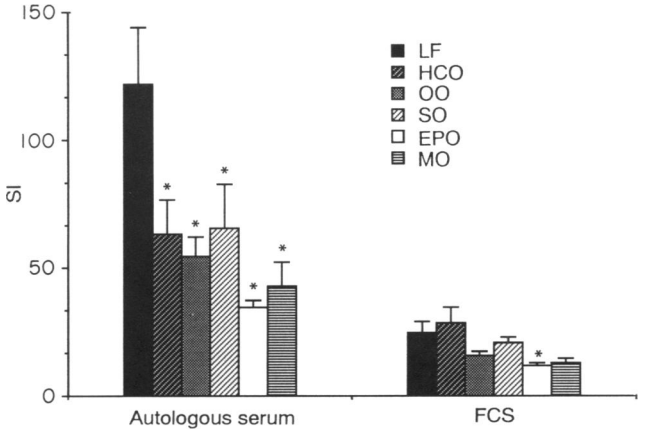

Figure 1. The effect of dietary lipid manipulation on the proliferation of Con A-stimulated spleen lymphocytes. Rats were fed for 10 weeks on one of the diets described in the Materials and Methods. Lymphocytes were prepared as described in Materials and Methods and were cultured for 66 hr in either 2·5% (v/v) autologous serum or 10% (v/v) FCS, in the presence of 5 μg/ml Con A. Proliferation was measured by [^{3}H]thymidine incorporation over the final 18 hr of culture. Data are mean ± SEM of three, four or five separate experiments. *Significant difference (for $P < 0.05$ using one-way analysis of variance) versus the LF diet.

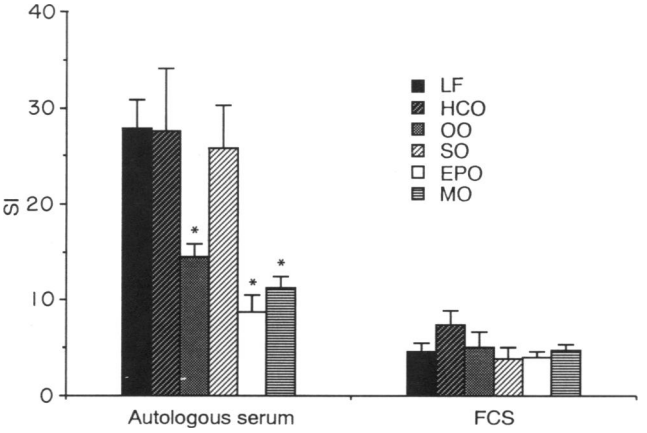

Figure 3. The effect of dietary lipid manipulation on the proliferation of Con A-stimulated lymph node lymphocytes. Rats were fed for 10 weeks on one of the diets described in the Materials and Methods. Lymphocytes were prepared from mesenteric lymph nodes as described in the Materials and Methods and were cultured for 66 hr in either 2·5% (v/v) autologous serum or 10% (v/v) FCS, in the presence of 5 μg/ml Con A. Proliferation was measured by [^{3}H]thymidine incorporation over the final 18 hr of culture. Data are mean ± SEM of three, four or five separate experiments. *Significant difference (for $P < 0.05$ using one-way analysis of variance) versus the LF diet.

Effects on spleen lymphocytes proliferation

The SI values for spleen lymphocytes cultured in 2·5% autologous serum, in the presence of Con A, were significantly lower for cells obtained from animals fed each of the high-fat diets (HCO, OO, SO, EPO, MO) compared with those fed the LF diet (Fig. 1). When the spleen lymphocytes were cultured in 10% FCS, lymphocytes from the OO-, EPO- or MO-fed rats had significantly lower SI values than those from rats fed the HCO diet (Fig. 1). Lymphocytes from rats fed the EPO diet also had significantly lower SI values than those from rats fed the LF or SO diets (Fig. 1).

Effects on thymic lymphocytes proliferation

Lymphocytes from the thymi of rats fed the high-fat diets had lower SI values than those from rats fed the LF diet when cultured in autologous serum (Fig. 2), although there were no statistically significant differences. There were no differences between any of the diet groups when thymic lymphocytes were cultured in FCS (Fig. 2).

Effects on lymph node lymphocyte proliferation

In response to the T-cell mitogen, Con A, mesenteric lymph node lymphocytes from rats fed the OO, EPO or MO diets had significantly lower SI values than those from rats fed the LF, HCO or SO diets, when the cells were cultured in autologous serum (Fig. 3). There were no significant differences between any of the diet groups when lymphocytes were cultured in FCS, suggesting that culturing in FCS completely masked the effect of dietary lipid manipulation (Fig. 3).

DISCUSSION

This study investigated the effect of dietary lipid manipulation on receptor and surface marker expression in freshly prepared and Con A-stimulated rat lymphocytes and on lymphocyte proliferation in response to Con A.

There was little effect of dietary lipid manipulation on the expression of CD4, CD8 or B-cell and macrophage markers by fresh lymphocytes from the different sources. There was a consistent trend for the MO diet to decrease expression of the transferrin receptor by mitogen-stimulated lymphocytes from lymph nodes and thymus and to decrease IL-2 receptor expression by mitogen-stimulated lymphocytes from the spleen and thymus.

The *ex vivo* proliferation of lymphocytes has previously been used to test the hypothesis that PUFA supplied by the diet are immunosuppressive. Differences in protocol make comparison between previous studies rather difficult. One factor which may explain previous contradictory results is the type of serum used in the cell culture. FCS provides ingredients that support the maintenance and growth of cells *in vitro*, but it also contains uncharacterized elements, including antibodies, growth factors and other potentially immunologically reactive substances, which may obscure assay results for specific responses.[33] FCS also contains lipids, including lipoproteins, phospholipids and non-esterified fatty acids. The changes in fatty acid composition of lymphocytes that occur as a result of dietary lipid manipulation are relatively subtle (P. Yaqoob, N. M. Jeffrey, D. J. Harvey, E. A. Newsholme and P. C. Calder, manuscript submitted) and an inherent problem with the use of even short periods of lymphocyte culture in tests of immune function following dietary supplementation is that the effects of dietary lipid manipulation on lymphocyte fatty acid composition may be partially reversed by culturing in medium containing lipid from a different source, particularly FCS, which may vary from batch to batch. Enzymes present in the cell culture system, for example those released by contaminating macrophages, may cause hydrolysis of the lipid in FCS, releasing fatty acids which could compete with the exogenously added fatty acids. The present study is the first to investigate the effects of a wide range of dietary lipids on the proliferation of lymphocytes cultured in either autologous serum or FCS.

It is clear that lymphocytes from different tissues respond differently to dietary lipid manipulation. Lymphocytes from the spleens of rats fed high-fat diets and cultured in autologous serum had significantly lower T-cell proliferation relative to those from rats fed the LF diet; the greatest inhibition of proliferation was seen with cells from rats fed the EPO and MO diets. The inhibitory effect of feeding the OO, EPO and MO diets was still observed when the cells were cultured in FCS. Feeding the OO, EPO and MO diets also led to inhibition of proliferation of lymph node lymphocytes, although the effect was masked when the cells were cultured in FCS. There was much less impact of dietary lipid manipulation on the proliferation of thymic lymphocytes; the HCO and OO diets tended to suppress proliferation of thymic lymphocytes when cultured in autologous serum, but these differences did not reach statistical significance. The lack of effect of dietary lipid manipulation on thymic lymphocyte proliferation is perhaps not surprising considering the very minor effect of feeding the experimental diets on the fatty acid composition of thymic lymphocytes (P. Yaqoob *et al.*, manuscript submitted).

The results presented here show that culturing lymphocytes isolated from rats fed the experimental diets in medium containing FCS can partially or totally mask the effects of dietary lipid manipulation on lymphocyte proliferation. Kelly and Parker[34] have investigated the effects of a variety of fatty acids on the proliferation of human lymphocytes cultured in medium containing delipidated FCS or human plasma. It was reported that low concentrations ($< 50\,\mu M$) of fatty acids enhanced lymphocyte proliferation, whereas high concentrations inhibited proliferation. When cells were cultured in either delipidated FCS or human plasma, the stimulatory effects of very low concentrations of fatty acids on lymphocyte proliferation were enhanced in the absence of serum lipid at the low mitogen concentrations, but the mitogen dose–response curves were extended so that higher concentrations of fatty acids were required to be inhibitory. This supports the hypothesis that when FCS is present in the culture medium, some of the inhibition of proliferation observed when single fatty acids are added is the result of lipid in the FCS.

In the present study, the response of lymphocytes to mitogen was greater when they were cultured in autologous serum than when they were cultured in FCS. This was likely owing to the fact that 10% (v/v) FCS is not the optimal concentration for proliferation and also because the autologous serum may provide specific growth factors, cytokines or other regulatory compounds that may have been absent, limiting or immunologically foreign in FCS. It is likely, however, that the

immunosuppression observed in rats fed some of the high-fat diets is partly due to changes in the fatty acid composition of the serum and partly due to changes in the fatty acid composition of the lymphocyte themselves. The suppression of lymphocyte proliferation by serum from animals fed diets high in various lipids has been observed in several studies.[26,35,36] However, the fact that most previous dietary studies have used FCS, either alone or in combination with autologous serum, in tests of lymphocyte proliferation, makes results from different studies difficult to compare. Some dietary studies (e.g. Friend *et al.*[36]) have used concentrations of autologous or allogeneic serum of up to 20%, which in the present study were toxic to the cells (data not shown).

The results from this study show clearly that some dietary lipids possess immunosuppressive properties; in particular, OO, EPO and MO diets produce consistent inhibition of rat spleen and lymph node lymphocyte proliferation. The effects of the MO diet may be related to changes in the expression of transferrin and IL-2 receptors, although this requires further investigation. The mechanism by which these diets suppress lymphocyte proliferation is not clear. The effect of the EPO diet is particularly interesting as the composition of the diet is remarkably similar to that of the SO diet, except for the small amount of γ-linolenic acid in the EPO diet (Table 1), yet the effects of these diets on lymphocyte proliferation can be remarkably different.

It is of importance to investigate whether supplementation or manipulation of the human diet, with regard to different types of lipid, produces similar immunosuppressive effects. Few studies have investigated the effect of dietary lipid manipulation on human lymphocyte functions. However, Meydani *et al.*[37] have shown that supplementing the diet of healthy adult females with capsules containing fish oil (about 2 g/day) for 8 weeks or more lowered the proliferative response of peripheral blood lymphocytes to phytohaemagglutinin, which is consistent with the effects of the MO diet in the present study. There is currently considerable interest in exploiting the immunosuppressive effects of dietary oils, particularly fish oils, to develop therapies for inflammatory disorders[9–11] and for patients undergoing organ transplantation.[38] Clearly the effects and mechanism of action of these lipids warrant further investigation.

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