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Polyunsaturated fatty acids suppress human peripheral blood lymphocyte proliferation and interleukin-2 production

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1. The effects of a variety of fatty acids on human peripheral blood lymphocyte proliferation stimulated by concanavalin A or purified protein derivative of *Mycobacterium tuberculosis* were studied.

2. The proliferative response to concanavalin A was inhibited by all of the polyunsaturated fatty acids tested (eicosapentaenoate, arachidonate, docosahexaenoate, linoleate and α-linolenate) and also by the saturated fatty acid, stearate. The greatest inhibition of proliferation (approximately 85%) was caused by eicosapentaenoate.

3. The proliferative response to the purified protein derivative of *Mycobacterium tuberculosis* was inhibited by all of the polyunsaturated fatty acids tested, except α-linolenate, and also by stearate. The greatest inhibition of proliferation (approximately 75%) was caused by eicosapentaenoate.

4. The pattern of inhibition of proliferation by fatty acids was similar to that previously reported for rat lymphocytes with one exception: oleate did not inhibit human lymphocyte proliferation.

5. The proliferation of T-lymphocytes is dependent upon their ability to synthesize and secrete the cytokine, interleukin-2. In the presence of mitogen the concentration of interleukin-2 in the culture medium increased markedly above that in the medium of non-stimulated cells.

6. All polyunsaturated fatty acids tested caused a decrease in the concentration of interleukin-2; the greatest decrease (approximately 90%) was caused by eicosapentaenoate.

7. There was a good correlation between lymphocyte proliferation in the presence of fatty acids and interleukin-2 concentration. However, stearate did not decrease the interleukin-2 concentration but did inhibit lymphocyte proliferation.

8. These observations suggest that, although fatty acid suppression of interleukin-2 production may play a role in the inhibition of proliferation, it is not the sole mechanism by which fatty acids act.

INTRODUCTION

There have been several reports of the effects of fatty acids on proliferative responses of human peripheral blood lymphocytes stimulated in culture with phytohaemagglutinin: myristate, palmitate, stearate, oleate, linoleate and arachidonate inhibit this process [1–7]. However, the large differences in the procedures used (e.g. exposure time of the cells to fatty acid, concentration of fatty acid, means of presenting fatty acid to the cells), the variations in the extent of inhibition observed between studies and the limited range of fatty acids used in each individual study complicate the interpretation of these findings. Recently, there has been considerable interest in the effects of fish-oil-derived $n-3$ polyunsaturated fatty acids (PUFAs) on the immune system [8], but, to our knowledge, there have been no reports of their effect upon the proliferation of human lymphocytes.

Interest in this general area has been stimulated by work which indicates that a PUFA-rich diet can result in immunosuppression [9–11]. There are reports that dietary supplementation with plant oils (rich in $n-6$ PUFAs) or fish oils (rich in $n-3$ PUFAs) improves the condition of patients suffering from diseases characterized by an overactive immune system, such as rheumatoid arthritis [12], multiple sclerosis [13] and psoraisis [14]. Such diets are also beneficial in some animal models of autoimmune disease [15]. However, a number of clinical trials of such diets in rheumatoid arthritis or multiple sclerosis have not provided clear evidence of a sustained improvement in the condition of the patients [16–20]. In view of this, it seemed important to provide more basic information about the effectiveness of a wide range of fatty acids on immune cell function and to investigate potential mechanisms of action of such fatty acids. Such information may permit more rational diets to be used to influence the immune system. Indeed, it was recently stated that "before further necessarily large studies of lipid supplementation in multiple sclerosis are

Key words: fatty acids, fish oils, interleukin-2, lymphocyte proliferation.

Abbreviations: Con A, concanavalin A; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; IL-2, interleukin-2; PPD, purified protein derivative of *Mycobacterium tuberculosis*; PUFA, polyunsaturated fatty acid.

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undertaken, more needs to be understood at a biochemical level about the possible role of these lipids in the disease and the effect of manipulations of intake" [21].

In this paper we report the effect of a large range of fatty acids on the proliferation of human peripheral blood lymphocytes. Furthermore, since interleukin-2 (IL-2) is an essential component of T-lymphocyte proliferation [22], the effect of fatty acids on the IL-2 concentration in the medium of mitogen-stimulated human lymphocytes was investigated. Fatty acids used in this study include the saturated fatty acids myristate (14:0), palmitate (16:0) and stearate (18:0), the $n-9$ monounsaturated fatty acid oleate (18:1), the $n-6$ PUFAs linoleate (18:2) and arachidonate (20:4), and the $n-3$ PUFAs α-linolenate (18:3), eicosapentaenoate (20:5) and docosahexaenoate (22:6).

MATERIALS AND METHODS

Materials

Materials were obtained from the sources described previously [23]. In addition, Leucoprep cell separation tubes were obtained from Becton Dickinson, Lincoln Park, NJ, U.S.A., the purified protein derivative of *Mycobacterium tuberculosis* (PPD) was a gift from Professor Siamon Gordon, Sir William Dunn School of Pathology, University of Oxford, Oxford, U.K., and recombinant human IL-2 (Cetus) and the CTLL-2 cell line were generous gifts from Dr Maggie Dallman, Nuffield Department of Surgery, John Radcliffe Hospital, Headington, Oxford, U.K.

Lymphocyte culture and proliferation assay

Peripheral blood was collected by trained personnel from five healthy female subjects (age range 22–51 years). The blood was transferred to Leucoprep tubes and the lymphocytes were purified by centrifugation (1500 *g* for 20 min). The lymphocytes were washed in RPMI culture medium before culturing. The cells were cultured at 37°C in an air/CO_2 atmosphere (19:1) in 96-well microtitre culture plates (approximately 2×10^5 cells/well and a final culture volume of 200 μl) in 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (Hepes)-buffered RPMI medium supplemented with 10% (v/v) foetal calf serum, 2 mmol/l glutamine and antibiotics (100 units of streptomycin/ml and 200 units of penicillin/ml). The cell culture medium also contained 15 μg of concanavalin A (Con A)/ml or 10 μg of PPD/ml and 100 μmol/l fatty acid (added as a 1:1 complex with BSA). The fatty acid–BSA complexes were formed as described previously [23]. After 48 h, [6-^{3}H]thymidine (0.2 μCi/well) was added and the cells were cultured for a further 18 h. The cells were then harvested on to glass fibre filters and were washed and dried using a Skatron Cell Harvester. [^{3}H]Thymidine incorporation was measured by liquid scintillation counting. The stimulation index was calculated as follows:

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

and was used to indicate Con A- or PPD-stimulated cell proliferation.

Assay for IL-2 in culture media

The concentration of IL-2 in lymphocyte culture media was determined using a bioassay involving the IL-2-dependent murine CTLL-2 cell line [24]. The cell line was maintained in a Hepes-buffered RPMI medium supplemented with 10% (v/v) foetal calf serum, 2 mmol/l glutamine, 25 μmol/l 2-mercaptoethanol and recombinant human IL-2. Before use in the bioassay, the cells were collected by centrifugation and were washed three times in IL-2-free medium.

After culture of lymphocytes for various times in the conditions described above, the medium was removed, diluted serially twofold and 100 μl aliquots were transferred to microtitre plate wells. CTLL-2 cells (1×10^4/100 μl) were added to each well. The plates were incubated for 18 h at 37°C and then [6-^{3}H]thymidine was added (0.5 μCi/well). After a further 6 h incubation, the cells were harvested on to glass fibre filters, washed and dried. Incorporation of [^{3}H]thymidine was measured in a liquid scintillation counter. Recombinant human IL-2 was used to construct a standard curve of [^{3}H]thymidine incorporation against IL-2 concentration.

Statistics

Unless otherwise indicated, data are presented as means ± SEM. Statistical comparisons between data were made by using the two-tailed Student's *t*-test.

RESULTS

Effect of fatty acids on Con A-stimulated proliferation of peripheral blood lymphocytes

Values for [^{3}H]thymidine incorporation into Con A-stimulated peripheral blood lymphocytes obtained on different days and from different subjects were (means ± SD, $n=6$): 66 883 ± 2625 (subject no. 1, day 1), 83 103 ± 16 597 (subject no. 1, day 2), 136 255 ± 7847 (subject no. 1, day 3), 18 125 ± 4820 (subject no. 1, day 4), 108 860 ± 6407 (subject no. 2), 122 010 ± 10 465 (subject no. 3) and 23 710 ± 1495 (subject no. 4) d.p.m. In the absence of Con A, [^{3}H]thymidine incorporation was low: it was always less than 3000 d.p.m. and usually less than 2000 d.p.m. The stimulation index, a measure of lymphocyte proliferation, was calculated from the [^{3}H]thymidine incorporation observed in the presence and absence of Con A (see the Materials and methods section). The values obtained were 34.9, 74.3, 46.7, 63.2 (for the four cell preparations from subject no. 1), 73.9 (subject no. 2), 57.4 (subject no. 3) and 35.1 (subject no. 4). The mean ± SEM stimulation index for the seven separate cell preparations was 55.1 ± 6.2.

The effects of fatty acids on the Con A-stimulated proliferation of each of the seven lymphocyte preparations are shown in Fig. 1. Several of the fatty acids consistently caused inhibition of lymphocyte proliferation:

docosahexaenoate, arachidonate and eicosapentaenoate decreased the stimulation index of all seven lymphocyte preparations, while linoleate, α-linolenate and stearate caused a lower response in five of the preparations (Fig. 1). In contrast, palmitate did not affect the proliferation of any of the lymphocyte preparations and oleate inhibited the response of two preparations. The data combined for all seven lymphocyte preparations are shown in Table 1. The PUFAs and stearate caused significant inhibition of lymphocyte proliferation in response to Con A; eicosapentaenoate caused the greatest inhibition (approximately 85%; Table 1). Oleate, palmitate and myristate did not cause significant inhibition (Table 1). There was no statistically significant difference in the lymphocyte response to Con A in the presence of eicosapentaenoate and arachidonate or docosahexaenoate and arachidonate (both $P > 0.05$). However, lymphocyte proliferation in the presence of eicosapentaenoate was significantly lower than in the presence of docosahexaenoate ($P < 0.05$).

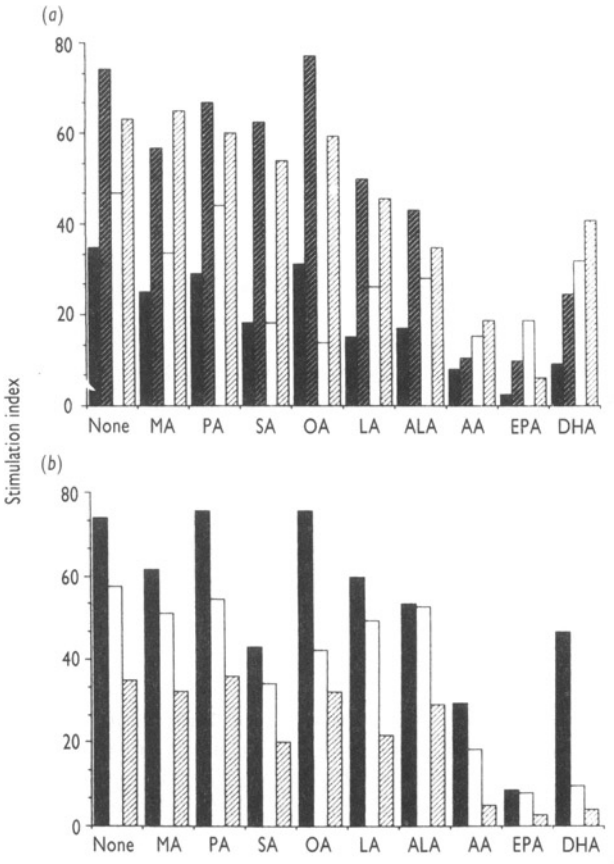

Fig. 1. Effect of fatty acids on Con A-stimulated proliferation of human lymphocytes. Human peripheral blood lymphocytes were cultured in the presence of 100 μmol/l fatty acid–BSA complexes in the absence or presence of Con A (15 μg/ml). [^{3}H]Thymidine incorporation into DNA was measured over the final 18 h of a 66 h culture period. Values for [^{3}H]thymidine incorporation in the presence of Con A but in the absence of fatty acids are given in the text (see the Results section). Stimulation indices in the absence and presence of fatty acids were calculated as described in the Materials and methods section and are shown for (*a*) four separate lymphocyte preparations (■, day 1; ▨, day 2; □, day 3; ▨, day 4) from subject no. 1 and (*b*) single lymphocyte preparations from subject nos. 2 (■), 3 (□) and 4 (▨). Each value is the mean of six determinations; in all cases SD was less than 10% of the mean. Fatty acids are abbreviated as follows: MA, myristate; PA, palmitate; SA, stearate; OA, oleate; LA, linoleate; ALA, α-linolenate; AA, arachidonate; EPA, eicosapentaenoate; DHA, docosahexaenoate.

Effect of fatty acids on PPD-stimulated proliferation of peripheral blood lymphocytes

The proliferative response of human lymphocytes to PPD was much less than the response to Con A: for cells from three subjects tested, [^{3}H]thymidine incorporation was 12% (subject no. 2), 16% (subject no. 3, day 1), 6% (subject no. 3, day 2), 10% (subject no. 3, day 3) and 19% (subject no. 5) of that measured in the presence of Con A. The values for [^{3}H]thymidine incorporation in response to PPD were (mean ± SD, $n = 6$): 13 063 ± 1809 (subject no. 2), 19 521 ± 481 (subject no. 3, day 1), 9153 ± 1444 (subject no. 3, day 2), 15 588 ± 1321 (subject no. 3, day 3) and 16 796 ± 1524 (subject no. 5) d.p.m. The stimulation indices for these lymphocyte preparations were 8.9, 9.2, 6.1, 8.9 and 13.5, respectively. The mean ± SEM stimulation index for the five separate cell preparations was 9.3 ± 1.1.

The effects of fatty acids on PPD-stimulated proliferation of each of the five lymphocyte preparations are shown in Fig. 2. As was observed for Con A-stimulated proliferation (Fig. 1), docosahexaenoate, arachidonate and eicosapentaenoate lowered the stimulation index of all five lymphocyte preparations (Fig. 2). Linoleate, α-linolenate, stearate and palmitate inhibited proliferation of some, but not all, of the preparations. Oleate lowered the response in two of the preparations, but myristate had no effect on the stimulation index of any of the five preparations (Fig. 2). The data are combined in Table 1;

Table 1. Effect of fatty acids on proliferation of human lymphocytes. Data are the means ± SEM of seven (Con A) or five (PPD) separate cell preparations. The stimulation index for each cell preparation is shown in Fig. 1 (Con A) and Fig. 2 (PPD). Statistical significance: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control cells (cultured in the absence of fatty acid). Numbers in parentheses are the stimulation indices expressed as percentages of the control.

Fatty acid added	Stimulation index			
	Con A		PPD	
None	55.1 ± 6.2	(100)	9.3 ± 1.1	(100)
Myristate (14:0)	46.4 ± 5.6	(84 ± 4)	8.6 ± 1.2	(90 ± 4)
Palmitate (16:0)	52.2 ± 5.9	(94 ± 2)	6.8 ± 1.2	(72 ± 4)
Stearate (18:0)	35.7 ± 6.2*	(62 ± 6)	5.5 ± 1.0*	(56 ± 7)
Oleate (18:1 *n* − 9)	47.4 ± 8.4	(83 ± 9)	7.7 ± 1.2	(82 ± 8)
Linoleate (18:2 *n* − 6)	38.3 ± 4.5*	(67 ± 5)	5.7 ± 1.1*	(61 ± 7)
α-Linolenate (18:3 *n* − 3)	36.9 ± 4.7*	(67 ± 6)	6.8 ± 1.1	(72 ± 4)
Arachidonate (20:4 *n* − 6)	15.1 ± 2.9***	(27 ± 3)	3.6 ± 0.7**	(39 ± 8)
Eicosapentaenoate (20:5 *n* − 3)	8.1 ± 1.9***	(15 ± 4)	2.3 ± 0.3***	(24 ± 2)
Docosahexaenoate (22:6 *n* − 3)	23.9 ± 5.9**	(41 ± 8)	4.6 ± 0.7**	(49 ± 9)

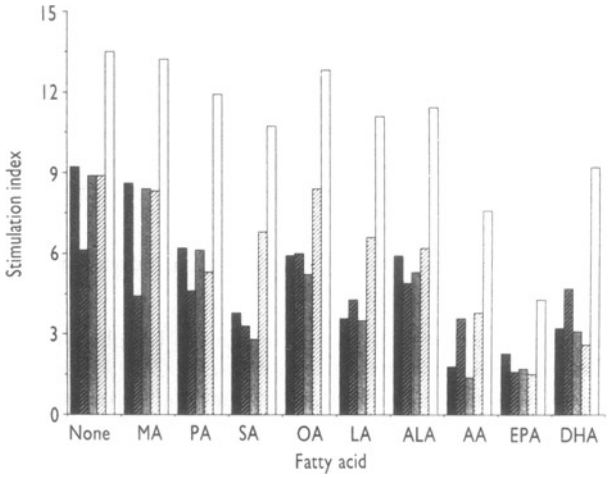

Fig. 2. Effect of fatty acids on PPD-stimulated proliferation of human lymphocytes. Human peripheral blood lymphocytes were cultured in the presence of 100 μmol/l fatty acid–BSA complexes in the absence or presence of PPD (10 μg/ml). [^{3}H]Thymidine incorporation into DNA was measured over the final 18 h of a 66 h culture period. Values for [^{3}H]thymidine incorporation in the presence of PPD but in the absence of fatty acids are given in the text (see the Results section). Stimulation indices in the absence and presence of fatty acids were calculated as described in the Materials and methods section and are shown for three separate lymphocyte preparations (■, day 1; ▨, day 2; ▩, day 3) from subject no. 3 and single lymphocyte preparations from subject nos. 4 (▨) and 5 (□). Each value is the mean of six determinations; in all cases SD was less than 10% of the mean. Fatty acids are abbreviated as follows: MA, myristate; PA, palmitate; SA, stearate; OA, oleate; LA, linoleate; ALA, α-linolenate; AA, arachidonate; EPA, eicosapentaenoate; DHA, docosahexanoate.

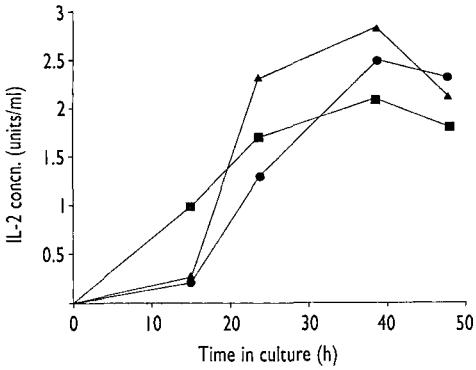

Fig. 3. IL-2 concentration in the culture medium of Con A-stimulated human lymphocytes. Human peripheral blood lymphocytes from subject nos. 1 (▲), 2 (■) and 5 (●) were cultured in the presence of 15 μg of Con A/ml. At various times the medium was removed, serially diluted twofold and the concentration of IL-2 was determined using the CTLL-2 cell line (see the Materials and methods section). Each point is the mean of three determinations; in all cases SD was less than 15% of the mean.

several of the fatty acids tested (eicosapentaenoate, arachidonate, docosahexaenoate, linoleate and stearate) caused significant inhibition of lymphocyte proliferation. Myristate, palmitate, oleate and α-linolenate did not cause significant inhibition of [^{3}H]thymidine incorporation (Table 1). There was no statistically significant difference in the response to PPD observed in the presence of eicosapentaenoate and arachidonate or docosahexaenoate and arachidonate (both $P > 0.05$). However, lymphocyte proliferation in the presence of eicosapentaenoate was significantly lower than in the presence of docosahexaenoate ($P < 0.02$).

Effect of fatty acids on the concentration of IL-2 in the culture medium of Con A-stimulated peripheral blood lymphocytes

IL-2 was not detected in the medium of peripheral blood lymphocytes cultured in the absence of mitogen for periods up to 48 h. In contrast, Con A stimulation of human lymphocytes caused an increase in the IL-2 concentration in the culture medium which reached a maximum after 39 h of culture (Fig. 3); in cells from subject no. 2, the increase in IL-2 concentration was linear up to 20 h, whereas for cells from subject nos. 1 and 5 there was a lag period of 15 h (Fig. 3). For cells from the three subjects, the IL-2 concentration (mean ± SEM) in the culture medium was 0.48 ± 0.24, 1.76 ± 0.29, 2.45 ± 0.21 and 2.01 ± 0.14 units/ml at 15, 24, 39 and 48 h, respectively.

Saturated fatty acids had no effect on the concentration of IL-2 after 24 or 48 h of culture (Table 2). However, PUFAs decreased the concentration of IL-2 markedly (Table 2). Different PUFAs decreased the concentration of IL-2 by 85–95% after 24 h of culture, and by 70–90% after 48 h of culture (Table 2). Eicosapentaenoate caused the greatest decrease at both time points (Table 2). Oleate decreased the IL-2 concentration after 24 h of culture, but its effect was not statistically significant after 48 h (Table 2).

DISCUSSION

The present study shows that PUFAs and stearate inhibit [^{3}H]thymidine incorporation into Con A- or PPD-stimulated human peripheral blood lymphocytes (Table 1). The effects of the different fatty acids were similar whether Con A or PPD was used to stimulate the cells. Of particular note is the very marked inhibition caused by either $n-3$ or $n-6$ PUFAs, but in all cases eicosapentaenoate caused the greatest inhibition. This study confirms previous observations that fatty acids can inhibit mitogen-stimulated human peripheral blood lymphocyte proliferation *in vitro* [1–7]. However, the present study has compared a greater range of fatty acids than previous studies and, to our knowledge, this is the first time that fish-oil-derived $n-3$ fatty acids have been shown to inhibit proliferation of these cells. All of these studies have been performed with cell populations containing a mixture of cell types, but the specificity of Con A (used in the present study) and phytohaemagglutinin (used in previous studies [1–7]) as T-cell mitogens [25] implies that the fatty acids are exerting their inhibitory effects on T-lymphocyte proliferation.

Table 2. Effect of fatty acids on the concentration of IL-2 in the culture medium of Con A-stimulated human lymphocytes. Human peripheral blood lymphocytes were cultured for 24 or 48 h in the presence of Con A and 100 μmol/l fatty acid-BSA complexes. The medium was removed, serially diluted twofold and the concentration of IL-2 was determined using the CTLL-2 cell line [24] (see the Materials and methods section). Data are the means ± SEM of three separate preparations. Statistical significance: $^{*}P<0.05$, $^{**}P<0.02$, $^{***}P<0.01$, $^{****}P<0.001$ compared with control cells (i.e. no fatty acid addition). Numbers in parentheses are the IL-2 concentrations presented as percentages of the control mean.

Fatty acid added	IL-2 concn. (units/ml)			
	24 h		48 h	
None	1.77 ± 0.29	(100)	2.07 ± 0.14	(100)
Myristate (14:0)	1.63 ± 0.39	(92)	2.17 ± 0.66	(105)
Palmitate (16:0)	1.60 ± 0.66	(90)	2.00 ± 0.36	(97)
Stearate (18:0)	1.63 ± 0.23	(92)	2.17 ± 0.14	(105)
Oleate (18:1 $n-9$)	0.60 ± 0.29*	(34)	1.40 ± 0.45	(68)
Linoleate (18:2 $n-6$)	0.20 ± 0.03***	(11)	0.40 ± 0.10****	(19)
α-Linolenate (18:3 $n-3$)	0.27 ± 0.16**	(15)	0.60 ± 0.12***	(29)
Arachidonate (20:4 $n-6$)	0.11 ± 0.04***	(6)	0.31 ± 0.10****	(15)
Eicosapentaenoate (20:5 $n-3$)	0.09 ± 0.05***	(5)	0.24 ± 0.11****	(12)
Docosahexaenoate (22:6 $n-3$)	0.30 ± 0.07***	(17)	0.63 ± 0.14***	(30)

We have previously shown that proliferation of rat lymph node lymphocytes is inhibited by a number of fatty acids [23]. A similar pattern of inhibition is observed with both rat and human cells: in both cases eicosapentaenoate and arachidonate were the most potent inhibitors and myristate and palmitate the least potent. One important difference, however, was the effect of oleate. This fatty acid inhibits Con A-stimulated rat lymph node lymphocyte proliferation to approximately the same extent as stearate [23], but it does not inhibit human blood lymphocyte proliferation, whereas stearate does (Table 1). The reason for this difference is not known; it may be due to a difference in the ability of different cells to take up and metabolize the fatty acids. This difference could be of value in further studies on the mechanism of action of fatty acids.

The proliferation of T-lymphocytes is dependent on their ability to synthesize and secrete the cytokine IL-2 [22]. This study shows that PUFAs decrease the concentration of IL-2 in the culture medium of Con A-stimulated cells (Table 2). This effect is presumably the result of inhibition of IL-2 synthesis or secretion of fatty acids. The greatest inhibition of IL-2 production was observed in the presence of eicosapentaenoate, arachidonate or linoleate. There is a good correlation between lymphocyte proliferation (i.e. stimulation index) in the presence of fatty acids and IL-2 concentration at 48 h ($r=0.768$ for Con A and $r=0.730$ for PPD; both $P<0.02$). This suggests that fatty acids inhibit lymphocyte proliferation by interfering with the production of IL-2 by stimulated lymphocytes. However, stearate did not affect the concentration of IL-2, whereas this fatty acid caused inhibition of proliferation of lymphocytes. Thus, although fatty acid suppression of the production of IL-2 may play a role in the observed inhibition of proliferation, it is unlikely to be the sole mechanism.

It is possible that the basic mechanism by which fatty acids exert their inhibitory effect upon lymphocyte proliferation may depend upon the incorporation of fatty acids into membrane phospholipids, which will result in changes in fluidity. It has recently been shown that, during culture of activated lymphocytes, specific changes in fatty acid composition and membrane fluidity occur [26]; these changes may be necessary for an optimal proliferative response. Since lymphocytes readily incorporate fatty acids into their lipids [27], the presence of an excess of one fatty acid may result in accumulation of that particular fatty acid in the membrane, leading to a change in the plasma membrane fatty acid composition that would be expected to affect fluidity. Such changes in membrane composition, if they occurred in the present study, could cause the observed decreases in IL-2 secretion (Table 2) and in proliferation (Table 1). In support of this, it has been shown that other agents which perturb membrane structure and/or fluidity also inhibit IL-2 production by activated T-lymphocytes. These agents include ethanol [28], cyclosporin A [29], sterols [30] and carcinogens [31, 32].

A number of serious diseases are due to an over-active immune system directed against host tissues. Activated T-lymphocytes appear to play a role in such autoimmune and inflammatory disorders: activated T-cells and IL-2 have been described at the site of injury in rheumatoid arthritis [33, 34], multiple sclerosis [35] and pulmonary sarcoidosis [36]. Furthermore, increases in the concentrations of IL-2 and IL-2 receptors in the circulation have been reported in rheumatoid arthritis [37, 38], multiple sclerosis [39] and systemic lupus erythematosus [40]. These observations indicate that factors that cause a decrease in the activation, and subsequent proliferation, of T-lymphocytes may be of therapeutic use in such disorders. The present results suggest that T-cell activation and hence immune responses may be modulated by dietary fatty acid intake. The reason why dietary manipulations using oils rich in PUFAs have not been successful in the treatment of some disorders [16–20] may be because the diets did not result in sufficient changes in the fatty acid composition of lymphocytes (and macrophages) to cause changes in membrane fluidity. To our knowledge, no measurements of the fatty acid composition of cells of the immune system (e.g. peripheral blood lymphocytes) have been made during the course of these trials. In the light of our current observations (Tables 1 and 2), it seems important that the fatty acid composition of, and IL-2 production by, lymphocytes from patients in trials using PUFA-enriched diets be determined. This could provide a relatively simple and useful means of monitoring the effect of the dietary manipulation upon the immune system.

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REFERENCES

1. Mertin, J., Hughes, D. & Stewart-Wynne, E. PHA transformation in M.S.: inhibition by linoleic acid. Lancet 1974; **i,** 1005–6.
2. Offner, H. & Clausen, J. Inhibition of lymphocyte response to stimulants induced by unsaturated fatty acids and prostaglandins in multiple sclerosis. Lancet 1974; **ii,** 1204–5.
3. Mihas, A.A., Gibson, R.G. & Hirschowtiz, B.I. Suppression of lymphocyte transformation by 16,(16)-dimethyl prostaglandin E_2 and unsaturated fatty acids. Proc. Soc. Exp. Biol. Med., 1975; **149,** 1026–8.
4. Mertin, J. & Hughes, D. Specific inhibitory action of polyunsaturated fatty acids on lymphocyte transformation induced by PHA and PPD. Int. Arch. Allergy Appl. Immunol. 1975; **48,** 203–10.
5. Weyman, C., Belin, J., Smith, A.D. & Thompson, R.H.S. Linoleic acid as an immunosuppressive agent. Lancet 1975; **ii,** 33.
6. Weyman, C., Morgan, S.J., Belin, J. & Smith, A.D. Phytohaemagglutinin stimulation of human lymphocytes: effect of fatty acids on uridine uptake and phosphoglyceride fatty acid profile. Biochim. Biophys. Acta 1977; **496,** 155–66.
7. Tsang, W.M., Weyman, C. & Smith, A.D. Effect of fatty acid mixtures on phytohaemagglutinin-stimulated lymphocytes of different species. Biochem. Soc. Trans. 1977; **5,** 153–4.
8. Simopoulos, A.P.S., Kifer, R.R. & Martin, R.E., eds. Health effects of polyunsaturated fatty acids in seafoods. Orlando, FL: Academic Press, 1986.
9. Gurr, M.I. The role of lipids in the regulation of the immune system. Prog. Lipid Res. 1983; **22,** 257–87.
10. Hwang, D. Essential fatty acids and the immune response. FASEB J. 1989; **3,** 2052–61.
11. Kinsella, J.E., Lokesh, B., Broughton, S. & Whelan, J. Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells — an overview. Nutrition 1990; **6,** 24–44.
12. Kremer, J.R., Jubiz, W., Michalek, A. et al. Fish oil fatty acid supplementation in active rheumatoid arthritis. Ann. Intern. Med. 1987; **106,** 497–503.
13. Swank, R.L. & Duggan, B.B. Effect of low saturated fat diet in early and late cases of multiple sclerosis. Lancet 1990; **ii,** 37–9.
14. Bittiner, S.B., Tucker, W.F.G., Cartwright, I. & Bleehen, S.S. A double-blind, randomised, placebo-controlled trial of fish oil in psoriasis. Lancet 1988; **i,** 378–80.
15. Robinson, D.R., Colvin, R.B., Hirai, A. & Steinberg, A.D. Dietary marine lipids modify autoimmune diseases. In: Simopoulos, A.P.S., Kifer, R.R. & Martin, R.E., eds. Health effects of polyunsaturated fatty acids in seafoods. Orlando, FL: Academic Press, 1986: 247–59.
16. Hansen, T.M., Lerche, A., Kassis, V., Lorenzen, I. & Sondergaard, J. Treatment of rheumatoid arthritis with prostaglandin E_1 precursors *cis*-linolenic acid and γ-linolenic acid. Scand. J. Rheumatol., 1983; **12,** 85–8.
17. Millar, J.H.D., Zilkha, K.J., Langman, M.J.S. et al. Double-blind trial of linoleate supplementation of the diet in multiple sclerosis. Br. Med. J., 1973; **i,** 765–8.
18. Bates, D., Fawcett, P.R.W., Shaw, D.A. & Weightman, D. Polyunsaturated fatty acids in treatment of acute remitting multiple sclerosis. Br. Med. J., 1978; **2,** 1390–1.
19. Paty, D.W., Cousin, H.K., Reid, S. & Adlakha, K. Linoleic acid in multiple sclerosis: failure to show any therapeutic effect. Acta Neurol. Scand., 1978; **58,** 53–8.
20. Bates, D., Cartlidge, N.E.F., French, J.M. et al. A double-blind controlled trial of long chain $n-3$ polyunsaturated fatty acids in the treatment of multiple sclerosis. J. Neurol. Neurosurg. Psychiatry 1988; **52,** 18–22.
21. Editorial. Lipids and multiple sclerosis. Lancet 1990; **ii,** 25–6.
22. Smith, K.A. Interleukin-2: inception, impact and implications. Science (Washington, D.C.) 1988; **240,** 1169–76.
23. Calder, P.C., Bond, J.A., Bevan, S.J., Hunt, S.V. & Newsholme, E.A. Effect of fatty acids on the proliferation of concanavalin A-stimulated rat lymph node lymphocytes. Int. J. Biochem. 1991; **23,** 579–88.
24. Gillis, S., Ferm, M.M., Ou, W. & Smith, K.A. T-cell growth factor: parameters of production and a quantitative microassay for activity. J. Immunol. 1978; **120,** 2027–32.
25. Stobo, J.D. Phytohaemagglutinin and concanavalin A: probes for murine T cell activation and differentiation. Transplant. Rev. 1972; **11,** 60–86.
26. Anel, A., Naval, J., Gonzalez, B. et al. Fatty acid metabolism in human lymphocytes. I. Time-course changes in fatty acid composition and membrane fluidity during blastic transformation of peripheral blood lymphocytes. Biochim. Biophys. Acta 1990; **1044,** 323–31.
27. Anel, A., Naval, J., Gonzalez, B., Uriel, J. & Pineiro, A. Fatty acid metabolism in human lymphocytes. II. Activation of fatty acid desaturase–elongase systems during blastic transformation. Biochim. Biophys. Acta 1990; **1044,** 332–9.
28. Kaplan, D.R. A novel method of immunosuppression mediated by ethanol. Cell. Immunol. 1986; **102,** 1–9.
29. LeGrue, S.J., Freidman, A.W. & Kahan, B.D. Binding of cyclosporine A by human lymphocytes and phospholipid vesicles. J. Immunol. 1983; **131,** 712–8.
30. Moog, C., Luu, B., Beck, J., Italiano, L. & Bischoff, P. Studies on the immunosuppressive properties of 7,25-dihydrocholeterol. I. Reduction of interleukin production by treated lymphocytes. Int. J. Immunopharmacol., 1988; **10,** 511–18.
31. House, R.V., Lauer, L.D., Murray, M.J. & Dean, J.H. Suppression of T-helper cell function in mice following exposure to the carcinogen 7,12-dimethylbenz[*a*]anthracene and its restoration by interleukin-2. Int. J. Immunopharmacol. 1987; **9,** 89–97.
32. Lyte, M., Blanton, R.H., Myers, M.J. & Bick, P.H. Effect of *in vivo* administration of the carcinogen benzo[*a*]pyrene on interleukin-2 and interleukin-3 production. Int. J. Immunopharmacol. 1987; **9,** 307–12.
33. Buchan, G., Barrett, K., Fujita, T., Taniguchi, T., Maini, R. & Feldmann, M. Detection of activated T-cell products in the rheumatoid joint using cDNA probes to interleukin-2 (IL-2), IL-2 receptor and IFN-γ. Clin. Exp. Immunol., 1988; **71,** 295–301.
34. Stamenkovic, I., Stegagno, M., Wright, K.A. et al. Clonal dominance among T-lymphocyte infiltrates in arthritis. Proc. Natl. Acad. Sci. U.S.A. 1988; **85,** 1179–83.
35. Konttinen, Y.T., Bergroth, V., Kinnunen, E., Nordstrom, D. & Kouri, T. Activated T-lymphocytes in patients with multiple sclerosis in clinical remission. J. Neurol. Sci. 1987; **81,** 133–9.
36. Konishi, K., Moller, D.R., Saltini, C., Kirby, M. & Crystal, R.G. Spontaneous expression of the interleukin 2 receptor gene and presence of functional interleukin 2 receptors on T lymphocytes in the blood of individuals with active pulmonary sarcoidosis. J. Clin. Invest. 1988; **82,** 775–81.
37. Symons, J.A., Wood, N.C., DiGiovini, F.S. & Duff, G.W. Soluble IL-2 receptors in rheumatoid arthritis: correlation with disease activity, IL-1 and IL-2 inhibition. J. Immunol. 1988; **141,** 2612–8.
38. Warrington, R.J. Interleukin 2 abnormalities in systemic lupus erythematosus and rheumatoid arthritis. A role for overproduction of interleukin 2 in human autoimmunity. J. Rheumatol. 1987; **15,** 616–20.
39. Merrill, J.E., Mohlstrom, C., Uittenbogaart, C., Kermani-Arab, V., Ellison, G.W. & Myers, L.W. Response to and production of interleukin 2 by peripheral blood and cerebrospinal fluid lympohocytes of patients with multiple sclerosis. J. Immunol. 1984; **133,** 1931–7.
40. Wolf, R.E. & Brelsford, W.G. Soluble interleukin-2 receptors in systemic lupus erythematosus. Arthritis Rheum. 1988; **31,** 729–35.