*Immunology* 1992 **75** 108–115

# The inhibition of T-lymphocyte proliferation by fatty acids is via an eicosanoid-independent mechanism

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*Accepted for publication 23 August 1991*

## SUMMARY

Eicosanoids, in particular prostaglandin $E_2$ ($PGE_2$), are potent inhibitors of a number of immune responses, including lymphocyte proliferation. We have previously shown that fatty acids, especially polyunsaturated fatty acids (PUFA), inhibit mitogen-stimulated proliferation of lymphocytes. One mechanism by which fatty acids could exert their inhibitory effect is via modulation of eicosanoid synthesis. This possibility was investigated in the present study. $PGE_2$ concentrations in the medium taken from lymphocytes cultured in the presence of a range of different fatty acids did not correlate with the inhibitory effects of the fatty acids upon lymphocyte proliferation. Although $PGE_2$ at concentrations above 10 nM caused inhibition of lymphocyte proliferation, $PGE_2$ at the concentration measured in lymphocyte culture medium (0·3–4 nM) was not inhibitory. $PGE_3$ did not inhibit lymphocyte proliferation, except at high concentrations (>250 nM). The maximal inhibition of proliferation caused by $PGE_2$ or $PGE_3$ was less than the inhibition caused by each of the fatty acids except myristic or palmitic acids. Inclusion of inhibitors of phospholipase $A_2$, cyclo-oxygenase or lipoxygenase in the culture medium did not prevent the fatty acids from exerting their inhibitory effect on lymphocyte proliferation. The eicosanoids present in lymph node cell cultures originate from macrophages rather than lymphocytes. Depletion of macrophages from the cell preparation by adherence did not prevent fatty acids from inhibiting proliferation. Proliferation of thoracic duct lymphocytes, which are devoid of macrophages, is inhibited by fatty acids to a similar extent as proliferation of lymph node lymphocytes. These observations provide convincing evidence that the inhibition of lymphocyte proliferation by fatty acids is independent of the production of eicosanoids. Therefore, other mechanisms must be investigated if the effect of fatty acids upon lymphocyte proliferation is to be understood at a biochemical level.

## INTRODUCTION

The eicosanoid family includes prostaglandins (PG), thromboxanes (TX), leukotrienes (LT), hydroxyeicoisatetraenoic acids (HETE) and hydroperoxyeicosatetraenoic acids (HPETE). These compounds are oxygenation products of polyunsaturated fatty acids (PUFA) derived from plasma membrane phospholipids. The principal substrate for eicosanoid biosynthesis is the $n-6$ PUFA arachidonic acid, which can be metabolized by cyclo-oxygenase to yield the 2-series PGs and TXs (Fig. 1). Arachidonic acid can also be metabolized to 4-series LTs by 5-lipoxygenase and to hydroxy and hydroperoxy fatty acids by 5-, 12- or 15-lipoxygenase (Fig. 1). There is now considerable evidence that various members of the eicosanoid family derived from arachidonic acid participate in inflammatory reactions and may act as regulators of the immune response.[1–3] In particular, E-series prostaglandins such as $PGE_1$ and $PGE_2$ suppress a number of T-cell functions including proliferation,[1,4–8] interleukin-2 secretion[9–11] and cytotoxicity.[5] Several other eicosanoids, including $LTB_4$, 15-HETE and 15-HPETE, also inhibit T-lymphocyte proliferation.[8,12–14]

Replacement of arachidonic acid in membrane phospholipids by the $n-3$ PUFA eicosapentaenoic acid leads to synthesis of a different family of eicosanoids[15–17] whose effects on immune functions such as lymphocyte proliferation remain to be investigated, although it has been reported recently that $PGE_3$ does not affect T-lymphocyte proliferation.[18,19]

We have shown that saturated, monounsaturated and polyunsaturated fatty acids including both $n-6$ and $n-3$ PUFA inhibit [$^3$H]thymidine incorporation into the DNA of concanavalin A (Con A)-stimulated rat lymph node lympho-

Abbreviations: BSA, bovine serum albumin; CA, caffeic acid; Con A, concanavalin A; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; IM, indomethacin; LT, leukotriene; NDGA, nordihydroguaiaretic acid; PG, prostaglandin; PHA, phytohaemagglutinin; PUFA, polyunsaturated fatty acid; QUIN, quinacrine; TX, thromboxane.

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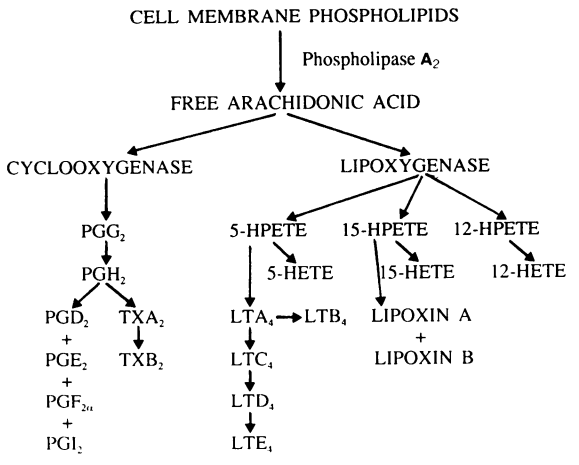


**Figure 1.** The pathway of eicosanoid synthesis from arachidonic acid.

cytes.[20,21] Unsaturated fatty acids were more inhibitory than saturated fatty acids; the greatest inhibition was caused by eicosapentaenoic and arachidonic acids and the least inhibition was caused by myristic and palmitic acids. Inhibition of lymphocyte proliferation by fatty acids, in particular PUFA, suggests that dietary lipid manipulation could potentially be used for treatment of disorders characterized, at least partially, by activated lymphocytes (e.g. rheumatoid arthritis, psoriasis, systemic lupus erythematosus, multiple sclerosis). However, it is considered that for a more rational design of such dietary therapies, the biochemical mechanism(s) of action of fatty acids upon cells of the immune system must be examined further.[22]

It is possible that the inhibitory effects of fatty acids upon lymphocyte proliferation are mediated via formation of PGs or other eicosanoids; the differences in inhibition between fatty acids may arise through different effects upon the pathways of eicosanoid metabolism. A number of compounds are available which have inhibitory effects at different points of eicosanoid synthesis: quinacrine (QUIN) is an inhibitor of phospholipase $A_2$;[23] indomethacin (IM) is a well-documented cyclo-oxygenase inhibitor, and nordihydroguaiaretic acid (NDGA) and caffeic acid (CA) are potent lipoxygenase inhibitors.[24,25] To test the possibility that fatty acid inhibition of lymphocyte proliferation is due to modulation of eicosanoid synthesis we investigated the effect of fatty acids upon lymphocyte proliferation in the presence of these inhibitors. In addition, since a major source of eicosanoids is the macrophage,[26,27] we investigated the effect of fatty acids upon lymphocyte proliferation in cell preparations from which macrophages were absent. Fatty acids used in this study were myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1 $n-9$), linoleic (18:2 $n-6$), α-linolenic (18:3 $n-3$), arachidonic (20:4 $n-6$), eicosapentaenoic (20:5 $n-3$) and docosahexaenoic (22:6 $n-3$).

## MATERIALS AND METHODS

*Animals and chemicals*

The source and housing of rats and sources of chemicals were as described previously.[21] In addition, $PGE_2$, QUIN, IM, NDGA and CA were obtained from Sigma Chemical Co., Poole, Dorset, U.K., $PGE_3$ was purchased from Cayman Chemical Co., Ann Arbor, MI. Bovine serum albumin (BSA)-fatty acid complexes were formed as described previously.[21] Monoclonal antibodies (MRC OX-42 and MRC OX-21) and peroxidase-conjugated rabbit anti-mouse IgG were gifts from the MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford.

*Cell culture*

Cervical lymph nodes were dissected from male rats (250–300 g), freed of adipose tissue and gently ground. Lymphocytes were collected by centrifugation and washed twice. The cells were cultured at 37° in an air:$CO_2$ (19:1) atmosphere at a density of $5 \times 10^5$ cells/well in HEPES-buffered RPMI supplemented with 10 mM-glucose, 2 mM-glutamine, 10% foetal calf serum, 100 units/ml streptomycin, 200 units/ml penicillin and 5 μg/ml Con A. Where indicated, the cell culture medium was also supplemented with BSA-fatty acid complexes (100 μM or 250 μM fatty acid; BSA:fatty acid ratio = 1:1) and/or eicosanoid synthesis inhibitors or $PGE_2$ or $PGE_3$ (see results section for concentrations used). After various times (see results) [6-$^3$H]-thymidine was added (0·2 μCi/well) and the cells incubated for a further 18 hr. The cells were then harvested onto glass fibre filters which were washed and dried using a Skatron Cell Harvester. Radioactive thymidine incorporation was measured by liquid scintillation counting and was used to indicate cell proliferation.[28]

*Macrophage depletion*

Macrophages were removed from the lymph node cell preparation by adhesion to plastic Petri dishes treated for tissue culture. Lymph node cells were prepared as described above and incubated at 37° at a density of $5 \times 10^7$ cells/plate in RPMI supplemented with 10 mM-glucose, 2 mM-glutamine, 10% foetal calf serum and antibiotics. Under these conditions it is known that macrophages adhere to the surface of the culture dish, whereas lymphocytes do not.[29] After 4 hr the non-adherent cells were collected and washed. The depletion of macrophages from the lymph node cell preparation using this technique was confirmed by immunocytochemistry using an anti-CD 11 monoclonal antibody (MRC OX-42) and peroxidase-conjugated rabbit anti-mouse IgG; approximately 4% of cells in the complete cell preparation stained positively, whereas in the depleted cell preparation approximately 1% of cells were stained. Background staining was determined using an anti-human C3b activator protein monoclonal antibody (MRC OX-21); 0·7–1% of cells stained positively in both the complete and depleted cell preparations.

*Collection of thoracic duct lymphocytes*

Lymphocytes were collected by cannulation of the thoracic duct as described in detail by Hunt.[29] Subsequent purification and culture of these cells were carried out as described above for lymph node lymphocytes.

*$PGE_2$ measurement*

The concentration of $PGE_2$ in cell culture media was assayed using a commercial radioimmunoassay kit, as described previously.[21]

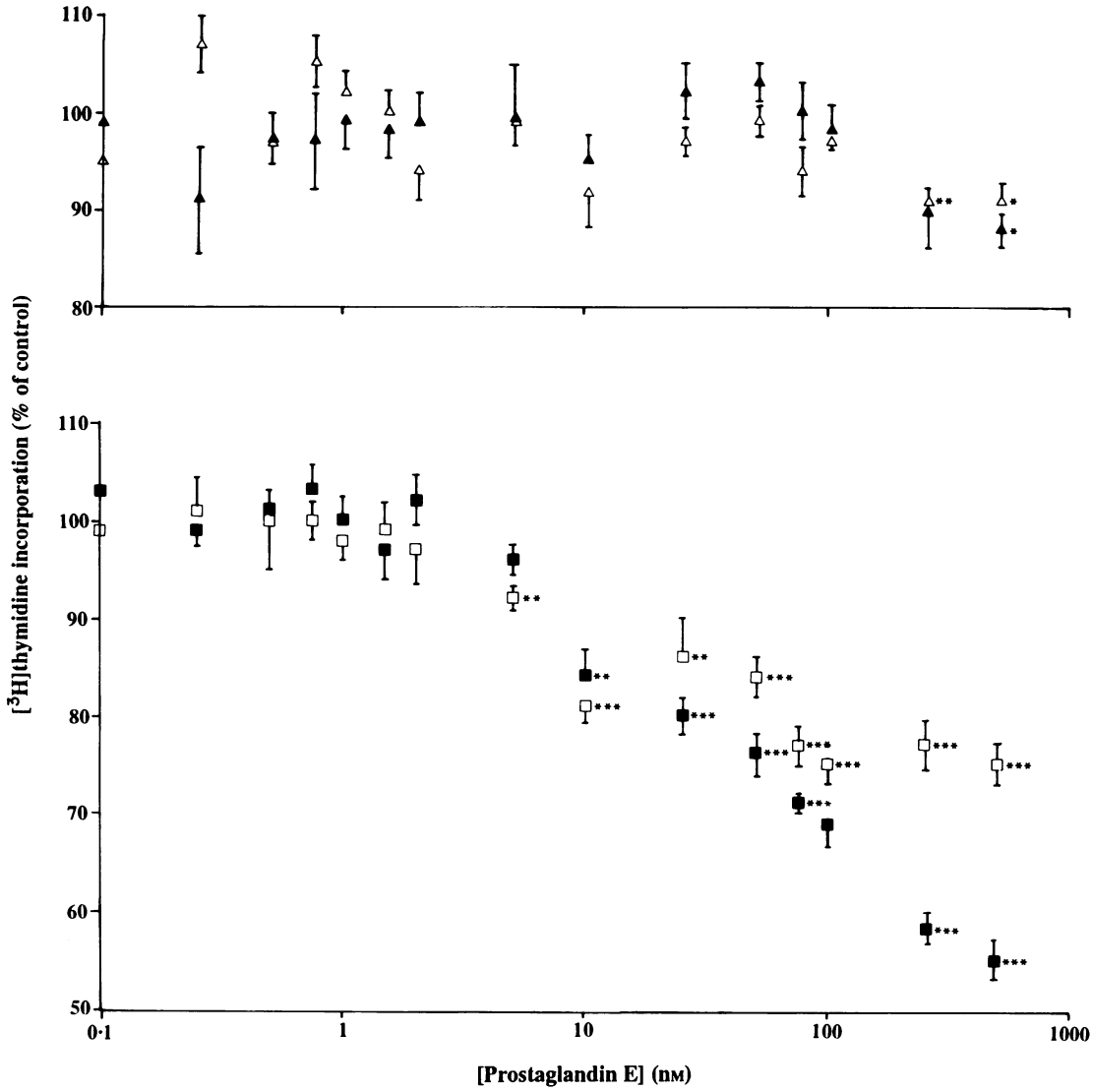


**Figure 2.** The effect of $PGE_2$ and $PGE_3$ on lymphocyte proliferation. Lymphocytes were cultured in the presence of Con A and various concentrations of $PGE_2$ (lower panel) or $PGE_3$ (upper panel) for 18 hr (open symbols) or 66 hr (closed symbols). Thymidine incorporation was measured over the final 18 hr of culture. Data are the mean ± SEM ($n=6$) thymidine incorporation expressed as percentage of control (i.e. no PG added) incorporation. Total thymidine incorporation (mean ± SEM, $n=6$) was 6340 ± 105 dpm at 18 hr and 162250 ± 3795 dpm at 66 hr. Statistical significance (Student's $t$-test) versus control: $*P<0{\cdot}02$, $**P<0{\cdot}01$, $***P<0{\cdot}001$.

## RESULTS

### $PGE_2$ concentrations in cell culture media

The $PGE_2$ concentration in the cell culture medium of lymphocytes cultured for 18 hr was 0·84 ± 0·04 nM for unstimulated cells and 1·23 ± 0·02 nM for Con A-stimulated cells (mean ± SEM, $n=6$). The presence of fatty acids (100 $\mu$M) in the cell culture medium caused an increase in the $PGE_2$ concentration in the medium; the highest concentration (3·81 ± 0·35 nM, $n=6$) was observed if arachidonic acid was added to the culture medium.

### The effect of $PGE_2$ on lymphocyte proliferation

To investigate whether $PGE_2$ at the concentrations measured in cell culture media could be responsible for the inhibition of lymphocyte proliferation observed in the presence of fatty acids, the effect of exogenously added $PGE_2$ upon lymphocyte proliferation was determined. Two different periods of culture were used (18 hr and 66 hr) and thymidine incorporation was measured over the final 18 hr of culture. For both times of culture, $PGE_2$ at a concentration of 10 nM or higher was a potent inhibitor of proliferation (Fig. 2). At 18 hr of culture, $PGE_2$ at a concentration of 5 nM also caused inhibition, but this concentration was not inhibitory at 66 hr of culture (Fig. 2). At either 18 or 66 hr of culture, $PGE_2$ concentrations of 2 nM or below did not inhibit proliferation (Fig. 2). Maximal inhibition of lymphocyte proliferation was approximately 25% after 18 hr of culture and 45% after 66 hr of culture. The effect of $PGE_2$ on thymidine incorporation (expressed as a percentage of the control) was not significantly different between the two culture periods except at $PGE_2$ concentrations of 250 or 500 nM ($P<0{\cdot}001$).

### The effect of $PGE_3$ on lymphocyte proliferation

The effect of $PGE_3$ on lymphocyte proliferation was investigated at two different periods of culture (18 hr and 66 hr); thymidine incorporation was measured over the final 18 hr of culture. For both times of culture, $PGE_3$ at a concentration of 100 nM or less did not inhibit proliferation (Fig. 2). At 18 hr of culture, $PGE_3$ at concentrations of 250 and 500 nM caused inhibition of proliferation, but only 500 nM $PGE_3$ was inhibitory at 66 hr of culture (Fig. 2). Maximal inhibition of proliferation was approximately 10% (Fig. 2). The effect of $PGE_3$ on thymidine incorporation (expressed as a percentage of the control) was not significantly different between the two culture periods. $PGE_2$ was significantly more inhibitory than $PGE_3$ at all concentrations at which $PGE_2$ inhibited thymidine incorporation.

### Effect of inhibitors of eicosanoid metabolism upon mitogen-stimulated lymphocyte proliferation

Over the first 18 hr of culture low concentrations (1–10 μM) of IM, NDGA or CA enhance lymphocyte proliferation.[30] This is consistent with the observations that most eicosanoids investigated to date inhibit lymphocyte proliferation (see Introduction) and suggests that in standard lymphocyte cultures products of both the cyclooxygenase and lipoxygenase pathways play a regulatory role.

Inhibitors of both cyclo-oxygenase (IM) and lipoxygenase (NDGA, CA) caused similar enhancement of proliferation after 18 hr of culture;[30] the greatest enhancement was observed using 10 μM IM or NDGA where thymidine incorporation was 1·7 times that measured in the absence of eicosanoid synthesis inhibitors. At concentrations of 1 and 2·5 μM, QUIN also enhanced proliferation but at concentrations of 5 μM or above, QUIN caused marked inhibition of lymphocyte proliferation;[30] this is likely to be due to the ability of this compound to inhibit flavoprotein enzymes, uncouple oxidative phosphorylation, inhibit ATPase and intercalate DNA.[31]

After culture for 18 hr, IM (1 or 10 μM) lowered the $PGE_2$ concentration in the medium of Con A-stimulated cells to $0{\cdot}32 \pm 0{\cdot}01$ nM ($P < 0{\cdot}001$ versus cells cultured in the absence of IM), indicating effective inhibition of PG synthesis. In the presence of arachidonic acid, IM lowered the $PGE_2$ concentration in the culture medium to $1{\cdot}05 \pm 0{\cdot}02$ nM ($P < 0{\cdot}001$ versus cells cultured in the absence of IM).

The stimulatory effect of eicosanoid synthesis inhibitors upon lymphocyte proliferation increased up to 30 hr of culture (Fig. 3) and disappeared after 42 hr of culture (Fig. 3). The inhibitory effects of concentrations of QUIN greater than 5 μM increased as the culture period was extended (data not shown). Low concentrations of QUIN did not, however, become inhibitory with extended culture (Fig. 3). The loss of an effect of these compounds during culture is most likely due to degradation of the inhibitor–enzyme complex and/or synthesis of new phospholipase $A_2$, cyclo-oxygenase and lipoxygenase. The latter would occur as a result of normal enzyme turn-over and possibly upregulation of enzyme synthesis due to the presence of the inhibitor.

### Effect of fatty acids upon lymphocyte proliferation in the presence of eicosanoid synthesis inhibitors

The effect of fatty acids (250 μM) upon lymphocyte proliferation was investigated in the presence of QUIN, IM, NDGA and CA

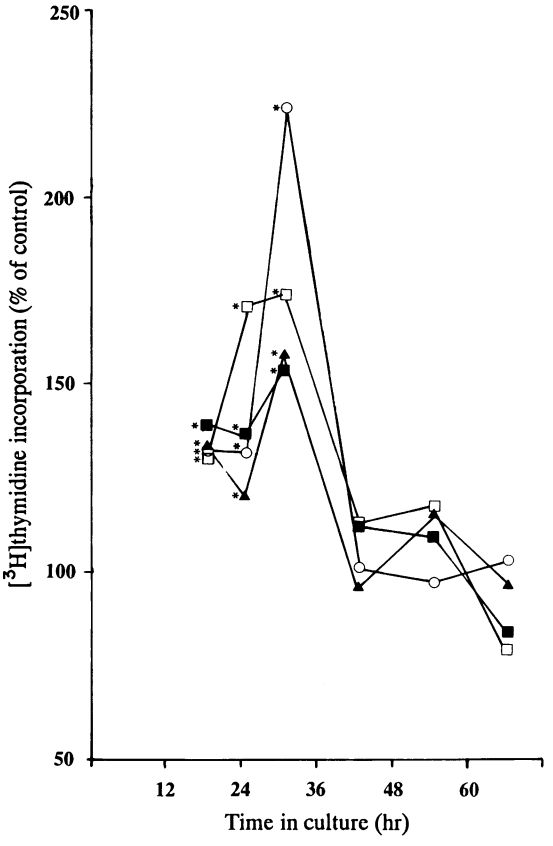


**Figure 3.** Time-course of the effect of inhibitors of eicosanoid synthesis upon lymphocyte proliferation. Lymphocytes were cultured in the presence of Con A and eicosanoid synthesis inhibitors for various times. Incorporation of [$^3$H]thymidine was measured over the final 18 hr of culture. Eicosanoid synthesis inhibitors used were 1 μM IM (□), 1 μM QUIN (■), 5 μM NDGA (▲) and 5 μM CA (○). Data are the mean thymidine incorporation (expressed as percentage of the incorporation observed in the absence of inhibitor) of six determinations; in all cases the SEM was less than 15% of the mean. Thymidine incorporation (mean ± SEM, $n = 6$) at various times was $2465 \pm 110$ (18 hr), $4550 \pm 220$ (24 hr), $12390 \pm 2785$ (30 hr), $76360 \pm 9030$ (42 hr), $133950 \pm 15690$ (54 hr), $93895 \pm 9550$ (66 hr). Statistical significance (Student's $t$-test) versus control (i.e. no inhibitor added): $^*P < 0{\cdot}05$.

(Fig. 4). Eicosanoid synthesis inhibitors had no effect on the inhibition of proliferation caused by fatty acids. Indeed, fatty acids were able to overcome the enhancement of proliferation caused by QUIN, IM, NDGA and CA (Fig. 4). For each fatty acid, thymidine incorporation in the presence of inhibitor was similar to that in its absence.

### Effect of fatty acids upon lymphocyte proliferation in the absence of macrophages

To investigate the effect of fatty acids upon lymphocyte proliferation in the absence of the cells that produce eicosanoids (i.e. macrophages), two experimental systems were used.

First, macrophages were depleted from the lymph node cell preparation by adherence to tissue culture plastic. As expected, the removal of adherent cells reduced the ability of the cell preparation to produce $PGE_2$: the $PGE_2$ concentration in the culture medium was $0{\cdot}36 \pm 0{\cdot}01$ nM (unstimulated cells) or $0{\cdot}52 \pm 0{\cdot}06$ nM (Con A-stimulated cells) (both $P < 0{\cdot}001$ versus

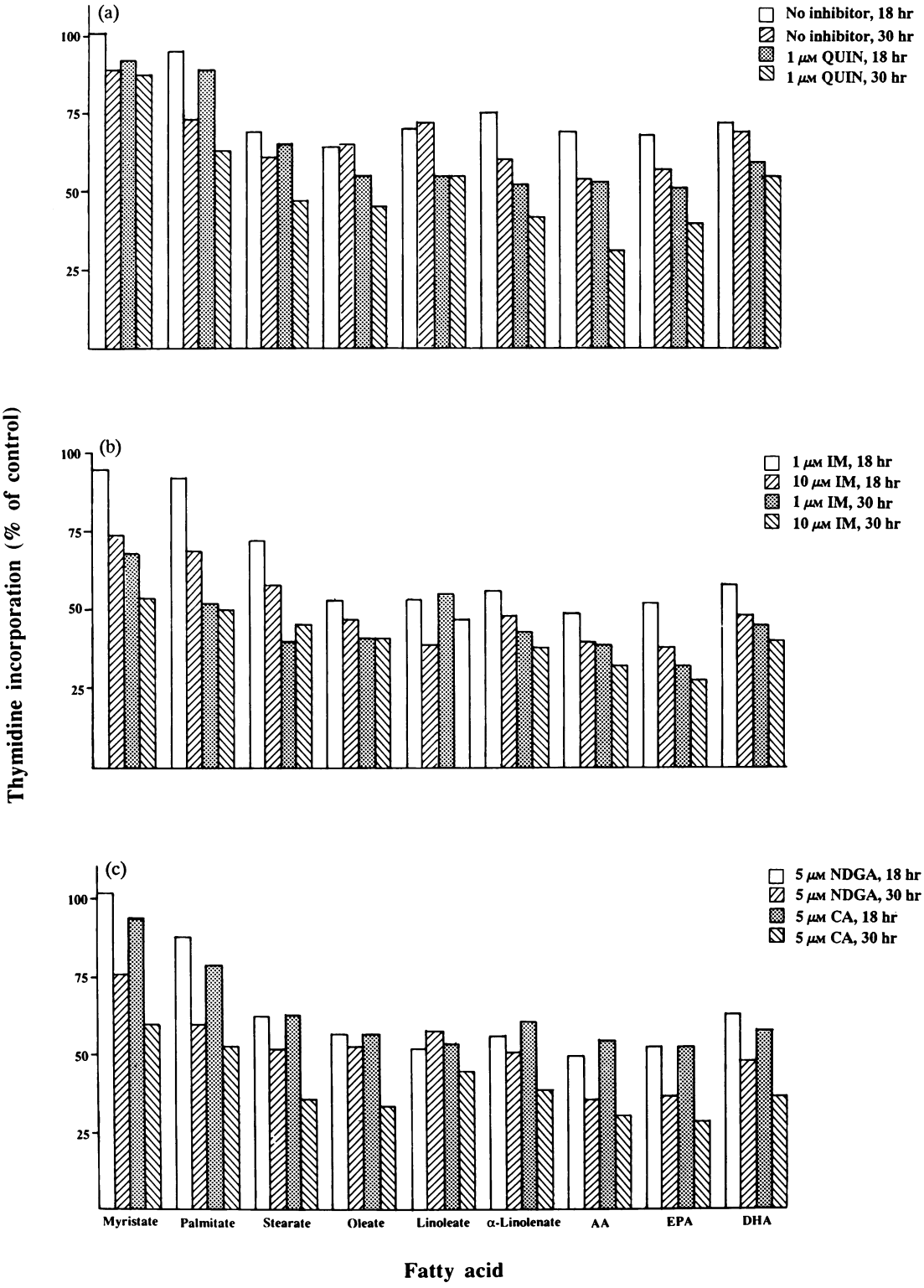


**Figure 4.** Effect of fatty acids on lymphocyte proliferation in the presence of inhibitors of eicosanoid synthesis. Lymphocytes were cultured in the presence of Con A, fatty acid (250 μM) and (a) QUIN (1 μM), (b) IM (1 or 10 μM), (c) NDGA or CA (5 μM) for 18 or 30 hr. Thymidine incorporation was measured over the final 18 hr of the culture period. Data are the mean thymidine incorporation (expressed as a percentage of the incorporation observed in the absence of added fatty acid) of six determinations; in all cases the SEM was less than 10% of the mean. Thymidine incorporation (mean, $n = 6$) was 6450 (18 hr; no inhibitor), 33560 (30 hr; no inhibitor), 8710 (18 hr; QUIN), 54030 (30 hr; QUIN), 8385 (18 hr; 1 μM IM), 57052 (30 hr; 1 μM IM), 10300 (18 hr; 10 μM IM), 61750 (30 hr; 10 μM IM), 7930 (18 hr; NDGA), 49330 (30 hr; NDGA), 8450 (18 hr; CA), 69130 (30 hr; CA). AA, EPA and DHA indicate arachidonic, eicosapentaenoic and docosahexaenoic acid, respectively.

**Table 1.** Effect of fatty acids upon lymphocyte proliferation in the presence and absence of macrophages. Macrophages were depleted from the lymph node cell preparation by adhesion (see Methods). The non-adherent cells were collected, washed, plated and cultured in the presence of Con A and fatty acid (100 $\mu$M) as described in Methods. Thoracic duct lymphocytes were collected by cannulation, washed and cultured as described in Methods. Thymidine incorporation was measured over the final 18 hr of a 66-hr culture period. Data are mean ± SEM ($n=6$). Values in parentheses are thymidine incorporation as % of the control (i.e. no fatty acid addition). Statistical significance (Student's $t$-test) versus control: $*P<0{\cdot}05$; $**P<0{\cdot}001$

| | | [$^3$H]thymidine incorporation (dpm/well) | | |
|---|---|---|---|---|
| Fatty acid | Con A | Complete | Depleted | Thoracic duct |
| None | – | 4560 ± 352 | 5705 ± 677 | 1005 ± 184 |
| None | + | 142804 ± 5797 (100) | 130309 ± 3489 (100) | 126608 ± 6235 (100) |
| Myristic | + | 125667 ± 4284* (88) | 119884 ± 6515 (92) | 111415 ± 6092 (88) |
| Palmitic | + | 105674 ± 5712** (74) | 100337 ± 3262** (77) | 79763 ± 6459** (63) |
| Stearic | + | 69974 ± 2586** (49) | 71669 ± 1313** (55) | 64570 ± 4036** (51) |
| Oleic | + | 75686 ± 7140** (53) | 79488 ± 4818** (61) | 74698 ± 5183** (59) |
| Linoleic | + | 41413 ± 2932** (29) | 33880 ± 2642** (26) | 82295 ± 2358** (65) |
| Linolenic | + | 57121 ± 2478** (40) | 48214 ± 5210** (37) | 84827 ± 4368** (67) |
| Arachidonic | + | 37129 ± 2296** (26) | 35183 ± 3125** (27) | 25321 ± 2554** (20) |
| Eicosapentaenoic | + | 22848 ± 3104** (16) | 19546 ± 2519** (15) | 18991 ± 2313** (15) |
| Docosahexaenoic | + | 52837 ± 2713** (37) | 49517 ± 3250** (38) | 54441 ± 1402** (43) |

control cell preparation). The depletion of adherent cells from the lymph node cell preparation did not affect the response to Con A (Table 1). Furthermore, the ability of fatty acids to inhibit Con A-stimulated proliferation was unaffected by removal of the adherent cells; the effect of fatty acids upon thymidine incorporation into both the control and macrophage-depleted cell preparations was very similar (Table 1). Indeed, in the presence of any given fatty acid, thymidine incorporation was almost identical in both control and depleted preparations (Table 1).

Secondly, lymphocytes were collected by cannulation of the thoracic duct; the cells present in such preparations are almost exclusively lymphocytes.[32] Each fatty acid tested, except myristic, inhibited Con A-stimulated incorporation of thymidine into thoracic duct lymphocytes (Table 1). The effect of each of the fatty acids was similar to the effect upon proliferation of lymph node lymphocytes (Table 1); the greatest inhibition of proliferation was caused by eicosapentaenoic and arachidonic acids and the least inhibition of proliferation was caused by myristic and palmitic acids (Table 1).

## DISCUSSION

We have shown previously that fatty acids, in particular PUFA, are able to inhibit Con A-stimulated lymphocyte proliferation.[20,21] One means by which fatty acids could cause inhibition of lymphocyte proliferation may be by acting as modulators of eicosanoid synthesis or by acting directly as precursors for the synthesis of eicosanoids, which themselves inhibit proliferation. All of the eicosanoids derived from arachidonic acid that have been tested inhibit lymphocyte proliferation *in vitro* (see Introduction). The most extensively studied of these eicosanoids is $PGE_2$. It must be noted, however, that the concentrations of $PGE_2$ employed in many studies of *in vitro* lymphocyte proliferation are rather high, often in excess of 1 $\mu$M. We have previously reported that the concentration of endogenously produced $PGE_2$ in the medium of lymphocytes cultured in the presence of 100 $\mu$M fatty acid and Con A for 48 hr was between 0·34 and 1·5 nM, depending upon the fatty acid present; the highest $PGE_2$ concentration was observed when arachidonic acid was present in the medium.[21] In the current study we observed higher $PGE_2$ concentrations in the cell culture medium after 18 hr than after 48 hr culture. However, the $PGE_2$ concentration was less than 4 nM for all cells including those grown in the presence of arachidonic acid. At this concentration $PGE_2$ did not inhibit proliferation either after 18 hr or 66 hr of culture (Fig. 2). Inhibition of lymphocyte proliferation was observed at $PGE_2$ concentrations higher than 5 nM (18 hr culture) or 10 nM (66 hr culture) (Fig. 2). The maximal inhibition of proliferation, observed after 66 hr culture in the presence of $PGE_2$ at a concentration of 500 nM, was 45% (Fig. 2). $PGE_3$ inhibited lymphocyte proliferation only at very high concentrations (> 250 nM; Fig. 2) and the maximal inhibition observed was 10% (Fig. 2). The percentage inhibition of proliferation caused by $PGE_2$ or $PGE_3$ is less than that caused by each of the fatty acids tested, except myristic and palmitic acids (see Table 1). In particular, the inhibition of proliferation caused by $PGE_2$ and $PGE_3$ is much less than that caused by their precursor fatty acids (arachidonic and eicosapentaenoic acids, respectively).

Despite the inability of $PGE_2$ to inhibit proliferation at the concentration measured in the cell culture medium, inhibitors of eicosanoid synthesis (IM, QUIN, NDGA, CA) were able to enhance lymphocyte proliferation during the first 30 hr of culture (Fig. 3). This suggests either that an eicosanoid other than $PGE_2$, or a combination of several eicosanoids including $PGE_2$, act to regulate lymphocyte proliferation in standard culture conditions. It is worth noting that inhibition of either cyclo-oxygenase or lipoxygenase enhanced proliferation, suggesting that products of either enzyme regulate lymphocyte proliferation. Fatty acids maintained their inhibitory effect upon lymphocyte proliferation in the presence of QUIN, IM, NDGA and CA (Fig. 4). Indeed, fatty acids were able to

overcome the enhancement of proliferation caused by these compounds.

Although there have been several reports of the production of eicosanoids by lymphocytes (see[26,27] for references), lymphocytes do not appear to possess the enzymes of eicosanoid synthesis; it is considered that these compounds are produced by macrophages present in the cell preparation.[26,27] Indeed, removal of adherent cells, including macrophages, markedly decreased the ability of the lymph node cell preparation to produce eicosanoids. Removal of adherent cells did not affect either the response to Con A or the effect of fatty acids upon Con A-stimulated thymidine incorporation (Table 1). Similarly, fatty acids inhibited the Con A-stimulated proliferation of thoracic duct lymphocytes which are devoid of macrophages (Table 1). Thus, even in the absence of the capacity to synthesize eicosanoids, fatty acids still cause potent inhibition of lymphocyte proliferation.

This study provides convincing evidence that fatty acids do not inhibit lymphocyte proliferation by an effect which is dependent upon the synthesis of eicosanoids. How then do fatty acids affect this process? During culture of mitogen-stimulated lymphocytes specific changes in fatty acid composition and membrane fluidity occur;[33] these changes may be necessary for an optimal proliferative response. Lymphocytes readily incorporate fatty acids into their lipids,[34] and the presence of an excess of one fatty acid may result in accumulation of that particular fatty acid, leading to modification of plasma membrane fatty acid composition and an alteration of membrane fluidity. Such changes could cause the observed decreases in proliferation in response to mitogen and, clearly, are independent of eicosanoid metabolism.

## ACKNOWLEDGMENTS

P.C.C. is a Nuffield Medical Fellow. S.J.B. is a recipient of an S.E.R.C. training Award. We would like to thank Dr S. V. Hunt for continued interest and helpful advice and Mr S. Simmonds, Sir William Dunn School of Pathology for providing thoracic duct lymphocytes.

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