Suppression of Interleukin 2-dependent Human T Cell Growth In Vitro by Prostaglandin E (PGE) and Their Precursor Fatty Acids

Evidence for a PGE-independent Mechanism of Inhibition by the Fatty Acids

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Abstract

PGE represent oxygenation products of polyunsaturated essential fatty acids and are important regulators of cell-mediated immune responses. Because oils enriched in such fatty acids reduce inflammation and tissue injury in vivo, we examined the effects of these PGE precursors on IL-2-driven growth of human T lymphocytes. Dihomogamma linoleic acid (DGLA), AA, and their metabolites (PGE_1 and PGE_2, respectively) strongly inhibited short- and long-term growth of IL-2-dependent T cell cultures; EPA was much less inhibitory and its product, PGE_3, failed to suppress IL-2 responses. Short-term pretreatment of the cells with DGLA or AA and removal of the fatty acids before the proliferation assay resulted in a smaller reduction in [^{3}H]TdR incorporation. PGE and fatty acids did not alter the number of high affinity IL-2 binding sites on the T cell cultures but reduced the percentage of cells expressing CD25 and HLA class II molecules. No PGE was detected in supernatants from the fatty acid-treated cultures. Moreover, indomethacin, a cyclooxygenase inhibitor, did not reverse the antiproliferative effects of the fatty acids. Together, these findings indicate that fatty acids can inhibit IL-2-driven T cell growth via a PGE-independent mechanism and might be relevant to inflammatory diseases associated with persistent T cell activation. (*J. Clin. Invest.* 1990. 85:424–432.) autoimmunity • dihomogammalinolenate • eicosanoids • inflammation • lymphokines

Introduction

Abundant experimental evidence supports the view that PG, thromboxanes, and leukotrienes, collectively termed eicosanoids, participate in development and regulation of immunological and inflammatory responses (1–6). E-series PG (PGE), in particular, are capable of suppressing diverse T cell functions, such as IL-2 production, proliferation, and cytotoxicity (1–5, 7). PGE are oxygenation products of polyunsaturated fatty acids, which are classified under two groups: the omega 6 (*n*-6) group is derived from linoleic acid (LA),[1] which can be converted via gamma linolenic acid (GLA) and dihomogamma linolenic acid (DGLA) into arachidonic acid (AA) (Fig. 1). The parent substance of the omega 3 (*n*-3) group of essential fatty acids is alpha linolenic acid (ALA) which can be converted to the longer chain, more highly saturated, eicosapentaenoic acid (EPA). DGLA, AA, and EPA are oxidized by means of a cyclooxygenase pathway to form PGE_1, PGE_2, and PGE_3, respectively (Fig. 1).

Several lines of evidence indicate that dietary fatty acids have antiinflammatory effects. We have shown that enrichment of diets with DGLA and EPA reduces inflammation and tissue injury in animal models (8). Dietary supplementation with fish oil, rich in EPA, and plant seed oils rich in GLA (precursor to DGLA), reduces inflammation and pain in patients with RA and with psoriasis (9–11). Very recently, Endres et al. (12) have shown that the synthesis of IL-1 and tumor necrosis factor, principal polypeptide mediators of inflammation, can be suppressed by dietary supplementation with fish oil. The immunoregulatory effects of dietary fatty acids are thought to occur through their oxygenation products, the eicosanoids (13). However, fatty acids constitute an important component of the cell membrane structure and confer upon membranes properties of fluidity and flexibility that are key determinants of the behavior of enzymes and receptors. Many of these functions are independent of the role of essential fatty acids as eicosanoid precursors. Most studies on the effects of fatty acids on lymphocyte function have been done with animal cells and have led to contradictory results. In human lymphocytes, AA, LA, and DGLA were shown to either enhance or suppress mitogen-induced [^{3}H]TdR incorporation depending on their concentration (14). To our knowledge, no information is available on the effects of fatty acids on the IL-2-driven proliferative phase of preactivated human T cells. In this report, we demonstrate that (*a*) DGLA, AA and their PGE derivatives suppress proliferation of IL-2-dependent human T lymphocytes, and (*b*) the inhibitory activity of the fatty acids is direct and does not require conversion to their metabolites. These findings indicate that small changes in the essential fatty acid profile of human T cells might have profound effects on their growth and function, regardless of the production of PGE in the system.

Methods

Establishment of IL-2-dependent T cell cultures. Peripheral blood mononuclear cells from healthy donors were separated on a Ficoll-Hypaque gradient, and incubated at 37°C in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (RPMI-FBS), glutamine, antibiotics and 1% phytohemagglutinin (PHA; Burroughs Wellcome, Greenville, NC). After 3 d, cells were washed extensively and expanded in the presence of recombinant human (rh) IL-2 (Amgen Biologicals, Thousand Oaks, CA). The established IL-2-dependent T cell lines were maintained in culture by biweekly addition of

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1. *Abbreviations used in this paper:* ALA, alpha linolenic acid; DGLA, dihomogamma linolenic acid; EPA, eicosapentaenoic acid; GLA, gamma linolenic acid; LA, linoleic acid; MS, multiple sclerosis; OA, oleic acid; PA, palmitic acid; rh, recombinant human.

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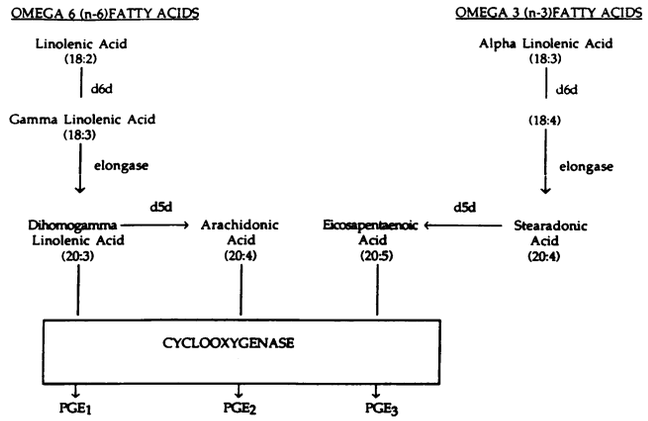

Figure 1. Metabolic pathways of essential fatty acids. The pathway is one of progressive desaturation alternating with elongation (addition of 2 carbons). d5d, delta 5 desaturase; d6d, delta 6 desaturase.

medium and of rhIL-2. Expression of T cell markers and activation antigens was monitored at various intervals, as described below.

Chemicals. The following reagents were purchased from Sigma Chemical Co., St. Louis, MO: PGE_1, PGE_2, PGE_3, $PGF_{2\alpha}$; DGLA, GLA, LA, AA, EPA, oleic acid (OA), palmitic acid (PA), and indomethacin. All the agents were dissolved in absolute ethanol and diluted in RPMI 1640 medium. The final concentration of ethanol in the cultures was $\leq 0.4\%$. The presence of this level of ethanol in control cultures did not affect their viability nor ability to proliferate in response to IL-2.

Proliferation assay. The effects of PG and fatty acids on the short-term proliferation of IL-2-dependent T cell lines were determined by measuring [^{3}H]TdR incorporation in cells seeded at $5\text{-}15 \times 10^4$/well in 96-well microtiter plates (Falcon, Becton Dickinson, Oxnard, CA) in the presence of rhIL-2 (0.1 to 5 U/ml) and indicated concentrations of PG (1 ng to 10 μg/ml, corresponding to 2.8×10^{-9} to 2.8×10^{-5} M) or fatty acids (1–20 μg/ml, equivalent to 3–60 μM). The effects of indomethacin (10^{-6} M), either alone or in combination with fatty acids, were also evaluated. After 2 or 3 d incubation at 37°C, 2 μCi of [^{3}H]TdR (2 Ci/mmol) was added to each well and incorporation measured 7–18 h later (15). All data are presented as mean counts per minute from triplicate cultures.

In order to investigate whether the continuous presence of PGE or fatty acids was necessary for the antiproliferative activity, in some experiments IL-2-dependent T cells were incubated in the presence of the eicosanoids for only 16 h, then washed and cultured for 2 more d with rhIL-2 alone before addition of isotope.

Growth curve analysis. The effects of fatty acids on the long-term growth of IL-2-dependent T cell cultures were evaluated by seeding the cells in RPMI-FBS containing rhIL-2 (1 U/ml) in the presence or absence of fatty acids at the indicated concentrations, and counting viable cells (by erythrosin B dye exclusion test) every 3 to 4 d. At these times, cells were split or refed by adding fresh medium and rhIL-2 (1 or 2 U/ml). In some experiments, fatty acids were added only on the first day of culture and removed 3 d later; in other experiments, fatty acids were added at weekly intervals but never left in the cultures longer than 3 d.

Phenotypic characterization. Expression of T cell markers and activation antigens on IL-2-dependent T cell cultures incubated for 2–3 d with IL-2 in the presence and absence of PG or fatty acids was measured by indirect immunofluorescence using an Ortho Cytofluorograf cell sorter, as described (15). The following monoclonal antibodies (MAbs) were used: OKT3, OKT4, and OKT8 (Ortho Pharmaceutical, Raritan, NJ), anti-Leu-12 (Becton Dickinson, Mountain View, CA), anti-Tac and XD5.A11 (gifts of Drs. T. Waldmann, National Institutes of Health, Baltimore, MD, and S. Radka, Genetic Systems Corp., Seattle, WA, respectively). Fluorescein isothiocyanate-conjugated $F(ab')_2$ fragments of goat antibodies to $F(ab')_2$ fragments of mouse IgG (Cappel Laboratories, Cochranville, PA) were used as a second antibody. A fluorescence intensity threshold was established at which 99% of the control cells were negative. The mean fluorescence intensity was determined in a range with an upper limit of 200.

Binding assay. The possibility that incubation with PGE or fatty acids would downregulate specific IL-2 receptor numbers on T cell cultures was analyzed by binding assay. Cells were incubated for 2–3 d in rhIL-2 in the presence and absence of these agents, washed, incubated for 2 h at 37°C in medium alone, and tested for ability to bind ^{125}I-labeled rhIL-2 (1 U/ml) in the presence of increasing concentrations of unlabeled rhIL-2 (0–100 U/ml). Alternatively, the direct effects of PGE and fatty acids on the IL-2 binding ability were analyzed by performing the assay in the presence of ^{125}I-labeled rhIL-2 (1 U/ml) and various concentrations of the eicosanoids. The binding assay was performed in tubes that had been pretreated with 1% bovine serum albumin (BSA) for 2–16 h to minimize nonspecific binding. Cells were incubated at 1×10^6/tube (triplicate tubes) for 2 h at 4°C in 0.2 ml serum-free RPMI medium containing 1% BSA and 0.02% NaN_3. After incubation, the cells were washed twice and transferred to counting vials. Iodination of highly purified rhIL-2 was performed by a slight modification of the chloramine T method (16). The specific activity of ^{125}I-rhIL-2 was 68,000 cpm/4×10^{-4} μg.

PG analysis. Total PGE produced by IL-2-dependent T cells was measured by RIA (17) using an antibody (Advanced Magnetics, Cambridge MA) which does not discriminate PGE_1 from PGE_2. The sensitivity of the assay is $\sim$ 30 pg/ml, and the intraassay variation is $< 10\%$.

Fatty acid analysis. Uptake of fatty acids by IL-2-dependent T cell cultures was measured as follows: cells were rinsed with ice-cold PBS to preserve cellular fatty acids (18). Total cell lipid was extracted according to Cohen et al. (19) for total fatty acid analysis. [^{3}H]14:0 myristic acid was added to each sample to determine percentage recovery of the fatty acids in extracted samples. Fatty acid methyl esters were prepared for gas liquid chromatography using methylene chloride in methanol (20). The fatty acid methyl esters were analyzed utilizing a Supelco Wax 10 capillary column. The temperature program was set up from 150° to 250°C, and increased 10°C/min using a Varian 3700 instrument equipped with a flame ionization detector. Peaks were integrated and compared to external standards (Nucheck Prep, Inc., Elysian, MN) using a Hewlett-Packard 3390 A integrator. Intrasample variability is $< 2\%$.

Results

Dose-dependent inhibition of T cell proliferation. As measured in short-term [^{3}H]TdR incorporation assays with a number of human IL-2-propagated cultures, PGE_1 and PGE_2, but not PGE_3 nor $PGF_{2\alpha}$, inhibited T cell proliferative responses to rhIL-2 in a dose-dependent fashion (Fig. 2). Half-maximal inhibition was achieved with doses of PGE_1 and PGE_2 lower than 1 μg/ml. Their immediate precursors DGLA and AA were also potent inhibitors when tested against the same cell lines; doses of 5–10 μg/ml were adequate to induce 50% reduction of isotope incorporation (Fig. 2). In contrast, EPA, precursor to PGE_3, was much less inhibitory even at a concentration of 20 μg/ml (Fig. 2). Also LA and GLA, from which DGLA is formed, inhibited T cell proliferation, but less efficiently than DGLA (data not shown); monounsaturated (OA) and saturated (PA) fatty acids at 10 μg/ml did not have any effect on IL-2-driven T cell proliferation (data not shown).

The requirement for the continuous presence of PGE and fatty acids during the proliferation assay was investigated. When PGE_1, PGE_2, DGLA, or AA were added to cells with IL-2 for 16 h and then washed out before carrying out the proliferation assay in the presence of IL-2 alone (1 U/ml) for 2

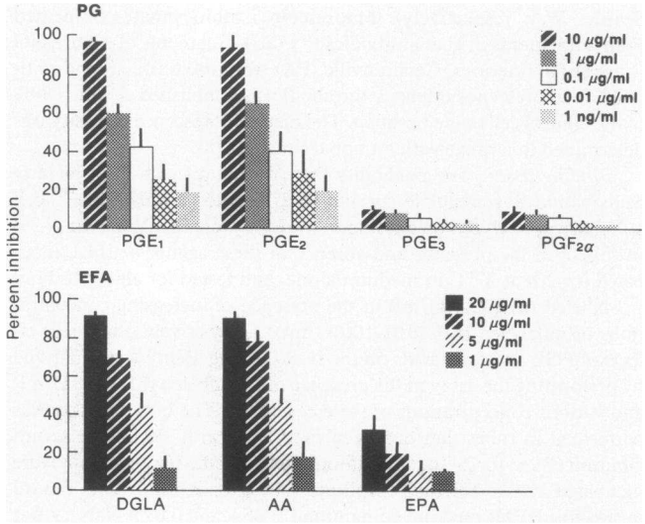

Figure 2. Dose-dependent inhibition of IL-2-dependent T cell proliferation induced by PG (1 ng to 10 μg/ml) and essential fatty acids (EFA) (1–20 μg/ml). These agents were present throughout the proliferation assay together with IL-2 (1 U/ml). Results indicate mean±SD from percent inhibition values obtained by testing three to five different IL-2-dependent target cell cultures.

more d, a substantial reduction in [^{3}H]TdR incorporation was still observed in all four experiments performed (Fig. 3). Pretreatment with EPA at 20 μg/ml caused < 15% inhibition and pretreatment with PGE_3 induced no inhibition at all (data not shown). In a single experiment (Fig. 4) in which cells were pretreated for 24 h with various concentrations of DGLA in the presence of 1 U/ml rhIL-2, washed extensively, and incubated further for 48 h in the presence of various doses of rhIL-2 (0.2 to 5 U/ml), reduction in [^{3}H]TdR incorporation was observed at all doses of IL-2 used. However, the inhibitory effects were about twofold higher in the culture in which DGLA had not been washed out. Thus, the continuous presence of fatty acids during the 3-d proliferation assay was not necessary for the inhibitory activity but was much more efficient than pretreatment and removal from the cultures.

Incorporation of fatty acids into IL-2-dependent lymphocytes. The ability of the fatty acids to inhibit IL-2-driven T cell proliferation was associated with their incorporation into cellular lipids. Fatty acid analysis of T cell cultures incubated with DGLA indicated a progressive dose-dependent enrichment of cells with the fatty acids added in vitro. When DGLA was added at 10 μg/ml, the percent total cellular fatty acid

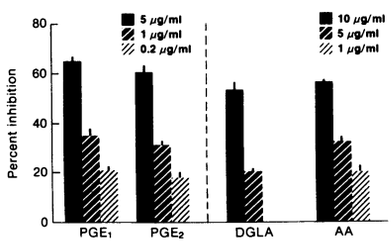

Figure 3. Inhibition of IL-2-dependent T cell proliferation (mean±SD from four experiments) induced upon a short-term (16 h) pretreatment of the target cells with PGE and fatty acids. After washing out these agents, rhIL-2 (1 U/ml) was added and isotope incorporation measured 2 d later.

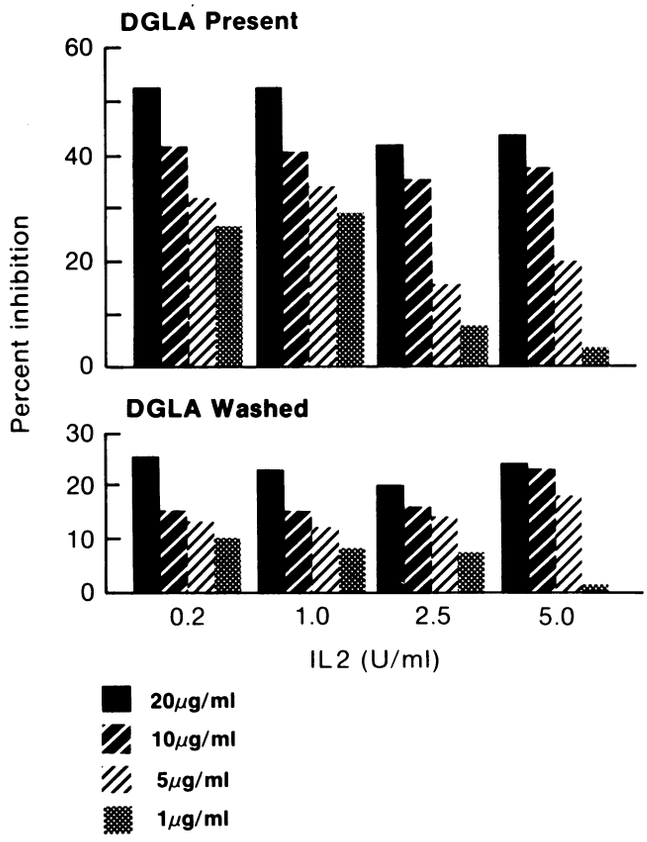

Figure 4. Inhibition of IL-2-dependent T cell proliferation induced by DGLA in cultures incubated with various doses of rhIL 2 (0.2 to 5 U/ml). DGLA was either added to the cultures for 24 h and washed out before a 2-d incubation in IL-2, or present together with IL-2 throughout the whole (3 d) incubation period.

increased ~ 2- to 10-fold between 2 and 48 h compared to the untreated cultures (Table I). With DGLA at 1 μg/ml, instead, there was a maximum incorporation of only 2.3-fold over control cultures at 48 h (Table I). Similar results were obtained in two more experiments using AA (data not shown).

Evidence for a direct antiproliferative effect of the fatty acids. The possibility that the inhibitory activity of DGLA and AA was mediated by conversion into their PGE products rather than being a direct effect of the fatty acids was investigated. As measured by RIA, no PGE was detectable in the supernatants of T cell cultures incubated for 3 d either in IL-2 alone (untreated) or in the presence of fatty acids even at the highest concentration (20 μg/ml) tested. Despite the absence of measurable PGE production, we tested the ability of indomethacin (10^{-6} M), a cyclooxygenase inhibitor, to affect the antiproliferative effects of the fatty acids. Results from proliferation assays carried out in the presence of various concentrations of fatty acids alone and in combination with indomethacin indicated that this agent blocked PGE production completely (not shown) but did not affect significantly the antiproliferative effects of DGLA and AA (Table II). These data could be reproduced in five out of five experiments performed.

Inhibition of long-term IL-2-dependent T cell growth. The long-term antiproliferative effects of the fatty acids on T cell cultures were assessed by adding these agents to cells at subop-

Table I. DGLA as Percent Total Cellular Fatty Acids Incorporated by IL-2-dependent Lymphocytes

Incubation period	Treatments		
	None	DGLA, *1 μg/ml*	DGLA, *10 μg/ml*
h			
0	3.6±0.6*	3.4±0.5	3.5±0.8
2	4.6±0.7	4.8±0.6	9.6±1.1
6	4.0±0.7	6.4±0.7	14.8±1.4
24	3.8±0.6	7.2±1.0	25.4±2.1
48	3.7±1.0	8.7±1.2	38.1±2.9

* Mean±SD from three experiments.

timal doses and monitoring cell counts and viability biweekly. At these times, fresh rhIL-2 and medium were added to keep cell concentrations in control cultures between 5 and 7×10^5/ml. Although Fig. 5 presents the results obtained with T cell cultures from two donors, similar results were obtained with IL-2-dependent cells from the peripheral blood of another healthy donor and the synovial fluid of two RA patients (data not shown). When DGLA, AA (both at 5 μg/ml) and EPA (10 μg/ml) were added only at the start of the cultures and removed 3 d later, inhibition of cell growth without significant changes in viability was observed within the first week of culture (Fig. 5, *top*); the DGLA- and EPA-treated cells recovered partially thereafter and kept dividing at a rate similar to that of control cells, whereas the AA-treated cultures ceased dividing, and by the end of the 2nd wk had a very poor viability. PGE_1

Table II. Effects of Indomethacin on Fatty Acid–induced Inhibition of T Cell Proliferation

Fatty acid	μg/ml	Indomethacin 10^{-6} M	[^{3}H]TdR Incorporation	
			cpm	% Inhibition
None		−	7,696*	
DGLA	1	−	6,670	13
		+	7,349	5
	5	−	3,835	50
		+	4,647	40
	10	−	1,891	76
		+	2,477	68
	20	−	520	93
		+	598	92
AA	1	−	6,920	10
		+	6,981	9
	5	−	4,845	37
		+	5,084	34
	10	−	2,565	67
		+	2,735	65
	20	−	786	90
		+	981	87

T cells cultured for 14 d in IL-2 were incubated at 10^5/well in microplates in the presence of rhIL-2 (1 U/ml), fatty acids, and indomethacin. Isotope incorporation was measured 3 d later.
* Mean from triplicate cultures.

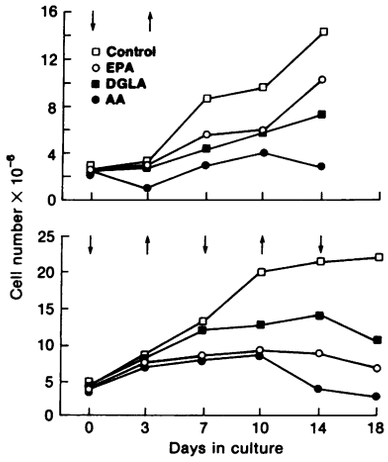

Figure 5. Effects of fatty acids on the long-term growth of IL-2-dependent T cells. Two different IL-2-dependent T cell lines were cultured in the presence and absence (control cells) of DGLA (5 μg/ml), AA (5 μg/ml), or EPA (10 μg/ml). Cells were monitored for number and viability, and refed with RPMI-FBS and rhIL-2 (1–2 U/ml) every 3 to 4 d. Fatty acids were either added only on the first day of incubation (top) or at weekly intervals (bottom). Down and upwards arrows indicate the days in which fatty acids were added to or removed from the cultures, respectively.

and PGE_2 (1 μg/ml) reduced T cell growth dramatically within 3 d and rendered the cells unable to divide thereafter. A similar result was observed upon removal of IL-2 from control cultures on day 3 (data not shown).

Periodic treatment with fatty acids induced inhibition of long-term T cell growth more efficiently than did one exposure only. A representative experiment in which fatty acids were added every 7 d, and each time removed 3 d later, is depicted in Fig. 5 (bottom). Similar data were obtained using T cell cultures from two more donors. Despite its weak inhibitory effects in short-term assays (Fig. 3), EPA at 10 μg/ml inhibited long-term growth as efficiently as the other fatty acids at 5 μg/ml. DGLA at 10 μg/ml inhibited T cell growth at a faster rate than at 5 μg/ml (data not shown). The presence of fatty acids at the concentrations used did not seem to induce toxic effects, as we never observed a marked increase in the number of cells taking up vital dyes shortly after treatment. Rather, the viability of the fatty acid-treated cells decreased gradually (within 10 to 14 d) as a consequence of diminished capacity to divide.

Effects of PGE and fatty acids on the IL-2 binding ability of the T cell cultures. We examined the possibility that the observed antiproliferative effects of PGE and fatty acids were due to a modulation of the number or affinity of IL-2 binding sites on the treated cells. T cell cultures previously propagated in IL-2 for at least 10 d were incubated in IL-2 (1 U/ml) in the presence and absence of DGLA and AA (10 μg/ml) for 3 more d and then tested for ability to bind ^{125}I-labeled rhIL-2 in the presence of increasing concentrations of unlabeled rhIL-2. Results (Table III) showed that the radioactivity bound by control cells was very similar to that bound by cells cultured with the fatty acids, and that the same dose of cold rhIL-2 (0.05 U/ml) was adequate to specifically displace 50% binding. Scatchard analysis revealed no difference between control and treated cultures in both the number of IL-2 binding sites (400–500 per cell) and the dissociation constant (K_d = 3.7 to 4.3×10^{-12} M; $r - 0.98$). Similar results were obtained when IL-2-dependent T cells had been incubated with PGE_1 (1 μg/ml) or GLA (10 μg/ml) (data not shown). These observations indicate that incubation of cells with PGE or fatty acids

Table III. Effects of Fatty Acids on the IL-2 Binding Ability of IL-2-dependent T Cells

Unlabeled IL-2 (U/ml) present during the binding assay	Cells precultured in the presence of		
	Medium alone	DGLA	AA
0	2,863±75*	2,705±59	2,991±101
0.01	2,447±53	2,304±48	2,567±77
0.05	1,568±48	1,269±35	1,432±48
0.1	968±67	909±33	1,019±51
0.5	372±24	401±41	505±33
1	150±18	146±27	162±50
10	39±5	47±11	51±14
100	5±1	<1	7±2

IL-2-dependent T cells were incubated in medium containing IL-2 and DGLA or AA (10 μg/ml). Control cultures contained no fatty acid. After 3 d, cells were harvested, washed and tested for ability to bind ^{125}I-labeled IL-2 (1 U/ml) in the presence of increasing concentrations of unlabeled IL-2.

* Values represent the mean cpm±SD of ^{125}I-labeled IL-2 bound during a 2-h incubation at 4°C in the presence of unlabeled IL-2 (0–100 U/ml) (1×10^6 cells/tube, triplicate tubes).

does not alter the number of IL-2 binding sites per cell nor the apparent affinity of these sites.

Another approach was to carry out binding assays in the presence and absence of various concentrations of PGE or fatty acids (as competitors) using IL-2-propagated T cells, which had not been pretreated with these compounds. Results in Table IV show that the counts per minute bound were very similar regardless of the dose of PGE_1 or fatty acids used, and were not statistically different from control values (medium alone).

Table IV. Effects of PGE_1 and Fatty Acids on the IL-2 Binding Ability of IL-2-dependent T Cell Cultures

Binding assay in the presence of	μg/ml	^{125}I-labeled IL-2 bound
Medium alone		1,597±34*
PGE_1	0.1	1,512±81
	1	1,715±45
	10	1,806±13
DGLA	1	1,391±53
	5	1,310±47
	10	1,410±38
	20	1,836±10
AA	1	1,699±13
	5	1,319±58
	10	1,374±61
	20	1,410±24
EPA	1	1,343±37
	5	1,561±25
	10	1,318±41
	20	1,948±11

IL-2-dependent T cells (1×10^6/tube) were incubated for 2 h at 4°C in the presence of ^{125}I-labeled IL-2 (1 U/ml) and various concentrations of PGE_1 or fatty acids.

* Mean cpm±SD from triplicate tubes.

Effects of PGE and fatty acids on expression of T cell markers and activation antigens. To identify the T cell subset affected by the antiproliferative activity of PGE and fatty acids and to investigate the effects on expression of activation antigens, FACS analysis was performed on IL-2-propagated T cells after 72 h incubation in the presence of rhIL-2 and these agents. Table V presents data from an experiment in which the effects of PGE_1, PGE_2, and the precursor fatty acids were examined all together on one T cell culture. Similar results were obtained in two more experiments in which different IL-2-dependent T cell lines were tested. The T cell profile of the IL-2-dependent cells was confirmed by the total absence of the B lymphocyte antigen Leu-12, and the presence of close to 100% $CD3^+$ cells in the control cultures containing 0.4% ethanol (Table V). Control cells incubated in IL-2 without ethanol expressed the same reactivity for all of the MAbs used as control cultures incubated in IL-2 and 0.4% ethanol (data not shown). Treatment with high concentrations of PGE_1, PGE_2, and especially DGLA, reduced the percentage of $CD3^+$, $CD4^+$, and $CD8^+$ cells in the cultures; AA, at 20 μg/ml, reduced only the percentage of $CD4^+$ cells, whereas EPA did not affect the expression of any T cell marker (Table V). When used at high doses, both PGE and all three fatty acids had inhibitory effects on the expression of activation antigens, such as the anti-Tac (CD25) and HLA class II molecules. The percentage of $CD25^+$ cells was particularly reduced on cultures treated with DGLA even at 5 μg/ml (Table V).

Discussion

Human T lymphocytes can be activated by interaction with different stimuli such as antigens, mitogenic lectins, and antibodies directed to cell surface membrane structures, the best-characterized of which are the CD3-TCR complex and the CD2 (T11, sheep E receptor) protein (21, 22). During activation, T cells acquire a number of cell surface glycoproteins some of which (IL-2, transferrin and insulin receptors, 4F2 and EA.1 antigens) appear early, even before DNA synthesis, and others (HLA-DR, CB.1, Ta-1, TLiSA1, T10, and VLA-1) appear later (23–30). The activation process also stimulates the production and secretion of IL-2 (31), which is accompanied by the upregulation of the Tac (CD25) antigen (32), and the ultimate expansion of sensitized T lymphocytes (33). The progressive loss of high affinity IL-2 receptors leads to termination of T cell growth in vitro.

Activated T cells appear to have a central role in the pathogenesis of many autoimmune and inflammatory diseases. Although measurements of IL-2 in RA patients have produced conflicting results (34), excessive production of IL-2, as well as B cell activation and high levels of soluble IL-2 receptors, have been observed in these patients (35, 36) and in patients with multiple sclerosis (MS) (37) and systemic lupus erythematosus (38). IL-2 responsive activated T cell clones have been described at the site of tissue injury in patients with RA (39, 40), MS (41), and pulmonary sarcoidosis (42). Thus, downregulation of IL-2 dependent T cell proliferation by benign means might be a useful therapeutic maneuver in these patients. Indeed, gold sodium thiomalate, in concentrations attainable during chrysotherapy for RA, significantly inhibits the proliferative responses of cultured human T cells stimulated by IL-2 (43).

Table V. Effects of PGE_1, PGE_2, and Fatty Acids on Surface Marker Expression of IL-2-dependent T Cell Cultures

		IL-2-dependent T cells incubated in medium containing*:									
			PGE_1		PGE_2		DGLA		AA		EPA	
	Specificity (CD group)	Ethanol 0.4%	1‡	5	1	5	5	20	5	20	5	20
OKT3	Mature T lymphocytes (CD3)	97§	93	80	95	82	89	57	95	93	97	97
		(162)	(139)	(116)	(150)	(112)	(143)	(93)	(145)	(118)	(161)	(156)
OKT4	Helper/inducer T cells (CD4)	43	35	18	38	13	25	13	41	26	42	43
		(92)	(85)	(67)	(78)	(68)	(90)	(81)	(80)	(64)	(94)	(81)
OKT8	Suppressor/cytotoxic T cells	60	54	44	57	53	51	26	55	53	58	56
	(CD8)	(167)	(164)	(106)	(180)	(119)	(162)	(86)	(158)	(138)	(178)	(172)
Anti-Leu-12	B lymphocytes (CD19)	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1
XD5.A11	HLA class II antigens	35	33	26	31	26	8	24	30	22	28	25
	(B cells, activated T cells,	(105)	(123)	(128)	(121)	(115)	(149)	(149)	(114)	(112)	(115)	(95)
	CFU-GEMM, monocytes)											
Anti-Tac	IL-2 receptor (CD25)	28	26	5	22	12	9	≤1	20	13	32	17
		(54)	(78)	(80)	(86)	(72)	(89)		(69)	(67)	(53)	(70)

* IL-2-dependent T cell cultures were maintained for 3 d in medium containing IL-2 and PG (1 and 5 μg/ml) or fatty acids (5 or 20 μg/ml). Control cells were incubated in medium containing 0.4% ethanol. ‡ μg/ml. § Percentage of cells reactive with each MAb as determined by indirect immunofluorescence using an Ortho Cytofluorograf cell sorter. In parentheses, the mean of fluorescence intensity in a range with an upper limit of 200.

We have recently described the ability of DGLA and AA, and to a lesser extent of EPA, to inhibit IL-2 production by mitogen-stimulated human T cells via a PGE-independent mechanism (44). Here, we demonstrate that these essential fatty acids can also inhibit IL-2 dependent human T cell proliferation and long-term growth without conversion into their cyclooxygenase pathway metabolites. In fact, little or no PG was produced by fatty acid-treated T cell cultures as compared to the untreated cultures, and indomethacin, a PG synthetase inhibitor, could not reverse the antiproliferative effects of the fatty acids. Controversy exists regarding the capacity of human T lymphocytes to produce PGE: studies by Goldyne and Rea (45) indicate that stimulated T lymphocytes and T cell lines do not release PGE_2, whereas Aussel et al. (46) reported that Jurkat cells are capable of PGE_2 synthesis. In our experiments, when PGE_2 measurements were corrected for PGE_2 in the medium and for the cross-reactivity of the anti-PGE antibody with AA (0.015%), no PGE production by the T cell cultures could be detected. Thus, the effect of the fatty acids on IL-2 dependent T cell proliferation is direct and not due to conversion to PGE. Between 2 and 48 h treatment, we demonstrated a rapid rate of incorporation of these fatty acids into cellular lipids, which could account for the reduced ability of fatty acid-treated T cells to respond to IL-2 even after a short-term exposure to the compounds and their removal from the cultures. However, both DGLA and AA and their metabolites inhibited IL-2-dependent T cell growth more efficiently when they were either present throughout the short-term proliferation assays or added weekly to long-term cultures, thus demonstrating the ability of such cultures to repristinate a normal IL-2-responsiveness upon removal of the eicosanoids.

The effects of PGE_2 on T cell proliferation have been studied extensively (47–58). This agent induces a profound inhibition of T-lymphocyte activation and proliferation in mitogen-stimulated cells (47, 48). The phenomenon is associated with (*a*) inhibition of IL-2 production (49–56), IL-2 receptor (54, 55) and transferrin receptor (55, 56) expression, (*b*) transient increase in intracellular levels of cyclic adenosine monophosphate (cAMP) (57, 58), and (*c*) modulation of protein kinase C activation pathway which prevents the increase in intracellular Ca^{2+} (56). The latter event occurs very early after mitogenic activation and precedes IL-2 and IL-2 receptor synthesis. All these observations point to the conclusion that PGE_2 interferes with early signal transduction mechanisms. In the system of T cell activation triggered by anti-CD3 MAbs (54), it was shown that PGE_2 added simultaneously with the MAb inhibits T cells at an early step of the activation process in a direct fashion and not through suppressor T cells nor through inhibition of accessory cell function as previously indicated by others (50). However, PGE_2 even at high doses (up to 3×10^{-6} M) did not affect IL-2-driven proliferation of T-cells after they had been preactivated with the anti-CD3 MAbs for 3 d (54). Here we show that PGE_1, PGE_2, and their precursors can inhibit both the short-term IL-2 proliferative responses and the long-term growth of cultures that are being propagated in this lymphokine for as long as 2 wk after the initial mitogenic stimulus. Thus, we conclude that these agents do not only inhibit at an early step of T cell activation but are also capable of suppressing the IL-2-driven proliferative phase of preactivated long-term T cell cultures.

To investigate the possibility that PGE and essential fatty acids interfere with the normal IL-2 binding ability of these T cell cultures, binding assays and immunofluorescence studies were performed. In agreement with a report by Chouaib et al. (55) in which T lymphocytes expressed IL-2 receptors normally after stimulation with either PHA or anti-CD3 in the presence of PGE_2, in our IL-2 propagated T cell cultures, high affinity binding to ^{125}I-labeled rhIL-2 was not affected by either preincubation with or addition of PGE and fatty acids. In another system where purified cells were incubated for only 24 h in the presence of PHA or anti-CD3 MAb (59), the simultaneous presence of PGE_2 and other reagents that increase in-

tracellular cAMP downregulated the cell membrane expression of CD25 and the concentration of CD25-specific mRNA. A marked reduction in the percentage of $CD25^+$ (Tac^+) T cells was also induced by fatty acids and their metabolites in our system dealing with IL-2-propagated cultures. The apparent discrepancy between our binding and immunofluorescence data showing, respectively, that PGE and fatty acids do not reduce the number of IL-2 binding sites but downmodulate the Tac molecule in the IL-2-propagated T cell cultures, can be explained based on the existence of three distinct affinities of IL-2 binding: low (Tac alone), medium (p70–75 alone), and high (the p70 + Tac dimeric complex) (60, 61). The anti-Tac MAb binds both the high and low affinity receptors, and not the p70–75 receptor for which MAbs are being developed. As one would normally expect from T cells that are maintained in IL-2 for 10–14 d after activation, the IL-2-propagated T cell cultures in our study expressed a relatively low number (400–500) of high affinity ($K_d \sim 4 \times 10^{-12}$ M), and, likely, a high number of low affinity IL-2 receptors. Therefore, our results suggest that fatty acids and PGE affect the expression of low affinity (Tac^+) binding sites only. Because it is currently accepted that the high affinity IL-2 receptor (formed by the association between p70–75 non-Tac molecule with Tac) functions in signal transduction for lymphocyte proliferation (62–69), we conclude that essential fatty acids and their metabolites do not inhibit IL-2-dependent T cell growth by interfering with the expression and binding of functional IL-2 receptor molecules.

Thus, the precise mechanism by which these agents suppress the in vitro growth of IL-2-dependent T cells remains to be elucidated. Since DGLA (three double bonds) and AA (four double bonds) have a similar capacity to suppress IL-2-driven T cell proliferation, and both fatty acids are more potent in this regard than EPA (five double bonds), it is not their degree of unsaturation per se which accounts for the antiproliferative effect of the fatty acids. However, in the bilayer of cell membranes, the shorter chain unsaturates (such as OA) and the saturated fatty acids (such as PA) induce a high degree of order (70) whereas the longer chain fatty acids might be expected to introduce a disordering effect in the region near the phospholipid head group. Such a change might alter expression of surface molecules and/or the response of cells to mitogenic stimulation. Another mechanism by which essential fatty acids might inhibit IL-2-driven T cell growth would be through the induction of an inhibitory factor which is secreted into the medium, analogous to recent observations with glucocorticoids in murine T cells (71). In this context, it has been shown that PGE_2 produced by macrophages can activate PGE_2 receptor-positive lymphocytes to induce suppressor cells, and promote the secretion of soluble suppressor factors (72, 73). Alternatively, E-series PG and their precursors might be involved in IL-2-mediated pathways of lymphokine secretion; in support of this, would be the recent observation that PGE_2 can act synergistically with IL-2 to induce granulocyte macrophage colony stimulating factor secretion by certain murine T helper clones while simultaneously inhibiting their proliferation (74).

Overall, our data indicate that PGE_1, PGE_2, and their immediate precursor fatty acids have potent suppressive effects on the IL-2-driven proliferative phase that follows T cell activation. It is very likely that the concentrations of fatty acids used in these in vitro studies can be obtained in vivo. For example, the concentration of DGLA in plasma from a volunteer who had ingested 1.4 g GLA/d (12 capsules of borage seed oil/d) for 4 wk was found in our studies (DeMarco, D., R. B. Zurier, unpublished observations) to be 34.4 μg/ml. Moreover, daily doses of GLA of 540 mg (9) to 1.1 g (75) appear to have a beneficial effect in patients with RA. These data indicate that minimal alteration of dietary fatty acids may have important effects on immune responses, especially in diseases in which uncontrolled T cell proliferation contributes (or leads) to overt autoimmune or inflammatory reactions.

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