

Prostaglandin E₂ control of T cell cytokine production is functionally related to the reduced lymphocyte proliferation in atopic dermatitis

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Past studies of peripheral blood mononuclear cells (PBMC) from patients with atopic dermatitis (AD) have demonstrated reduced proliferation. We have studied phytohemagglutinin-induced lymphocyte proliferation in the context of interleukin-4 (IL-4), interferon-gamma (IFN- γ), and prostaglandin E₂ (PGE₂) production in cultures of PBMC from patients without and with AD. The proliferation index was found to correlate proportionally to IFN- γ production and inversely to T-cell IL-4 and monocyte PGE₂ production. Assays in parallel cultures showed significantly increased PGE₂ production by purified AD monocytes. The proliferation index in PBMC from persons with AD was significantly reduced compared with normal PBMC. This difference was normalized in the presence of extrinsic IFN- γ but exaggerated when IL-4 was added. Increased AD monocyte production of inflammatory factors (e.g., PGE₂) and cytokines appears to increase IL-4 production by Th2 while suppressing IFN- γ production by Th1. Restoration of the normal proliferation of PBMC by the addition of IFN- γ may represent one mechanism for the clinical efficacy of IFN- γ treatment of AD. (J ALLERGY CLIN IMMUNOL 1996;97:85-94.)

Key words: Prostaglandin E₂, T cell, cytokine, proliferation, atopic dermatitis

The transformation, thymidine uptake, and proliferation of lymphocytes formed the basis for early laboratory measurements of immune responsiveness. One of the first in vitro immunologic studies of atopic dermatitis (AD) demonstrated a marked reduction in the phytohemagglutinin (PHA)-stimulated lymphocyte transformation test in two patients during severe exacerbations of their dermatitis.¹ This was confirmed by several other investigators,²⁻⁴ along with the observation that when the lymphocytes were held in culture for 48 to 96 hours before stimulation the response was normalized.⁴ The mechanism for this reversible, subnormal lymphocyte proliferative activity in AD remained unclear.

Abbreviations used

AD:	Atopic dermatitis
FCS:	Fetal calf serum
IFN- γ :	Interferon-gamma
IL-4:	Interleukin-4
LTC ₄ :	Leukotriene C ₄
PBMC:	Peripheral blood mononuclear cells
PBS:	Phosphate-buffered saline
PHA:	Phytohemagglutinin
PI:	Proliferation index
PGE ₂ :	Prostaglandin E ₂

Recent studies have suggested that eicosanoids may play a role in modulating the proliferation of T cells.^{5,6} Betz and Fox⁵ showed that prostaglandin E₂ (PGE₂) may act as an immunoregulator, suppressing murine type I CD4⁺ T cell (Th1) clonal proliferation and expression of interferon-gamma (IFN- γ). Another report suggested an indirect mechanism for PGE₂ regulation of lymphocyte proliferation in AD.⁷ We recently demonstrated that PGE₂ production is increased, and that this increase accounts for reduced IFN- γ production

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in cultured peripheral blood mononuclear cells (PBMC), in patients with AD.⁸ In the present study we examined lymphocyte proliferation in the context of interleukin-4 (IL-4) and IFN- γ and PGE₂ synthesis in 24-hour cultures of PBMC taken from persons without AD and patients with AD and show that the reduced lymphocyte proliferation results from IL-4 exposure and that this may be reversed by the addition of IFN- γ .

METHODS

Subjects

Blood donors called "normal" in this article had no personal history of atopic diseases and were taking no medication at the time of the study. Patients with AD were chosen according to well-defined criteria.⁹ All were adult patients with chronic, moderate-to-severe dermatitis who had used no systemic corticosteroids for at least 2 months and no topical corticosteroids for 1 week. Donors received no aspirin or nonsteroidal antiinflammatory agent for at least 1 week and consumed no beverage containing caffeine or methylxanthine for at least 12 hours before the morning blood drawing.

Leukocyte separation

PBMC were separated by means of Ficoll-Hypaque density gradient centrifugation (Histopaque; Sigma Chemical Co., St. Louis, Mo.). Cells recovered from the plasma-Ficoll interface were washed three times with normal saline solution and resuspended in medium RPMI-1640, supplemented with 10% heat-inactivated fetal calf serum (FCS).¹⁰ To obtain monocytes, 10 ml of PBMC suspension at 4×10^6 cells/ml was allowed to adhere in 16×100 mm petri dishes for at least 2 hours at 37°C. The nonadherent cells were decanted and washed three times with warm Gey's balanced salt solution (Gibco, Gaithersburg, Md.). The adherent monocytes were quantitated, after removal with a sterile rubber policeman, by Coulter counter and by total protein determination by using a BioRad kit (BioRad, Richmond, Calif.). This procedure yielded approximately 25×10^6 monocytes from 100×10^6 PBMC subjected to adherence harvesting, yielding more than 90% recovery of monocytes. The PBMC compositions, determined by means of Stat Stain (Volu-Sol; Logos Scientific, Henderson, Nev.), contained 10% to 40% monocytes, 60% to 70% lymphocytes, 2% or less polymorphonuclear leukocytes, and 5% or less platelets. The nonadherent cells were typically at least 95% CD₃⁺ lymphocytes. Cell viability, monitored by means of the trypan blue exclusion test, was always more than 98%. Monocyte purity in the adherent cell preparations, confirmed by alpha-naphthyl acetate esterase and Factor XIII immunoperoxidase staining, was 95% or greater.

T cells were separated, using T cell-recovery columns (Biotex Laboratories, Edmonton, Alberta, Canada). Briefly, nonadherent PBMC (10^8 cells/ml) were applied

to the columns, which were freshly pre-coated with goat antihuman serum. T cells were eluted by washing the columns with 20 ml of phosphate-buffered saline (PBS) solution, and B cells were retained in the columns. Purity of T cells was at least 97% CD₃⁺ lymphocytes with less than 3% B cells (detected by means of immunofluorescence after treating cells with fluorescein isothiocyanate-labeled human IgG) and less than 1% monocytes (confirmed by alpha-naphthyl acetate esterase staining).¹¹

Lymphocyte proliferation assay

PBMC, at 1×10^6 cells/ml, were cultured for 24 hours in RPMI 1640/10% FCS with or without PHA (0.01 to 10 μ g/ml; Gibco). We also carried out comparison cultures stimulated with anti-CD₃ (10 ng/ml; Hoffmann La-Roche, Nutley, N.J.), which was also used for stimulating cytokine production in PBMC cultures (see below). Eight hours before harvest, tritiated thymidine (1 μ Ci/ml) was added to cultures, cells were harvested by means of a MASH III Harvester (Microbiologics, Boston, Mass.), and thymidine uptake was quantitated by scintillation counting and expressed as counts per minute (cpm). Proliferative index (PI) was calculated by cpm stimulation divided by cpm in control cultures.

Cytokine production

Cytokine production was assessed in two different cell preparations, PBMC and purified T cells. PBMC, at 2×10^6 /ml, were incubated for 24 hours at 37°C in the presence of anti-CD₃, under identical conditions to the parallel proliferation assay. Cells were then spun, and supernatants were stored frozen for cytokine assays. Purified T cells were stimulated with phorbol myristate acetate plus calcium ionophore A23187, which directly activates T-cell cytokine production,^{8,12} in contrast to anti-CD₃, which requires antigen-presenting cells. Monocytes, the major PGE₂-producing cells in the PBMC preparations,⁸ must be eliminated from preparations to study PGE₂ effects on T cells. Purified T cells, at 2×10^6 cells/ml of RPMI medium, supplemented with 10% FCS, $\pm$ 500 ng/ml phorbol myristate acetate, and 10 ng/ml calcium ionophore A23187, were cultured for 24 hours at 37°C. PGE₂ (0.1 to 10 μ M/L) was added to some T-cell cultures to monitor its effect on cytokine production. Supernatants were harvested by pelleting cells at 700 g and stored at -70°C for cytokine assays. Adherent monocytes were similarly cultured at 1×10^6 /ml in 10% FCS/RPMI for spontaneous PGE₂ production.

Immunoassays

PGE₂ was assayed by means of radioimmunoassay using culture supernatants containing 0.05 mmol/L indomethacin as blank. PGE₂ antisera were produced in rabbits according to the method of Jaffe and Behrman.¹³ PGE₂ was conjugated to porcine thyroglobulin by the mixed anhydride method before immunization of the

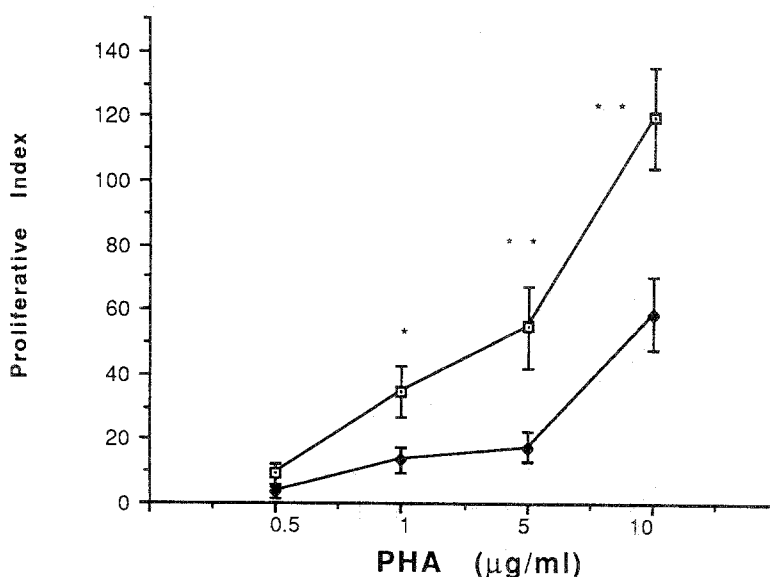


FIG. 1. Phytohemagglutinin-stimulated proliferation indices of normal and AD lymphocytes. Normal (open) and AD (solid) PBMC, in RPMI medium 1640/15% FCS, were incubated with PHA (0.5 to 10 μ g/ml) for 24 hours. Tritiated thymidine uptake was added for the last 8 hours. Values represent the mean $\pm$ SEM of three experiments (* p < 0.05; ** p < 0.01).

rabbits. At a dilution of 1:6000, the PGE₂ antiserum had a sensitivity of 10 pg/0.1 ml sample and the following cross-reactivities at B/B₀ 50%: PGD₂, 0.8%; PGF_{2 α} , 1%; PGF_{1 α} , 0.3%; PGE₁, 9.3%; 6-keto-PGF_{1 α} , 2.2%; and 6-keto-PGE₂, 2%, as previously reported.¹⁴ Labeled tracers ([³H] PGE₂) were obtained from NEN Research Products, Boston, Mass., and unlabeled standards (PGE₂ and LTC₄) were obtained from Cayman Chemical Company, Ann Arbor, Mich. Each assay was performed in duplicate according to standard protocols. The sensitivity of the radioimmunoassay measurement of PGE₂ was 1 pg/ml.

IFN- γ was measured by means of ELISA-protocol version 2.1 (kindly provided by Brian Paasch, Genentech, S. San Francisco, Calif.) Appropriate cell culture media were used as controls. Human recombinant IFN- γ (specific activity = 19) was kindly provided by Dr. Susan Kramer (Genentech) and dissolved in stock solutions of PBS with 0.1% bovine serum albumin. The sensitivity of the assay was 0.1 pg/ml. Results were expressed as units pg/ml supernatant.

The quantitative determination of human IL-4 was performed with ELISA kits from R & D System, Minneapolis, Minn., with minor modification of manufacturer's protocol. Briefly, 96-well polystyrene microtiter plates were coated with a monoclonal mouse recombinant anti-human IL-4 antibody (anti-*rh*IL-4). Supernatants from PBMC cultures or IL-4 standards were applied to the coated wells in duplicates and allowed to incubate for 2 hours at room temperature. After the plates were washed, polyclonal rabbit anti-human IL-4 conjugated to horseradish peroxidase was added and incubated for another 2 hours at room temperature.

Colorimetric quantitation was possible after reaction with peroxide and tetramethylbenzidine. A Microplate Auto-reader (Bio-Tek Instruments, Inc., Winooski, Vt.) was used for colorimetry. Standard curves were generated by the serial dilution of highly purified *rh*IL-4. The sensitivity of the assay was 5 pg/ml.

Statistical interpretation

Data are presented as mean $\pm$ SE. Statistical analyses were performed using Student's *t* test, for comparing subject groups. Data on cpm were log-converted to normalize distribution for *t* test determinations.

RESULTS

PHA-stimulated proliferation in normal and atopic PBMC

The effect of PHA was monitored by tritiated thymidine uptake and calculated as PI. Both AD and normal PBMC showed dose-dependent responses to stimulation by PHA over a concentration range from 0.5 μ g/ml to 10 μ g/ml (Fig. 1). The PI was lower for AD lymphocytes at all PHA concentrations, and the differences were significant at 1 μ g/ml (p < 0.05) and at 5 and 10 μ g/ml (p < 0.01, n = 3). These results were similar to those in our past studies using 72-hour culture systems.⁴ The differences between the mean PI values of normal and AD cells were not due to a higher level of basal activation. As may be seen from uptake data in Table I, there was no difference between unstimulated normal and AD preparations, but

TABLE I. Tritiated thymidine uptake (cpm) by peripheral blood mononuclear leukocytes from normals and patients with atopic dermatitis (AD) after 24-hour stimulation with phytohemagglutinin (PHA, 10 μ g/ml) or anti-CD₃ (10 ng/ml)

	Normal	AD	<i>p</i> Value
Control	515 465 1,119	331 316 1,014	
Mean $\pm$ SEM	699 $\pm$ 121	553 $\pm$ 132	0.101
PHA	65,263 67,662 12,253	22,794 28,765 46,522	
Mean $\pm$ SEM	85,156 $\pm$ 10,799	32,627 $\pm$ 4,113	0.0035
Anti-CD ₃	7,564 21,896 54,368	987 3,301 17,719	
Mean $\pm$ SEM	27,942 $\pm$ 799	7,336 $\pm$ 3,021	0.0273

TABLE II. Proliferation indices and cytokine production by PBMC from patients without (normal) and with AD. Values represent the mean $\pm$ SE of three experiments.

	Normal	AD	<i>p</i> Value*
Proliferation index			
Stimulated with PHA (10 μ g/ml)	122 $\pm$ 9.23	59 $\pm$ 13.3	0.023
Stimulated with anti-CD ₃ (10 ng/ml)	39.94 $\pm$ 12.7	13.25 $\pm$ 3.8	0.027
IFN- γ production (pg/ml)	1765 $\pm$ 372	765 $\pm$ 52	0.048
IL-4 production (ng/ml)	10.87 $\pm$ 1.2	24.4 $\pm$ 4.3	0.027

**p* values for differences between normal and AD cells are shown.

after stimulation with PHA, AD values were consistently much lower (mean 32,627 $\pm$ 4113 cpm) compared with normals (85,156 $\pm$ 10,799 cpm; p < 0.005). Because we have previously routinely used anti-CD₃ as a stimulant for cytokine production,^{8,12} we also tested its effect on lymphocyte proliferation and likewise found lower uptake in AD preparations (p < 0.05).

AD and normal IFN- γ and IL-4 production

We found significantly greater IL-4 production and decreased IFN- γ production in anti-CD₃-stimulated AD PBMC-cultured parallel to the proliferation assays described above (Table II). These results were consistent with our previous studies of anti-CD₃-stimulated cytokine production, which showed that IFN- γ was consistently lower and that levels of IL-4 were consistently higher in the AD cells.^{8,12}

Comparing cytokine production with proliferation indices, we found that the decreased PI in AD cells corresponded with decreased IFN- γ and increased IL-4 levels as shown by the significant

correlation coefficients generated by linear regression plots (r = 0.99 and r = 0.65, respectively; Fig. 2). These data suggested that the reduced proliferation of AD lymphocytes may relate to a deficit of IFN- γ -producing Th1 cells or to an inhibitory effect of excessive IL-4 produced by Th2 cells. We tested this relationship by adding these cytokines to the cultures and measuring proliferation.

Effect of cytokines on lymphocyte proliferation

PBMC cultures were incubated in parallel to those above for 24 hours with IFN- γ , 100 U/ml. This concentration was previously shown to have an optimal differential influence on AD and normal cells.¹⁵ Fig. 3 shows the effects of IFN- γ treatment on AD lymphocyte proliferation (superimposed on the untreated AD and normal proliferation results presented in Fig. 1). In the presence of IFN- γ , proliferation of AD cells was significantly increased at each PHA concentration above 0.5 μ g/ml (p < 0.05). The greatest increase occurred at 10 μ g/ml PHA where, as shown in Tables

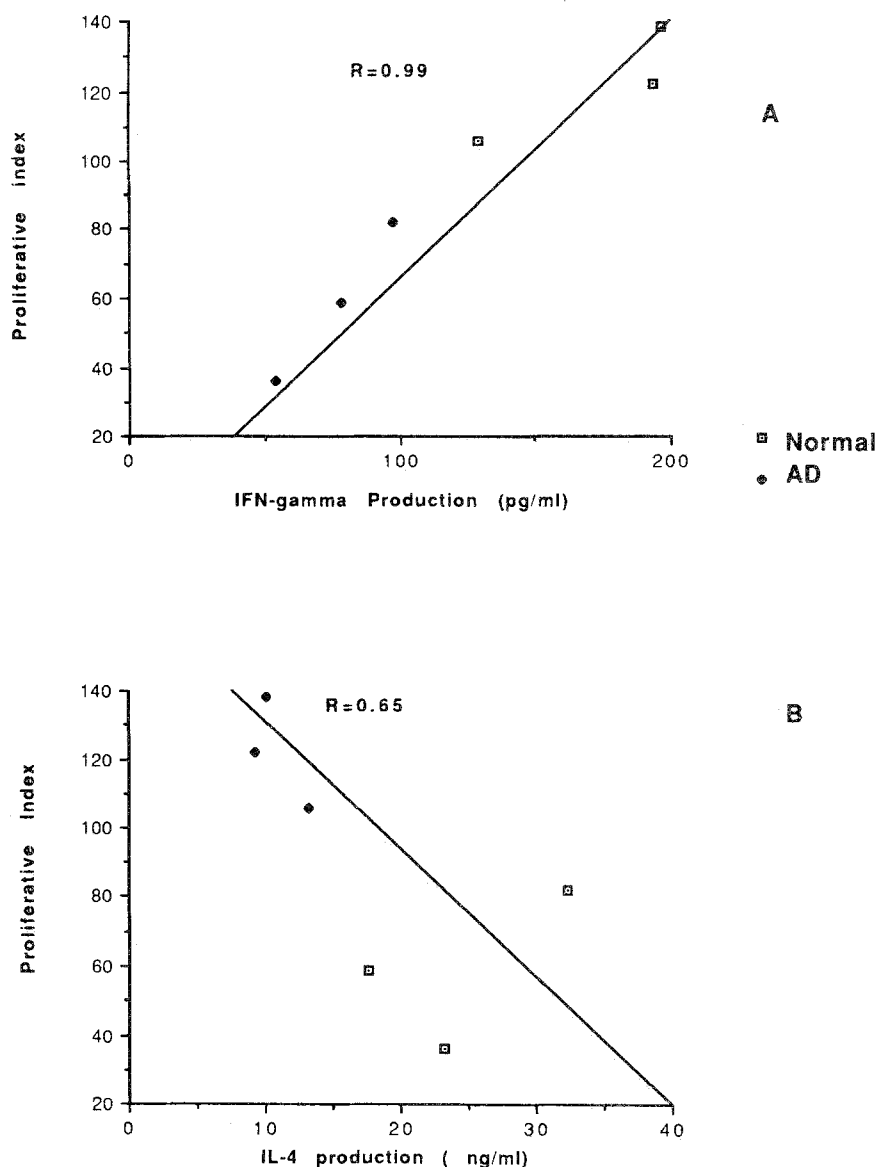


FIG. 2. Correlation between proliferative index and T cell cytokine production. Coefficient of correlation (r) was determined by linear regression plots for PI versus IFN- γ (A) and PI versus IL-4 (B) in normal (open) and AD (solid) PBMC cultures.

I and III, IFN- γ caused increased AD cell proliferation from $32,627 \pm 4113$ cpm to $(63,655 \pm 7893$ cpm; $p = 0.046$), approaching that of normal cells ($78,273 \pm 14,691$ cpm; $p = 0.103$). Comparing mean values in Tables I and III, IFN- γ had no effect on PHA-stimulated normal cells cultured in parallel ($p = 0.115$) or on unstimulated AD or on normal cell proliferation.

We next compared the effect of IL-4 (100 U/ml) on proliferation of PBMC cultures stimulated with the same 10 μ g/ml PHA dose (Tables I and III). IL-4 significantly reduced the AD proliferation from

$32,627 \pm 4113$ cpm (PI = 59 ± 13) to $12,166 \pm 1372$ cpm (PI = 22 ± 3), $p = 0.025$. As with IFN- γ , IL-4 had no effect on the proliferation of normal lymphocytes, which had mean PI values of 122 ± 9 and 157 ± 22 in the presence and absence of IL-4 ($p > 0.2$) (Fig. 4). As with IFN- γ , IL-4 alone in medium had no effect on PBMC proliferation (Table III). Thus in the presence of IL-4 the difference between AD and normal proliferation widened (Table III; $p = 0.006$), whereas IFN- γ caused normalization of AD proliferation.

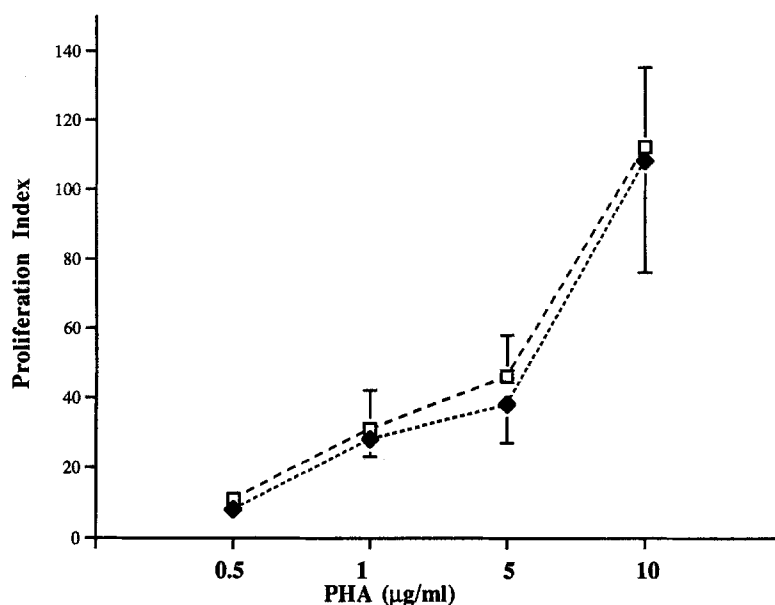


FIG. 3. Effect of IFN- γ on proliferation index. PBMC were incubated with varying concentrations of PHA (0.5 to 10.0 μ g/ml) in the presence of IFN- γ (100 U/ml). (Open squares = normal, closed squares = AD; * p < 0.05, comparing AD control to IFN- γ treated AD (n = 3); controls are shown in Fig. 1.)

TABLE III. Tritiated thymidine uptake (mean cpm $\pm$ SEM) by PBMC from normals and patients with AD after 24-hour stimulation with PHA (10 μ g/ml) and treatment with IFN- γ (100 U/ml) or IL-4 (100 U/ml). Untreated controls are the same as in Table I for comparison

	Normal	AD	<i>p</i> Values
Control	699 $\pm$ 121	554 $\pm$ 132	
IFN- γ	683 $\pm$ 71	578 $\pm$ 120	0.459 (normal)* 0.249 (AD)*
IFN- γ + PHA	78,273 $\pm$ 14,691	63,655 $\pm$ 7893	0.103†
IL-4	603 $\pm$ 56	590 $\pm$ 154	0.283 (normal)* 0.209 (AD)*
IL-4 + PHA	109,586 $\pm$ 889	12,166 $\pm$ 1372	0.00575†

*Compared with controls.

†Comparing normals with patients with AD.

PGE₂ Synthesis

Because of our recent finding that PBMC from patients with AD spontaneously produce increased PGE₂,⁸ we assayed for PGE₂ production in unstimulated 24-hour AD and normal monocytes. Purified monocyte cultures from AD donors showed significantly increased spontaneous PGE₂ synthesis compared with normal monocytes (p < 0.05; Table IV).

Effect of PGE₂ on T-cell cytokine production

As shown in Figs. 3 and 4, cytokines modulated proliferation in these 24-hour AD cell cultures. We considered that increased AD monocyte PGE₂

synthesis might account for abnormal proliferation by regulating intrinsic cytokine production in these cultures. Therefore we examined the effect of PGE₂ on cytokine production in 24-hour cultures of purified T cells, using PMA/ionophore to directly stimulate T cells in the absence of monocytes. The presence of monocytes may interfere with assessment of the extrinsic PGE₂ effect. Added PGE₂ (0.01 to 10 μ mol/L) inhibited IFN- γ production in a dose-dependent manner with a maximal effect at 10 μ mol/L, which was used in subsequent studies. PGE₂ significantly reduced the IFN- γ production level in both normal (p < 0.05) and AD (p = 0.002) T cell cultures (Fig. 5).

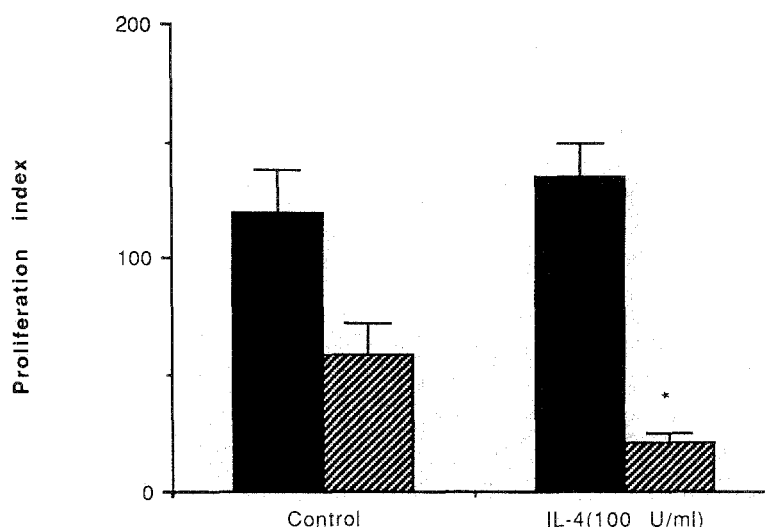


FIG. 4. Effect of IL-4 on proliferation index. Normal (solid columns) and AD (hatched columns) PBMC were incubated with IL-4 (100 U/ml) simultaneously with PHA (10 μ g/ml) or as described in Methods. The data are the mean $\pm$ SE of three experiments. PI of IL-4 treated PBMC from normal versus AD donors. (*Difference between IL-4 treated and untreated AD cells; * $p < 0.05$.)

We also assessed the PGE₂ influence on IL-4 production in these cultures. Fig. 6 shows the effects of PGE₂ on PMA/ionophore-stimulated IL-4 production in cultured AD and normal T cells. IL-4 production was significantly higher in AD cultures ($p = 0.0127$, $n = 7$). IL-4 production was further increased in AD cells ($p = 0.0478$) by PGE₂ (10 μ mol/L), but PGE₂ had no effect on normal IL-4 production. The T cells in four of these experiments were from the same donors as the monocytes shown in Table IV. The increased PMA/ionophore-stimulated IL-4 production from these purified T cells correlated closely with the high spontaneous PGE₂ production by the isolated monocytes ($r = 0.98$).

DISCUSSION

In this study we have demonstrated reduced PHA- and anti-CD₃-stimulated lymphocyte proliferation in 24-hour cultures of lymphocytes from patients with AD—responses similar to past results in cultures carried out for longer periods.^{1,4} The cause of this reduced proliferation has never been clear. Our findings again demonstrate no evidence of in vivo lymphocyte proliferative activation associated with AD. Both this and our previous study clearly show no increased basal proliferative activity by AD cells⁴; thus the decreased PI is not caused by a higher denominator value. Some have speculated that reduced IL-1 production by atopic mononuclear leukocytes might underlie the deficit. Studies have shown reduced in vitro IL-1 produc-

TABLE IV. Spontaneous PGE₂ production by monocytes from patients without (normal) and with AD

	Normal	PGE ₂ (pg/0.1 ml) AD	<i>p</i> Value*
Monocytes	65 $\pm$ 17	211 $\pm$ 89	0.026

Adherence purified monocytes ($n = 8$) were incubated in medium RPMI with 10% FCS for 24 hours. Values represent mean $\pm$ SE.

*Probability values for the difference between the normal and AD cells are shown.

tion by mononuclear leukocytes from persons with atopy.¹⁶⁻¹⁸ However, Thestrup-Pedersen et al.¹⁹ have shown that purified AD monocytes actually produce increased IL-1 levels, making an IL-1-related mechanism unlikely.

We next compared cytokine production with proliferative activities in the AD and normal cultures. Parallel 24-hour PBMC cultures stimulated with anti-CD₃ demonstrated reduced IFN- γ production by AD cells, consistent with other reports,^{8,20,21} and this correlated directly with the reduced proliferation. In contrast, the increased IL-4 production correlated inversely with proliferation in those cultures. Based on these correlations, we then added IFN- γ to the AD cell cultures and were able to significantly increase proliferation to that in the normal control cultures. One interpretation of these data is that the reduced proliferative index is due to a reduced growth response

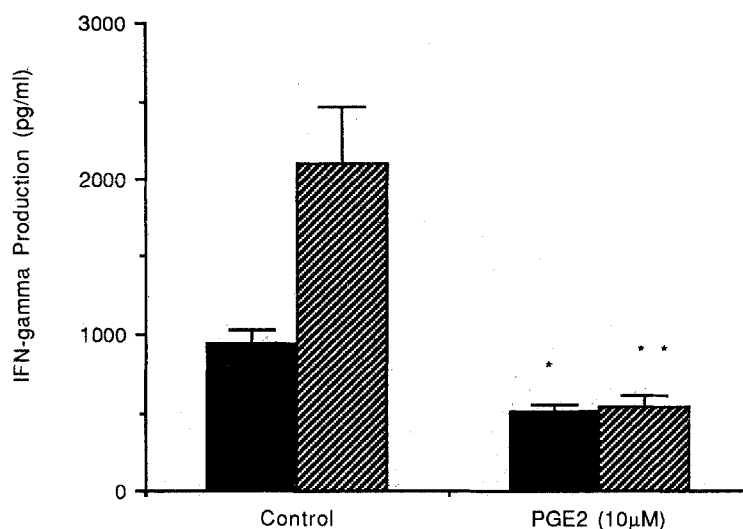


FIG. 5. Effect of PGE₂ on T cell IFN-γ production. Normal (solid columns) and AD (hatched columns) T cells at 2×10^6 cells/ml were stimulated with PMA (500 ng/ml) and calcium ionophore A23187 (100 ng/ml) in the absence or presence of 10 μmol/L PGE₂ for 24 hours. The values represent the mean $\pm$ SE of seven experiments. IFN-γ levels were determined by means of ELISA as described in Methods. (* $p < 0.05$, control vs PGE₂-treated normal cells; ** $p = 0.002$, control vs PGE₂-treated AD cells.)

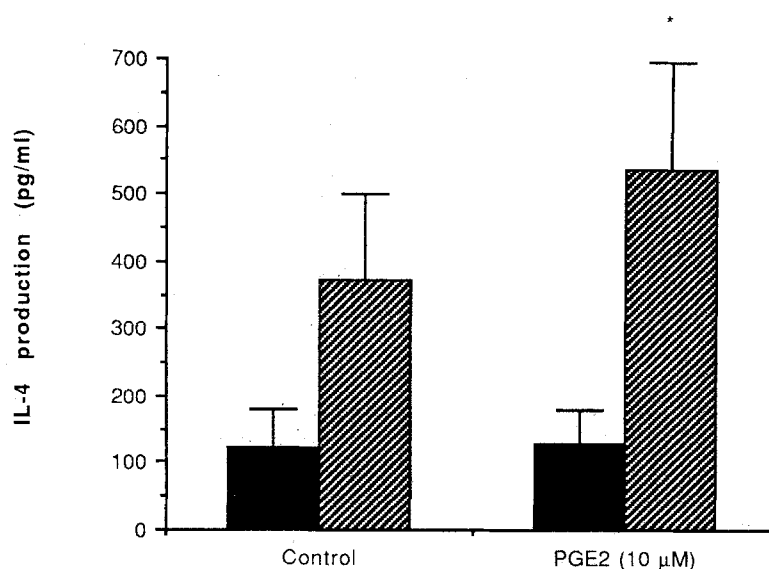


FIG. 6. Effect of PGE₂ on T cell IL-4 production. Normal (solid columns) and AD (hatched columns) T cells at 2×10^6 cells/ml were incubated with PMA (500 ng/ml) and calcium ionophore A23187 (100 ng/ml) $\pm$ 10 μmol/L PGE₂ for 24 hours. The values represent the mean $\pm$ SE of seven experiments. IL-4 levels were determined by means of ELISA as described in Methods. (* $p = 0.0478$, control vs PGE₂-treated AD cells.)

of Th1 cells. Addition of IFN-γ normalized atopic T-cell proliferation, whereas IL-4 reduced it, suggesting that the reduced proliferative index is due to reduced growth of Th1 cells and greater proportions of Th2 cells.

These findings may also indicate a mechanism by which human recombinant IFN-γ affects clinical improvement when given to patients with severe AD.²² Increasing proportions of IFN-γ-producing Th1 cells might correct a disproportionate Th2

influence and improve cellular immune responses that are otherwise reduced in AD.^{1,2} It might be considered surprising that IL-4 inhibits net proliferative responses, because some studies have demonstrated that IL-4 enhances T-cell proliferation.²³ Conversely, others have shown that IFN- γ inhibits Th2 proliferation.²⁴ Those studies, however, were carried out in model systems consisting of Th1 or Th2 clones. In contrast, our peripheral blood T-cell populations have only a small proportion of Th2 cells as determined by *in situ* hybridization studies of IL-4 gene expression (Brown MA, Chan SC, unpublished observation, 1994). Thus it is not surprising that the major proliferative influence comes from the IFN- γ , presumably by enhancing growth of the more abundant Th1 cells. The normalization suggests a reciprocal relationship for the regulation of Th1 and Th2 cell growth through their specific cytokines.

This interpretation would be consistent with our demonstration of excessive PGE₂ production by AD monocytes and with recent reports showing PGE₂ inhibition of IFN- γ production.^{5, 7, 8, 25} PGE₂ has been shown to inhibit murine T cell clonal proliferation, particularly in the Th1 subtype.⁵ Others have demonstrated PGE₂ inhibitory effects on IFN- γ production in human PBMC and T cell clones.^{7, 25} The resultant downward shift in the Th1/Th2 ratio leads to decreased IFN- γ production and, as we have previously shown,⁸ this effect is reversed by indomethacin. This may be a direct PGE₂ effect or may be caused by IL-4 inhibition. Our data suggest the former, though, because of previous studies showing IFN- γ downregulation of IL-4,^{26, 27} the IL-4-enhancing effect of PGE₂ in PBMC cultures might be mediated through reduced IFN- γ . However, the IL-4 enhancing effect of PGE₂ may be the result of reduced IFN- γ in the T-cell culture. Because our present results and previous data have shown that both IFN- γ and IL-4 levels were elevated in AD T cell cultures, it is unlikely that IL-4 is regulating IFN- γ production.

The interrelationships between Th1 and Th2 populations will remain speculative until we have the ability to directly quantitate these cells with specific markers. Our findings do indicate a lesser proliferative potential for atopic lymphocytes, a defect possibly associated with reduced cellular immune responsiveness. IFN- γ , an important mediator of cellular immunity, corrects the proliferative defect *in vitro* and might have similar effects when used therapeutically.

REFERENCES

1. Lobitz WC Jr, Honeyman JF, Winkler MW. Suppressed cell-mediated immunity in two adults with atopic dermatitis. *Br J Dermatol* 1972;86:317-28.
2. McGeady SJ, Buckley RH. Depression of cell-mediated immunity in atopic eczema. *J ALLERGY CLIN IMMUNOL* 1975;56:393-406.
3. Strannegård I-L, Lindholm L, Strannegård Ö. T lymphocytes in atopic children. *Int Arch Allergy Appl Immunol* 1976;50:684.
4. Elliott ST, Hanifin JM. Lymphocyte response to phytohemagglutinin in atopic dermatitis. Enhancement after *in vitro* culture. *Arch Dermatol* 1979;115:1424-6.
5. Betz M, Fox BS. Prostaglandin E₂ inhibits production of Th-1 lymphokines but not of Th-2 lymphokines. *J Immunol* 1991;146:108-13.
6. Phipps RP, Stein SH, Roper RL. A new view of prostaglandin E regulation of the immune response. *Immunology Today* 1991;12:349-52.
7. Jakob T, Hudspeth BN, Latchman YE, Rycroft R, Brostoff J. Depressed lymphocyte transformation and the role of prostaglandins in atopic dermatitis. *Clin Exp Immunol* 1990;79:380-4.
8. Chan SC, Kim J-W, Henderson WR, Hanifin JM. Altered prostaglandin E₂ regulation of cytokine production in atopic dermatitis. *J Immunol* 1993;151:3345-52.
9. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1980;92:44-7.
10. Chan SC, Reifsnnyder D, Beavo JA, Hanifin JM. Immunochemical characterization of the distinct monocyte cyclic AMP-phosphodiesterase from patients with atopic dermatitis. *J ALLERGY CLIN IMMUNOL* 1993;91:1179-88.
11. Holden CA, Chan S, Hanifin JM. Monocyte localization of elevated cAMP phosphodiesterase activity in atopic dermatitis. *J Invest Dermatol* 1986;87:372.
12. Chan SC, Li S-H, Hanifin JM. Increased interleukin 4 production by atopic mononuclear leukocytes correlates with increased cyclic AMP-PDE activity and is reversible by PDE inhibition. *J Invest Dermatol* 1993;100:681-4.
13. Jaffe BM, Behrman HR. Prostaglandins E, A, and F. In: Jaffe BM, Behrman HR, editors. *Hormone radioimmunoassay*. New York: Academic Press, 1974:19.
14. Geissler FT, Kuzan FB, Faustman EM, Henderson WR Jr. Lipid mediator production by post-implantation rat embryos *in vitro*. *Prostaglandins* 1989;38:145.
15. Li S-H, Chan SC, Kramer SM, Hanifin JM. Modulation of leukocyte cyclic AMP phosphodiesterase activity by recombinant interferon-gamma: evidence for a differential effect on atopic monocytes. *J Interferon Res* 1993;13:197-202.
16. Mizoguchi M, Furusawa S, Okitsu S, Yoshino K. Macrophage-derived interleukin 1 activity in atopic dermatitis. *J Invest Dermatol* 1985;84:303.
17. Kapp A, Kirnbauer R, Luger TA, Schöpf E. Altered production of immuno-modulating cytokines in patients with atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1989;144:97-9.
18. Räsänen L, Reunala T. Langerhans' cell antigen presentation and interleukin-1 production in atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1989;(suppl 144):64-6.
19. Thestrup-Pedersen K, Larsen CS, Zachariae C, Kristensen M, Beleuran B, Larsen CG. Interleukin release from peripheral blood mononuclear cells and soluble interleukin-2 receptors in serum from patients with atopic dermatitis. Immunological and pharmacological aspects of atopic

- and contact eczema. In: Czernielewski JM, editor. Pharmacology and the skin. Karger, Switzerland: Basel, 1991;4:188.
20. Reinhold U, Wehrmann W, Kukel S, Kreysel HW. Evidence that defective interferon-gamma production in atopic dermatitis patients is due to intrinsic abnormalities. *Clin Exp Immunol* 1990;79:374.
 21. Jujo K, Renz H, Abe J, Gelfand EW, Leung DYM. Decreased interferon-gamma and increased interleukin 4 production in atopic dermatitis promotes IgE synthesis. *J ALLERGY CLIN IMMUNOL* 1992;90:323-31.
 22. Hanifin JM, Schneider LC, Leung DYM, et al. Recombinant interferon-gamma therapy for atopic dermatitis. *J Am Acad Dermatol* 1993;28:189-97.
 23. van Reijssen FC, Bruynzeel-Koomen CAFM, Kalthoff FS, et al. Skin derived aeroallergen-specific T cell clones of Th2 phenotype in patients with atopic dermatitis. *J ALLERGY CLIN IMMUNOL* 1992;90:184-92.
 24. Gajewski TF, Fitch FW. Anti-proliferative effect of IFN- γ in immune regulation, I: IFN- γ inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. *J Immunol* 1988;140:4245-52.
 25. Snijdwint FGM, Kaliński P, Wierenga EA, Bos JD, Kapsenberg ML. Prostaglandin E₂ differentially modulates cytokine secretion profiles of human T helper lymphocytes. *J Immunol* 1993;150:5321-9.
 26. Peleman R, Wu J, Fargeas C, Delespesse G. Recombinant interleukin 4 suppresses the production of interferon- γ by human mononuclear cells. *J Exp Med* 1989;170:1751.
 27. Vercelli D, Jabara HH, Lauener RP, Geha RS. IL-4 inhibits the synthesis of IFN- γ and induces the synthesis of IgE in human mixed lymphocyte cultures. *J Immunol* 1990; 144:570-3.

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