SUPPRESSION OF HUMAN T-CELL MITOGENESIS BY PROSTAGLANDIN

Existence of a Prostaglandin-Producing Suppressor Cell*

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Prostaglandins (PGs)[1] of the E series have been shown to inhibit many in vitro measurements of immune function in experimental animals. These include macrophage inhibitory factor production in guinea pigs (1, 2), direct cytolysis in murine lymphocytes (3), hemolytic plaque formation by murine leukocytes (4), and mitogen-induced stimulation of murine lymphocytes (5). In the human, Lomnitzer et al. (6) have shown that PGE_1 and PGE_2 cause reduction in leukocyte inhibitory factor production by phytohemagglutinin(PHA)-stimulated human lymphocytes. Smith et al. (7) demonstrated significant inhibition of [^{3}H]thymidine incorporation into PHA-stimulated human lymphocytes by PGE_1, E_2, A_1, and $F_{1\alpha}$. As in the study by Lomnitzer, the final concentration of PGs was relatively high, 10^{-5}–10^{-4}M. Ferraris and Derubertis have reported (8) that stimulation of 10^6 human luekocytes with optimal concentrations of PHA produced $\sim 10^{-8}$M PGE.

These data suggest that PGs of the E series might act as endogenous modulators in human immune reactions. PGE_1 is produced in the mitogen stimulation of human leukocytes, and may in turn inhibit this stimulation. Thus, data are accumulating to support the hypothesis of Bourne et al. (9) that PGs are important regulators of immune function. One troublesome aspect of the studies on human leukocytes is the great disparity between the amount of PGE produced during mitogen stimulation ($\sim 10^{-8}$M or 5 ng/ml) and the amount required to suppress mitogen stimulation when added to cultures ($\sim 10^{-5}$M or 5 μg/ml).

In the present paper we describe the effects of lower, more physiologic concentrations of PGs on mitogen stimulation of human lymphocytes and lymphocyte subpopulations. We also describe the effect of PG synthetase inhibitors on mitogen-induced stimulation of these cells. We have identified a population of glass adherent mononuclear cells that suppress T-cell mitogenic activity through production of PGs of the E series.

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[1] *Abbreviations used in this paper*: Con A, concanavalin A; EDTA, ethylenediamine-tetraacetate; ETOH, ethyl alcohol; FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; PBS, phosphate-buffered saline; PG, prostaglandin; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SD, standard deviation.

Materials and Methods

Subjects. 25 young adult men and women were used as blood donors for the studies. Most were studied more than once, and a few were used more than 10 times over the course of several months.

Preparation of Lymphocytes. Peripheral venous blood was drawn in syringes containing preservative-free heparin. Mononuclear cells were isolated by centrifugation over Ficoll-Hypaque and were washed three times with phosphate-buffered saline (PBS).

Removal of Glass Wool Adherent Cells. Glass wool (Pyrex wool; Corning Glass Works, Corning, N. Y.) was packed to 8 ml in 10-ml plastic syringes and equilibrated with Hanks' balanced salt solution (HBSS) supplemented with 10% fetal calf serum (FCS; Grand Island Biological Co., Grand Island, N. Y.). 5 ml of a cell suspension (5×10^7 cells) was incubated on the column at 37°C for 30 min, then eluted with 30 ml of warm HBSS and washed (10). The yield of nonadherent cells was generally 50% with > 95% viability as measured by trypan blue exclusion. In some experiments, the cells and the column were equilibrated and eluted with PBS without HBSS or FCS.

Recovery of Glass Adherent Cells. After elution of the nonadherent cells with HBSS, 10 ml of 10^{-4} M ethylenediamine-tetraacetate (EDTA) in PBS was added, and the columns were incubated for an additional 30 min. The cells were then eluted with 30 ml cold PBS with gentle agitation of the glass wool and were washed three times in HBSS. The recovery of the adherent cell population was generally 20–30% of the cells initially put on the column, with >90% viability as measured by trypan blue exclusion.

The percentage of monocytes in the various cell fractions was as follows: peripheral blood mononuclear cells, 20–40%; glass nonadherent cells, < 4%; and glass adherent cells, 20–40%. Wright's stain of both the glass adherent and glass nonadherent populations revealed 100% mononuclear cells, with no identifiable platelets. Wright's stain of the peripheral blood mononuclear cell preparations revealed 96–98% mononuclear cells, 2–4% polymorphonuclear leukocytes and variable numbers of platelets. Previous work in our laboratory has demonstrated no difference between the adherent and nonadherent population in percentage of cells bearing Fc receptors, C_3 receptors, surface immunoglobulin, or cells forming E-rosettes with sheep erythrocytes (11).

Preparation of Enriched T-Lymphocyte Suspensions. Enriched T-cell suspensions were prepared by the passage of lymphocytes over double-layer (antihuman IgG-IgG) columns composed of coated Degalan V-26 plastic beads (Degussa Wolfgang, A. G., Hannau am Main, Germany). The preparation of such columns has been previously described (12). The effluent contained > 90% lymphocytes which formed E-rosettes with sheep erythrocytes (13) and < 1% of cells with surface immunoglobulin or receptors for IgG (14).

Drugs. PG A_1, E_1, E_2, $F_1\alpha$, and $F_2\alpha$ were a gift of Dr. John Pike (Upjohn Co., Kalamazoo, Mich.); indomethacin was a gift of Dr. Clement Stone (Merck Sharp & Dohme, West Point, Pa.); and D,1-6-chloro-α-methyl-carbozole-α-acetic acid (RO-20-5720) was a gift of Dr. W. E. Scott (Hoffmann-La Roche Inc., Nutley, N. Y.). RO-20-5720 is an experimental drug developed at Roche Laboratories, the only known action of which is reversible inhibition of PG synthetase (15). All drugs were dissolved in 95% ethyl alcohol (ETOH) at 10 mg/ml and diluted with PBS. This resulted in final concentrations of 0.01% ETOH in the cultures. ETOH concentration of 0.0001–0.2% had no effect on control cultures.

Cell Cultures. The various lymphocyte preparations were cultured in minimal essential media (Microbiological Associates, Bethesda, Md.) supplemented with L-glutamine, penicillin-streptomycin, and 20% FCS. Dose-response curves to PHA (Difco Laboratories, Detroit, Mich.), concanavalin A (Con A, Sigma Chemical Co., St. Louis, Mo.) and pokeweed mitogen (PWM; Grand Island Biological Co.) were obtained, and all further experiments were performed at optimal concentrations of the three mitogens. Cells were cultured in microtiter plates, 1×10^5 cells in 200 μl. Mitogens and drugs were added directly to the wells. The final volume in all the cultures was adjusted to 240 μl. Cells were incubated at 37°C in 5% CO_2 for 72 h. The cultures were pulsed with [^{3}H]thymidine (New England Nuclear, Boston, Mass.; 5×10^{-7} Ci per well) at 48 h and harvested on glass wool filters at 72 h using a Mash II Harvester (Valcor Engineering Corp., Kenilworth, N. J.). The filters were counted in a liquid scintillation counter. All cultures were performed in sextuplicate. Percent inhibition of [^{3}H]thymidine incorporation caused by PGs was calculated by dividing the net counts per minute of the mitogen cultures with PG by the net

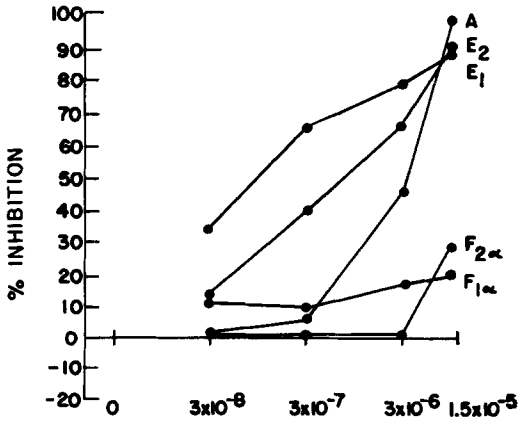

FIG. 1. Percent inhibition of optimal PHA (10 μg/ml) response of unfractionated lymphocytes caused by PGA, E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$ at various concentrations.

counts per minute of the mitogen cultures without PG. That fraction was expressed as a percent and subtracted from 100% to obtain percent inhibition. Net counts per minute were calculated as counts per minute of cells plus mitogen plus drug (PG, indomethacin, RO-20-5720, or nothing) minus counts per minute of cells plus drug.

The Con A-activated suppressor cell was prepared in a manner similar to that described by Shou et al. (16). Suspensions of mononuclear cells, 2×10^6/ml in minimal essential media and 20% FCS, were cultured for 18 h with or without the presence of Con A (20 μg/ml). The cells were then harvested, washed, treated with mitomycin C, and washed an additional four times in PBS. These Con A-, mitomycin C-pretreated lymphocytes were then added to Con A-stimulated cultures of freshly prepared lymphocytes from the same or different donors. The percent inhibition of [^{3}H]thymidine incorporation caused by the Con A-activated cells was calculated from the formula of Shou et al.: $(1 - [(C_M^{CA} - C^{CA})/(C_M - C)]) \times 100\%$, where C_M^{CA} is counts per minute of normal lymphocytes plus Con A-, mitomycin C-pretreated cells plus mitogen; C^{CA} is counts per minute of normal lymphocytes plus Con A-, mitomycin C-pretreated cells; C_M is counts per minute of normal lymphocytes plus mitomycin C-pretreated cells incubated without Con A, plus mitogen; C is counts per minute of normal lymphocytes plus mitomycin C-treated cells incubated without Con A.

To determine whether the Con A-activated suppressor cell acted via PG production, we added indomethacin (1 μg/ml) to the second incubation and measured the effect on percent inhibition. All cultures were performed in sextuplicate.

Measurement of PGE Production in Cultures. The various cell suspensions were prepared as described above and were incubated in 1-ml aliquots (5×10^5 cells/ml) with PHA (10 μg/ml) and in some cases, indomethacin (1 μg/ml). At 48 h the cell suspensions were centrifuged and the supernates assayed for PGE_2 concentrations. The extraction procedures and the radioimmunoassay techniques have been previously described (17). The assays were done on duplicate samples.

Results

Effect of PGs on PHA- and PWM-Stimulated [^{3}H]Thymidine Incorporation in Peripheral Mononuclear Cells. The effects of PGA_1, E_1, E_2, $F_1\alpha$, and $F_2\alpha$ on PHA- and PWM-induced [^{3}H]thymidine incorporation in peripheral blood lymphocytes are shown in Figs. 1 and 2. Data given are from one representative experiment. Similar results were obtained with two other normal donors. Small concentrations of PGE_1 and E_2 (3×10^{-8} M) inhibited PHA stimulation of mononuclear cells, whereas PGs had little effect on PWM stimulation until 100-fold greater concentrations were used. The effect of PGE_2 on PHA and PWM stimulation in 15 normals is shown in Fig. 3. PHA-induced [^{3}H]thymidine

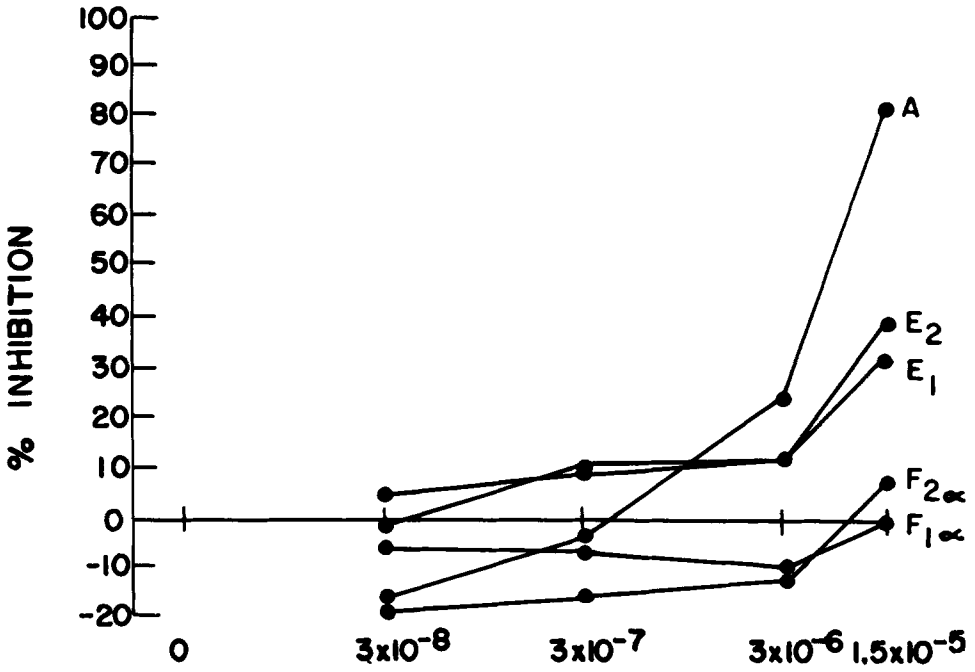

FIG. 2. Percent inhibition of optimal PWM (50 μg/ml) response of unfractionated lymphocytes caused by PGA, E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$ at various concentrations.

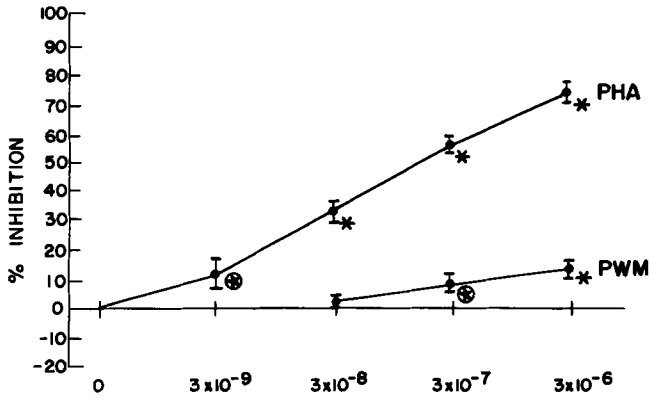

FIG. 3. Percent inhibition of optimal PHA (10 μg/ml) and PWM (50 μg/ml) response of unfractionated lymphocytes caused by PGE_2. Each point represents the mean ± SEM of data from 15 individuals. Inhibition is significantly greater than 0; * indicates $P < 0.001$ and ⊛ indicates $P < 0.02$.

incorporation was inhibited by as little as 3×10^{-9} M PGE_2 ($P < 0.02$) with a 50% inhibition at $\sim 10^{-7}$ M. PWM-induced stimulation, however, was inhibited only slightly by PGE_2, and only at higher concentrations. These experiments were done at optimal PHA and PWM concentrations. In similar experiments done with three subjects, we found that PGE_1 and PGE_2 inhibited Con A(20 μg/ml)-induced [^{3}H]thymidine incorporation to the same extent that it inhibited PHA.

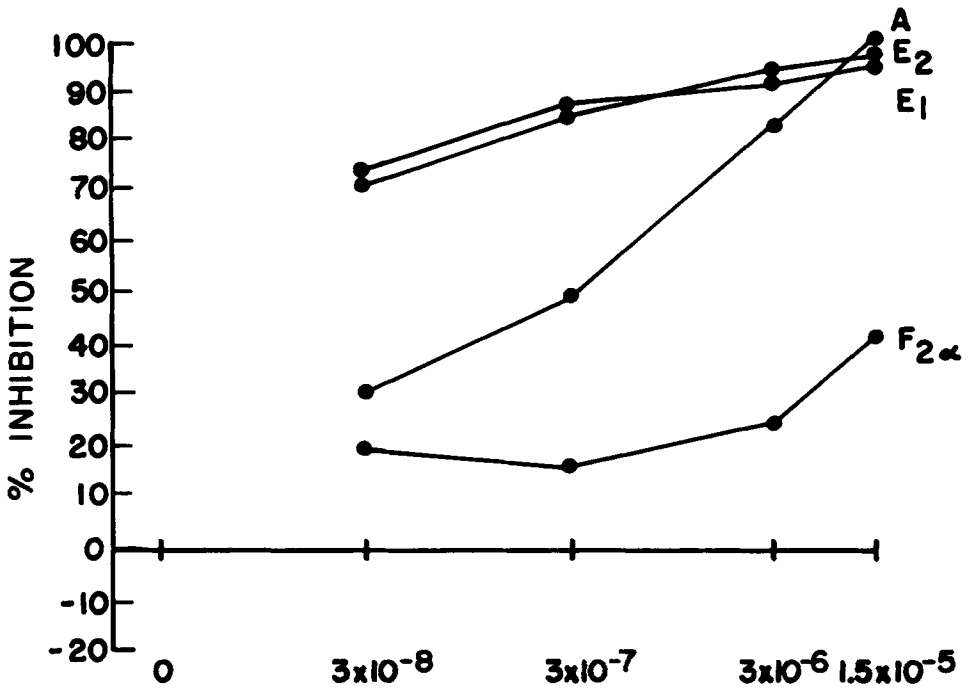

FIG. 4. Percent inhibition of optimal PHA (10 μg/ml) response of T-enriched cells by PGA, E_1, E_2, $F_{2\alpha}$.

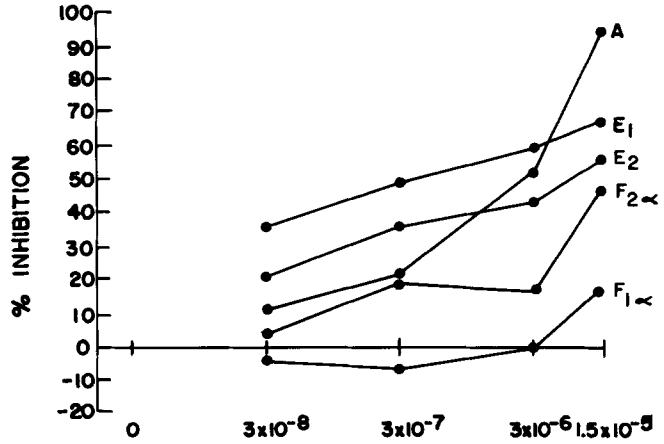

FIG. 5. Percent inhibition of optimal PWM (50 μg/ml) response of T-enriched cells by PGA. E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$.

Inasmuch as PHA and Con A are primarily T-cell mitogens, and PWM stimulates both B and T cells in the human (18), these data suggest that PGE_1 and PGE_2 inhibit T-cell but not B-cell mitogenic activity. To investigate this further, we studied the effects of PGs on PHA and PWM stimulation of enriched T-cell preparations. These preparations contained >90% T cells by E-rosette assay and <1% B cells by Fc receptor and surface immunoglobulin assay. Representative experiments are shown in Figs. 4 and 5. In the experi-

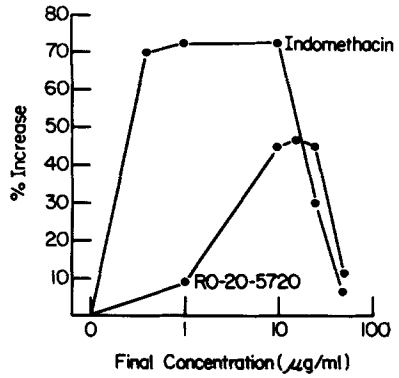

FIG. 6. Effect of PG synthetase inhibitors on optimal PHA (10 μg/ml) stimulation. Data from one subject showing percent increase in [^{3}H]thymidine incorporation at various concentrations of indomethacin and RO-20-5720.

ments performed with enriched T cells, PGE_1 and PGE_2 were better inhibitors of PHA stimulation than they were with whole lymphocyte preparations (Fig. 4 vs. Fig. 1). The enriched T-cell preparations responded less well to PWM than did the whole lymphocyte preparations (stimulation index of ~20 compared to ~40), but they were much more suppressible with PGE_1 and PGE_2 (Fig. 5 vs. Fig. 2). In all of these experiments, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ had little effect on mitogen stimulation, and PGA inhibited only at high concentrations.

Effect of PG Synthetase Inhibitors In Vitro on Mitogen Stimulation. Panush has reported that indomethacin in vitro inhibits mitogen stimulation of human lymphocytes (19), but high and possibly toxic doses of the drug were used (20). Because small amounts of PGs are produced in mitogen-stimulated cultures (8), we reasoned that in vitro blockade of their synthesis might actually increase mitogen stimulation. Fig. 6 demonstrates this phenomenon in one subject with varying concentrations of two PG synthetase inhibitors: iodomethacin and RO-20-5720. Both drugs enhanced stimulation. The effect of indomethacin was greatest over the range of 0.5–10 μg/ml, which is the same concentration as required to inhibit PG synthetase in vitro (15, 21, 22). In this concentration range there was no effect of indomethacin on cell viability after 72-h cultures. At higher concentrations, the enhancing effect of indomethacin was diminished, presumably because of its known toxicity to human leukocytes at levels of 40 μg/ml and greater (20, 23). In all subsequent experiments, a final concentration of 1 μg/ml was used. At this concentration, indomethacin produced a 58 ± 28% (mean ± SD, $P < 0.001$) increase in [^{3}H]thymidine incorporation in PHA-stimulated lymphocytes from 15 normal men and women, aged 20–40 yr. A similar enhancement was seen at suboptimal concentrations of PHA, but at supraoptimal concentrations the effect was diminished (Table I). The magnitude of the increase seen with indomethacin did not correlate with the magnitude of the base-line PHA stimulation ($r = 0.05$, $P > 0.8$); that is, the increase caused by indomethacin did not vary in relation to the size of the PHA response. In six patients tested, indomethacin enhanced Con A-induced [^{3}H]thymidine incorporation in a manner similar to its effect on PHA stimulation (81 ± 45% increase, $P < 0.01$).

TABLE I

Percent Increase in [^{3}H]Thymidine Incorporation Caused by Indomethacin at Different PHA Concentrations

PHA concentration	Before indomethacin	Increase with indomethacin
μg/ml	*cpm*	*%*
−0−	142 ± 47	70 ± 48 ($P < 0.001$)
2	4,078 ± 5,852	55 ± 46 ($P < 0.001$)
10	12,630 ± 6,448	58 ± 28 ($P < 0.001$)
20	13,334 ± 7,573	46 ± 28 ($P < 0.001$)
50	7,984 ± 7,657	16 ± 16 ($P < 0.01$)

Each point represents mean ± SD of 15 subjects. The cells were cultured as described in Materials and Methods with or without the addition of indomethacin (1 μg/ml).

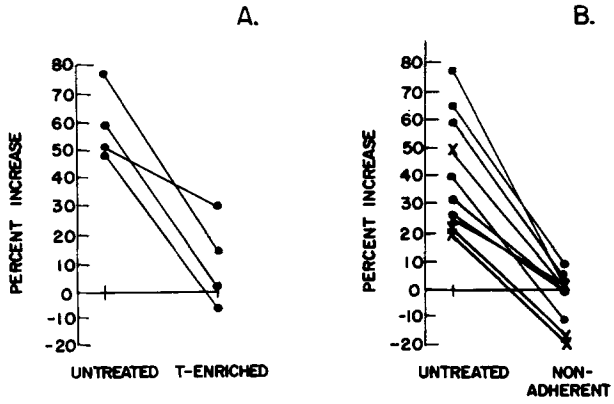

FIG. 7. Effect of PG synthetase inhibitors on PHA stimulation of unfractionated lymphocytes vs. T-enriched or glass nonadherent lymphocytes. Lines connect points representing percent increase in PHA (10 μg/ml) stimulation caused by indomethacin (1 μg/ml) or RO-20-5720 (40 μg/ml) in a subject's lymphocytes before and after T-cell enrichment or passage over glass wool. X, RO-20-5720; ●, indomethacin.

Indomethacin caused less enhancement of PWM-stimulated peripheral lymphocytes (13 ± 5.5% increase in 10 subjects, $P < 0.01$), as would be expected from the data above showing little inhibiting effect of PGs on PWM-stimulated unfractionated lymphocytes.

Studies Using Cell Fractions. The effect of indomethacin on PHA stimulation of subfractions of lymphocytes is shown in Fig. 7. After passage of the lymphocytes over glass wool columns or after T-cell enrichment by passage over IgG anti-IgG-coated Degalan beads, the enhancing effect of indomethacin and RO-20-5720 on the mitogen response was greatly reduced. In six subjects tested, indomethacin caused an increase in PHA stimulation of 49.8 ± 20.7% in untreated lymphocytes and 0.8 ± 7.2% (mean ± SD) increase in lymphocytes depleted of glass wool adherent cells. With RO-20-5720 there was a 27.8 ± 11.3% increase in untreated lymphocytes vs. a 7.4 ± 10.8% decrease after glass wool passage. In both cases the differences in the response of treated vs. untreated lymphocytes were significant with $P < 0.001$. Clearly, the glass wool

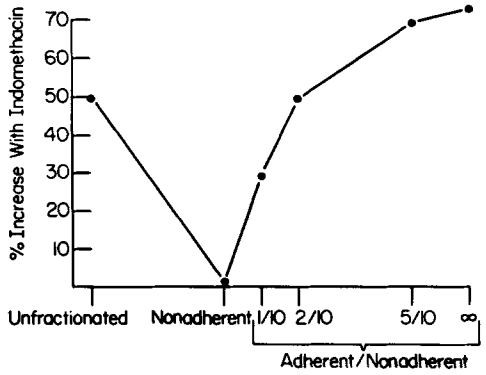

FIG. 8. Effect of readdition of glass adherent cells to glass nonadherent cells on enhancement of PHA (10 μg/ml) response caused by indomethacin (1 μg/ml). The PHA response of the unfractionated peripheral blood mononuclear cells increases 50% with indomethacin. This enhancement decreases to 2% with the nonadherent cells and then increases with the readdition of adherent cells. Total cell number per culture was kept constant at 10^5 in 240 μl.

is removing a cell population upon which the PG synthetase inhibitors were acting to cause enhancement of [^{3}H]thymidine incorporation. Depletion of glass wool adherent cells had a variable effect on the base-line PHA stimulation without indomethacin. In six experiments, optimal PHA stimulation gave 20,424 ± 11,136 cpm before glass wool adherence and 10,586 ± 3,330 after. In four experiments, total counts per minute decreased after removal of glass wool adherent cells; in one experiment the counts per minute increased; and in one the counts per minute were unchanged. Thus, passage over glass wool has effects other than removal of suppressor cells.

We next investigated the effects of indomethacin on the PHA response of glass nonadherent cells with the readdition of glass adherent cells to the culture. Fig. 8 shows the results of such an experiment, demonstrating an increasing effect of indomethacin with increasing numbers of glass adherent cells. At an adherent/nonadherent ratio of 2/10, indomethacin increased the PHA stimulation by 50%, which was identical to the increase in the unfractionated lymphs. This experiment was performed three times in different donors with similar results.

These glass wool adherent suppressor cells could be monocytes, B cells, or glass adherent T cells. It is doubtful that either polymorphonuclear leukocytes or platelets are involved, because the recovered adherent cell population which contains the suppressor cell does not contain those blood elements. In an attempt to further characterize this suppressor cell, we performed a linear regression to obtain a coefficient of correlation between the percent increase obtained with indomethacin in a culture and the percent of monocytes in that culture. There was no correlation ($r = 0.20$, $P > 0.5$). We also found that monocyte depletion performed on glass wool columns or in glass Petri dishes, without calcium in the media, reduced the percentage of monocytes in the culture without substantially changing the response to indomethacin. In four experiments, monocytes decreased from 27 ± 4 (mean ± SD) to 9 ± 4% monocytes, whereas the percent increase with indomethacin went from 52 ± 7

TABLE II
Effect of Indomethacin on the Inhibition of Mitogen Stimulation Caused by the Con A-Activated Suppressor Cell

Experiment	Con A pretreatment	Without indomethacin	% inhibition	With indomethacin	% inhibition
		cpm		*cpm*	
1	−	5,421	25	6,494	32
	+	4,084		4,447	
2	−	8,271	21	9,037	44
	+	7,562		5,066	
3	−	7,015	26	22,360	26
	+	5,173		16,585	
4	−	5,527	25	5,882	38
	+	4,130		3,659	
5	−	8,808	39	12,104	54
	+	5,361		5,521	
6	−	6,564	15	20,883	14
	+	5,599		17,865	

In experiments 1, 2, and 3 the Con A-, mitomycin C-pretreated cells were added (10^5 cells/well) to Con A-stimulated cultures (50 μg/ml) of fresh allogeneic cells (10^5 cells/well). In Experiments 4, 5, and 6 the Con A-, mitomycin C-pretreated cells were added to cultures of fresh syngeneic cells. Indomethacin (1 μg/ml) was added to the second incubation. See Materials and Methods for details.

to 41 ± 4%. This represents a 67% decrease in monocytes with only a 21% decrease in indomethacin stimulation. This does not rule out the possibility that a small subpopulation of monocytes contained the PG-producing suppressor cell.

Comparison to the Con A-Activated Suppressor Cell. Shou et al. (16) and Hubert et al. (24) have recently described a human suppressor cell population that is activated by preincubation with Con A or PHA and inhibits subsequent mitogen, antigen, or mixed lymphocyte culture activation of allogeneic or syngeneic lymphocytes. To determine whether this Con A-activated suppressor cell acted through PG production, we added indomethacin to the cultures in an attempt to block the inhibition. As seen in Table II, indomethacin did not significantly alter the percent inhibition caused by the Con A-activated suppressor cell. Thus, the Con A-activated suppressor cell does not inhibit mitogen response via PG production, as does the PG-producing suppressor cell.

Direct Measurement of PG Production. We next measured the endogenous production of PGs in PHA-stimulated cultures of unfractionated lymphocytes, lymphocytes plus indomethacin, and in glass wool nonadherent lymphocytes. As shown in Table III, PHA stimulation of unfractionated lymphocytes produces greater than 10^{-8} M PGE in 48 h. Addition of indomethacin to the culture decreases PGE production to <20% of control values, and PHA stimulation of glass nonadherent cells results in PGE production equal to <10% of that produced in cultures of unfractionated lymphocytes. Thus, the amount of PGE produced by PHA stimulation of unfractionated lymphocytes is in the range that inhibits [^{3}H]thymidine incorporation when the PGE is added exogenously,

TABLE III
PGE_2 Production in PHA Cultures

Untreated lymphocytes	Lymphocytes + indomethacin	Glass nonadherent cells
8,472 (2.5×10^{-8} M)	1,599 (5×10^{-9} M)	556 (10^{-9} M)
5,018 (1.5×10^{-8} M)	609 (10^{-9} M)	414 (10^{-9} M)

Data is from two experiments, and is expressed as picograms per milliliter. Approximate molar concentrations are given in parentheses. 5×10^5 cells in 1 ml media were cultured for 48 h with PHA (10 μg/ml) with or without indomethacin (1 μg/ml). Results given as mean of duplicate samples.

TABLE IV
Percent Inhibition of PHA Stimulation by PGE_2 in Untreated Lymphocytes, Lymphocytes Plus Indomethacin, Nonadherent Cells, and T-Enriched Lymphocytes

PGE_2 concentration	Percent Inhibition			
	Untreated lymphocytes	Plus indomethacin	Nonadherent cells	T-enriched
3×10^{-9} M	9.5 ± 9.5	18.3 ± 0.6*	29.0 ± 3.9	43.5 ± 29.0
3×10^{-8} M	26.1 ± 12.1	51.6 ± 15.3	52.2 ± 9.2	78.0 ± 7.9
3×10^{-7} M	48.9 ± 13.1	70.2 ± 13.9	70.2 ± 10.1	89.3 ± 3.2
3×10^{-6} M	63.3 ± 8.5	74.7 ± 4.9	80.5 ± 12.1	92.0 ± 0.8

Data given as mean inhibition ± SD. A total of 12 subjects was studied. Lymphocytes from all 12 were studied untreated. Lymphocytes from each subject were also studied with one of the treatments: addition of indomethacin (four subjects), nonadherent cells (five subjects), and T-enriched cells (three subjects).

* Not significant. All other points significantly different from untreated lymphocytes at $P < 0.01$, by using one-tailed t-test.

and the cells that are producing the PGE are 90% removed by passage over glass wool.

Finally, we studied what effect removing endogenously produced PGs might have on the inhibitory effect of exogenously added PGE_2. It was probable that endogenously produced PGs were diluting the inhibitory effect of exogenous PGE_2, and that when we added PGE_2 to a PHA culture of peripheral lymphocytes, we were starting with an already inhibited culture. Three methods of decreasing endogenous PG production were tried: the removal of glass wool adherent cells, removal of cells by IgG-anti-IgG Degalan column, and the addition of indomethacin before the addition of exogenous PGE_2. Table IV shows the effect of these manipulations on inhibition of PHA stimulation by PGE_2. In all cases, the sensitivity to exogenous PGE_2 was significantly enhanced. With this data, we calculate that approximately 10^{-8} M PGE_2 is required for 50% inhibition of PHA stimulation of human lymphocytes.

Discussion

We draw several conclusions from the data presented above. First, low concentrations of PGE_1 and PGE_2 suppress mitogen stimulation of human lymphocytes. Second, these PGs suppress mitogen stimulation of T cells, but probably not B cells. Third, PGs produced endogenously in mitogen cultures suppress [^{3}H]thymidine incorporation by T cells in those cultures. Fourth, the

endogenous PGs appear to be produced by a subset of mononuclear cells that can be depleted by adherence to glass wool or by T-cell enrichment on IgG anti-IgG-coated Degalan beads.

Smith et al. (7) showed that PGs, along with aminophylline and isoproterenol, would inhibit PHA stimulation of human lymphocytes. Further work by that group (25), as well as by others (9), suggests that all agents that inhibit mitogen response do so by raising intracellular cAMP. The first section of our results is a confirmation of their data, the main difference being that much lower concentrations of PGE_1 and PGE_2 were needed to show inhibition. We found consistent reproducible inhibition with PGE_1 and PGE_2 at concentrations of 1 ng/ml (3×10^{-9} M) and 10 ng/ml (3×10^{-8} M; Fig. 3 and Table IV). Inasmuch as these concentrations of PGE are similar to what is produced in mitogen cultures of human lymphocytes (8), it is no surprise that PG synthetase inhibitors increase mitogen response. The concentration range of PGE_2 (1–100 ng/ml) that we found inhibitory in mitogen cultures is also the range of concentrations found in inflammatory fluids (26, 27).

We have no final explanation for why we found significant inhibition of PHA stimulation at PGE_1 and PGE_2 levels three orders of magnitude lower than the concentrations employed by Smith et al. Berenbaum and his co-workers (28) have shown significant inhibition of PHA response in human lymphocytes by 10^{-7} M PGE_2. Their assay employed unseparated peripheral blood. Higher concentrations may have been needed in their assay because of binding of PGE_2 to blood elements and plasma proteins (29).

The finding that none of the PGs tested suppressed PWM stimulation of peripheral leukocytes except in high concentration is interesting, for it suggests that these compounds do not inhibit mitogenic activity in human B lymphocytes. Greaves et al. (18) have shown that the responding population to PWM stimulation of human spleen cells is predominantly composed of B cells, but when B cells are removed the T cells respond well. In our experiments when T cells were enriched (and B cells depleted) by passage over plastic bead columns, the inhibition curves of PGE_1 and PGE_2 on PWM approached the curves of the effects of these compounds on PHA-stimulated cells. There is some evidence in animals that PGs affect B-cell function, but there are no data in man (4, 30–33). Further investigation of PG effects with other mitogens and on B-cell subpopulations may clarify this issue.

We have assumed throughout this paper that the enhancing effect of indomethacin is secondary to inhibition of PG synthetase. We have based this assumption on three facts. First, although indomethacin has many actions in vitro, the only known action of the drug at the low concentrations (1 μg/ml) we employed is PG synthetase inhibition (34). Second, we obtained a similar but smaller enhancement using RO-20-5720, an experimental drug entirely unrelated structurally to indomethacin. The only known action of RO-20-5720 is PG synthetase inhibition (15). It is a competitive inhibitor of PG synthetase, whereas indomethacin is an irreversible inhibitor (15). We feel this is the reason for the smaller enhancement of PHA stimulation we found with RO-20-5720. The third reason for assuming that indomethacin works through PG synthetase inhibition in our assay system is that we and others (8) have demonstrated that PGE is produced in sufficient quantities in mitogen cultures

to suppress [^{3}H]thymidine incorporation, and that this production of PGE is blocked by the addition of indomethacin (Table III). We have also shown that the PGE-producing cells are glass adherent, and that removal of the PG-producing cells by passage over glass wool results in loss of the enhancing effect seen with indomethacin. Readdition of glass adherent cells to cultures of glass nonadherent cells restores the enhancing effect of indomethacin.

Our estimate of PGE_2 production is a conservative one, for it does not take into account any possible in vitro metabolism of the PGE_2 produced. We could find little data on the stability of PGE_2 in this system or any similar systems. Endogenously produced PGE_2 is stable in cultures of human rheumatoid synovium (35), and very unstable when incubated with dog or cat serum (36). PGE_2 accumulates over time in mitogen cultures of mouse spleen cells (8), demonstrating that whatever metabolism there may be is slower than the synthetic rate. Further studies on the time-course of PGE_2 production and stability in cultures of human leukocytes appear warranted.

One might anticipate that removing glass wool adherent cells, and thereby removing the suppressor cells, would result in an enhanced mitogen response. We did not find this, however. There has been considerable recent interest in the helper effect of monocytes in mitogen responses (37–39). The mechanism of this interaction is not understood, but different workers have clearly demonstrated that removal of monocytes from mitogen cultures causes depression of the response. We feel, therefore, that there is a balance in PHA cultures between the helper effects of monocytes and the inhibiting effects of suppressor cells. Because they are both removed by glass wool, the PHA stimulation will increase or decrease with glass wool adsorption, depending on which cell type is dominant. In normals, it appears that most PHA cultures give decreased stimulation with glass wool adsorption (37); that is, the helper function is dominant. In some disease states, however, the suppressor cell is dominant. We have recently reported that glass wool adsorption of mononuclear cells from six patients with Hodgkin's disease resulted in an increased response to mitogens (40). PHA-stimulated cultures of Hodgkin's disease mononuclear cells produced approximately fourfold more PGE_2 than normal. Blockade of PGE_2 production with indomethacin resulted in a 182 ± 60% increase in [^{3}H]thymidine incorporation for the Hodgkin's disease cells vs. 44 ± 18% increase in normals. After elimination of PG production, either by the addition of indomethacin or by removal of the glass adherent cells, the originally depressed response to PHA of the Hodgkin's disease cells increased to normal. Thus, increased activity of the PG-producing suppressor cell appears responsible for the depressed response to PHA in Hodgkin's disease. Preliminary studies in our laboratory would indicate that the suppressor cell function is also dominant in adult-acquired agammaglobulinemia and in the relative anergy of old age.

The functional role of suppressor cells in the control of the immune response has been the subject of intensive investigation over the past several years. Investigators employing experimental animal models have identified suppressor cells that influence many aspects of T- and B-cell function (for review, see 41). Furthermore, T-cell, B-cell, and macrophage subpopulations have been implicated as suppressor cells (38, 39, 42).

These cells have been suggested as etiologic or contributory in the pathogenesis of several diseases, including common variable hypogammaglobulinemia (43), IgA deficiency (44), multiple myeloma (45), Hodgkin's disease (11, 40, 46), and systemic lupus erythematosus (47). Work in this area has been hindered, however, by lack of a suitable assay of suppressor cell activity. Human suppressor cell activity was shown by adding circulating lymphocytes from patients with those diseases to normal lymphocytes and demonstrating an inhibition of various in vitro T- and B-cell functional assays. More recently, several groups have reported on assays for suppressor cell activity in peripheral blood leukocytes from normal humans (16, 24, 47). These assays are similar and involve stimulating normal lymphocytes with Con A for 24 h, then adding them to fresh mitogen-stimulated or mixed lymphocyte cultures from the same or different donors. The "prestimulated" cells cause a 20–50% inhibition in the subsequent cultures. These Con A-activated suppressor cells inhibit subsequent Con A and PHA cultures better than they inhibit PWM cultures (16). Waksman and his co-workers (10, 38, 48) have thoroughly studied a similar system in mice and rats and have characterized an inhibitor of DNA synthesis produced by Con A-pretreated spleen cells. This is a large molecular weight glycoprotein and appears to mediate the actions of the Con A-activated suppressor cell in the rat and mouse. In this present study, we have shown that the PG-producing suppressor cell is clearly different from the Con A-activated cell in humans.

Although ours is the first report identifying the PGs as mediators of suppressor cells in humans, there is already good evidence that these compounds function as suppressors in experimental animals. Plescia et al. (33) demonstrated that addition of syngeneic mouse tumor cells to suspensions of mouse spleen cells inhibited antibody response to sheep erythrocytes. This inhibition could be mimicked by addition of PGE_2 and blocked by PG synthetase inhibitors. Webb and Jamieson (5) have described a mouse, glass adherent, splenic cell that suppresses PHA and Con A stimulation of the nonglass adherent population. This cell can be neutralized with antithymocyte serum plus complement, and its suppression can be blocked by PG synthetase inhibitors. Indeed, this mouse suppressor cell shares many characteristics with the human suppressor cell described here. It is glass adherent; its effects are mediated through PG; and it inhibits T-cell function.

Summary

Small amounts of PGE inhibit mitogen-induced [^{3}H]thymidine incorporation in human peripheral lymphocytes. The 50% inhibitory concentration is $\sim 10^{-7}$ M, and this is reduced to $\sim 10^{-8}$ M when endogenous PGE production is blocked. PGE inhibits PHA- and Con A-stimulated cultures much better than PWM cultures, suggesting a differential effect of PGE on T-cell vs. B-cell function. In vitro blockade of PG synthesis results in ~50% increase in [^{3}H]thymidine incorporation in PHA cultures. PGE is produced endogenously in PHA cultures by glass adherent suppressor cells.

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