Prostaglandin Suppression of Mitogen-Stimulated Lymphocytes In Vitro

CHANGES WITH MITOGEN DOSE AND PREINCUBATION

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ABSTRACT In this study we further characterize the properties of the prostaglandin-producing suppressor cell. Overnight preincubation of peripheral blood mononuclear cells results in an increased response of the cells to phytohemagglutinin or Concanavalin A compared to the response of fresh cells. This increase in mitogen response with preincubation was similar in magnitude to the increase in mitogen response of fresh cells after the addition of indomethacin. The two manipulations were not additive; that is, after preincubation, indomethacin caused much less enhancement of mitogen stimulation of peripheral blood mononuclear cells (100±12% increase before preincubation vs. 12±6% after preincubation; mean±SEM, $P < 0.001$). Preincubated cells also lose sensitivity to inhibition by exogenous prostaglandin E_2. It requires the addition of 100- to >1,000-fold more exogenous PGE_2 to produce comparable inhibition of phytohemagglutinin-stimulated preincubated cells than is required for inhibition of phytohemagglutinin-stimulated fresh cells.

The enhancing effect of indomethacin increases with decreasing doses of phytohemagglutinin. Indomethacin causes a 1,059±134% increase in [^{3}H]thymidine incorporation at the lowest dose of phytohemagglutinin (0.2 μg/ml), and a 4±3% increase at the highest dose (20 μg/ml). This increase in response to indomethacin with a lower dose of phytohemagglutinin is due to increased sensitivity to inhibition by PGE_2 at lower mitogen doses.

The prostaglandin-producing suppressor cell assay and the short-lived suppressor cell assay measure overlapping phenomena. The increased suppressive effect of the prostaglandin-producing suppressor at suboptimal mitogen dose must be taken into account in the interpretation of any study where the response to a range of mitogen doses is studied.

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INTRODUCTION

The role of suppressor cells in the regulation of humoral and cellular immunity has received much attention in this decade. Investigators employing animal models have identified suppressor cells that influence many aspects of T- and B-cell function (1). Furthermore, T-cell (2), B-cell (3), and macrophage (4) subpopulations have been implicated as suppressor cells. These cells have been suggested as etiologic or contributory in the pathogenesis of several diseases, including common variable hypogammaglobulinemia (5), immunoglobulin A (IgA) deficiency (6), multiple myeloma (7), Hodgkin's disease (8–10), and systemic lupus erythematosus (11). 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 cells in peripheral blood mononuclear cells (PBMC)[1] in normal humans. Shou et al. (12) and Hubert et al. (13) have described a concanavalin A (Con A)-activated suppressor cell in normal

[1] *Abbreviations used in this paper*: Con A, concanavalin A; FCS, fetal calf serum; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PG, prostaglandin; PHA, phytohemagglutinin.

humans similar to the cell described by Waksman and his co-workers in mice (2, 14). This assay involves stimulating normal lymphocytes with Con A for 24 h, then adding them to fresh mitogen-stimulated or mixed lymphocyte cultures from the same or a different donor. The "prestimulated" cells cause a 20–50% inhibition in the subsequent cultures. Bresnihan and Jasin (11) described a "short-lived suppressor cell" in normal peripheral blood. They found that lymphocytes preincubated overnight in media alone responded better to suboptimal doses of T-cell mitogens than did fresh lymphocytes. They ascribed this increase in reactivity to the death or deactivation of a "short-lived suppressor cell."

We have described a prostaglandin (PG)-producing suppressor cell that inhibits human lymphocyte activation by T-cell mitogens in vitro (15). This cell secretes PGE_2 and inhibits phytohemagglutinin (PHA)- or Con A-induced [^{3}H]thymidine incorporation in normal human lymphocytes. Addition of PG synthetase inhibitors to the mitogen-containing cultures decreases PGE_2 production to 10% of normal and leads to an $\cong$50% increase in [^{3}H]thymidine incorporation. When the endogenously inhibited PGE_2 is replaced by comparable amounts of exogenous PGE_2 ($\cong$10 nM), [^{3}H]thymidine incorporation returns to normal. This PG-producing suppressor cell appears to be responsible for the defect in cellular immunity seen in Hodgkin's disease. PHA cultures of lymphocytes from Hodgkin's disease patients produce fourfold more PGE_2 than do cultures of normal lymphocytes (10). Addition of PG synthetase inhibitors eliminates this production and restores the depressed mitogen response to normal.

In the present study, we further characterize the properties of the PG-producing suppressor cell. It appears that the PG-producing suppressor cell assay and the short-lived suppressor cell assay measure overlapping phenomena.

METHODS

Subjects. 15 young adult men and women were used as blood donors for the studies. Most were studied more than once. Blood samples were obtained in the morning 1 h after breakfast, with avoidance of prior heavy exercise.

Preparation of lymphocytes. Peripheral venous blood was drawn in syringes containing preservative-free heparin. Mononuclear cells were isolated by centrifugation over Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N. J.) 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 supplemented with 10% fetal calf serum (FCS; Grand Island Biological Co., Grand Island, N. Y., lot E 064421). 5 ml of a cell suspension (20×10^6 cells) was incubated on the column at 37°C for 30 min, then eluted with 30 ml of warm Hanks' balanced salt solution and washed. The yield of nonadherent cells was generally 50% with >90% viability as measured by trypan blue exclusion.

Drugs. PGE_2 was a gift of Dr. John Pike (Upjohn Co., Kalamazoo, Mich.); indomethacin was a gift of Dr. Clement Stone (Merck Sharp & Dohme, Div. of Merck & Co., Inc., Westpoint, Pa.). The drugs were dissolved in 95% ethyl alcohol at 10 mg/ml and diluted with PBS. This resulted in final concentrations of 0.01% ethyl alcohol in the cultures. Ethyl alcohol concentrations of 0.0001–0.2% had no effect on control cultures. PHA (lot 3110-56, Difco Laboratories, Detroit, Mich.) and Con A (lot 65C-5022, Sigma Chemical Co., St. Louis, Mo.) were used as mitogens.

Cell cultures. The various lymphocyte preparations were cultured in minimal essential media (Microbiological Associates, Walkersville, Md.) supplemented with L-glutamine, penicillin-streptomycin, and 20% FCS. 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.; 0.5 μCi per well) at 48 h and harvested on glass wool filters at 72 h with a Mash II Harvester (Valcor Engineering Corp., Kenilworth, N. J.). In the assay of the short-lived suppressor cell, the cells were added to the wells and incubated overnight; mitogen and/or PGE_2 and/or indomethacin were added at 24 h. The cultures were pulsed at 72 h and harvested at 96 h. In some experiments the cells were preincubated in round-bottom plastic tubes (type 2054, Falcon Plastics, Div. BioQuest, Oxnard, Calif.), 2×10^6 cells in 1 ml of media per tube. These suspensions were combined and washed twice with PBS after overnight incubation. They were then prepared in the culture plates as described above. The filters were counted in a liquid scintillation counter. All cultures were performed in sextuplicate. Net counts per minute (cpm) were calculated as cpm of cells plus mitogen plus drug (PG, indomethacin, or nothing) minus cpm of cells plus drug. Percent stimulation of [^{3}H]thymidine incorporation was calculated by dividing the net cpm of the mitogen cultures with indomethacin by the net cpm of the mitogen cultures without indomethacin. This number was expressed as a percent and 100% was subtracted from it to obtain percent stimulation. Percent inhibition of [^{3}H]thymidine incorporation caused by PGE was calculated by dividing the net cpm of the mitogen cultures with PGE by the net cpm of the mitogen cultures without PGE. The fraction was expressed as a percent and subtracted from 100% to obtain percent inhibition. Whenever we measured the inhibiting effect of exogenously added PGE_2, we also added indomethacin (1 μg/ml) to those cultures to remove the confounding variable of endogenously produced PGs (14). The lots of PHA, Con A, and FCS used in these experiments were different from those used in our previous reports (10, 14).

Measurement of PG production in cultures. Cell suspensions (5×10^5 per ml in minimal essential medium with 20% FCS) were prepared as described above and 1-ml aliquots were incubated in 1 × 7.5-cm round-bottom plastic tubes with PHA in varying concentrations. A blank assay consisting of minimal essential medium and 20% FCS without cells was also established. These tubes were then placed in a 5% CO_2 incubator at 37°C. At various times thereafter, duplicate tubes were withdrawn, the cells resuspended, and then centrifuged at 400 *g* for 10 min. The supernates were then withdrawn for measurement of "total PG" and of PGE_2. The assay for PG was performed as previously described (10, 15, 16), using an antiserum that recognized both PGE and PGA (10). The cell supernates were extracted for PG determination by previously described methods (16). Aliquots of the extracted supernates

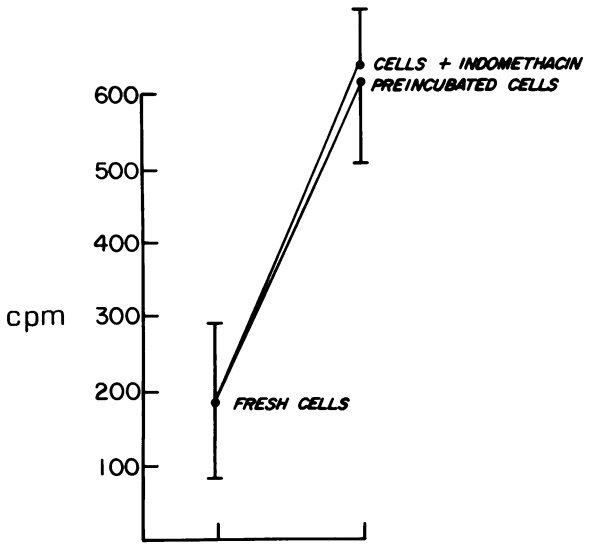

FIGURE 1 Effect of preincubation vs. addition of indomethacin on the response of PBMC to a suboptimal concentration of Con A (1 μg/ml). Data is expressed as cpm (mean±SEM) from three subjects. Both addition of indomethacin (1 μg/ml) and preincubation of cells result in significant increases in [^{3}H]thymidine incorporation in PBMC stimulated by Con A ($P < 0.01$). The increases caused by the two manipulations are essentially identical.

were subjected to silicic acid chromatography, and the eluted fraction containing PGE_2 was collected. Then both chromatographed and unchromatographed samples were assayed for PG. The results for the unchromatographed samples, called "total PG," represent PGE plus PGA with little or no contribution from PGF or PG metabolites, as these compounds gave <0.5% cross-reactivity with PGE_2 (10). The results for the chromatographed samples were called PGE_2 measurements. The total PG and PGE_2 levels in the blank tubes (without cells) were subtracted from the PG values of the tubes with cells to obtain a net "total PG" and net PGE_2. Each duplicate supernate sample was assayed in triplicate in the radioimmunoassay.

RESULTS

Comparison of indomethacin and preincubation on the response of leukocytes. We first investigated whether indomethacin and preincubation had similar quantitative effects on the mitogen response. As shown in Fig. 1, both treatments increased the response to a suboptimal dose of Con A (1 μg/ml). The increases were essentially equal. To determine whether indomethacin and preincubation affected similar cells, indomethacin was added to preincubated cells. The enhancing effects of the two procedures were not additive (Table I). The increase obtained with both was usually no greater than that seen with the addition of indomethacin to fresh cells. In 10 experiments at various PHA concentrations, addition of indomethacin to fresh cells caused a 100±12% increase in [^{3}H]thymidine incorporation; this fell to a 12±6% increase when indomethacin was added to cells that had been preincubated in the microtiter plates overnight (mean ±SEM, $P < 0.001$). To control for the possible effect of an accumulated metabolite in the preincubated cultures, we also preincubated leukocytes overnight and then washed them twice in PBS before putting them in the culture plates. These preincubated and washed cells also were significantly less enhanced by the addition of indomethacin (20±12% increase, $P < 0.001$).

Fig. 2 graphs the enhancement caused by addition of indomethacin to PHA-stimulated cultures of PBMC as a function of time of preincubation and as a function of concentration of PHA. The ability of indomethacin to enhance [^{3}H]thymidine incorporation decreases rapidly with preincubation and is almost totally abolished

TABLE I
Effect of Addition of Indomethacin, Preincubation of Cells, or Both on Response to Mitogens

Mitogen*	Number of experiments	Fresh cells		Preincubated cells‡		Increase in cpm§		
		Alone	Plus indomethacin	Alone	Plus indomethacin	With indomethacin	With preincubation	With both
		cpm	*cpm*	*cpm*	*cpm*		%	
Con A, 1 μg/ml	3	188±106	640±74	621±127	784±78	240	230	317
Con A, 5 μg/ml	5	2,484±386	7,589±1,638	5,790±1,203	7,572±1,794	205	133	204
PHA, 10 μg/ml	4	57,586±2,271	77,679±3,273	70,684±2,464	77,585±3,069	35	23	35

* Data is presented from two suboptimal concentrations of Con A and an optimal concentration of PHA. cpm are mean±SEM of the three to five subjects tested.

‡ Cells preincubated in microtiter plates for 18 h before addition of mitogen.

§ Mean percent increase in cpm of the subjects tested. At 5 μg/ml Con A and at 10 μg/ml PHA, the mean percent increase caused by both preincubation and addition of indomethacin was no greater than the increase caused by indomethacin alone. At 1 μg/ml Con A, the mean increase with both treatments was higher than that with indomethacin alone, but this difference was not significant ($t = 1.31$, $P > 0.2$ by two-tailed t test).

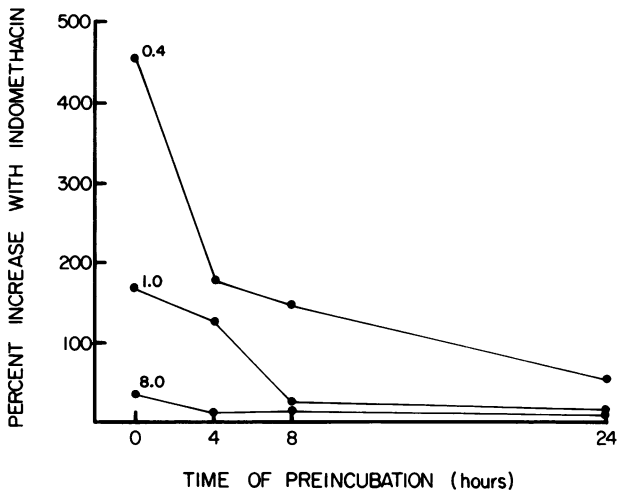

FIGURE 2 Enhancement in [^{3}H]thymidine incorporation caused by addition of indomethacin (1 μg/ml) as a function of duration of preincubation and as a function of concentration of PHA. Data are given as the mean of two experiments, and show that sensitivity to enhancement by indomethacin falls rapidly with preincubation at 37°C. The cells were preincubated in the microtiter plates.

by 24 h. It is also clear from this figure that indomethacin causes greater enhancement of cultures with lower concentrations of PHA.

As in our previous studies (10, 15), the effects of RO-20-5720, a PG synthetase inhibitor chemically unrelated to indomethacin (17), were similar to the effects of indomethacin mentioned above.

One possible explanation for the loss of sensitivity to indomethacin with preincubation is that preincubation shifts the kinetics of the PHA response, so that enhancement with indomethacin might be seen if [^{3}H]-thymidine incorporation were measured at different times after initiation of the cultures. The data shown in Table II indicate that this is not the case. In PHA-stimulated cultures of fresh cells, indomethacin enhances [^{3}H]thymidine incorporation throughout a 5-day culture; after preincubation indomethacin has a much diminished effect throughout the 5-day culture.

Prostaglandin production in mitogen cultures. Two possible explanations for the loss of enhancing effect of indomethacin after preincubation of cells are apparent. First, the PG-producing suppressor cell could be "short-lived" and stop producing PGE_2. Second, the cells normally inhibited by the endogenously produced PGE_2 could become refractory to its inhibiting action. To examine the first possibility, we measured production of PGE_2 in PHA-stimulated cultures over a 48-h period. The data presented in Fig. 3 demonstrate that both PGE and total PG (PGE plus PGA) accumulate over time in mitogen cultures. Thus the PG-producing suppressor cell is still active after 24 h of incubation. Inasmuch as it is probable that the PGA accumulated in cell cultures is a nonenzymatic breakdown product of PGE (18), the total PG production might be a closer estimate of the PGE produced in the culture (18, 19).

Table III presents data on PG production in cultures of PBMC as a function of PHA concentration. Total PG and PGE_2 production both increase with increasing doses of PHA. PBMC cultured without the addition of PHA still produce an appreciable amount of PG. Cultures of PBMC without PHA accumulate PGE and total PG over time in a manner parallel to that shown in Fig. 3 for cultures with PHA.

Loss of sensitivity to PGE_2 after preincubation. We next examined the effect of exogenous PGE_2 on mitogen stimulation of fresh vs. preincubated leukocytes. As shown in Table IV, after overnight incubation PHA-stimulated leukocytes are much less sensitive to the inhibiting effects of exogenous PGE_2. It required 100- to 1,000-fold higher amounts of exogenous PGE_2 in cultures of preincubated cells to achieve levels of in-

TABLE II

Kinetics of Indomethacin Enhancement of [^{3}H]Thymidine Incorporation in Fresh vs. Preincubated PBMC Stimulated by PHA (4.0 μg/ml)

Fresh cells				Preincubated cells			
Hours of pulse*							
24–48	48–72	72–96	96–120	24–48	48–72	72–96	96–120
cpm†							
10,377	40,330	48,369	48,843	13,104	43,280	44,148	21,542
Percent increase of cpm with indomethacin							
65	43	49	50	14	17	12	5

* Hours after addition of PHA and indomethacin to the cultures. The preincubated cells were cultured for 20 h before addition of PHA and indomethacin.
† Mean cpm of [^{3}H]thymidine incorporation in PBMC from two subjects stimulated by PHA (4.0 μg/ml) without indomethacin in the culture.

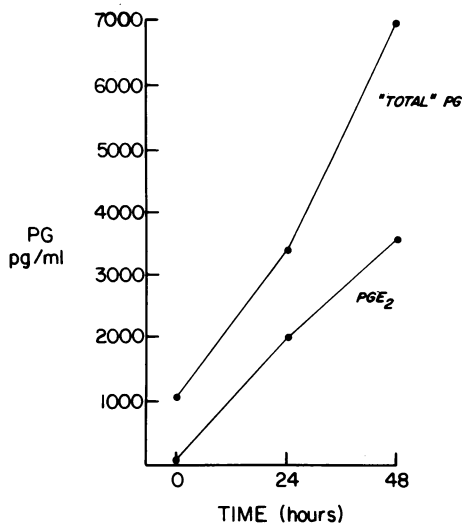

FIGURE 3 PG production in a PHA (0.2 μg/ml) culture of PBMC. Both total PG (PGA and PGE) and PGE_2 concentrations in the supernate appear to increase linearly with time. PG concentration is expressed as picograms per milliliter in the culture supernate. 1,000 pg/ml ≅ 3 nM.

TABLE IV
Percent Inhibition of [³H]Thymidine Incorporation by PGE_2 in Cultures of Fresh vs. Preincubated Cells

PGE_2 concentration	Inhibition*		
	Fresh cells	Preincubated cells	Preincubated and washed cells
		%	
3 nM	23±5	7±2	8±2
30 nM	38±5	13±2	0±9
0.3 μM	58±3	17±2	17±2
3 μM	70±4	17±4	37±2
Radioactivity, *cpm*	34,786±4,681	41,362±5,622	44,277±5,044

* Data are expressed as mean±SEM of the percent inhibition of PHA (4.0 μg/ml)-stimulated [³H]thymidine incorporation in cultures of PBMC from eight subjects. The amount of inhibition with the preincubated or preincubated and washed cells is significantly less than the inhibition with fresh cells, $P < 0.01$ at each concentration of PGE_2.

hibition obtained in cultures of fresh cells. Fig. 4 graphs the loss of sensitivity to PGE_2 in PHA cultures as a function of time of preincubation. PBMC rapidly lose their susceptibility to inhibition by PGE_2 with preincubation. These curves for inhibition by PGE_2 as a function of time of preincubation are similar to the curves for enhancement by indomethacin as a function of time of preincubation (Fig. 2). Table V presents data on the kinetics of PGE_2 inhibition of PHA-stimulated fresh vs. preincubated cells. Addition of PGE_2 inhibits [³H]thymidine incorporation throughout a 5-day PHA-stimulated culture of fresh PBMC, whereas PGE_2 has little effect on PHA cultures of preincubated cells, regardless of when [³H]thymidine incorporation is measured.

The above data would suggest that although the PG-producing suppressor cell acts as it if were "short-lived," in actuality the responding population of cells has short-lived suppressibility. This loss of sensitivity of cells to PGE_2 would appear to be an active process, for preincubation of cells overnight at room tempera-

TABLE III
Prostaglandin Production in Cultures of PBMC as a Function of PHA Concentration

PHA concentration	PG synthetase inhibitor	Total PG*	PGE_2
μg/ml	μg/ml		
0	—	6,222	4,349
0.2	—	8,045	5,840
1.0	—	10,823	7,393
5.0	—	12,997	9,088
20.0	—	14,114	10,200
1.0	Indomethacin (1)	572	<300
1.0	RO-20-5720 (50)	840	<300

* Data are expressed as total PG or PGE_2 in picograms per milliliter produced in 48 h in cultures of 5×10^5 PBMC in 1 ml. Each value is the mean of two experiments on different individuals.

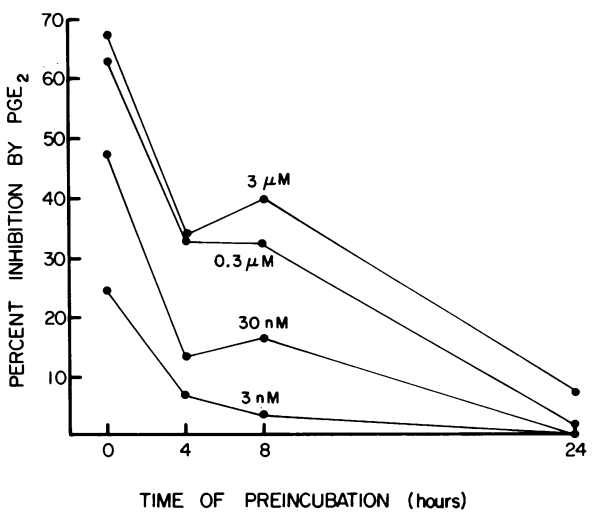

FIGURE 4 Percent inhibition of [³H]thymidine incorporation in PHA (0.4 μg/ml)-stimulated cultures caused by four concentrations of PGE_2 as a function of duration of preincubation of the cultures. Data are given as the mean of two experiments, and show that sensitivity to inhibition by PGE_2 decreases as the cells are preincubated at 37°C. The cells were preincubated in the microtiter plates.

TABLE V

Kinetics of PGE_2 Inhibition of [^3H]Thymidine Incorporation in Fresh vs. Preincubated Peripheral Blood Mononuclear Cells Stimulated by PHA (0.4 mg/ml)

PGE_2	Inhibition*							
	Fresh cells				Preincubated cells			
	%							
3 nM	29	25	29	28	−3	−2	24	17
30 nM	60	47	70	76	−40	−1	18	17
0.3 μM	78	67	73	76	−40	−7	34	15
3 μM	90	67	70	73	−3	6	32	16
Pulse, h‡	24–48	48–72	72–96	96–120	24–48	48–72	72–96	96–120
cpm§	896	6,219	14,137	12,423	306	8,100	14,084	14,264

* Mean percent inhibition of [^{3}H]thymidine incorporation caused by the four concentrations of PGE_2 at different times after addition of mitogens and drugs to the cultures. The values for 24–48 h are unreliable due to the low cpm obtained.

‡ Hours after addition of PHA (0.4 μg/ml), indomethacin, and PGE_2 to the cultures. The preincubated cells were cultured for 20 h before addition of PHA, indomethacin, and PGE_2.

§ Mean cpm of [^{3}H]thymidine incorporation in PBMC from two subjects stimulated by PHA (0.4 μg/ml) plus indomethacin (μg/ml).

ture rather than 37°C did not result in loss of sensitivity (data not shown).

Increasing sensitivity to PGE_2 with decreasing dose of mitogen. In our initial report of the PG-producing suppressor cell, we had found that the percent increase in [^{3}H]thymidine incorporation caused by indomethacin in PHA-stimulated leukocyte cultures did not vary significantly with dose until supraoptimal concentrations of PHA were used (15). We have reexamined that question using a wider range of suboptimal doses of PHA and found that indomethacin caused a greater percent increase in [^{3}H]thymidine incorporation as the dose of PHA decreased. This is demonstrated in Table VI, which represents a summary of the enhancement of [^{3}H]thymidine incorporation caused by indomethacin at seven concentrations of PHA in PBMC from three subjects. The percent increase in [^{3}H]thymidine incorporation with addition of indomethacin ranges from 1,059±134% at the lowest dose of PHA (0.2 μg/ml) to 4±3% at the highest dose (20 μg/ml).

One possible explanation for this varying response to indomethacin is that the PBMC might be more sensitive to the inhibiting effects of endogenous PGE_2 at lower concentrations of mitogen. This indeed appears to be the case. Fig. 5 graphs the percent inhibition caused by four concentrations of PGE_2 of [^{3}H]thymidine incorporation induced by different concentrations of mitogen. A family of curves is generated, cultures with the lowest concentrations of PHA showing the greatest sensitivity to exogenously added PGE_2. Table VII summarizes data from experiments from five subjects comparing sensitivity to PGE_2 at two suboptimal concentrations of PHA (0.2 and 1.0 μg/ml). Even the small reduction in PHA concentration from 1.0 to 0.2 μg/ml results in significantly increased sensitivity to exogenous PGE_2.

DISCUSSION

The findings of this investigation are the following. First, the enhancement of mitogen stimulation by indomethacin is gradually lost as the responding cells are preincubated. Second, this loss of response is due to a loss of sensitivity to PGE_2 in preincubated cells. Third, the increase in [^{3}H]thymidine incorporation in

TABLE VI

Percent Increase in [^3H]Thymidine Incorporation Caused by Indomethacin as a Function of Dose of Mitogen

PHA concentration	Without indomethacin	With indomethacin	Increase with indomethacin
μg/ml	cpm*		%
0.2*	411±213	4,764±3,155	1,059±134
0.4	4,823±1,556	16,069±1,724	233±86
1.0	18,164±3,410	36,533±6,281	101±33
2.0	27,768±5,569	47,205±5,591	70±10
4.0	33,079±5,876	56,630±7,378	71±10
8.0	43,428±8,923	60,777±11,753	40±6
20.0‡	52,111±16,428	54,196±17,197	4±3

* This concentration was supraoptimal in two of the three subjects tested; i.e., the cpm at this concentration was lower than the cpm at 8.0 μg/ml PHA in two of the subjects.

‡ The data is expressed as mean±SEM of cpm, and of percent increase in [^{3}H]thymidine incorporation, in three experiments on different subjects.

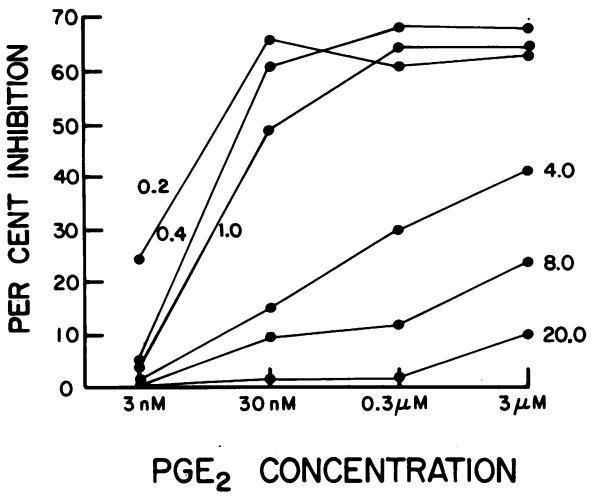

FIGURE 5 Percent inhibition of [^{3}H]thymidine incorporation caused by PGE_2 at six concentrations of PHA. Data is from one experiment, and shows that, at any given level of PGE_2, more inhibition is caused in PHA cultures as the concentration of the mitogen is decreased. In this experiment, the PHA concentration of 4.0 μg/ml gave optimal stimulation.

response to suboptimal doses of mitogen after preincubation of cells is due, at least in part, to this loss of sensitivity to PGE_2. Thus, the "short-lived suppressor cell" assay and the "PG-producing suppressor cell" assay measure overlapping phenomena. Fourth, sensitivity to PGE_2 and enhancement by indomethacin increases in mitogen cultures as the dose of mitogen decreases.

The finding that sensitivity of lymphocytes to PGE_2 increases with decreasing doses of mitogens explains why earlier investigators found a suppressive effect only with an amount of PGE three orders of magnitude higher than we employed to suppress the mitogen response (20). The earlier reports used optimal doses of mitogens whereas we have employed slightly suboptimal levels. As shown in Fig. 2, changing from 1 to 4 μg/ml in PHA concentration results in a 100-fold decrease in suppressibility of the lymphocytes by PGE_2. These data also stress the fact that effects of the endogenous PG-producing suppressor cells are relatively greater with suboptimal mitogen stimulation and must be considered in the interpretation of all experiments that involve a differential response to a range of mitogen doses. At the lowest dose of PHA, the lymphocyte response is >90% inhibited by endogenously produced PGs. Thus, one would expect that diseases associated with increased activity of the PG-producing suppressor cell (10) would show relatively greater suppression of responses to suboptimal doses of mitogen compared to normals, with less effect seen at optimal mitogen levels.

It is not yet clear why sensitivity to PGE increases at lower concentrations of mitogen. It would not appear

TABLE VII

Percent Inhibition by PGE_2 of [^{3}H]Thymidine Incorporation Stimulated by Two Suboptimal Concentrations of PHA

PGE_2 concentration	Inhibition* PHA 0.2 μg/ml	PHA 1.0 μg/ml	Significance‡
	%		*P*
3 nM	39±9	22±4	<0.10
30 nM	72±7	40±7	<0.01
0.3 μM	76±7	54±8	<0.05
3 μM	79±5	60±7	<0.02

* Data is expressed as mean±SEM of the percent inhibition of PHA (0.2 and 1.0 μg/ml)-stimulated [^{3}H]thymidine incorporation in cultures of PBMC from five subjects.

‡ *P* values obtained using one-tailed *t* test, comparing difference in percent inhibition caused by each of four concentrations of PGE_2 at two concentrations of PHA.

that PGE binds directly to PHA and Con A, for this would not explain the loss of sensitivity to PGE after preincubation of cells. Addition of exogenous PGE_2, together with mitogen, to preincubated cells does not decrease the response, whereas the same procedure with fresh cells results in a marked decrease (Table IV). We have recently described a high affinity binding site for PGE_2 on human PBMC.[2] It is possible that the loss of sensitivity of lymphocytes to PGE_2 is caused by a loss of these receptors, or the loss of sensitivity could be mediated at a step distal to PGE binding to membrane receptors.

Ferraris and DeRubertis (21) have reported that mitogen- or antigen-stimulated mouse splenocytes produced substantial amounts of PG in culture. It is of interest that a substantial amount of PGE_2 is produced in cultures of human PBMC without the addition of PHA (Table III). Other work in our laboratory has identified the PG-producing suppressor cell as a monocyte or macrophage, using double labeling experiments with fluorescein-tagged anti-PGE_2 antibodies and ingestion of polystyrene beads.[3] This cell apparently is capable of a substantial unstimulated PGE_2 production, which then increases when increasing concentrations of PHA are added. But while PGE_2 production increases, sensitivity to PGE_2 decreases with increasing PHA concentrations, so that, in balance, PGE_2 plays a greater role in cultures stimulated with small amounts of PHA.

Over the past several years, several other suppressor cell systems have been described in experimental animals and man. It is possible and perhaps even likely

[2] Goodwin, J. S., A. Wiik, M. Lewis, A. D. Bankhurst, and R. C. Williams, Jr. Manuscript submitted for publication.

[3] Bankhurst, A. D., J. S. Goodwin, R. P. Messner, and R. C. Williams, Jr. Manuscript in preparation.

that some of these systems work through PGE_2 production. The Con A-activated suppressor cell described in animals by Pierce and co-workers (22) and also by Waksman et al. (2, 14) is also present in humans (12, 13). We have found that this cell does not work via PG synthesis in humans (15). This is in keeping with findings in experimental animals describing a glycoprotein as the mediator for this cell (14). On the other hand, Stobo et al. (23) has described a circulating suppressor cell in the peripheral blood of some patients with chronic fungal diseases. The macrophages of these patients liberate a soluble material that will suppress the mitogen response of normal cells. Preincubation of either normal or patient cells renders them insensitive to the suppressing effects of this soluble material (24). Thus, this suppressor cell behaves similarly to the PG-producing suppressor cell. Baird and Kaplan (25) have studied the suppression of mitogen cultures by macrophages in mice. This suppression is also mediated through a soluble substance (26). Olding et al. (27) have recently reported that fetal cells secrete a dializable substance that suppresses the response of maternal lymphocytes to mitogens. These mediators have not been identified, but the possibility that they could be PGE_2 should be investigated.

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