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Prostaglandins (PG) of the E series, PGE_1 and PGE_2 (PGEs), can induce elevations of intracellular cyclic AMP (cAMP) among thymus-derived (T) lymphocytes (T cells) and inhibit their reactivity. For example, 0.1 μM of PGEs induces a two- to threefold increase of intracellular cAMP among human peripheral blood T cells and a 20-30% suppression of their blastogenic response to phytohemagglutinin. However, this suppression actually represents the net reactivity of T-cell populations demonstrating quite different responses to PGEs. Fractionation of T-enriched populations on a discontinuous density gradient yields a population of high density cells whose phytohemagglutinin-induced blastogenic response is suppressed 60%; a population of intermediate density cells whose response is suppressed 20%; and a population of low density T cells whose response is not suppressed, but is enhanced 20% by both of the PGEs. The diametrically opposite responses of low and high density T cells to the PGEs is not related to any difference in their intrinsic mitogen reactivity nor is it influenced by interactions with other T cells, bone marrow-derived (B) cells, or monocytes. Moreover, the distinct blastogenic response of low and high density T cells to PGEs does not simply correlate with PGE-mediated activation of adenylate cyclase. PGE_2 induced comparable absolute and identical relative increases of intracellular cAMP among the low and high density T cells. Cholera toxin, a potent […]

Prostaglandin E Modulation of the Mitogenic Response of Human T Cells

DIFFERENTIAL RESPONSE OF T-CELL SUBPOPULATIONS

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ABSTRACT Prostaglandins (PG) of the E series, PGE_1 and PGE_2 (PGEs), can induce elevations of intracellular cyclic AMP (cAMP) among thymus-derived (T) lymphocytes (T cells) and inhibit their reactivity. For example, 0.1 μM of PGEs induces a two- to threefold increase of intracellular cAMP among human peripheral blood T cells and a 20–30% suppression of their blastogenic response to phytohemagglutinin. However, this suppression actually represents the net reactivity of T-cell populations demonstrating quite different responses to PGEs. Fractionation of T-enriched populations on a discontinuous density gradient yields a population of high density cells whose phytohemagglutinin-induced blastogenic response is suppressed 60%; a population of intermediate density cells whose response is suppressed 20%; and a population of low density T cells whose response is not suppressed, but is enhanced 20% by both of the PGEs. The diametrically opposite responses of low and high density T cells to the PGEs is not related to any difference in their intrinsic mitogen reactivity nor is it influenced by interactions with other T cells, bone marrow-derived (B) cells, or monocytes. Moreover, the distinct blastogenic response of low and high density T cells to PGEs does not simply correlate with PGE-mediated activation of adenylate cyclase. PGE_2 induced comparable absolute and identical relative increases of intracellular cAMP among the low and high density T cells. Cholera toxin, a potent activator of adenylate cyclase, and exogenous 8-bromo cAMP mimicked the effects of the PGEs on these two T-cell populations. These data demonstrate that T cells are heterogeneous with regard to their response to the PGEs. Thus, PGEs should be considered as potential regulators rather than as universal suppressors for T-cell reactivity. Moreover, the effect of PGEs on the blastogenic response of a given T-cell population depends upon intracellular events which occur subsequent to elevations of cAMP.

INTRODUCTION

Prostaglandins (PG)[1] represent a family of 20 carbon, unsaturated fatty acids which may modulate function in a variety of biologic systems (1, 2). Their potential importance as modulators of immune reactivity is suggested by two observations. First, cells of the monocyte/macrophage lineage are crucially involved in regulating immune function and also constitute one of the major PGE-secreting classes of immunocytes (3–5). Second, it has been directly demonstrated that PG of the E series (PGE_1 and PGE_2 [PGEs]) can modulate immune function, presumably through their ability to alter intracellular levels of cyclic nucleotides (6–8). Most of these studies suggest that the PGEs suppress immune reactivity. For example, PGEs elevate intracellular cyclic AMP (cAMP) among thymus-derived (T) lymphocytes (T cells) and inhibit their mitogen-induced proliferation as well as their production of certain lymphokines (9–11). However, evidence also exists to suggest that PGEs may augment T-cell reactivity. Yoneda and Mundy (12) have recently demonstrated that PGE_1 enhances the mitogen-induced production of osteoclast-activation factor by T cells. It is possible therefore, that PGEs may not be universally

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[1] *Abbreviations used in this paper*: BSA, bovine serum albumin; cAMP, cyclic AMP; ETOH, ethanol; FCS, fetal calf serum; PBMC, peripheral blood mononuclear cells; PG, prostaglandins; PGEs, PGE_1 and PGE_2; PHA, phytohemagglutinin; 8-Br, 8-bromo.

suppressive for T-cell reactivity. Instead, their immunoregulatory effects may depend on the T-cell reactivity being assayed and thus reflect differential responses of individual T-cell populations.

In this paper, we describe experiments which demonstrate that T cells are indeed heterogeneous with regard to their response to the immunomodulating effects of PGEs. Although PGEs inhibit the phytohemagglutinin (PHA) -induced blast transformation occurring among whole peripheral blood T cells, this represents the contribution by T-cell populations manifesting distinctly different responses to PGEs. Fractionation of T-enriched populations on a 5-step, discontinuous bovine serum albumin density gradient yields a population of high density T cells whose mitogen reactivity is suppressed by both PGEs. In contrast, both PGEs augment the mitogen reactivity of low density T cells. PGE_2 induces comparable increases of intracellular cAMP among both low and high density T cells, and the effect of PGE_2 on their mitogen-induced blastogenesis can be mimicked by other agents capable of activating adenylate cyclase as well as by exogenous 8-bromo (8-Br) cAMP. These studies support the concept that PGEs function as modulators rather than as universal suppressors of T-cell reactivity and further suggest that this modulation results from intracellular events occurring subsequent to activation of adenylate cyclase.

METHODS

Cell population and culture. Peripheral blood mononuclear cells (PBMC) (70±4% T, 4±2% immunoglobulin [Ig] bearing, and 20±8% esterase positive); populations enriched for T cells (93±5% T, 2±1% Ig bearing, and 3±2% esterase positive); and populations enriched for bone marrow-derived (B) cells and monocytes (10±3% T, 40±10% Ig bearing, and 40 ±8% esterase positive) were obtained as previously described (13). T-enriched populations were fractionated on a discontinuous bovine serum albumin (BSA) density gradient that consisted of layers of 10/23/26/29/33% BSA (Pathocyte 5, Miles Laboratories, Inc., Kankakee, Ill. [13]). The recovered gradient-fractionated cells were divided into three populations by pooling cells sedimenting in the top two (10/23% and 23/26% BSA) interfaces; keeping cells of intermediate density separate (26/29%) interface; and by pooling cells sedimenting in the bottom interface and pellet. This was done to obtain numbers of cells sufficient for the experiments. 70% of the T cells originally placed on the gradient were recovered with 50±8.2 (mean±SE), 25±4.8, and 25±4.9% of the recovered cells obtained in the three fractions, respectively.

To determine the effects of PGEs on T-cell proliferation, PGEs (10 mg/ml in 100% ethanol [ETOH]) were added in a final concentration of 0.1 μM and 10 nM to T cells suspended in RPMI 1640 (Cell Culture Facility, University of California, San Francisco, San Francisco, Calif.) that contained 10% heat-inactivated (56°C for 30 min) fetal calf serum (FCS). Controls consisted of cell suspensions which contained an equivalent final concentration of ETOH (0.01 and 0.001%) without PGEs. 50,000 cells of each mixture were cultured in triplicate, round-bottom microtiter wells at 37°C in a humidified atmosphere of 95% air, 5% CO_2 for 4 d with medium only or with three concentrations of PHA (0.3, 1.0, and 3.0 μg/ml final concentrations; Burroughs Wellcome Co., Triangle Park, N. C.). Reactivity was determined by assaying the amount of tritiated thymidine (2.0 Ci/mM, New England Nuclear, Boston, Mass.) incorporated into DNA during the final 12 h of a 4-d culture (13). The mean change in counts per minute (counts per minute in stimulated cultures minus counts per minute in nonstimulated cultures) was calculated and results are expressed as:

$$\% \text{ inhibition} = \left[1 - \left(\frac{\Delta\text{cpm in cultures containing PGEs}}{\Delta\text{cpm in cultures containing ETOH}}\right)\right] \times 100,$$

or as

$$\% \text{ augmentation} = \left[\left(\frac{\Delta\text{cpm in cultures containing PGEs}}{\Delta\text{cpm in cultures containing ETOH}}\right) - 1\right] \times 100.$$

In initial experiments, it was ascertained that the regulatory effects of PGEs as determined by incorporation of tritiated thymidine correlated with those determined by morphologic evaluation of blast transformation.

Determination of intracellular cAMP. To determine the ability of PGEs to alter intracellular cAMP, cells to be assayed were first incubated in 5% FCS in the presence 0.1 mM of a phosphodiesterase inhibitor (RO-20-1724) for 45 min at 37°C. PGEs or appropriate concentrations of ETOH were then added, and the cells were incubated at 37°C in a humidified atmosphere of 95% air, 5% CO_2. At various intervals, aliquots were removed and centrifuged at 1,800 *g* for 30 s in a microfuge (Beckman Instruments, Inc., Spinco Div. Palo Alto, Calif.); and then the cell pellet was suspended in 20 mM sodium acetate, 0.1 mM methylisobutyl-xanthine, pH 4, and boiled for 5 min. Cellular cAMP was determined with the competitive protein binding assay of Gilman (14). Allowance was made for residual media in the pellet. Results are expressed as picomoles of cAMP/10^7 cells.

RESULTS

Inhibition of proliferation among whole and fractionated T cells by PGEs. In unpublished studies, we demonstrated that adherent human peripheral blood monocytes, but not T cells, synthesize PGE.[2] Quantitative gas-liquid chromatography/mass spectrometry analysis of culture fluids obtained from 1×10^6 adherent monocytes incubated for 48–72 h in 0.5 ml indicated the accumulation of 1.1 ng of PGE_1 and 16.9±2.1 ng of PGE_2. On the basis of these data, PGEs were used in final concentrations of 0.1 μM and 10 nM to test their effects on the proliferative response of T-enriched populations to three concentrations of PHA (these as well as all subsequent proliferative assays

[2] Goldyne, M., and J. Stobo. Manuscript submitted for publication.

were performed in the presence of 1 μM of indomethacin to block endogenous PGE synthesis by the small number of monocytes present in the T-enriched population). The results (Fig. 1) are consistent with previous reports demonstrating that PGEs suppress the PHA-induced proliferation among human peripheral blood T cells (11).

To investigate the possibility that distinct populations of T cells respond differently to PGE, advantage was taken of the fact that subpopulations of peripheral blood T cells can be delineated by differences in their density (13, 15). T-enriched populations were fractionated on a discontinuous BSA gradient, and the recovered three fractions were assayed for PHA reactivity in the presence of 0.1 μM of PGE_1 or PGE_2 (Fig. 2). Both PGEs markedly suppressed PHA-induced proliferation among high density cells. This suppression (≅60%) was substantially greater than that noted when comparable concentrations of PGEs were added to unfractionated T cells (30–40%, Fig. 1). In contrast, neither PGE inhibited the proliferative response among low density T cells. Instead, PGEs effected a small, but significant ($P < 0.01$), augmentation of reactivity to all three concentrations of PHA. PGEs caused ≅20% suppression of the PHA reactivity among cells of intermediate density. The absolute counts per minute for low and high density cells tested in the presence of 0.1 μM and 10 nM PGE_2 are presented in Table I. (Three representative experiments are shown.)

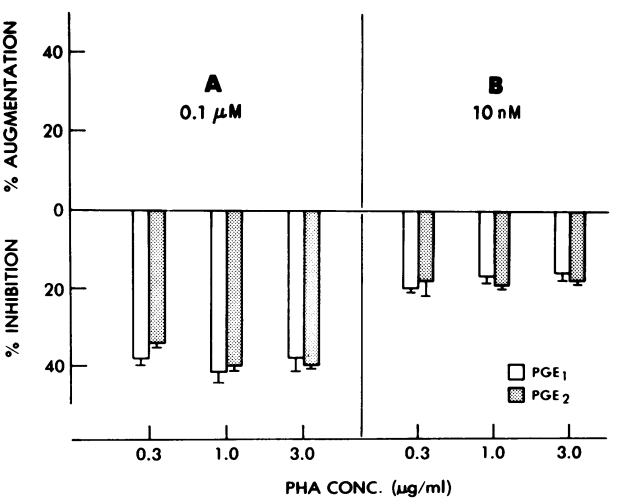

FIGURE 1 Effects of PGEs on the PHA reactivity of unfractionated T cells. T-enriched populations were tested for their reactivity to the indicated three final concentrations of PHA in the presence of 0.1 μM and 10 nM PGE_1 or PGE_2. Reactivity was measured by the incorporation of [^{3}H]thymidine with the results presented as percent augmentation or percent suppression (reactivity in the presence of PGE/reactivity in the presence of ETOH). The data represent the arithmetic mean±SE obtained for 10 experiments. Conc., Concentration.

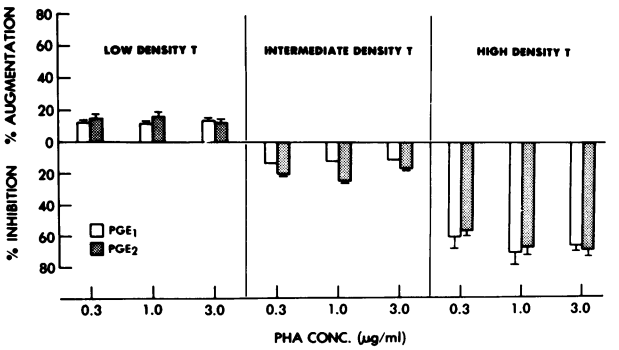

FIGURE 2 Effects of PGEs on the PHA reactivity of fractionated T cells. T-enriched populations were fractionated on a discontinuous BSA density gradient, and the recovered cells were pooled into three fractions. The reactivity of these cells to three concentrations of PHA was assayed in the presence of 0.1 μM PGE_1 or PGE_2. The results are presented as the mean percent augmentation or percent suppression ±SE noted for six experiments.

Several points concerning these studies should be emphasized. First, incubation of low density T cells in concentrations of BSA comparable to those present in the high density fractions, and vice versa, did not alter their subsequent response to PGEs (data not shown). Second, the differential effect of the PGEs on low and high density T cells was not dependent upon the time at which DNA synthesis was determined. PGE_2 also augmented reactivity among low and suppressed reactivity among high density cells when tritiated thymidine incorporation was measured after 72 or after 120 h of culture (data not shown). Third, PGE_2-mediated modulation of low and high density T cells was not substantially altered by the addition of B cells and monocytes. PGE_2 (0.1 μM) effected a 14% mean augmentation of maximal PHA reactivity among cultures containing 50,000 low density T cells and 50,000 cells from autologous populations enriched for B cells and monocytes. Similarly, PGE_2 suppressed by 47% the maximal PHA reactivity of cultures containing comparable proportions of high density T cells and B cells plus monocytes. (The maximal PHA reactivity of B cells and monocytes tested alone in the presence or absence of 0.1 μM PGE_2 was meager: 8,752 and 9,934 cpm, respectively.) Finally, PGE-mediated interactions with high density T cells did not, in turn, result in influences capable of suppressing low density populations (Fig. 3). Addition of increasing proportions of high density T cells to the low density population in the presence of PGE_2 (0.1 μM) resulted in a relatively linear decline in maximal PHA reactivity. This was similar to the situation noted when the reactivity of the low density population was simply diluted by the intrinsically unresponsive B cells and monocytes. The sum of these findings indicates the existence of T-cell populations manifesting distinct differences in their

TABLE I

Effect of PGE_2 on the PHA Reactivity of Fractionated T Cells

Source of T cells	Media	Concentration of PHA μg/ml... 3	1	0.3
Low density	*cpm*		*cpm*	
+ ETOH	844 ±258	52,594 ±18,734	63,874 ±18,659	39,890 ±11,033
+ PGE_2, 0.1 μm	760 ±130	62,321 ±19,036	79,112 ±21,994	52,762 ±14,308
+ PGE_2, 10 nM	513 ±227	57,633 ±18,515	66,923 ±19,546	40,483 ±19,513
High density				
+ ETOH	217 ±91	51,158 ±7,840	48,372 ±13,040	29,113 ±8,378
+ PGE_2, 0.1 μM	218 ±91	15,416 ±3,760	16,310 ±5,526	11,467 ±6,999
+ PGE_2, 10 nM	341 ±124	34,205 ±6,673	35,198 ±10,728	20,367 ±6,164

T-enriched populations were fractionated on a discontinuous BSA density gradient. Low density (fractions 1 and 2) as well as high density (fractions 4 and 5) cells were tested for their reactivity to three concentrations of PHA in the presence of ETOH (0.001%) or two concentrations of PGE_2 (0.1 μM, 10 nM). The response of nonstimulated cells (media) is also shown. Reactivity was measured by the incorporation of tritiated thymidine into DNA with results presented as mean counts per minute±SE for three experiments.

response to PGEs. Furthermore, they suggest that this differential response is not related to the BSA used in the density gradient, occurs independently of the duration of culture, and is not altered by interactions with other mononuclear cells.

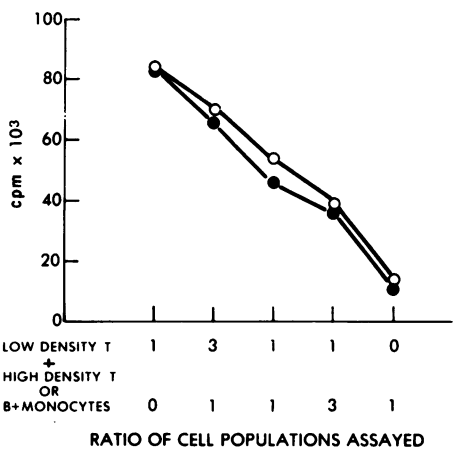

FIGURE 3 Effect of high density T cells or B cells and monocytes on the PGE_2-modulated reactivity of low density T cells. Low density T cells were mixed in the indicated proportions with either high density T cells (●) or with populations enriched for autologous B cells and monocytes (○). 100,000 cells from each mixture were then tested for their reactivity to three concentrations of PHA in the presence of 0.1 μM PGE_2. Results are presented as maximal counts per minute noted in response to PHA and is representative of two experiments.

PGE-induced modulation of intracellular levels of cAMP among fractionated T cells. PG can increase intracellular cAMP among intact lymphocytes, and it is presumbaly through this mechanism that they exert their inhibitory effects on lymphocyte proliferation (16, 17). One might postulate that the different PGE-initiated metabolic events occurring among low and high density T cells would be reflected by differences in PGE-induced elevations of this cyclic nucleotide. To determine this, both cell populations were incubated with 0.1 μM PGE_2, and intracellular cAMP levels were determined. The results of a typical experiment are graphically presented in Fig. 4. PGE_2-induced detectable increases of intracellular cAMP after 3 min of incubation (data not shown) with maximal increases among both populations occurring after 6 min. PGE_2 caused substantial absolute increases in cAMP among both low and high density T cells (five experiments, 486±140 and 238±51 pmol cAMP/10^7 low and high density T cells, respectively). Moreover, the relative (i.e., stimulated/nonstimulated) increase in cAMP assayed at 6 min was virtually identical among both populations (2.6±0.48 vs. 2.5±0.34 [Fig. 4]). These data suggest that the differential responses of low and high density T cells to the PGEs reflect differences in intra-

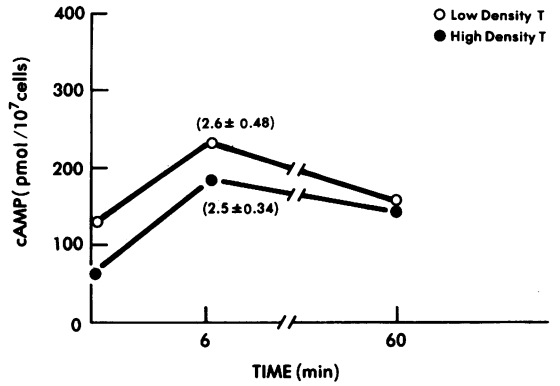

FIGURE 4 PGE_2-induced elevations of intracellular cAMP among low and high density T cells. 0.1 μM of PGE_2 was mixed with either low (○) or high density (●) T cells in the presence of an inhibitor of phosphodiesterase. At the indicated intervals, the cells were removed and assayed for intracellular cAMP. The results are presented as picomoles of cAMP/10^7 cells and are representative of the actual values obtained for five experiments (lines) or as the mean±SE relative increase (picomoles cAMP/10^7 cells at 6 min/picomoles cAMP/10^7 cells at time 0) noted for five experiments. No significant change in nonstimulated levels of intracellular cAMP among low or high density cells occurred during 60 min of incubation.

TABLE II

Effect of Cholera Toxin and 8-Br cAMP on the PHA-induced Blastogenesis among Fresh or Cultured Low and High Density T Cells

Populations tested	Concentration of PHA		
	μg/ml . . . 0.3	1.0	3.0
	Reactivity		
Fresh T cells			
Low density			
Cholera toxin	↑9	↑13	↑10
8-Br cAMP	↑9	↑10	↑16
High density			
Cholera toxin	↓53	↓47	↓49
8-Br cAMP	↓27	↓24	↓22
Cultured T cells			
Low density			
Cholera toxin	↑38	↑45	↑33
8-Br cAMP	↑29	↑48	↑43
High density			
Cholera toxin	↓64	↓43	↓49
8-Br cAMP	↓39	↓40	↓29

T-enriched populations were fractionated on a 5-step discontinuous BSA density gradient. The reactivity of fresh low density (fractions 1 and 2) and fresh high density (fractions 4 and 5) T cells to three concentrations of PHA was tested in the presence of cholera toxin (5 μg/ml, final concentration) and 8-Br cAMP (1 μM, final concentration). The same low and high density T cells were then incubated for 24 h at 37°C in 5% FCS. The cells were then washed, resuspended in fresh culture media, and tested for their PHA reactivity in the presence of cholera toxin or 8-Br cAMP. Reactivity is presented as percent augmentation (↑) or as percent suppression (↓) when compared with the reactivity of cells tested in the absence of cholera toxin or 8-Br cAMP and represents the mean of three paired experiments (i.e., the fresh and cultured populations were obtained from the same individual).

cellular events occurring subsequent to increases of intracellular cAMP. This is supported by the data presented in Table II. Cholera toxin, another activator of adenylate cyclase, and exogenous 8-Br cAMP augmented PHA-induced proliferation among low density and suppressed proliferation among high density T cells. Thus, both these agents mimicked the effects of the PGEs on PHA-induced blastogenesis occurring among the two T-cell populations.

Effect of PGE on cultured cells. It has been reported that short-term (24–48 h) culture of PBMC yields a residual population which is resistant to the suppressive effects of PGEs (18). This phenomenon is demonstrated by data presented in Fig. 5. The subsequent PHA response of T cells initially incubated in 5% FCS for 4, 24, and 48 h was progressively more resistant to PGE-mediated suppression; a process which was abrogated if 24 μg/ml of cycloheximide, an inhibitor of protein synthesis, was present during the initial culture (Fig. 5, this concentration of cycloheximide inhibited protein synthesis of T cells by 90%, as determined by the incorporation of [^{3}H]leucine into TCA-precipitable protein; data not presented). The data presented in Fig. 6 indicate that this represents an apparent, rather than an actual, loss of PGE-suppressible T cells. No significant difference was noted in the ability of PGE_2 to suppress PHA reactivity among fresh vs. cultured high density T cells. In contrast, whereas PGE_2 induced a 5–8% increase in the reactivity of fresh, low density cells, it caused a 30–40% augmentation of reactivity among cultured low density T cells. These cultured low density cells were also more sensitive to the enhancing effects of cholera toxin and 8-Br cAMP, whereas the reactivity of cultured high density cells was inhibited by both agents to an extent comparable to that noted for fresh, high density populations (Table II). The culture-dependent change in the response of low density cells did not occur if cycloheximide was present during the initial incubation (Fig. 6).

DISCUSSION

Several investigators with a variety of systems have indicated a dual role for PGEs in modulating biologic function. Bonta et al. (19) suggested that PGEs inhibit the proliferative, but augment the exudative phase of

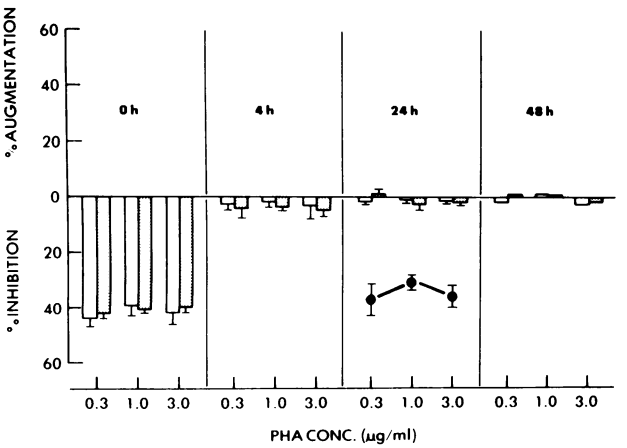

FIGURE 5 PGE modulation of the PHA reactivity of unfractionated, cultured T cells. T-enriched populations were incubated in either 5% FCS (bars) or 5% FCS containing 24 μg/ml of cyclohexamide (●). At the indicated intervals, the recovered cells were removed, washed, resuspended in fresh culture, mediated, and tested for their reactivity to three concentrations of PHA in the presence of 0.1 μM PGE_1 (▨) or PGE_2 (□). Results are presented as the mean±SE. Percent augmentation or percent suppression (reactivity in the presence of PGE/reactivity in the presence of ETOH) noted for two to six experiments.

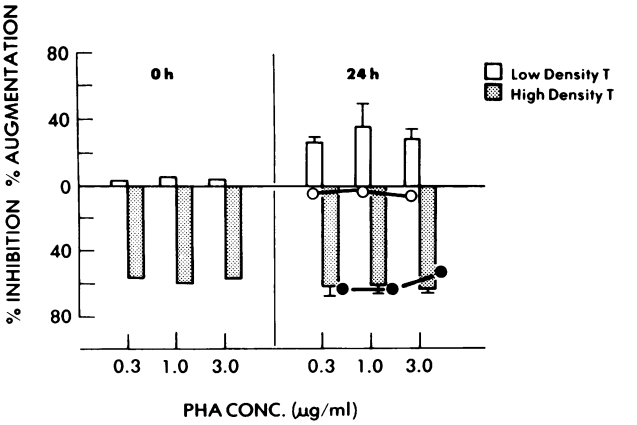

FIGURE 6 PGE_2 modulation of the PHA reactivity among cultured low and high density cells. Low or high density T cells were incubated either in 5% FCS (bars) or in 5% FCS containing 24 μg/ml of cyclohexamide (●). At the indicated intervals, the recovered cells were washed, resuspended in fresh media, and tested for their reactivity to three concentrations of PHA in the presence of 0.1 μM PGE_2.Results are represented as mean±SE. Percent augmentation or percent suppression (reactivity in the presence of PGE/reactivity in the presence of ETOH) noted for two to four experiments.

inflammatory reactions. Whereas PGEs enhance production of collagenase by macrophages, they inhibit their secretion of plasminogen activator (20).[3] PGEs inhibit the liberation of some lymphokines (migration inhibition factor, for example) but enhance the production of others (osteoclast-activating factor and a factor affecting vascular permeability [9, 10, 12, 21]). Our data suggest a similar dual role for PGEs in modulating PHA-induced T-cell proliferation. PGEs augment proliferation among a population of low density T cells and suppress proliferation among higher density T cells. Novogrodsky et al. (22) also suggested the existence of populations of T cells differing in their response to PGE modulation. These authors demonstrated that PGE effected relatively minor suppression among T cells responsive to some mitogens (PHA and concanavalin A) but marked suppression of T cells responsive to others (peanut agglutinin and soybean agglutinin).

At least three explanations might account for the noted differences in the response of the T-cell populations to PGE. First, it could simply be related to changes induced by the fractionation procedure used to obtain the T-cell populations. However, it was not possible to alter the PGE-modulated response of T-cell populations by incubating them in different concentrations of BSA. Recombination of low or high density T cells with B cells and monocytes did not substantially alter their response to PGEs, although it did blunt PGE-mediated suppression among the high density populations (47% suppression among mixed populations vs. 60% suppression among high density T cells tested alone). Additionally, high density T cells did not alter the PGE-mediated response of low density cells, at least when compared with the effects of B cells and monocytes. When considered together, these data suggest that the distinct reactivity of fractionated T cells to PGEs reflects intrinsic differences relevant to their response as they exist among whole PBMC.

The second explanation which might be pertinent to the different responses to PGEs concerns the ability of PGEs to modulate intracellular levels of cyclic nucleotides. Because agents which elevate intracellular cAMP inhibit T-cell proliferation, we hypothesized that PGE would increase intracellular cAMP among high but not among low density T cells. Instead, PGE_2 initiated comparable absolute and identical relative elevations of this cyclic nucleotide among both populations. In data not presented, it could be demonstrated that PGE_2 also induced comparable relative increases in cAMP among both populations when determinations were performed in the absence of an inhibitor of phosphodiesterase. This result rules against possible gross differences in the catabolism of cAMP existing among the two T-cell populations. Most importantly, the modulating effects of the PGEs on both T-cell populations could be mimicked by another agent capable of activating adenylate cyclase (cholera toxin) as well as

[3] Werb, Z. Personal communication.

by exogenous 8-Br cAMP. In preliminary experiments, there was no difference in PGE_2-induced activation of cAMP-dependent protein kinease among the T-cell populations.[4] We did not assay for other metabolic events which may be important in determining immunocyte activation (intracellular cyclic guanosine monophosphate, influx of divalent cations, and alterations among distinct pools of intracellular cAMP). Nonetheless, we feel that these data are consistent with the thesis that the differential response of T-cell populations to PGE is related to intracellular events occurring subsequent to elevations of intracellular cAMP. Novogrodsky et al. (22) also noted that T-cell populations responded differently to agents which increased intracellular cAMP.

The third explanation for the observed results could be that the differential response to PGEs is not a constant feature of distinct T-cell populations but rather a variable property related to different cell-cycle stages existing within a single T-cell line. We cannot rule out this possibility. Clearly, the responses of other homogeneous cell lines to PGEs have been observed to vary with cell-cycle stages (23). The experiments outlined in Fig. 6 do not directly bear on this point because nonstimulated cells may not move from one stage of the cell cycle to another. These experiments simply indicate that the differential response of low and high density cells to PGEs is consistent even after they have been incubated for 24 (Fig. 6) or 72 (data not shown) h. Even if the differential PGE responsiveness reported here is related to a function of the cell cycle, it does not detract from the notion that PGE is a modulator rather than a universal inhibitor of T-cell reactivity.

The experiments outlined in Fig. 5 emphasize the need for considering this concept of modulation in interpreting data concerning PGE-mediated regulation of heterogeneous cell populations. Goodwin et al. (18) recently reported that T-cell reactivity among cultured PBMC was resistant to PGE-mediated suppression. The data presented in Fig. 5 confirm this. However, this only represents an apparent resistance arising from an increase in the enhancing effect of the PGEs on the mitogenic response of low density T cells rather than any real loss of PGE-suppressible high density cells (Fig. 6, Table II). In other words, it is crucial to consider the possibility that net PGE-mediated effects on whole cell populations may represent the algebraic sum of different responses occurring among individual subpopulations.

Recently, there has been much interest in the immunosuppressive effects of PGEs. Likewise, recent data suggest that excessive production of PGEs by adherent cells may be etiologically related to clinically apparent T-cell dysfunction (24). Our data indicates that PGEs are not universally suppressive for T cells and suggests that their immunoregulatory capabilities may depend on the functional T-cell populations assayed. The pertinence of this in vitro modulation to in vivo biologic consequences of PGE-mediated interactions between macrophages and regulatory or effector T-cell populations remains to be determined.

[4] Kennedy, M., and J. Stobo. Unpublished observations.

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