JCI The Journal of Clinical Investigation

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J Clin Invest. 1989;83(4):1436-1440. https://doi.org/10.1172/JCI114035.

Research Article

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Prostaglandin E_2 Selectively Increases Interferon Gamma Receptor Expression on Human $CD8^+$ Lymphocytes

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Abstract

We previously reported that prostaglandin E_2 (PGE_2) at a physiologic concentration (10^{-8} M) and interferon gamma (IFNγ), acting sequentially, were required for the differentiation of suppressor cells in mitogen-stimulated cultures. The present study was designed to test whether PGE_2 might mediate IFNγ-dependent effects on $CD8^+$ cells by altering the number and/or affinity of their IFNγ receptors. $CD8^+$ and $CD4^+$ cells when cultured for 18 h expressed comparable numbers of IFNγ receptors of a single high affinity. Incubation with 10^{-8} M PGE_2 for 18 h, however, increased the number of IFNγ receptors on $CD8^+$ cells without affecting the binding affinity. Similar effects were not observed with $CD4^+$ cells, nor when $CD8^+$ cells were cultured in 10^{-8} M PGD_2. Concentrations of PGE_2, which were ineffective in the induction of IFNγ-dependent suppressor cell differentiation, also did not affect IFNγ receptor expression on $CD8^+$ cells. This observation of a specific stimulatory effect of PGE_2 on the display of IFNγ receptors of $CD8^+$ cells suggests a novel mechanism for eicosanoid function through tissue-specific regulation of hormone receptors.

Introduction

Both PGE_2 and IFNγ play roles in downregulation of the immune response. Data from several laboratories suggest that these agents act in large measure through induction of T suppressor cells that suppress many manifestations of T lymphocyte function including mitogen responsiveness (1–5). Previously we reported that differentiation of T suppressor cells in pokeweed mitogen (PWM) stimulated cultures required PGE_2 plus IFNγ, acting in sequence, for the generation of T suppressor cells (6, 7). That is, treatment with PGE_2 was required first, followed by IFNγ. Our preliminary studies also indicated that $CD8^+$ cells treated with PGE_2 bound greater quantities of IFNγ than cells treated with medium alone. These observations suggested possible effects of PGE_2 on IFNγ receptor expression.

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Received for publication 14 December 1988.

J. Clin. Invest.

0021-9738/89/04/1436/05 $2.00
Volume 83, April 1989, 1436–1440

A single high affinity IFNγ receptor has been described on both cultured and resting human T cells (8, 9). Upon activation of T lymphocytes, however, the number of IFNγ receptors has been reported to decrease by twofold (10). Since previous studies demonstrated that biological responses of IFNγ directly correlated with the amount of IFNγ bound to its specific cell receptors (8), the present study was designed to explore possible differences in the regulation of IFNγ receptors of $CD8^+$ and $CD4^+$ cells in response to PGE_2. Such differences may relate to the initial PGE_2 requirement of $CD8^+$ cells in the IFNγ-dependent differentiation of T suppressor cells.

Methods

Leukocytes. Peripheral blood mononuclear cells (PBMC) were obtained from heparinized whole blood. Some of the cells were stored in liquid nitrogen in 7.5% dimethyl sulfoxide until used.

Reagents. Monoclonal antibodies were obtained from Ortho Diagnostic Corp. (Raritan, NJ), Coulter Immunology (Hialeah, FL), and Becton Dickinson Monoclonal Center, Inc. (Mountain View, CA). Anti-CD28 MAb 9.3 (11), was kindly supplied by Dr. J. A. Hansen (Puget Sound Blood Center, Seattle, WA). Prostaglandins were purchased from Sigma Chemical Co. (St. Louis, MO) and human recombinant IFNγ was purchased from Genzyme (Boston, MA) and Amgen Biologicals (Thousand Oaks, CA).

^{125}I-IFNγ labeling. rIFNγ was radiolabeled using the Boulton-Hunter reagent (New England Nuclear, Boston, MA) as previously described (12). Unconjugated iodine was separated from protein-bound iodine over a Sephadex G-25 fine column (Pharmacia Fine Chemicals, Piscataway, NJ). Iodinated rIFNγ eluted from the gel column as a dimer with a M_r of ~ 34,000. The initial specific activity of the ^{125}I-IFNγ of different preparations varied between 18 and 34 μCi/μg, as counts per minute per microgram of biologically active IFNγ. IFNγ activity of radiolabeled preparations was measured by their ability to protect WISH cells (human amnion cell line CCL23; American Type Culture Collection, Rockville, MD) from lysis by vesicular stomatitis virus.

T cell separation and analysis. Techniques for fractionation of PBMC into subpopulations have been previously described (6). Briefly, fresh PBMC were depleted of adherent cells by plastic adherence. Nonadherent cells were further separated into B and T cell populations by passing over nylon wool columns. Purified $CD4^+$ and $CD8^+$ cell populations were prepared from the T cell-enriched fractions (nylon wool nonadherent cells) by negative selection using MAb and C treatment. Indirect immunofluorescence analysis indicated < 5% contamination with T cells of the reciprocal phenotype. In some experiments $CD8^+$ cells were further fractionated by panning into $CD28^-$ and $CD28^+$ subpopulations; flow cytometric analysis showed that positively selected cells were > 90% positive and negatively selected cells were < 5% positive for the antigen. Cell size was determined using forward angle light scatter on a Coulter EPICS V flow cytometer.

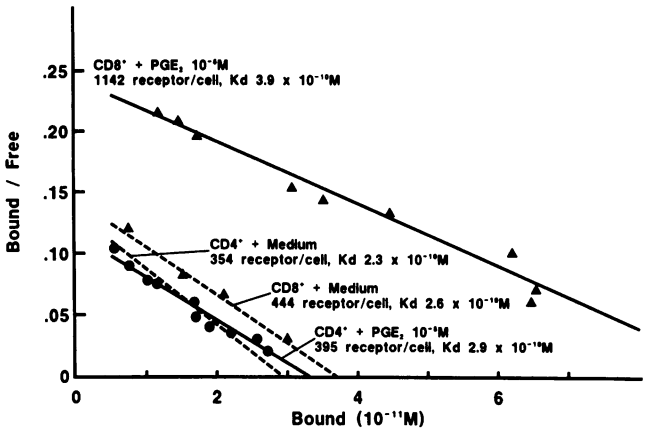

Figure 1. 10^{-8} M PGE_2 selectively increases the number of IFNγ receptors on $CD8^+$ cells. $CD8^+$ cells were cultured in medium (— ▲ —) or 10^{-8} M PGE_2 (—▲—) for 18 h at 37°C. $CD4^+$ cells from the same donor were similarly cultured in medium (— ● —) or 10^{-8} M PGE_2 (—●—).

Cell cultures. 2–3 × 10^6 $CD8^+$ or $CD4^+$ cells/ml were cultured in RPMI 1640 supplemented with 10% pooled human AB^+ heat-inactivated serum, 2 mM glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin. The cells were incubated at 37°C in 5% CO_2 for 18 h and then washed twice with RPMI containing 2% pooled human AB^+ heat-inactivated serum.

^{125}I-IFNγ binding assay. The binding assay was performed according to published protocols (12). Briefly, the cells were resuspended in RPMI 1640 supplemented with 2 mM glutamine and 2% pooled human AB^+ heat-inactivated serum. Duplicates of samples of a fixed number of lymphocytes were incubated with increasing concentrations of radiolabeled rIFNγ in Eppendorf tubes for 2 h at 4°C with occasional shaking. The cells were then washed twice using cold medium, and layered over a cushion of phthalate oils. After centrifugation, the radioactivity in the cell pellet was measured. Nonspecific binding, assessed by measuring the binding of ^{125}I-IFNγ in the presence of a 200–400-fold excess of unlabeled rIFNγ, varied between 10 and 33% of total isotope bound. Computer analysis of the best fit for the data points was performed using the Grapher program (Golden Software Inc., Golden, CO). Data in the figures represent the calculated specific binding per 5 × 10^6 cells in 0.1 ml. Specific binding was analyzed using the method of Scatchard (13). Statistical analysis of the data was performed using paired-sample *t* tests with two-tailed hypotheses and Bonferroni's method to adjust for multiple comparisons (14).

Results

Effect of prostaglandin E_2 on the number of IFNγ receptors of $CD8^+$ and $CD4^+$ cells. We previously reported that 10^{-8} M PGE_2, a concentration that falls in the physiological range (15), induced IFNγ-dependent differentiation of $CD8^+$ suppressor cells (7). We therefore investigated whether treatment of $CD8^+$ and $CD4^+$ cells with PGE_2 at this concentration would affect the number and/or affinity of IFNγ receptors. Purified $CD8^+$ cells and $CD4^+$ cells obtained from the same donor were cultured in medium alone or in 10^{-8} M PGE_2 for 18 h. Scatchard analysis of IFNγ specific binding resulted in linear plots (Fig. 1); the calculated number of IFNγ receptors/$CD8^+$ cell cultured in medium ranged from 315 to 399 (Table I). After treatment with 10^{-8} M PGE_2, however, the number of IFNγ receptors was significantly increased. In marked contrast to the effect on $CD8^+$ cells, treatment of $CD4^+$ cells with 10^{-8} M PGE_2 did not significantly change the number of IFNγ receptors. We reported previously that 10^{-8} M PGD_2 unlike PGE_2, could not replace the PGE_2 signal in IFNγ-dependent T suppressor differentiation (7). Similarly, when $CD8^+$ cells were treated with 10^{-8} M PGD_2 rather than PGE_2 there was no effect on expression of IFNγ receptors (Table I).

Table I. Effect of PGE_2 on Expression of IFNγ Receptors on $CD8^+$ and $CD4^+$ Lymphocytes

	Lymphocyte subset	No. of experiments	18-h culture	Receptors/cell*	$K_d \times 10^{-10}$ M*
A	$CD8^+$	5	Medium	367±64	3.0±1.2
	$CD8^+$	3	PGE_2 10^{-6} M	509±109	4.5±1.7
	$CD8^+$	5	PGE_2 10^{-8} M	1,089±187‡	4.2±1.4
	$CD8^+$	3	PGE_2 10^{-10} M	427±146	3.9±2.0
	$CD8^+$	3	PGE_2 10^{-8} M	426±170	4.2±2.1
B	$CD4^+$	4	Medium	308±36	2.5±0.5
	$CD4^+$	3	PGE_2 10^{-6} M	332±126	3.2±1.8
	$CD4^+$	4	PGE_2 10^{-8} M	340±98	3.7±1.7
	$CD4^+$	2	PGE_2 10^{-10} M	357 (264, 450)	2.1 (1.3, 3.0)

* Mean±SD or mean (range).
‡ Statistically significant ($P < 0.012$).

Dose-response analysis of PGE_2 effects on IFNγ receptor expression of $CD8^+$ and $CD4^+$ cells. Unlike 10^{-8} M PGE_2, higher (10^{-6} M) and lower (10^{-10} M) concentrations of PGE_2 failed to cause IFNγ-dependent differentiation of $CD8^+$ suppressor cells (7). We therefore examined whether the stimulatory effect of the 10^{-8} M concentration on IFNγ receptor expression could be observed at these concentrations. Purified $CD8^+$ and $CD4^+$ cells were cultured in medium alone or in 10^{-6}, 10^{-8}, or 10^{-10} M concentrations of PGE_2 for 18 h. As shown in Fig. 2 and Table I, the stimulatory effect of PGE_2 on IFNγ receptors of $CD8^+$ cells was exhibited only at the 10^{-8} M concentration. At no concentration tested was the affinity of the receptors significantly affected nor was the size of the cells altered compared to cells cultured in medium alone (data not shown), leading us to conclude that the observed increase in binding to $CD8^+$ cells was the result of increased numbers of

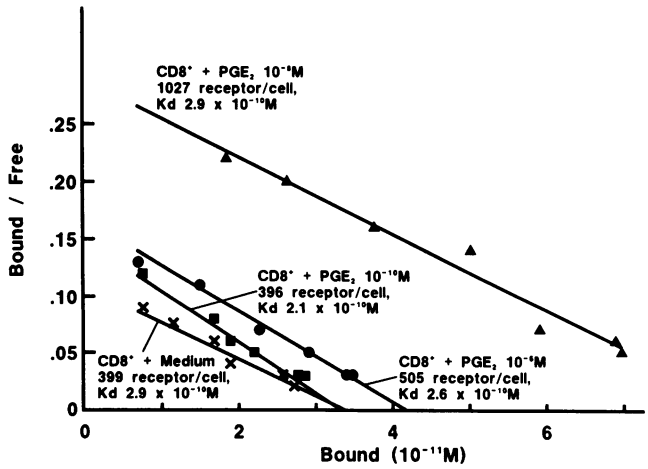

Figure 2. Dose-response analysis of the effect of PGE_2 on IFNγ receptors of $CD8^+$ cells. $CD8^+$ cells were cultured for 18 h at 37°C in medium (×), 10^{-6} M (●), 10^{-8} M (▲), or 10^{-10} M (■) concentrations of PGE_2.

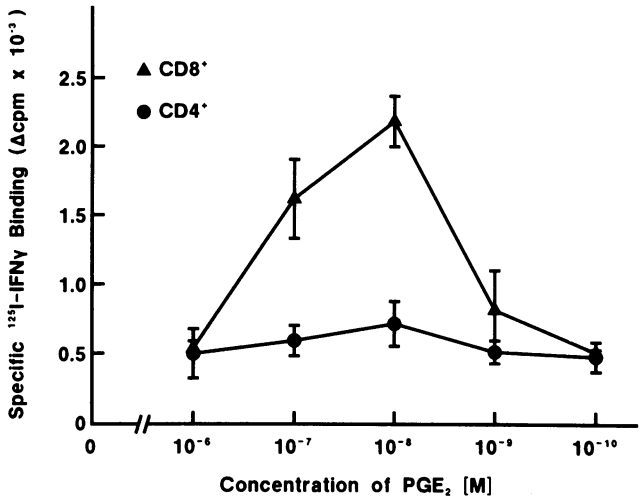

Figure 3. Effects of varying concentrations of PGE_2 on specific binding of ^{125}I-IFNγ to $CD8^+$ and $CD4^+$ cells. $CD8^+$ (▲) and $CD4^+$ (●) cells were cultured in increasing concentrations of PGE_2 for 18 h at 37°C. Each binding activity represents the mean±SD of triplicate measurements of specific binding of a single concentration of ^{125}I-IFNγ to 5×10^6 cell. Using two donors, we performed the experiment twice with similar results.

IFNγ receptors. No significant effects of PGE_2 were observed on $CD4^+$ cells at any concentration tested (Table I), confirming the selective effect of PGE_2 on IFNγ receptors of $CD8^+$ cells. It was not technically feasible to establish more extensive dose-response curves for both $CD4^+$ and $CD8^+$ cells in the same experiment using saturation binding curves for each PGE_2 concentration tested. Therefore, we compared the mean of triplicate measurements of specific binding of a single concentration of ^{125}I-IFNγ to cells treated with increasing concentration of PGE_2 for 18 h. As demonstrated in Fig. 3 the maximal effect of PGE_2 appeared at 10^{-8} M concentration, and at lower or higher concentrations the binding declined towards control levels.

IFNγ receptor expression on $CD8^+$ subpopulations. Expression of CD28, identified by the MAb 9.3, has been reported as a phenotypic marker of $CD8^+$ cytotoxic lymphocytes (CTL), but not $CD8^+$ T suppressor lymphocytes (11). Our earlier studies similarly demonstrated that the suppressor-effector activity of PWM-activated $CD8^+$ cells was predominantly in the $CD28^-$ subpopulation of $CD8^+$ cells (6). We therefore investigated whether PGE_2 had a preferential stimulatory effect on $CD8^+$ $CD28^-$ versus $CD8^+$ $CD28^+$ cells. Analysis of Scatchard plots of $CD8^+$ cells cultured in medium alone or in PGE_2 10^{-8} M for 18 h and then fractionated into $CD28^+$ and $CD28^-$ subpopulations revealed an increase in IFNγ receptors on both subsets (Fig. 4 and Table II), leading us to conclude that these two subsets of $CD8^+$ lymphocytes did not differ significantly in their responses to this treatment.

Discussion

This manuscript describes a PGE_2-induced increase in receptors for IFNγ that was selective for $CD8^+$ lymphocytes. Since PHA-stimulation of T cells results in downregulation of IFNγ receptors (10), the selective stimulatory effect of 10^{-8} M PGE_2 may represent a crucial difference between $CD4^+$ and $CD8^+$ cells in the regulation of IFNγ receptors and ultimately in their differentiation. Supporting this hypothesis, previous studies have shown that biological responses of IFNγ directly correlate with the amount of IFNγ bound to specific cell receptors (8). Furthermore, dexamethasone has been shown to increase levels of IFNγ receptors on monocytes and to enhance IFNγ functions (16). As noted, we have also shown that IFNγ-induced differentiation of PGE_2-treated $CD8^+$ cells exhibited a dose dependence (7) similar to that for increased receptor expression.

Little is known about the regulation of IFNγ receptors, however, there are data in other systems that may be relevant to our findings. For example, Akahoshi et al. recently reported that interleukin-1 (IL1) stimulates its own receptor expression on human fibroblasts through the endogenous production of PGE_2 (17). These authors also reported that addition of exogenous PGE_2 or analogues of AMP increased the specific binding of ^{125}I-labeled human IL-1α by more than twofold without affecting the binding affinity. Analogous to our results, the augmented IL1 receptor expression was maximal after 18 h incubation (17). The PGE_2 effect on IL1 receptors, however, was observed in a dose-dependent manner and was maximal at the supraphysiological concentration of 10^{-6} M (17). A role for a monocyte-secreted product in the differential expression of IL2 receptors on helper and suppressor T cells, which could not be replaced by IL1, was also reported by Malek et al. (18). In these studies, a monocyte-mediated signal induced expression of IL2 receptors on $CD4^+$ helper T cells, but not on $CD8^+$ suppressor cells. Since monocytes are major producers of PGEs, a role for PGs in this process is also possible. In addition, treatment with IFNγ which, like PGE_2, is a potent inducer of cAMP, increases expression of cellular receptors for tumor necrosis factor, IL2 and IgG Fc receptor (19).

Although we observed selectivity of PGE_2 effects on $CD8^+$ versus $CD4^+$ cells, similar selective effects were not observed among $CD8^+$ subpopulations defined by expression of CD28.

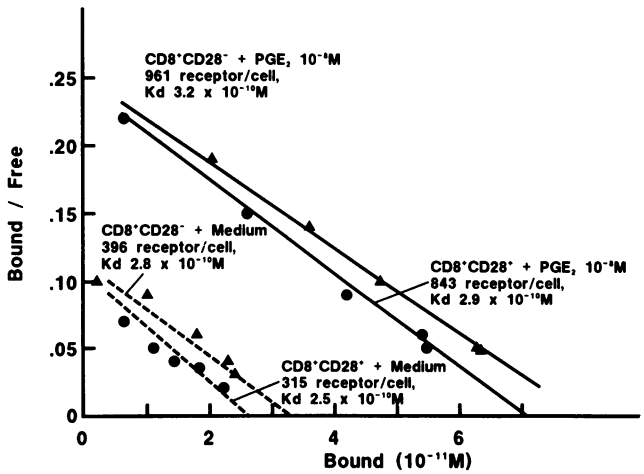

Figure 4. Effect of PGE_2 on IFNγ receptor expression on $CD8^+$ subpopulations. $CD8^+$ cells were cultured in medium alone or in PGE_2. Cells cultured in medium were then fractionated by panning into $CD28^+$ (— ● —) and $CD28^-$ (— ▲ —) subpopulations. Similarly, $CD8^+$ cells cultured in 10^{-8} M PGE_2 were fractionated by panning into $CD28^+$ (—●—) and $CD28^-$ (—▲—) subpopulations.

Table II. Expression of IFNγ Receptors on $CD8^+CD28^-$ and $CD8^+CD28^+$ Subpopulations in Response to 10^{-8} M PGE_2

Lymphocyte subset	No. of experiments	18-h Culture	Receptors/cell*	$K_d \times 10^{-10}$ M*
$CD8^+CD28^+$	2	Medium	302 (289; 315)	2.9 (2.5; 3.2)
$CD8^+CD28^-$	2	Medium	346 (295; 396)	3.5 (2.8; 4.1)
$CD8^+CD28^+$	2	PGE_2 10^{-8} M	883‡ (843; 922)	3.7 (2.9; 4.5)
$CD8^+CD28^-$	2	PGE_2 10^{-8} M	982‡ (961; 1002)	2.5 (1.9; 3.2)

* Mean (range). ‡ Statistically significant ($P < 0.03$).

An interesting possibility is that the increase in IFNγ receptors on the surface of $CD8^+$ $CD28^+$ cells is related to the stimulatory effect of IFNγ on differentiation of $CD8^+$ cytotoxic cells (20), perhaps reflecting the dual effects on this process of agents that increase intracellular cAMP concentrations (21). An increase in cAMP in the first 12 to 24 h of cultures enhanced CTL activity, whereas CTL generation was inhibited when intracellular cAMP concentrations were elevated for longer than 24 h (21). Thus, the early enhancement of CTL activity by an increase in cAMP correlates with the reported 24-h life span of PGE_2 receptors on the surface of lymphocytes (22), with our observation that PGE_2 increased IFNγ binding to $CD8^+$ cells and with the stimulatory effect of IFNγ on CTL generation (20). The reason that we (7) and others (3) failed to observe differentiation of CTL in response to PGE_2 treatment may reflect the ability of PGE_2 to inhibit IL2 production (1), which is required in this process (20). Our findings may also indicate differential responses of $CD8^+$ $CD28^-$ and $CD8^+$ $CD28^+$ subpopulations to intracellular events occurring subsequent to PGE_2 binding. For example, it is possible that the differences in the suppressor-effector function of these two subpopulations reflect a difference in the amounts of cAMP generated in response to IFNγ or in the response of these two subpopulations to an increase in cAMP. Indeed the studies of Goodwin et al. (23) indicate that T lymphocyte subsets differ in responses to agents that increase cAMP, such as IFNγ (24), with greater increases in cAMP levels of T suppressor cells as compared to T helper cells.

This study provides new insights into mechanisms of action of a class of arachidonic acid metabolites that is known for its important modulatory effects on a broad range of physiologic responses. The evidence for tissue-specific differences in the consequences of eicosanoids on hormonal receptors may have important implications for other systems affected by these agents and may lead to clearer understanding of the diversity of their immunoregulatory activities.

Acknowledgments

We thank Dr. D. Lewis, Dr. L. Birnbaumer, and Dr. R. Mattera for advice, Kathy Deemer for her technical assistance and Anna Moore for typing the manuscript.

This work was supported in part by U. S. Public Health Service grants AI15394 and AI21289. Computational assistance was provided by the CLINFO Project through U. S. Public Health Service grant RR00350.

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