Brief Definitive Report

PROSTAGLANDIN E INHIBITS THE PRODUCTION OF HUMAN INTERLEUKIN 2*

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A substantial body of evidence implicates prostaglandins (PG) as potent local regulators of the immune response (1, 2). Inhibition of human T cell proliferation by PG of the E type (PGE) is well established (3), but the mechanism is poorly understood. The discovery of interleukin 2 (IL-2), the lymphokine required for T cell proliferation (4, 5), has permitted further elucidation of this inhibitory event. Previously, it was shown (6, 7) that exogenous PGE inhibited lymphokine production by guinea pig lymph node lymphocytes, and recently it was reported (8) that PGE suppressed IL-2 production by murine lymphocytes. We report here that PGE inhibits the production of IL-2 by normal human peripheral blood lymphocytes, whereas PG of the type F (PGF) or A (PGA) do not. Further, we found that PG synthetase inhibitors raised IL-2 above normal levels. Removal of glass-adherent cells from mononuclear cell populations also led to increased IL-2 production by nonadherent cells. These cells were less responsive to stimulation by PG synthetase inhibitors but remained sensitive to inhibition by PGE. The results indicate that PGE, secreted by an adherent cell population, regulates the production of IL-2.

Materials and Methods

Separation of Lymphocytes. Peripheral venous blood, drawn from normal fasting men and women into heparinized Vacutainer tubes (Becton Dickinson & Co., Rutherford, NJ) was centrifuged at 1,200 *g* for 4 min to reduce volume and achieve partial separation of cells. After removal of plasma, the white cell layer was recovered and diluted with an equal volume of Hanks' balanced salt solution (HBSS). Mononuclear cells were isolated by Ficoll-Paque (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) density gradient centrifugation. The resultant lymphocyte-rich bands (unfractionated lymphocytes) were washed three times in HBSS and suspended in Hepes-buffered RPMI 1640 medium (M. A. Bioproducts, Walkersville, MD) containing 100 μg/ml streptomycin, 100 U/ml penicillin, 2.5 μg/ml Fungizone, and 5×10^{-5} M 2-mercaptoethanol (complete medium). Cell viability was >90% as determined by the trypan blue exclusion method. In some experiments, the blood was first passed through two successive glass wool columns (Pyrex wool; Corning Glass Works, Corning, NY) (600 g/10-cc syringe) to remove adherent cells. This adherent cell-depleted suspension is referred to as the nonadherent mononuclear cell population.

Production of IL-2. Unfractionated or nonadherent mononuclear cells were cultivated at a concentration of 10^6 cells/ml in complete medium supplemented with 2% heat-inactivated human AB serum. Cultures containing 1 μg/ml phytohemagglutinin (PHA) (Wellcome Reagents, Ltd., Beckenham, England) were incubated in the presence and absence of various compounds (PG, indomethacin or fentiazac) for 48 h at 37°C in a humidified atmosphere of 5% CO_2. After incubation, culture supernatants (conditioned medium [CM]) were sterilized by

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passage through 0.45-μm Acrodisc filters (Gelman Sciences, Inc., Ann Arbor, MI) and stored frozen at or below −20°C until assayed.

Prostaglandins E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, A_1, and A_2 (Sigma Chemical Co., St. Louis, MO) were dissolved in absolute ethanol and diluted in complete medium. The final concentration of ethanol in the cultures was ≤0.1%. Controls established that this level of ethanol did not interfere with the production or assay of IL-2. Indomethacin (Merck Sharp and Dohme, West Point, PA) and fentiazac (Wy-21,894; Wyeth Laboratories, Philadelphia, PA) were dissolved in phosphate-buffered saline, pH 7.2, and diluted in complete medium.

Measurement of IL-2. IL-2 levels were determined by measuring the ability of CM to stimulate proliferation of sensitized normal human lymphocytes. The assay cells (0.3×10^6 per ml) were cultured in complete medium with 20% heat-inactivated fetal calf serum (Δ-FCS) (Gibco, Grand Island, NY) and 1 μg/ml PHA for 7 d at 37° in a humidified atmosphere of 5% CO_2. Before assay, the cells were washed three times with HBSS, counted, and suspended (10^6 cells per ml) in complete medium containing 40% Δ-FCS. Aliquots of 10^5 cells (100 μl) were distributed in the wells of Linbro microtiter plates (Flow Laboratories, Inc., Hamden, CT), and test samples (100 μl per well) were added in quadruplicate. The plates were incubated for 48 h at 37° in a humidified atmosphere of 5% CO_2. The cultures were then incubated with 1 μCi/well of [^{3}H]thymidine (New England Nuclear, Boston, MA), and 16–18 h later they were harvested on glass fiber filters using a Titertek cell harvester (Flow Labortories, Inc., McLean, VA). The incorporated radioactivity retained by the filters was determined using a liquid scintillation counter. Data were evaluated using the following equation:

$$\left(\frac{\text{cpm test sample}}{\text{cpm control}} - 1\right) \times 100,$$

where the control sample represents CM produced by PHA alone, and the test sample represents CM produced by PHA and PG or drug. To rule out proliferation because of PHA present in the samples, assay cells were routinely tested for their response to fresh PHA (1 μg/ml) and found to be consistently unresponsive.

Results and Discussion

Using human mononuclear cells isolated by Ficoll-Paque gradient centrifugation, we investigated the effect of several PG on the production of IL-2. CM were prepared from PHA-stimulated lymphocyte cultures incubated for 48 h in the presence or absence of PGE_1, PGE_2, $PGF_{1\alpha}$, $PGF_{2\alpha}$, PGA_1, and PGA_2. Data from these and subsequent experiments were evaluated by analysis of variance (9). When tested over a dose range of 0.01–10 μg/ml, PGE_1 and PGE_2 inhibited IL-2 production in a dose-dependent manner, and they were inhibitory at physiological concentrations (10 ng/ml) (Fig. 1). In contrast, $PGF_{1\alpha}$, $PGF_{2\alpha}$, PGA_1, and PGA_2 (not shown) did not significantly inhibit IL-2 production, except that in this case, $PGF_{2\alpha}$ and PGA_1 inhibited at high concentrations (Fig. 1). Inhibition by $PGF_{1\alpha}$ and PGA_1 at these levels was not consistently observed, whereas in 10 out of 12 donors, PGE_2, at 0.1 μg/ml, inhibited IL-2 production by >50% ($P < 0.01$).

Because PG of the E type specifically inhibited IL-2, we cultivated mononuclear cells in the presence of two PG synthetase inhibitors, indomethacin (10) and fentiazac (11). When PHA-induced lymphocyte cultures were incubated in the presence of optimum concentrations of either inhibitor (1–5 μg/ml for indomethacin; 1–10 μg/ml for fentiazac), IL-2 levels increased 100–500% (Fig. 2). Thus, treatment with PG synthetase inhibitors augmented production of IL-2. Inouye et al. (12) also demonstrated that indomethacin enhanced IL-2 production by human mononuclear cells (as did removal of adherent cells) in a study evaluating methods for increasing the potency of IL-2.

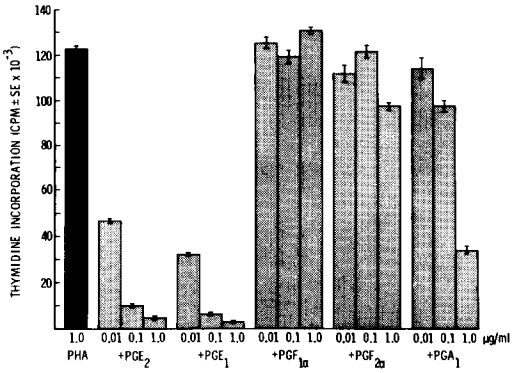

FIG. 1. Effect of various prostaglandins (PG) on PHA-induced production of interleukin 2 by lymphocytes from a representative normal human donor. IL-2 levels of CM, prepared in the presence of PHA and varying concentrations of different PG, were determined as described in Materials and Methods. The response of assay cells to PHA (1 μg/ml), complete medium, and to 48-h supernatant from resting lymphocyte cultures was 4,329 ± 124 cpm, 1,698 ± 51 cpm, and 1,330 ± 46 cpm, respectively. The response of assay cells to the various PG was similar to the response to medium or to supernatant from resting lymphocyte cultures.

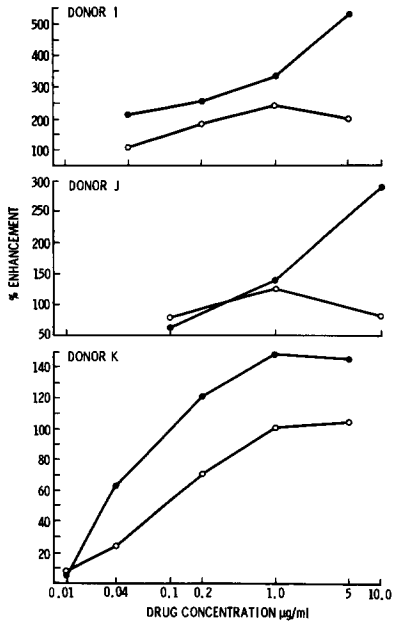

FIG. 2. Effect of the PG synthetase inhibitors, indomethacin and fentiazac, on the production of interleukin 2 by unfractionated mononuclear cells. CM obtained from cells cultivated in the presence of PHA (1 μg/ml) and varying concentrations of indomethacin or fentiazac were assayed as described in Materials and Methods. The results from three separate donors are reported as percent enhancement relative to interleukin 2 production in the presence of PHA alone. Significant enhancement ($P < 0.05$) was observed for all drug concentrations at or above 0.04 μg/ml. The response of assay cells to PHA-induced CM was 14,092 ± 541 cpm for donor I; 44,373 ± 1,084 cpm for donor J; and 32,691 ± 1,082 cpm for donor K. The responses of assay cells to (*a*) medium; (*b*) medium containing fresh PHA (1 μg/ml); and (*c*) 48-h culture supernatant from corresponding resting lymphocyte cultures were: (*a*) 1,085 ± 14 cpm, (*b*) 1,617 ± 91 cpm, and (*c*) 1,920 ± 130 cpm for donor I; (*a*) 3,783 ± 68 cpm, (*b*) 8,166 ± 271 cpm, and (*c*) 4,235 ± 140 cpm for donor J; and (*a*) 661 ± 72 cpm, (*b*) 957 ± 47 cpm, and (*c*) 1,147 ± 29 cpm for donor K. Fentiazac, ●; indomethacin, O.

TABLE I

Effect of the Removal of Adherent Cells from Mononuclear Cell Populations on the Production of Interleukin 2

Donor	Interleukin 2-mediated lymphocyte transformation [^{3}H]thymidine incorporation*	
	Unfractionated mononuclear cells‡	Nonadherent mononuclear cells§
	cpm ± SE	
A	34,062 ± 1,159	87,711 ± 2,433
B	54,870 ± 4,497	78,880 ± 3,475
C	14,092 ± 541	135,336 ± 2,825
D	8,080 ± 164	61,187 ± 2,667
E	6,367 ± 426	66,587 ± 3,433
F	6,781 ± 418	166,093 ± 7,084
G	3,344 ± 234	88,400 ± 2,759
H	30,085 ± 865	155,308 ± 2,390

* 18–20 h before harvest the cells were labeled with 1 μCi/ml [^{3}H]thymidine (>15 Ci/mmol). The results represent the mean of quadruplicate determinations for each condition. The mean response of assay cells to medium and to medium containing fresh PHA (1 μg/ml) was 1,556 ± 356 cpm and 3,646 ± 949 cpm, respectively, over all experiments. The response of assay cells to conditioned medium from donor-paired unfractionated and nonadherent cell populations was found to be significantly different by two-way analysis of variance ($P < 0.001$) for all donors.

‡ The mean response of assay cells to supernatants from resting unfractionated lymphocytes was 1,864 ± 244 cpm ($n = 8$).

§ The mean response of assay cells to supernatants from resting nonadherent lymphocytes was 1,797 ± 348 cpm ($n = 8$).

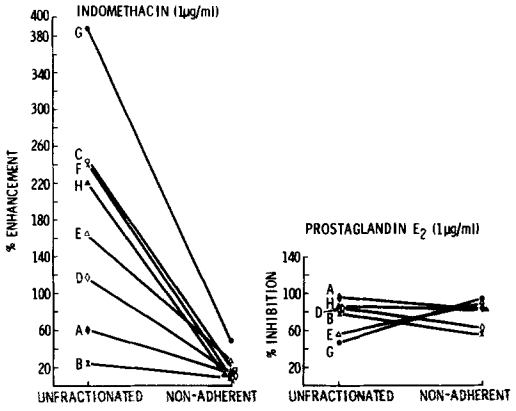

FIG. 3. Effect of indomethacin and PGE_2 on production of IL-2 by unfractionated and nonadherent mononuclear cell populations. PHA-stimulated cultures of unfractionated and nonadherent cells, corresponding to those described in Table I, were incubated with indomethacin (1 μg/ml) or PGE_2 (1 μg/ml) for 48 h at 37°C in a humidified atmosphere of 5% CO_2. CM were prepared and assayed as described in Materials and Methods. The results are given as percent enhancement (for indomethacin) or percent inhibition (for PGE_2) relative to IL-2 production in the presence of PHA alone. Statistical evaluation of the data by two-way analysis of variance showed a significant difference in the response of unfractionated and nonadherent cells to indomethacin ($P < 0.003$) but showed no significant difference in the response of both cell populations to PGE_2 ($P > 0.5$).

Goodwin et al. (13) identified a population of human glass-adherent cells that produce PGE_2 and suppress T cell mitogenesis. We studied IL-2 production after removing such cells. A comparison of IL-2 production by nonadherent and unfractionated mononuclear cell populations from several donors revealed that removal of

adherent cells significantly promoted IL-2 production (Table I). It is possible that donor to donor variation in IL-2 production, observed before removal of adherent cells (Table I), may be due in part to variation in endogenous PGE_2 levels. In addition, nonadherent cells were far less sensitive to stimulation by indomethacin (or fentiazac), but they were equally sensitive to inhibition by PGE_2 (Fig. 3). This observation suggests that PG synthetase inhibitors act preferentially on adherent cells. The increase in production of IL-2 by nonadherent cells is probably not the result of an increased density of these cells in nonadherent vs. unfractionated cell populations because the number of monocytes initially present in peripheral blood is only 4% (14). Nor could the enhancement in IL-2 production be attributed to effects of indomethacin, fentiazac, or PHA on the assay cells, because these compounds did not significantly stimulate [^{3}H]thymidine incorporation. Rather, the data indicate that removal of a PG-producing adherent cell enhances IL-2 production by eliminating the major source of PGE_2. This would explain the observation that adherent cell-depleted cultures were less responsive to indomethancin than were unfractionated cultures.

Recently, several other investigators (15–19) have demonstrated a requirement for the macrophage product, interleukin 1 (IL-1), in the production of IL-2 by murine lymphocytes. Moreover, Maizel et al. (20) reported that human monocyte-produced IL-1 augments lectin-stimulated mitogenesis of human T cells purified from peripheral blood. In contrast, we found that in vitro production of IL-2 by peripheral human lymphocytes is not dependent on an adherent cell being present. Indeed, removal of adherent cells resulted in enhanced IL-2 production. The difference between the findings may stem from the use by Maizel et al. of dialyzed macrophage-derived CM and/or purified IL-1 to demonstrate stimulation of T cell proliferation: such materials would be depleted of PGE produced by adherent cells. Although our results do not contradict the evidence of a T cell requirement for IL-1, they do suggest that peripheral blood lymphocytes do not require *de novo* synthesis of IL-1 for the production, secretion, and utilization of IL-2 in vitro.

The present findings support the concept that PGE_2, produced by an adherent cell population, plays a major role in the down-regulation of IL-2. Moreover, the fact that this mechanism acts across species lines underscores the relevance of animal models in the development of new drug therapies for modulation of PGE and T cell proliferative responses in human disease.

Summary

Prostaglandins of the E type specifically inhibited the production of interleukin 2 (IL-2) by normal human lymphocytes, whereas PG synthetase inhibitors such as indomethacin and fentiazac raised IL-2 production above normal levels. Removal of adherent cells from mononuclear cell populations also resulted in enhanced IL-2 production. The resultant nonadherent cell population lost sensitivity to the enhancement effect of PG synthetase inhibitors, suggesting that a PGE-producing adherent cell plays a major role in the regulation of IL-2.

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