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STIMULATION OF PROSTAGLANDIN E_2 AND THROMBOXANE B_2 PRODUCTION BY HUMAN MONOCYTES IN RESPONSE TO INTERLEUKIN-2

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Interleukin 2 (IL-2)[1] is a potent lymphokine involved in the regulation of immune responses and is classically regarded as a stimulus for the activation and growth of T-cells. Recent reports have demonstrated the IL-2 dependent activation of human peripheral blood lymphocytes into lymphokine activated killer cells capable of lysing tumor cells both in vitro and in vivo. In this study we report data which clearly show IL-2 may also act to down-regulate the immune response by inducing the synthesis of arachidonic acid metabolites with known immunosuppressive actions. Stimulation of peripheral human blood monocytes with IL-2 caused an increased production of prostaglandin E_2 (PGE_2) and thromboxane (TXB_2) in a dose-dependent manner. Kinetic analysis showed no increase above controls after 6 hours and maximal levels by 10 hours; elevated levels were maintained after 45 hours of incubation. After 20 hours of stimulation with 2000 U/ml IL-2, the level of PGE_2 and TXB_2 were greater than three-fold above controls, 0.7 and 19 ng/10^6 cells, respectively. The stimulation was relatively specific in that neither prostacyclin nor leukotrienes were produced in response to IL-2. These data demonstrate that IL-2 acts on human monocytes to induce the secretion of PGE_2 and TXB_2.

Interleukin 2 (IL-2) is a T-lymphocyte product necessary for the activation and long-term growth of various lymphocyte populations, including both T-cells and natural killer cells (1,2). The production of IL-2 is stimulated by accessory cells via the secretion of interleukin 1, and represents one of the final lymphokine-dependent activation steps in the elicitation of cell-mediated immune responses. Although extensive investigations have demonstrated that IL-2 has potent effects directed at

Abbreviations used in this paper:
IL-2, interleukin 2; PGE_2, prostaglandin E_2; 6-keto $PGF_{1\alpha}$, 6-keto prostaglandin $F_{1\alpha}$; TXB_2, thromboxane B_2; LPS, lipopolysaccharide.

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lymphocytes, relatively few studies have examined the effects of IL-2 on other immune cell populations. In this report we demonstrate that human recombinant IL-2 will stimulate human peripheral blood monocytes to secrete specific cyclooxygenase-derived metabolites, including prostaglandin E_2 (PGE_2) and thromboxane (TXB_2). This production occurred in a dose-dependent fashion and was suppressed by the cyclooxygenase inhibitor indomethacin. In addition, leukotriene synthesis was not induced by IL-2. This study has significant implications regarding the endogenous regulation of IL-2 production and lymphocyte activation.

Materials and Methods

Isolation of cells - Heparinized peripheral blood was obtained by venipuncture from normal volunteers, diluted 1:1 with normal saline and the mononuclear cells separated by Ficol-Paque (Pharmacia, Piscataway, NJ). The mononuclear cells were further separated on Sepracell-MN according to the manufacturer's instructions (Sepratech Corp., Oklahoma City, OK). The cells were washed 3 times, suspended in RPMI 1640 with 10 mM HEPES buffer, 2 mM L-glutamine and 100 units penicillin 100 µg/ml streptomycin (Hazelton Research Products, Denver, PA) at a concentration of 3×10^6/ml in 35 mm tissue culture plates (Costar, Cambridge, MA). The cells were allowed to adhere for 2 hrs at 37°C in 5% CO_2 and the non-adherent cells were removed by washing with warm media.

Stimulations - Purified human recombinant IL-2 (des-ala-ser_{125}) was the generous gift of Cetus Corp., Emeryville, CA. The material was dissolved in pyrogen-free sterile water and all dilutions were made with pyrogen-free normal saline. Indomethacin (Sigma Chemicals, St. Louis, MO) was prepared at 10^{-2}M in dimethyl sulfoxide (Sigma). Stock lipopolysaccharide (LPS) (Sigma) was prepared at 200 µg/ml in RPMI 1640. Stimulations were done for the times and doses indicated in the figure legends.

Quantitation of Arachidonic Acid Metabolites - The supernatants were collected, cells removed by centrifugation, and the samples extracted to remove protein and free fatty acids. Radioimmunoassays (RIA) using antibodies and methods developed by Fitzpatrick et al. (3) were used to quantitate prostaglandin E_2 (PGE_2), 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) and thromboxane B_2 (TXB_2). Bound ligand was separated from free ligand by the use of dextran-coated charcoal. The limits of sensitivity for PGE_2, 6-keto $PGF_{1\alpha}$, and TXB_2 were 8 picrograms, 4 picrograms, and 2 picrograms, respectively. Leukotrienes were measured by RIA for the following lipoxygenase products: LTC_4 (New England Nuclear, Boston, MA, 2.0 pg/ml sensitivity) and LTB_4 (Amersham, Arlington Heights, IL, 12.5 pg/ml sensitivity).

Controls - Control samples consisted of unstimulated cells processed in the same manner as those treated with the stimulants. Controls were obtained at each time point to account for spontaneous release of arachidonic acid metabolites. Lipopolysaccharide levels, as determined by the supplier of IL-2, was less than 2.5 fentograms/2000 Units of IL-2.

Statistics - Least squares linear regression was done to show a dose response to the IL-2 for Figure 1. The difference between the control and IL-2 treated cells in the kinetics study was determined by analysis of variance (Figure 2), as was the data for the indomethacin suppression

(Figure 3). The p value for the difference between the control and treated cells in Table 1 was calculated by the Student's t test, unless the variances were markedly dissimilar; in which case the p value was calculated by the non-parametric Wilcoxon rank sum test. p values were calculated compared to controls.

Results

As Shown in Figure 1, IL-2 induced peripheral blood monocytes to secrete PGE_2 and TXB_2 in both a dose-dependent and kinetic fashion. Neither PGE_2 nor TXB_2 were detectable at any IL-2 concentration tested prior to 6 hours, while maximum secretion of both PGE_2 and TXB_2 (at a dose of 2000 units/ml) reached a plateau 10 hours post-stimulation. At this time point, 2000 units/ml of IL-2 elevated monocyte-derived PGE_2 and TXB_2 by three-fold over control values. In contrast, there was no elevation above control values at any time point for either 6-keto-$PGF_{1\alpha}$ (the stable metabolite of prostacyclin) or LTB_4 or LTC_4 (data not shown).

A more detailed kinetics study was next conducted keeping the dose of IL-2 constant at 2000 units/ml (Figure 2). In this study, the secretion of PGE_2 and TXB_2 became maximally elevated by 10 hours and remained elevated compared to controls through the length of the investigation (45

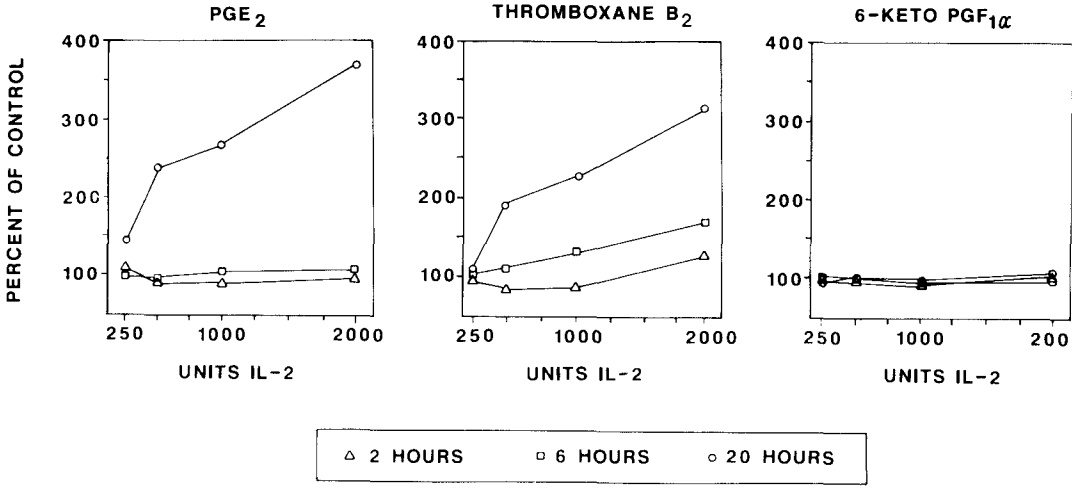

Figure 1. Production of PGE_2, TXB_2 and 6-keto $PGF_{1\alpha}$ by IL-2. Human peripheral blood monocytes were stimulated for the indicated times and doses; a non-stimulated control sample was run at each time point. For the 20 hour stimulation, linear regression showed a dose response curve for production of PGE_2 and TXB_2 ($p<.05$) but not for 6-keto $PGF_{1\alpha}$.

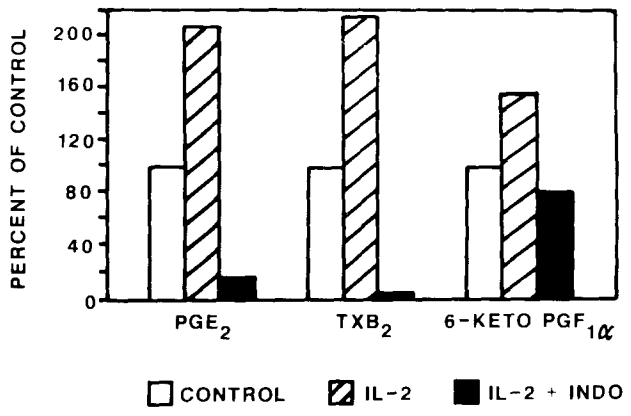

Figure 2. Kinetic analysis of PGE_2 and TXB_2. Human peripheral blood monocytes were stimulated with 2000 U/ml of IL-2 for the indicate times. Analysis of variance showed a significant difference between the control and stimulated populations ($p<.05$).

hours). There was no change in the amount of 6-keto-$PGF_{1\alpha}$ at any time point compared to controls. The secretion of PGE_2 or TXB_2 could be completely inhibited by the cyclooxygenase inhibitor indomethacin at a concentration of 10^{-6}M, as demonstrated in Figure 3. The suppression below control levels indicates that adhered monocytes tonically produce PGE_2 and TXB_2 and both the spontaneous production and the IL-2 stimulated production were suppressed. The IL-2 induction of arachidonate

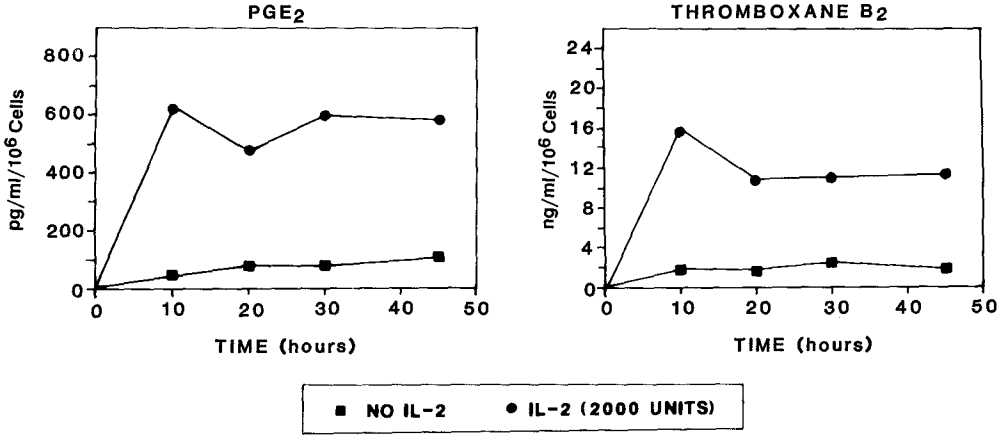

Figure 3. Indomethacin suppression of IL-2 induced prostaglandin synthesis. Indomethacin (10^{-6}M) significantly suppressed the production of PGE_2 and TXB_2 ($p<.05$ for both by analysis of variance), but not 6-keto $PGF_{1\alpha}$, in response to 4000 U IL-2/ml for 20 hours.

Table I. Stimulation of arachidonic acid metabolism by IL-2 and LPS

	Arachidonate Acid Metabolites ($pg/10^6$ cells) Mean ± SEM (Range)		
	Control	IL-2 2000 Units	LPS 10 μg
PGE_2	207 ± 8 (50-564)	770 ± 12* (160-1250)	2495 ± 3* (2333-2720)
TXB_2	7587 ± 55 (1265-2130)	19,138 ± 84** (2553-39100)	40,009 ± 52** (27,361-51,500)
6-Keto-$PGF_{1\alpha}$	91 ± 2 (65-115)	107 ± 3 (71-153)	342 ± 1** (325-365)

Human monocytes were stimulated for 20 hours and the supernatant assayed for activity. *$p<.01$ by Wilcoxon rank sum test. **$p<.03$ by Students t-test.

metabolism was relatively specific for the cyclooxygenase pathway since LTB_4 and LTC_4, as assessed by radioimmunoassay, were not elevated above control levels.

The IL-2 dependent production of PGE_2 and TXB_2 on a per cell basis is shown in Table I. For comparison, the data for LPS-induced monocyte production of PGE_2, TXB_2, and 6-keto $PGF_{1\alpha}$ is also presented, since LPS is an effective stimulus for cyclooxygenase-derived products (4). While not as potent a stimulus as LPS, the data clearly demonstrate the specific production of PGE_2 and TXB_2 in response to IL-2. The elevated production of TXB_2 monocytes in response to IL-2 is in agreement with a previous study (5) showing TXB_2 to be the major metabolite of stimulated human monocytes. Although LPS proved to be an effective stimulus for 6-keto $PGF_{1\alpha}$ production, elevating levels three-fold above background, IL-2 had no effect on its synthesis.

Discussion

A well-developed cell-mediated response is dependent upon the bi-directional communication that occurs via various immune cell-derived

mediators. These mediators are important in directing the progression of the immune response through several steps including: i) antigen uptake and processing by macrophages, ii) expression of Ia antigens on the surface of MØ and secretion of soluble mediators (7), iii) activation of lymphocytes with secretion of IL-2 ,and iv) clonal expansion of antigen-specific T-cells (7,8). While several studies have examined the various chemical signals that actively drive the immune response, there is limited data on events and mediators that down-regulate immune reactions. In this paper we demonstrate the novel ability of IL-2 to stimulate the production of PGE_2 and TXB_2 by human peripheral blood monocytes. The secretion of these arachidonic acid metabolites occurred in both a dose-dependent and kinetic fashion. PGE_2 and TXB_2 were identified as the major metabolites, as neither 6-keto $PGF_{1\alpha}$ (an additional cyclooxygenase product), nor the lipoxygenase products LTC_4 or LTB_4 were synthesized above control levels. The ability of monocyte/macrophage-derived PGE_2 to act as an immunomodulating compound is well established (9,10). In this study we further document a bi-directional communication circuit between IL-2 and PGE_2 whereby IL-2 can induce the production of PGE_2 from blood monocytes initiating a classic negative feedback inhibition loop. This elevation in PGE_2 could serve as an effective means to down-regulate immune responsiveness by suppressing Ia antigen expression (11), and regulating the production of IL-1 (12), tumor necrosis factor (TNF) (13) and IL-2, itself (14).

The ability of IL-2 to interact with mononuclear phagocytic cells via specific IL-2 receptors (IL-2R) has been previously documented (15), but the function of this IL-2R was not clear. The data in this paper demonstrate that IL-2 will cause monocytes to synthesize specific cyclo-oxygenase-derived metabolites; thus, a possible role the IL-2R on monocytes is to allow the induction of a feedback signal to quell immune responsiveness. In addition, recent reports have demonstrated that IL-2 may induce the production of TNF by peripheral blood mononuclear cells in

vitro (16) and, TNF has been shown to induce prostaglandin synthesis both in vitro (13) and in vivo (17). Therefore, it is possible that the IL-2 is having an indirect effect on the induction of arachidonate acid metabolism by acting through the production of TNF. Experiments are underway to address this question.

These observations have extremely important implications in the use of IL-2 as a biologic response modifier in cancer therapy. At a dose comparable to that used in our experiments, IL-2 has been used to generate lymphokine-activated killer cells (LAK) for the use in treating cancer patients (18). These LAK cells are generated from crude mononuclear cell preparations containing mononuclear phagocytic cells that will be induced to secrete PGE_2. Since PGE_2 has been shown to inhibit the generation of phytohemagglutinin killer cells (19), as well as the function of natural killer cells (20) (the probable precursor of LAK cells [21]), the endogenous production of IL-2 dependent PGE_2 may well inhibit the formation of maximally-active LAK cells.

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