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Research Article

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Influence of Natural and Recombinant Interleukin 2 on Endothelial Cell Arachidonate Metabolism

Induction of De Novo Synthesis of Prostaglandin H Synthase

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Abstract

We studied the effects of natural and recombinant human IL-2 (rIL-2) on secretion of prostacyclin (PGI₂), vWf, and tissue-type plasminogen activator (tPA). IL-2 elicited a steady increase in PGI₂ synthesis by cultured human umbilical vein endothelial cells (HUVECS) and bovine aortic endothelial cells but had no effect on vWf or tPA. Both purified natural IL-2 (nIL-2) and rIL-2 induced significant PGI₂ synthesis. Substitution of the cysteine residue at position 125 of rIL-2 with serine or alanine led to loss of PGI₂-stimulatory activity in HUVECS without affecting thymidine incorporation in lymphocytes. HPLC analysis of arachidonate metabolites detected predominantly 6 keto-PGF_{1α} (6KPGF_{1α}) peak. Treatment of cultured endothelial cells with cycloheximide and actinomycin D resulted in inhibition of 6KPGF_{1α} synthesis. The Western blot using a polyclonal antibody against PGH synthase revealed an increment in the 70-kD subunit of PGH synthase by nIL-2 and rIL-2, but not by alanine-substituted rIL-2. We conclude that IL-2 stimulates sustained PGI₂ production by a mechanism that includes the de novo synthesis of PGH synthase. This mechanism for regulating AA metabolism probably has important physiologic implications.

Introduction

IL-2 is a 15-kD glycoprotein secreted by lymphocytes. It was first described as a lymphokine capable of promoting the long-term growth of activated T lymphocytes in vitro (1, 2). It has subsequently been shown to modulate the functions of several subtypes of lymphocytes including cytotoxic T cells (3, 4), natural killer cells (5, 6), activated B cells (7, 8), and lymphokine-activated killer cells (9, 10). The biological activity of IL-2 is, however, not entirely limited to lymphocytes. Previous studies from our laboratory have shown that purified natural IL-2 (nIL-2)¹ has a significant stimulatory effect on prostacy-

clin (PGI₂) synthesis by bovine endothelial cells (11). In an effort to elucidate the mechanism by which IL-2 elicits sustained stimulation of eicosanoid synthesis, we investigated the effects of recombinant (r) and (n)IL-2 on endothelial cell AA metabolism as well as on the release of vWf and tissue-type plasminogen activator (tPA) from cultured human endothelial cells (EC). Our data indicate that IL-2 induces de novo synthesis of PGH synthase. By contrast, it has no effect on vWf or tPA generation.

Methods

Materials. Human nIL-2 was obtained from Cellular Products, Inc. (Buffalo, NY). Nonglycosylated rIL-2 (NG rIL-2), natural sequence analogue expressed in *Escherichia coli*, was from Amgen Biologicals (Thousand Oaks, CA). It has the entire 133 amino acids with addition of a methionine residue at the amino terminus. Two NG rIL-2 preparations that had a substitution of the cysteine at amino acid residue 125 (Cys¹²⁵) were obtained. One NG rIL-2 from Amgen Biologicals had substituted Cys¹²⁵ with alanine, while the other preparation from Cetus Corp. (Berkeley, CA) had substituted it with serine. The first alanine residue at the NH₂ terminus was also absent in the latter compound. Glycosylated rIL-2 (G rIL-2) expressed in yeast was obtained from Genzyme Corp. (Boston, MA). Endotoxin was determined by the Limulus amoebocyte lysate assay (Whittaker M. A. Bioproducts, Walkersville, MD). It was below the detectable level (< 0.1 ng/ml) in each IL-2 preparation. Purified PGH synthase was obtained from Oxford (Ann Arbor, MI). Affinity-purified polyclonal antibodies directed against purified ram seminal vesicle PGH synthase were obtained from Dr. H. H. Tai and also prepared in our laboratory. Both antibodies had similar activities on the Western blotting. 1-[¹⁴C]AA (56 mCi/mmol sp act) and all the tritiated eicosanoid standards (PGD₂, PGE₂, PGF_{2α}, 6 keto-PGF_{1α} [6KPGF_{1α}], TXB₂, 12-hydroxyheptadecatrienoic acid [HHT], 15-hydroxyeicosatetraenoic acid [HETE], 12 HETE, and leukotriene B₄) were obtained from New England Nuclear (Boston, MA).

Culture of endothelial cells. Human umbilical vein endothelial cells (HUVECS) were prepared from freshly obtained umbilical veins as previously described (12, 13). The cells were cultured on porcine skin gelatin (1 mg/ml)-coated flasks and maintained on medium 199 (Gibco Laboratories, Grand Island, NY) containing 20% heat-inactivated FCS, 50 μg/ml endothelial cell growth factor, 100 U/ml heparin, 50 μg/ml streptomycin, and 100 U/ml penicillin. In addition, multiple vitamin supplement (BME vitamin solution; Sigma Chemical Co., St. Louis, MO) containing sodium chloride, biotin, folic acid, niacinamide, D-pantothenate, riboflavin, thiamine, pyridoxal HCl, choline chloride, and myo-inositol. Bovine aortic endothelial cells (BAECS) were obtained from freshly excised bovine aorta as previously described (14). The cells were maintained in RPMI medium 1640 (Hazelton Research Products, Denver, PA) supplemented with 20% heat-

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1. *Abbreviations used in this paper:* BAECS, bovine aorta endothelial cells; CTC, cultured T cells; EC, cultured endothelial cells; G rIL-2, glycosylated rIL-2; HETE, hydroxyeicosatetraenoic acid;

HHT, 12-hydroxyheptadecatrienoic acid; HUVECS, human umbilical vein endothelial cells; 6KPGF_{1α}, 6 keto-prostaglandin F_{1α}; NG rIL-2, nonglycosylated rIL-2; nIL-2, purified natural IL-2; PGI₂, prostacyclin; rIL-2, recombinant IL-2; tPA, tissue-type plasminogen activator.

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inactivated FCS and the same antibiotics added to the HUVEC cultures. We routinely used HUVEC of passage 2–4 and BAEC of passage 5 in all experiments.

Immunoassays. To determine the effects of IL-2 on endothelial cell PGI₂, tPA, and vWf synthesis, we incubated HUVEC or BAEC (2×10^6 cells/flask) for various times at 37°C in the presence and absence of IL-2. At each time point, the medium was decanted and its 6KPGF_{1 α} , vWf, and tPA contents were measured by respective immunoassays. 6KPGF_{1 α} was measured by a highly sensitive and specific RIA as previously described (15). vWf was assayed by an ELISA using a monoclonal antibody against human vWf (American Bioproducts, Parsippany, NY). tPA was also measured by an ELISA using a monoclonal antibody against human tPA (American Diagnostica, Greenwich, CT). Both ELISA assays are highly sensitive and specific. The level of detection for vWf and tPA was 0.4% and 0.5 ng/ml, respectively.

Analysis of eicosanoids by HPLC. Endothelial cells (2×10^6 cells/flask) were prelabeled with 1 μ Ci [¹⁴C]arachidonate at 37°C for 2 h. The cells were washed gently to remove free radiolabeled arachidonate. Labeled cells were then incubated at 37°C with medium containing IL-2 or ionophore A23187 (Sigma Chemical Co.). The incubation medium was removed after 10 min and the eicosanoids were extracted using a C18 Sep-Pak cartridge (Waters Associates, Millipore Corp., Milford, MA) as previously described (15). Recovery of eicosanoids was ~ 95%. The eicosanoids were analyzed by reverse phase HPLC (Vista 5500; Varian Associates, Inc., Palo Alto, CA). Briefly, the prostanooids were separated using an isocratic solvent system of 35% acetonitrile in acidified water. After the initial isocratic period the solvent composition was changed to 50% acetonitrile in acidified water and run for 10 min to elute HHT, LTB₄, and HETEs. Next, a slight convex gradient was run from 50–75% acetonitrile for 8 min and then to 100% acetonitrile (15). Fractions were collected and the radioactivity was counted in a β -counter. Tritiated standards were used to determine the relative retention time of each eicosanoid. The detection limit for these compounds using HPLC is ~ 2 ng.

Measurement of lymphocyte proliferative activity. The effect of IL-2 on thymidine incorporation by human cultured T cells (CTC) was

evaluated as previously described (16, 17). In brief, 14-d-old CTC were plated at a density of 10^5 /well in 96-well microtiter plates. Serial half-log dilutions of IL-2 were added in a checkerboard pattern and cultured for 44 h. [³H]Thymidine was then added and the lymphocytes were harvested 4 h later onto glass fiber filters using a mini-MASH (M. A. Bioproducts, Walkersville, MD). Quadruplicate samples were counted in a liquid scintillation counter. The data were expressed as cpm/ 10^5 cells.

Western immunoblots. We used an affinity-purified polyclonal antibody to determine PGH synthase on Western blot. The antibody that was raised in rabbits against purified seminal vesicle PGH synthase recognized the 70-kD subunit of PGH synthase. HUVECS (T-75 flask, 6×10^6 cells/flask) or BAECs (6×10^6 cells/flask) were lysed in PBS containing 0.1% Triton X-100, 1 mM PMSF, 0.01% EDTA, and 0.03% leupeptin. The lysates were boiled for 3 min and centrifuged at 15,000 rpm for 10 min in an Eppendorf microfuge. The supernatant that contained the solubilized PGH synthase was concentrated and 50 μ l of the sample was applied to an 8% SDS polyacrylamide gel as described by Laemmli (18). After electrophoresis, the proteins were electrophoretically transferred to nitrocellulose sheets at 0.2 A for 4 h in 25 mM Tris, 190 mM glycine, and 20% (vol/vol) methanol. The blot was saturated with 5% BSA for 3 h and then the nitrocellulose papers were incubated with the anti-PGH synthase antibody (10 μ g/ml) at room temperature overnight. After washing, the papers were treated with goat anti-rabbit IgG conjugated with horseradish peroxidase (1:2,000; Bio-Rad Laboratories, Richmond, CA). The blots were treated with 4-chloro-1-naphthol (Bio-Rad Laboratories) for color development and visualization.

Statistical analysis. Data generated from multiple experiments were expressed as mean \pm SD. The *t* test was used to determine whether differences were statistically significant.

Results

The effects of nIL-2 and rIL-2 on HUVEC PGI₂ synthesis and CTC thymidine incorporation were evaluated concurrently

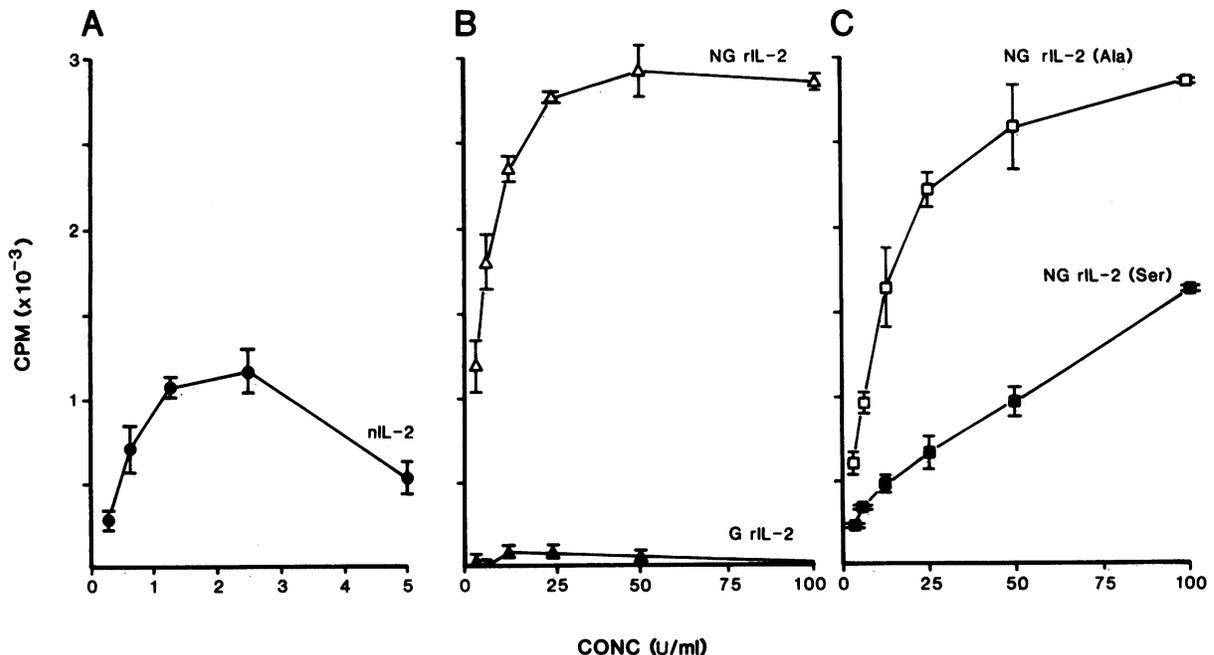


Figure 1. Dose-related stimulation of thymidine incorporation into cultured T cells by various IL-2 preparations. (A) nIL-2, (B) NG rIL-2 and (C) two NG rIL-2 with substitution of residue cysteine-125 with alanine (Ala) or serine (Ser). T cells were incubated

with IL-2 or control for 44 h, [³H]thymidine was added, and lymphocytes were harvested 4 h later. The data represent mean \pm SD of three to four experiments done in quadruplicate. The results were expressed as cpm/ 10^5 cells.

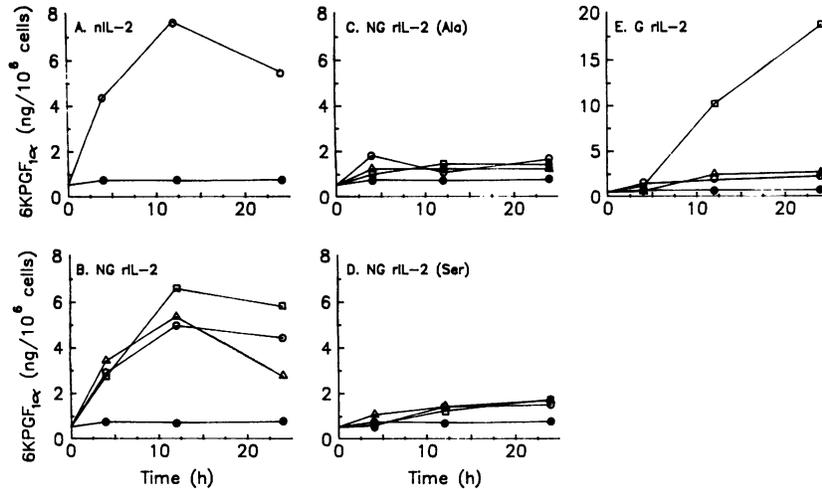


Figure 2. Time- and concentration-related stimulation of HUVEC PGI₂ synthesis by IL-2. PGI₂ was measured as its stable metabolite, 6KPGF_{1α}, by RIA. HUVECS (T-25 flask, passage 2–4) were treated with various concentrations of IL-2 and at designated time points medium was decanted and 6KPGF_{1α} was measured. The data represent mean values of duplicate determinations. ●, medium control; ○, 1 U/ml; △, 10 U/ml; □, 50 U/ml of IL-2.

using the identical IL-2 preparations freshly obtained. The nIL-2 exhibited the expected stimulatory activities on lymphocyte thymidine uptake (Fig. 1A) and HUVEC PGI₂ synthesis (Fig. 2A). The NG rIL-2 as well as its alanine and serine analogues enhanced thymidine incorporation (Fig. 1, B and C). The order of potency was NG rIL-2 > alanine analogue > serine analogue. G rIL-2, on the other hand, had no stimulating effect (Fig. 1B). By contrast, NG rIL-2 and G rIL-2 stimulated HUVEC PGI₂ synthesis while the substituted NG rIL-2s (alanine or serine substitution) were unable to stimulate PGI₂ production (Fig. 2, B and D). Stimulation of the HUVECS by NG rIL-2 and G rIL-2 was both time dependent and concentration dependent. 1 U/ml of NG rIL-2 increased 6KPGF_{1α} levels 4 h after its addition to HUVECS (Fig. 2B),

while 50 U/ml of G rIL-2 was required to elicit an increase in 6KPGF_{1α} (Fig. 2E). The time course of stimulation also differed between these two recombinant compounds (Fig. 3). Generally, stimulation by NG rIL-2 reached a maximum around 12 h after the addition of IL-2 although in some experiments 6KPGF_{1α} generation continued to increase after 12 h. Stimulation by G rIL-2, on the other hand, always gave a steady rate of 6KPGF_{1α} increase over the entire 24 h investigated (Fig. 3). BAECs released a large quantity of 6KPGF_{1α} when stimulated with nIL-2 (Table I). Their response to rIL-2 was not as substantial. However, the G rIL-2 appeared to be more potent than the NG rIL-2 (Table I).

To determine whether other arachidonate metabolites were also stimulated, we analyzed the eicosanoids by reverse-phase HPLC. The HPLC profile of BAECs treated with IL-2 for 10 min (Fig. 4A) was indistinguishable from that of EC

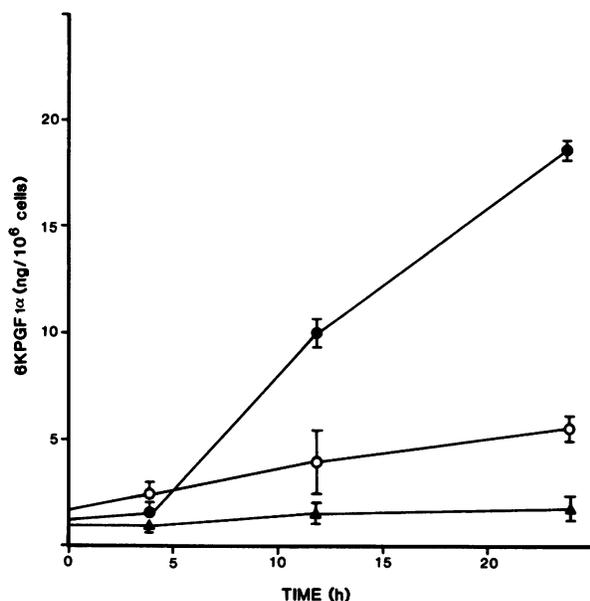


Figure 3. Comparison of PGI₂-stimulating activity of NG rIL-2 and G rIL-2. HUVECS (T-25 flasks, passage 2–4) were incubated with IL-2 or control and at designated time points medium was decanted and 6KPGF_{1α} content was measured. The data represent mean ± SD of four experiments. ○, NG rIL-2 (50 U/ml); ●, G rIL-2 (50 U/ml); ▲, medium control. The differences between NG rIL-2 and G rIL-2 after 12 and 24 h of stimulation were statistically significant ($P < 0.01$).

Table I. Stimulation of PGI₂ Synthesis in BAECs by rIL-2 and nIL-2

| Treatment | 6KPGF _{1α} | | |
|----------------|---------------------|-----------|-----------|
| | 6 h | 12 h | 24 h |
| | ng/ml | ng/ml | ng/ml |
| NG rIL-2* | | | |
| 5 U/ml | 0.3±0.1 | 1.3±0.3 | 1.9±0.4 |
| 10 U/ml | 0.4±0.2 | 1.5±0.3 | 2.1±0.9 |
| 50 U/ml | 0.8±0.2 | 1.9±0.1 | 1.7±0.6 |
| G rIL-2† | | | |
| 5 U/ml | — | — | 6.5±1.5 |
| 10 U/ml | — | — | 21.7±7.8 |
| 50 U/ml | — | — | 25.5±7.4 |
| n IL-2 | | | |
| 1 U/ml | 3.6±0.7 | 32.4±12.5 | 67.3±26.7 |
| Medium control | 0.4±0.2 | 0.4±0.1 | 0.7±0.1 |

BAEC (T-25 flasks, passage 5) were incubated with 4 ml of buffer containing IL-2 or control at 37°C. At 6, 12, and 24 h the medium was removed and the 6KPGF_{1α} content was measured by RIA. The data represent mean ± SD of five experiments performed in duplicate. * expressed in *E. coli*; † expressed in yeast.

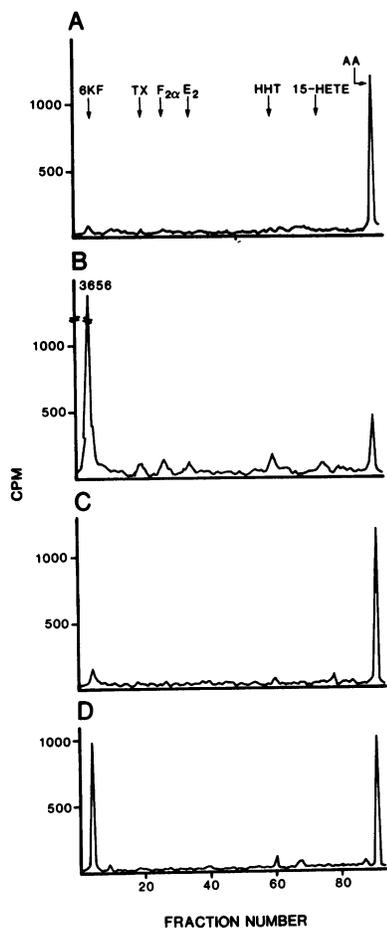


Figure 4. Analysis of eicosanoid production by reverse-phase HPLC. BAECs (2×10^6 cells/flask, passage 5) pre-labeled with [^{14}C]-arachidonate were treated with nIL-2, ionophore, or medium. The conditioned medium was removed at designated time points. Eicosanoids were extracted and analyzed by HPLC as described in Methods. The arrows refer to the relative retention time of each eicosanoid as determined by tritiated standards. (A) BAECs treated with nIL-2 (1 U/ml) for 10 min. (B) Treatment with nIL-2 (1 U/ml) for 24 h. (C) Treatment with medium without nIL-2 for 24 h. (D) Treatment with calcium ionophore A23187 (10 μM) for 30 min.

treated with medium without IL-2 for 10 min (data not shown). Essentially only the AA peak was noticeable. By contrast, BAECs treated with IL-2 for 24 h yielded a prominent 6KPGF $_{1\alpha}$ peak accompanied by apparent TXB $_2$, PGF $_{2\alpha}$, PGE $_2$, HHT, 15-HETE, and AA peaks (Fig. 4B) while the 24-h medium control showed a small 6KPGF $_{1\alpha}$ peak and a prominent AA peak (Fig. 4C). BAECs incubated with 10 μM ionophore A23187 for 30 min exhibited a large 6KPGF $_{1\alpha}$ peak (Fig. 4D), and incubation of BAECs with the ionophore for longer periods of time (up to 24 h) did not change the HPLC profile (data not shown).

Stimulation of endothelial cell PGI $_2$ synthesis by AA has been shown to be a self-limited process because of an autoinactivation of the cyclooxygenase activity and to a lesser extent the PGI $_2$ synthase activity (19–21). That IL-2 stimulation is sustained over a prolonged period of time implies that de novo synthesis of PGH synthase has occurred to circumvent the autoinactivation process. To test this hypothesis, we first determined the effects of specific inhibitors of protein synthesis on PGI $_2$ stimulation. Endothelial cells were pretreated with several concentrations of cycloheximide. nIL-2-induced 6KPGF $_{1\alpha}$ production was inhibited by cycloheximide in a dose-related manner (Table II). Actinomycin D, a specific inhibitor of RNA synthesis, also exerted a dose-related inhibition of PGI $_2$ synthesis (Table II). The data support the notion that protein synthesis is required for the sustained stimulation of PGI $_2$ synthesis. To directly evaluate whether IL-2 induces de novo synthesis of PGH synthase, we determined the influence

Table II. Inhibition of PGI $_2$ Synthesis by Cycloheximide and Actinomycin D

| Treatment | 6KPGF $_{1\alpha}$ ng/ml | Inhibition % |
|--|-----------------------------|-----------------|
| Media control | 0.39 \pm 0.20 | |
| Media + cycloheximide (3.6 μM) | 0.26 \pm 0.11 | |
| nIL-2 (1 U/ml) | 87.30 \pm 50.2 | |
| nIL-2 (1 U/ml) + cycloheximide | | |
| 0.18 μM | 24.93 \pm 19.43 | 71 |
| 0.36 μM | 14.59 \pm 10.72 | 83 |
| 1.8 μM | 3.96 \pm 0.30 | 95 |
| 3.6 μM | 2.25 \pm 1.16 | 97 |
| nIL-2 (1 U/ml) + actinomycin D | | |
| 10 nM | 56.75 \pm 23.18 | 35 |
| 40 nM | 16.32 \pm 11.05 | 81 |
| 160 nM | 13.65 \pm 3.36 | 84 |

BAECs (2×10^6 cells, passage 5) were incubated with nIL-2 in the presence and absence of cycloheximide or actinomycin D at 37°C for 24 h. The samples were removed for 6KPGF $_{1\alpha}$ assay. The data represent the mean \pm SD of four experiments. The cells remained viable at 24 h in the presence of cycloheximide (0.18–3.6 μM) or actinomycin D (10–160 nM) and there were no apparent morphological changes.

of IL-2 on PGH synthase accumulation in EC by the Western blot technique using a cross-reacting polyclonal antibody against ram seminal vesicle PGH synthase. As shown in Fig. 5A, the polyclonal antibody recognized the 70-kD subunit of purified ram seminal vesicle PGH synthase in a dose-related manner. A protein migrating coincident with the purified PGH synthase (70 kD) was present in the HUVECS incubated with medium alone (Fig. 5 B, lane 3). This band was enhanced by treatment of HUVECS with 50 U/ml NG rIL-2 for 2 h (Fig. 5 B, lane 2) and the enhanced effect of IL-2 was abolished by cycloheximide (Fig. 5 B, lane 4). Fig. 6 shows the 70-kD band in HUVECS incubated 2–4 h with medium containing various preparations of rIL-2. HUVECS treated with medium alone expressed the 70-kD band, and the intensity of the band appeared increased from 2–4 h (lanes 1 and 6). The 70-kD band was enhanced by 2-h treatment with G rIL-2 (lane 3) and NG rIL-2 (lane 5), and further enhanced at 4 h (G rIL-2, lane 7; NG rIL-2, lane 9). The alanine-substituted NG rIL-2 had no apparent enhancing effect on the 70-kD band at either 2 or 4 h of incubation (lanes 4 and 8). The polyclonal antibody also recognized the 70-kD subunit of BAECs incubated with medium in the absence and presence of IL-2. Similar to the HUVEC data, the 70-kD band was increased by nIL-2 in a time-related fashion (Fig. 7).

Experiments were then performed to determine whether physiologically important proteins such as vWf and tPA were also stimulated by IL-2. HUVECS were treated with rIL-2, and medium was removed at various time periods and assayed for vWf and tPA by highly specific ELISAs. 6KPGF $_{1\alpha}$ assays were concurrently performed. Despite a time-related increment in 6KPGF $_{1\alpha}$ levels after IL-2 stimulation, the levels of vWf and tPA in the IL-2-treated samples were not different from those of the control samples treated with media alone (Fig. 8).

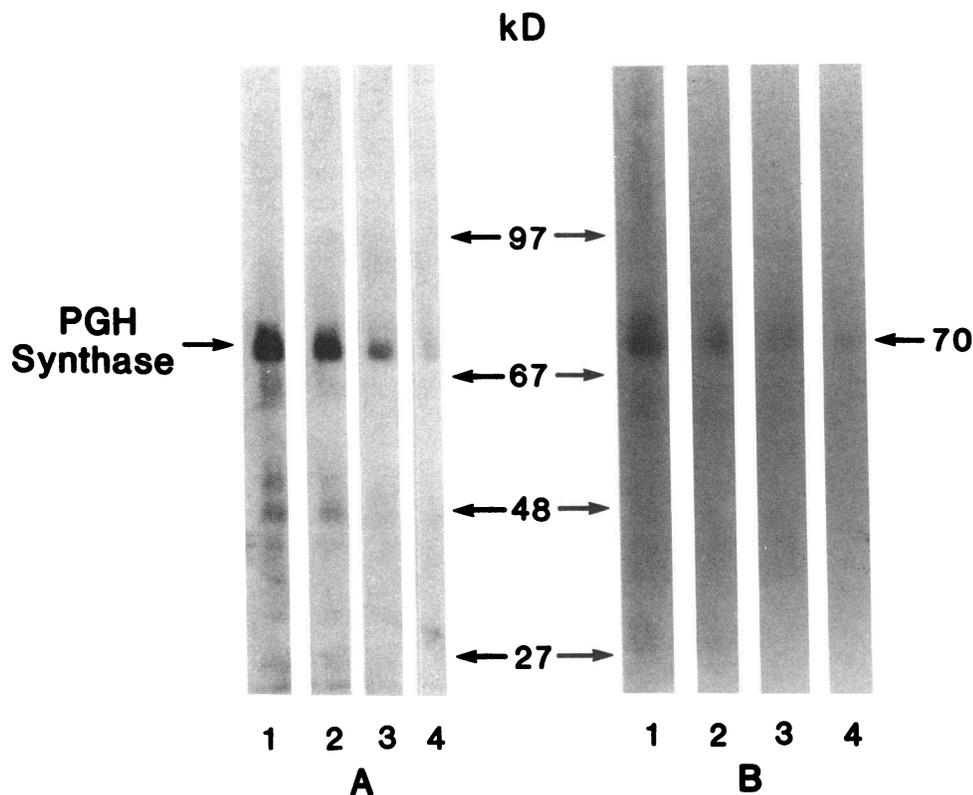


Figure 5. Western blot of PGH synthase. Molecular weight markers and the 70-kD subunit of PGH synthase are shown by arrows. (A) Different concentrations of purified seminal vesicle PGH synthase: (lane 1) 40 ng; (lane 2) 20 ng; (lane 3) 10 ng; (lane 4) 5 ng. (B) HUVEC lysates: (lane 1) PGH synthase standard, 10 ng; (lane 2) NG rIL-2, 50 U/ml, treatment for 2 h; (lane 3) medium without IL-2 for 2 h; (lane 4) 2 μ M cycloheximide coincubated with IL-2 for 2 h.

Discussion

This study demonstrates that IL-2 has a stimulatory effect on endothelial cell PGI₂ synthesis. The stimulatory activity does not start until the cells have been treated with IL-2 for at least 2 h, and the effect is sustained over a prolonged period of time. This stimulatory pattern is in striking contrast to that exerted by agonists such as ionophore A23187 and bradykinin (22). These agonists induce a rapid release of AA leading to PGI₂ and other eicosanoid synthesis usually within minutes after their addition to the cells. PGI₂ production reaches its peak at

30 min and declines thereafter (22). The self-limitation of PGI₂ synthesis stimulated by these agonists is thought to be due to autoinactivation of the cyclooxygenase activity of the PGH synthase molecule (19, 20). The autoinactivation process is rapid and irreversible. As these agonists are incapable of stimu-

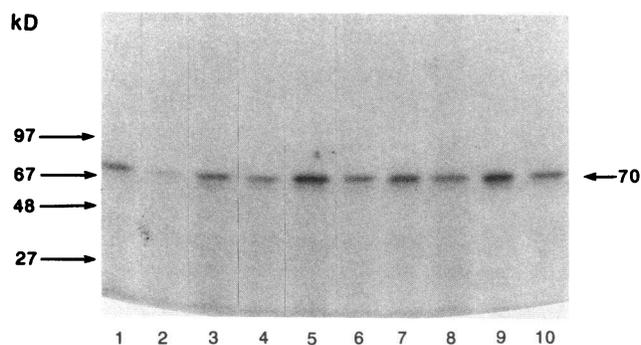


Figure 6. The Western blot shows the 70-kD bands in HUVECS incubated with medium for 2–4 h in the absence and presence of several rIL-2 preparations. (Lane 1) medium alone for 2 h; (lane 2) medium plus 2 μ M cycloheximide; (lane 3) 50 U/ml G rIL-2 for 2 h; (lane 4) NG rIL-2 (alanine substitution, 50 U/ml) for 2 h; (lane 5) NG rIL-2 for 2 h; (lane 6) medium alone for 4 h; (lane 7) 50 U/ml G rIL-2 for 4 h; (lane 8) NG rIL-2 (alanine substitution) for 4 h; (lane 9) NG rIL-2 for 4 h; (lane 10) 10 ng standard PGH synthase.

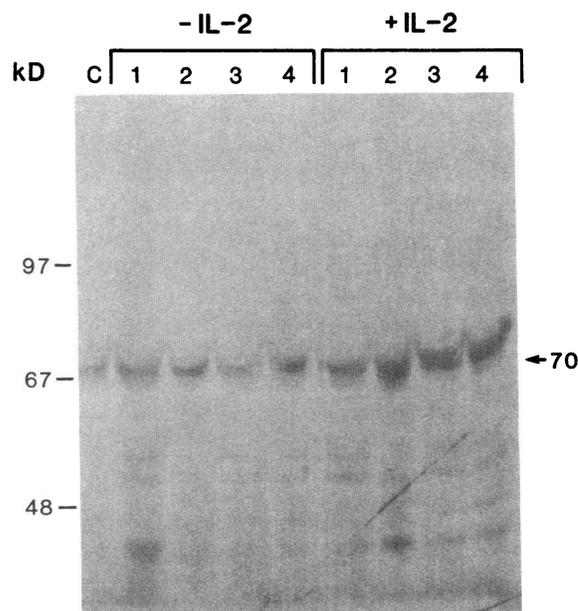


Figure 7. Western immunoblot of BAEC PGH synthase. The numbers (1–4) in the brackets under –IL-2 and +IL-2 refer to Western blotting performed 1–4 h after the addition of fresh medium without purified nIL-2 and with nIL-2 (1 U/ml), respectively.

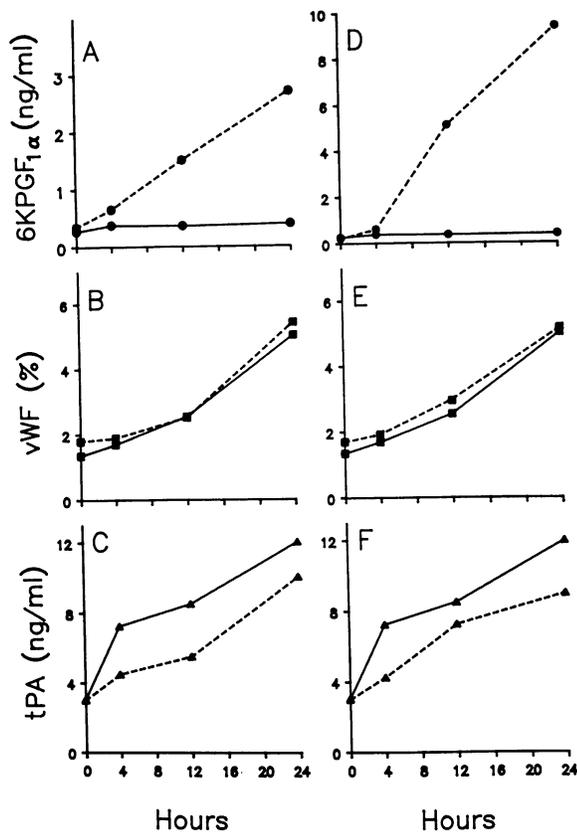


Figure 8. Effects of IL-2 on PGI₂, vWf, and tPA production by HUVECS. HUVECS (T-25 flask, passage 2–4) were incubated with 50 U/ml of NG rIL-2 (natural sequence) (A–C), or 50 U/ml of G rIL-2 (D–F). Incubation medium was removed at various time points and its 6KPGF_{1α} (A, D), vWf (B, E), and tPA (C, F) contents were measured by immunoassays. vWf content was expressed as percentage of a standard. Dotted lines refer to IL-2-treated samples, while the solid lines refer to the medium controls. Each point represents the mean of two experiments.

lating the regeneration of cyclooxygenase, eicosanoid production is not sustained. By contrast, IL-2 not only causes release of AA but also induces de novo synthesis of PGH synthase. Several lines of evidence from our experiments support this notion. First, stimulation of PGI₂ production was blocked by cycloheximide and actinomycin D indicative of a requirement of protein synthesis for continuous PGI₂ stimulation. Second, the Western blot reveals that IL-2 induces an increment in the 70-kD subunit band of PGH synthase. This increment was abolished by pretreating the cells with cycloheximide. Cycloheximide at 2 μM concentration almost entirely abolishes the stimulation of PGH synthase and PGI₂ productions. At the same concentration range, cycloheximide also inhibits the endothelial cell tPA production stimulated by phorbol 12-myristate 13-acetate while it does not block vWf synthesis (unpublished observations). The different sensitivities of various protein syntheses to cycloheximide in endothelial cells can be attributed at least in part to the turnover rates of these proteins in the cells. PGH synthase is considered to have a rapid turnover rate (23) and is quite susceptible to cycloheximide treatment. Third, we have previously shown that IL-2 is capable of stimulating aspirin-treated EC to convert exogenous AA into PGI₂ 2–3 h after aspirin treatment, while the control cells

remain unable to metabolize exogenously added AA into eicosanoids even at 24 h after the addition of aspirin (11). The time course for converting AA eicosanoid production in the aspirin-treated cells after IL-2 induction showed a sustained increment in PGI₂ synthesis (11). Taken together, these findings provide convincing evidence that IL-2 enhances PGI₂ synthesis in endothelial cells via induction of de novo synthesis of PGH synthase.

Our study reveals a major difference between endothelial cells and lymphocytes in response to rIL-2 stimulation. Substitution of rIL-2 Cys¹²⁵ has no demonstrable influence on its ability to stimulate lymphocyte thymidine incorporation. Using site-directed mutagenesis, Ju et al. have provided a valuable structure–function analysis of human IL-2 (24). Site-directed mutagenesis of the cysteine residues indicates that the cysteines at positions 58 and 105 must be maintained for rIL-2 bioactivity, while a substitution at Cys¹²⁵ has no drastic effects. They postulated that Cys⁵⁸ and Cys¹⁰⁵ form an intramolecular disulfide bond that stabilizes the IL-2 molecule in the bioactive conformation. By contrast, Cys¹²⁵ is critical for maintaining the bioactivity of rIL-2 toward cultured endothelial cells. Substitution of Cys¹²⁵ with alanine or serine leads to a drastic reduction of its PGI₂-stimulating activity. The alanine-substituted rIL-2 failed to enhance the PGH synthase band on the Western blot. By inference, our data imply that the contact site of rIL-2 with endothelial cell IL-2 receptors requires Cys¹²⁵ to maintain its bioactive conformation. BAECs produce a much higher quantity of PGI₂ than HUVECS when stimulated with human IL-2. This may reflect a species difference in IL-2 receptor interaction with its ligand.

Endothelial cells synthesize and release a number of proteins important in modulating platelet and vascular reactivity. Since IL-2 stimulates PGI₂ production via an increase in PGH synthase, we reasoned that it might exert an influence on the production of other physiologically important proteins. We chose to measure the release of two such proteins. vWf is a multimeric adhesive protein that mediates the adhesion of platelets to denuded vessel wall (25). tPA converts plasminogen into plasmin which is pivotal for lysing fibrin clots (26). Both proteins can be accurately measured by ELISA with monoclonal antibodies. While rIL-2 induces a steady increase of PGI₂ production in the culture medium, it has little effect on vWf or tPA production. The stimulating effect of IL-2 on PGI₂ synthesis does not simply represent a general stimulation of protein synthesis but appears more selective in stimulating the synthesis of PGH synthase. PGI₂ is a potent autacoid that causes vasodilatation, increases vascular permeability, inhibits platelet aggregation, and suppresses immune responses (27, 28). Selective stimulation of PGI₂ production is likely to have important physiologic and pathophysiologic implications, particularly at sites of active contact between lymphocytes and vascular endothelium.

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