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22 March 1985; accepted 22 May 1985

Prostacyclin Synthesis Induced in Vascular Cells by Interleukin-1

Abstract: *Supernatants from cultures of human monocytes that had been stimulated with endotoxin or silica induced the synthesis of prostacyclin in endothelial and smooth muscle cells. The lymphokine mediating these effects on the cells of the blood vessel wall was identified as interleukin-1; interferons and interleukin-2 were inactive. Interleukin-1–induced prostacyclin synthesis represents a new aspect of the interaction between the immune system (as well as other tissues) and the vessel wall and may serve as a basis for the development of new strategies in antithrombotic therapy.*

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Although there is evidence of a bidirectional interaction between immunocompetent and vascular cells, the factors mediating this interaction are largely unknown (*1*). Products of lymphocytes or macrophages regulate various aspects of vascular cell function, including proliferation, procoagulant activity, expression of class II histocompatibility (Ia) antigens, and production of colony-stimulating factor (*2–6*). In vivo, stimulated macrophages induce new blood vessel formation, and capillary endothelial cells proliferate at sites of cell-mediated immune reactions (*7*). We showed earlier that lymphokine-containing supernatants of stimulated blood mononuclear cells induce prostacyclin (PGI_2) synthesis in vascular cells (*8*). Prostacyclin, the major metabolite of arachidonic acid in vascular cells, may have a crucial role in the physiology and pathology of blood vessels (*9*).

Selective modulation of arachidonic acid metabolism in platelets as compared to that in vascular cells is a major goal of antithrombotic therapy (*9*). It is therefore important to identify the lymphokine that induces PGI_2 secretion in vascular cells. We now report that interleukin-1 (IL-1) induces PGI_2 synthesis in endothelial and smooth muscle cells, whereas other leukocyte mediators, including the interferons (IFN's) and interleukin-2 (IL-2), are inactive.

Culture supernatants of Percoll-purified (*10*) monocytes (2×10^6 cells per milliliter of RPMI 1640 medium with 5 percent fetal bovine serum) stimulated with lipopolysaccharide (LPS) (25 μg/ml) or silica (50 μg/ml) served as a source of IL-1 (*11*). The supernatants were precipitated with 75 percent ammonium sulfate and dialyzed. We refer to the resulting material as crude IL-1. Crude IL-1 was fractionated on a Sephacryl S-200 column or by chromatofocusing. Highly purified IL-1 (Ultrapure IL-1; Genzyme) was obtained from supernatants of *Staphylococcus albus*–stimulated monocytes by adsorption with rabbit antibodies coupled to a Sepharose 4B column. After elution from the column, the material, further purified by chromatography on Sephadex G-50, showed a homogenous band on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (*12*).

Interleukin-1 activity was evaluated in a costimulator assay, with C3H/HeJ thymocytes as responding cells and phytohemagglutinin (PHA) as stimulus. A partially purified (*13*) IL-1 preparation was assigned a value of 1000 units per milliliter and used as standard in each assay.

Results from triplicate cultures were plotted as a linear regression against the standard preparation and expressed in units per milliliter. Rat aortic smooth muscle cells and human umbilical vein endothelial cells, which are both components of vessel walls, served as indicators (*8, 14*). Briefly, intact confluent monolayers (2.5×10^5 to 3.5×10^5 cells in a 2-cm^2 culture well) were washed with 2 ml of phosphate-buffered saline (PBS) and then cultured for 24 hours at 37°C in the presence or absence of IL-1. In some experiments, acetylsalicylic acid (500 μ*M*) was added to cells throughout the incubation period. The amount of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) was measured by radioimmunoassay (*15*).

Vascular smooth muscle cells from rat aorta or endothelial cells from human umbilical vein were exposed to IL-1 for 24 hours, and PGI_2 production in the supernatant was measured as immunoreactive 6-keto-$PGF_{1\alpha}$ (Table 1). These vascular cells produce PGI_2 as their major arachidonic acid metabolite, as also assessed by thin-layer chromatography and mass spectrometry (*8, 16, 17*). Interleukin-1 preparations induced considerable release of 6-keto-$PGF_{1\alpha}$ in both rat aortic smooth muscle cells and human umbilical vein endothelial cells. Endothelial cells were consistently more sensitive to the stimulatory effect of IL-1, whether measured as minimal active concentration (usually 0.1 unit/ml) or amount of 6-keto-$PGF_{1\alpha}$ induced.

In six experiments with endothelial cells, IL-1 (1 unit/ml for 24 hours) induced the synthesis of 127.3 ± 25 pmol per 10^5 cells [mean ± standard error (S.E.M.); range 63 to 170] compared to baseline levels of 24.4 ± 9 (range 7.5 to 53). In six experiments with aortic smooth muscle cells, IL-1 (10 unit/ml for 24 hours) induced the synthesis of 6.6 ± 1.5 pmol of 6-keto-$PGF_{1\alpha}$ per 10^5 cells (mean ± S.E.M.; range 3 to 11.7) compared to baseline levels of 0.5 ± 0.1 (range 0.3 to 0.9). Induction of PGI_2 production by IL-1 was dependent on the integrity of the cyclooxygenase pathway of arachidonic acid metabolism, as shown by the complete blockade caused by acetylsalicylic acid (Table 1). Crude IL-1 preparations from LPS-stimulated monocytes contained LPS. The following observations suggest that LPS did not play an appreciable role in induction

of PGI_2 synthesis by IL-1: (i) Crude IL-1 from silica-stimulated monocytes in the absence of LPS [<0.1 μg/ml as detected in the Limulus assay (Sigma)] or highly purified IL-1 in the absence of LPS (Table 1) induced PGI_2 synthesis as effectively (per IL-1 unit) as LPS-contaminated preparations; (ii) LPS at concentrations up to 8 μg/ml had little effect on endothelial cell PGI_2 synthesis after 24 hours of exposure [8.7 ± 0.8 pmol per 10^5 cells (mean ± S.E.M.); four replicates)] and no effect on smooth muscle cells (0.03 ± 0.001 pmol per 10^5 cells; four replicates); (iii) the lipid A–reactive antibiotic polymyxin B (8 μg/ml) had no statistically significant effect on IL-1–induced PGI_2 synthesis, with 86.1 ± 8 pmol per 10^6 cells (four replicates) of 6-keto-$PGF_{1\alpha}$ produced in endothelial cells exposed to crude IL-1 at 1 unit/ml, compared to 58.1 ± 5 pmol per 10^6 cells produced in the presence of polymyxin B.

The results presented in Table 1 were obtained after cells were exposed to IL-1 for 24 hours. Previous experiments with crude supernatants of stimulated mononuclear cells had shown that induction of PGI_2 synthesis by lymphokines required a relatively long (more than 6 hours) interaction with vascular cells (*8*), and this time course was confirmed with crude and with purified IL-1 (data not shown). The requirement for a relatively long exposure distinguishes the action of lymphokine on vascular cells from that of other agents that stimulate PGI_2 synthesis such as thrombin, arachidonic acid, or the calcium ionophore A23187, all of which act in a few minutes (*14*).

To further characterize the mediator involved in the induction of PGI_2 secre-

Table 1. Effect of crude and purified IL-1 on vascular cell PGI_2 production. Crude IL-1 was obtained from culture supernatants of Percoll-purified monocytes stimulated with LPS (*Salmonella enteritidis*; Difco) or silica (particle size <5 μm, D. Q. 12; Wirschaftsverband Asbestzment E. V.). Purified IL-1 was obtained by immunoabsorption and Sephadex chromotography (*12*) from *Staphylococcus albus*–stimulated human monocyte supernatants (Genzyme). The absence of endotoxin was shown by a Limulus assay in silica-stimulated crude IL-1 and in pure IL-1. Human umbilical vein endothelial cells (HEC) were cultured in Medium 199 supplemented with 20 percent newborn calf serum and used at the time of the first passage at confluence (*14*). Rat smooth muscle cells (SMC), obtained from thoracic aorta (*8, 22*), were cultured in Eagle's minimum essential medium supplemented with 10 percent calf serum and used between the 6th and 22nd passages at confluence. After the growth medium was removed, the cells were washed once with PBS and cultured for 24 hours at 37°C with 300 μl of culture medium in the presence or absence of IL-1. In some experiments, acetylsalycylic acid (ASA) [in the form of its soluble lysine salt Flectadol (Maggioni)] was included in the medium during the incubation with IL-1. At the end of the incubation period the supernatant was removed and stored at −20°C until tested for 6-keto-$PGF_{1\alpha}$. The values are means of duplicate results and were similar to those found in six other experiments.

Addition (U/ml)	6-keto-$PGF_{1\alpha}$ (pmol/10^5 cells)	
	In HEC	In SMC
Medium	12.3	0.9
LPS-induced crude IL-1		
0.01	14.7	
0.1	58	1.5
1	171	1.5
10	231	11.7
100	330	23.4
100 plus ASA	<0.3	<0.3
Silica-induced crude IL-1		
0.1	45	
Purified IL-1		
1	104	
10	400	

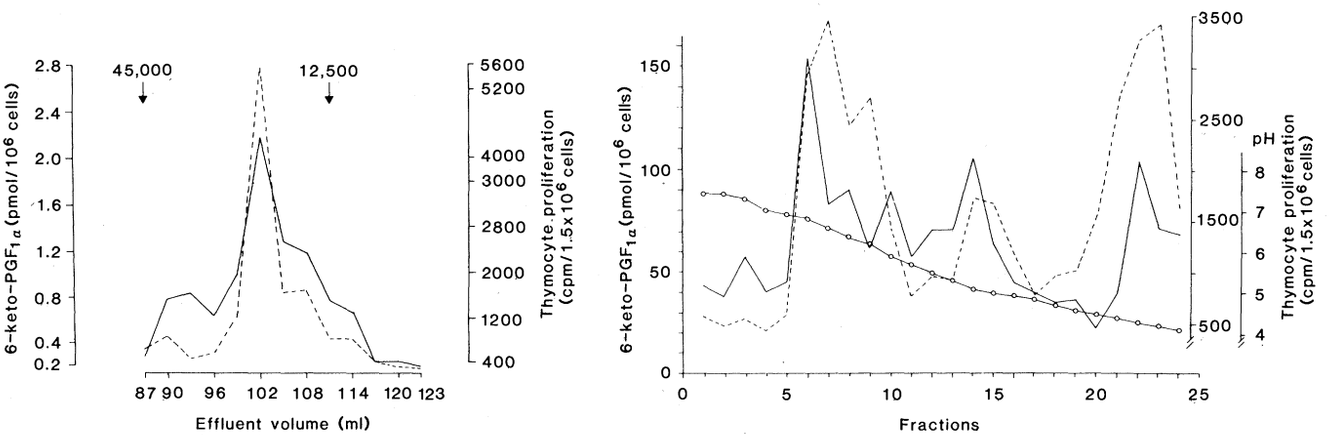

Fig. 1 (left). Coelution of thymocyte-costimulating (- - -) and PGI_2-inducing activity (——) during gel filtration on Sephacryl S-200. Percoll-purified human peripheral blood monocytes were cultured for 48 hours with LPS. The supernatant was precipitated with 75 percent ammonium sulfate and extensively dialyzed to produce crude IL-1. A Sephacryl S-200 (Pharmacia) column (1 by 90 cm) was calibrated with bovine serum albumin (64,000 daltons), ovalbumin (45,000 daltons), and cytochrome C (12,500 daltons). Crude IL-1 (3 ml) was applied to the column and eluted with PBS at a flow rate of 20 ml/hour. Fractions (3 ml) were collected, filtered, and assayed for induction of PGI_2 synthesis in aortic smooth muscle cells and for thymocyte-costimulating activity. Induction of PGI_2 secretion (measured as 6-keto-$PGF_{1\alpha}$) was evaluated as described in Table 1. For thymocyte-costimulating activity, thymus cells from 6- to 8-week-old C3H/HeJ mice (1.5 × 10^6 cells per well containing RPMI 1640 medium with 5 percent fetal bovine serum) were cultured for 72 hours in 96-well flat-bottomed plates (Costar 3596) with PHA (HA16, Wellcome) at 0.5 μg/ml. Cultures were pulsed for the last 6 hours of incubation with [^{3}H]thymidine (5 Ci/mmol, Amersham) at 0.5 μCi per well and harvested on filters. Results are presented for a 1:3 dilution of the various fractions. This experiment was repeated twice on rat aortic smooth muscle cells and twice on human umbilical vein endothelial cells with similar results. Fig. 2 (right). Coelution of thymocyte-costimulating (- - -) and PGI_2-inducing activity (——) on chromatofocusing, at *p*H gradient (0). Crude IL-1 (1.5 ml) equilibrated by dialysis on 0.025*M* imidazole-HCl, *p*H 7.4, was applied to a 0.7 by 20 cm column packed with Polybuffer Exchanger PBE94 (Pharmacia) and eluted with 100 ml of Polybuffer 74–HCl at *p*H 4 (Pharmacia). Fractions (3 ml) were collected and, after measurement of *p*H, extensively dialyzed against PBS at 4°C until the *p*H reached 7.4 in all fractions. Thymocyte-costimulating activity was evaluated in the various fractions as described in Fig. 1. Production of PGI_2 in human umbilical vein endothelial cells was evaluated as described in Table 1. This experiment was repeated twice on human umbilical vein endothelial cells and twice on rat aortic smooth muscle cells with similar results.

tion, we subjected crude IL-1 preparations to chromatofocusing and gel filtration. The activity on thymocytes (costimulator assay) and on vascular cells (6-keto-$PGF_{1\alpha}$ production) was evaluated in the various fractions in parallel. After separation by size (Sephacryl S-200) or charge (chromatofocusing), no appreciable dissociation was found between costimulator activity on thymocytes and modulatory activity on vascular cell arachidonic acid metabolism. After fractionation with Sephacryl S-200, both the costimulator activity on thymocytes and the modulation of arachidonic acid metabolism eluted as single peak corresponding to an apparent molecular mass of approximately 15,000 daltons (Fig. 1). As expected (*18*), costimulator activity on chromatofocusing was heterogenous with three peaks. The capacity to induce 6-keto-$PGF_{1\alpha}$ production in endothelial cells showed a similar pattern (Fig. 2). Possibly because of the lower sensitivity of aortic smooth muscle cells (Table 1), no consistent activity was found in the more acidic *p*H region when these cells were used as vascular indicators (data not shown).

The results discussed so far indicate that IL-1, or a monocyte product closely associated with it, induced 6-keto-$PGF_{1\alpha}$ production in vascular cells. We used culture supernatants of stimulated mononuclear cells for original observations on lymphokine modulation of arachidonic acid metabolism in vessel walls (*8*). In view of the variety of mononuclear cell products present in such preparations, we investigated the possibility that human lymphokines other than IL-1 might interact with vessel wall cells. Recombinant IL-2 (1 to 10 U/ml), IFN-γ and IFN-α (recombinant A and hybrid A/D), and natural IFN-β (at 500 to 1000 U/ml) had no effect on 6-keto-$PGF_{1\alpha}$ production by human endothelial or rat aortic smooth muscle cells (data not shown). These results are in agreement with those of Eldor *et al.* (*19*), who did not observe stimulation of the spontaneous release of prostacyclin by human IFN-β on bovine endothelial cells.

Our results show that IL-1, or a material closely associated with this lymphokine, induces PGI_2 synthesis in human umbilical vein endothelial cells and in rat aortic smooth muscle cells. Modulation of vascular cell arachidonic acid metabolism was found with both crude and highly purified IL-1 preparations. Moreover, after chromatofocusing and gel filtration, there was copurification of IL-1 activity, as detected in the thymocyte costimulator assay, and of IL-1 activity as detected by modulation of PGI_2 synthesis in cells of the vessel walls. Thus induction of PGI_2 production adds to the list of nonimmune effects of IL-1 (*18, 20*). Elucidation of the molecular basis of the pleiotropic effects of IL-1 will involve cloning of the gene encoding this mediator, or family of mediators, and the production of large quantities of homogenous material.

The in vivo relevance of the modulation of vessel wall arachidonic acid metabolism by IL-1 is unclear. Since vessel wall PGI_2 plays a major role in the regulation of important biological functions such as platelet aggregation and vascular tone (*9*), IL-1–induced PGI_2 secretion might contribute to vascular changes at sites of delayed hypersensitivity reactions (*7*). Inasmuch as various cell types can produce IL-1 (*18, 20*), this mediator could serve as a communication signal between tissues and blood vessels. Considerable effort has been devoted to designing pharmacological approaches to thrombosis by selectively inhibiting arachidonic acid metabolism in platelets as opposed to that in vascular cells (*9, 21*). Elucidation of the "slow" pathway through which IL-1 induces PGI_2 synthesis in vascular cells may offer clues for the development of new strategies in antithrombotic therapy.

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23. Supported by the Italian National Research Council, Progetto Finalizzato "Medicina Preventiva," Sottoprogetto "Malattie Degenerative," contract 83.02603.56, and Progetto Finalizzato Oncologia. F.B. is the recipient of a fellowship from Avv. F. Maironi. We thank G. de Gaetano for stimulating and helpful discussions; M. J. Buchanan, McMaster University, Hamilton, Ontario, Canada, for the antibody to 6-keto-$PGF_{1\alpha}$; and G. Scala, Naples, Italy, for a partially purified IL-1 preparation. G. B. Pisoni, V. de Ceglie and F. de Ceglie, and G. Scalvini helped prepare the manuscript.

3 January 1985; accepted 15 March 1985

Inhibition of Interferon-Gamma May Suppress Allograft Reactivity by T Lymphocytes in Vitro and in Vivo

Abstract. *The addition to mixed-leukocyte reactions of monoclonal antibodies to interferon-γ abrogated alloantigen recognition and induction of cytotoxic T lymphocytes by inducing early and highly effective suppressor T lymphocytes. This inhibitory activity was not confined to in vitro models, since daily injection of the antibodies into CBA/J mice blocked the usual rejection of allogenic tumor cells.*

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T-lymphocyte activation requires both presentation of the antigen in the context of the proper major histocompatibility complex (MHC) products and non–antigen-specific signals provided by interleukins (*1*). Of these, immune interferon (IFN-γ), mainly released by helper T lymphocytes on activation by alloantigens (*2*), seems to be endowed with many immunoregulatory effects besides its antiviral activity (*3*). IFN-γ obtained by recombinant DNA technology is a potent inducer of MHC antigen expression on a great variety of cells (*4*), a helper and maturation factor in B-cell antibody production (*5*), and an activator of macrophage functions (*6*). It may therefore play a critical role in triggering antigen recognition and allograft rejection.

To investigate its possible contribution to the mixed-leukocyte reaction (MLR) and allograft rejection, we performed a functional ablation of IFN-γ by using the