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# **Monocyte-Derived Interleukin 1:** Effects on Norepinephrine-Stimulated Aortic Contraction and Phosphoinositide Turnover

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Medium conditioned by silica-stimulated human peripheral blood monocytes expresses vascular suppressive activity. Rat aortic rings, after incubation in conditioned medium, exhibited comprised contraction to stimulation by norepinephrine (NE). Maximal contraction (300 ± 51 mg tension/mg tissue) and sensitivity (−5.91 ± 0.23 M [log $EC_{50}$]) were both reduced in comparison to contraction (762 ± 66) and sensitivity (−7.42 ± 0.11) displayed by rings after incubation in control medium. A polyvalent antibody (Ab) against human interleukin 1 (Il-1) neutralized the suppressive activity in conditioned medium. Rings incubated in conditioned medium containing Ab exhibited normal maximal contraction (722 ± 46) and a partial restoration of sensitivity to NE (−6.91 ± 0.13). In contrast, incubation of rings in control medium supplemented with recombinant human Il-1 resulted in a dose-dependent suppression of aortic contraction to NE that was analogous to the defects induced by monocyte-conditioned medium. No significant differences in NE-stimulated phosphoinositide hydrolysis were present between rings incubated in Ab-treated or untreated conditioned or control media. The data suggest that monocyte-derived Il-1 may have a significant influence on vascular contractile function and that the mechanism by which Il-1 induces vascular dysfunction cannot be demonstrated to involve inhibition of NE-stimulated phosphoinositide metabolism.

**Key words: sepsis, polyvalent antibody, vascular contraction, phorbol**



## INTRODUCTION

Septic or endotoxin-treated rats exhibit diminished vascular contractile responses to multiple contraction agonists, including norepinephrine (NE), angiotensin II, lysine vasopressin, and the prostaglandin endoperoxide analog U-46619, both in vivo and in vitro [1–4]. The causes of vascular contractile hyporesponsiveness in sepsis or after endotoxin treatment, in terms of proximate disorders of intracellular

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homeostasis or the identity of extracellular agents that initiate altered vascular function, are not well characterized. Vascular intracellular second messengers that exhibit modified activity in sepsis or after endotoxin treatment include cytosolic $Ca^{2+}$ metabolism and $\alpha_1$-adrenoceptor–coupled hydrolysis of phosphoinositides (PI). Bovine aortic smooth muscle cells show diminished $Ca^{2+}$ uptake by mitochondria and sarcoplasmic reticulum when treated with large doses of endotoxin in vitro [5]. An alternative, although not mutually exclusive, potential disorder in vascular second-messenger activity was suggested in a report that vascular $\alpha_1$-adrenoceptor–coupled basal and NE-stimulated hydrolysis of PI are reduced by approximately 50% in septic rats [6]. Endotoxin, which can cause vascular dysfunction when administered in vivo, may do so via mediator(s) in addition to possible direct influences on the vasculature. Treatment of isolated vascular tissue with endotoxin at concentrations greater than those observed in experimental bacteremia or sepsis [7,8] produced no change in contractile responses to NE or epinephrine by rabbit aortic tissue [9,10], although much larger doses do have direct effects on bovine aortic tissue [5].

Recent experiments in our laboratory demonstrated that rat peritoneal macrophages, stimulated by endotoxin in vitro, release product(s) that suppress contractile responses to NE by isolated rat aortas incubated in the macrophage-conditioned media [11]. In the current experiments, we characterized the influence of vascular suppressive activity released by silica-stimulated human monocytes in culture on vascular contraction and $\alpha_1$-adrenoceptor–mediated phosphoinositide hydrolysis. We also examined whether small doses of recombinant human interleukin 1 (Il-1) possessed suppressive actions on vascular contraction.

## MATERIALS AND METHODS

### Monocyte Culture

Blood was collected from healthy adult donors after obtaining informed consent. The blood was heparinized (0.75 U/ML) and mixed in equal volumes with 2% dextran in normal saline. After red cell sedimentation, mononuclear leukocytes were isolated by centrifugation (150*g* for 10 min at 20°C) on lymphocyte separation medium (Organon Teknika, Durham, NC). Leukocytes were washed three times with cold Hank's balanced salt solution (HBSS) without $Ca^{2+}$ or $Mg^{2+}$ (150*g*, 10 min, 5°C) to remove platelets. Autologous serum, which improves monocyte yield, purity, and cytotoxic activity [12], was filtered (0.2 μm), and 1 ml was placed in each well of a 24 well tissue culture plate. Washed cells were resuspended in Dulbecco's minimal essential medium (DMEM) containing 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 10% fetal calf serum (FCS). Autologous serum was aspirated from the wells, and the cells were added ($1 \times 10^7$ cells/well) and incubated in a humidified chamber at 37°C under 95% air–5% $CO_2$. Nonadherent cells were removed after 1–2 hr by three washes with HBSS containing $Ca^{2+}$ and $Mg^{2+}$. The cell viability and the proportion of adherent cells that were monocytes were both >95% by trypan blue and nonspecific esterase staining. One milliliter of DMEM (constituted as described above) was added to the wells containing adherent cells and to empty serum-coated wells to provide control media. Fifty micrograms of sterile silica particles (0.014 to 5 μm in size) was added to all wells, and the cells were incubated for 24 hr. Silica particles, instead of endotoxin, were utilized to

stimulate monocytes to avoid any possible confounding of the experiments with direct effects of endotoxin on vascular function. Monocyte-conditioned and control supernatants were collected, centrifuged at 900*g* for 10 min, and stored at −80°C until processed. The supernatants were dialyzed (3,500 molecular weight cutoff) for 72 hr at 4°C against Medium 199 (100 vol) containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Aliquots of the dialyzed media were serially diluted with fresh medium and assayed for vascular suppressive activity (vide infra). Individual preparations of conditioned medium retained the ability to suppress vascular contraction by at least 50% after 4- to 12-fold dilution. The remaining medium was frozen at −80°C.

### Incubation of Aortic Rings

Contraction by isolated rat aortic tissue, after exposure to medium conditioned by activated monocytes or by Il-1, was used as a bioassay for the effect of monocyte products on vascular contractile performance. The influence of conditioned medium on NE-stimulated hydrolysis of PI was also examined in rings prepared from the same rats.

Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing approximately 200 g were killed by decapitation, the thoracic aorta was immediately dissected and cleaned of adventitia, and each aorta was sectioned into four rings 3.5 mm in length. All four rings from each rat were distributed into one of four different medium preparations: 1) Medium 199 containing monocyte-conditioned medium sufficient to cause an approximately 50% decrease in aortic ring contractile performance, 2) Medium 199 containing 50 neutralizing units/ml of rabbit antibody against human Il-1, 3) Medium 199 containing monocyte-conditioned medium (50% suppression) plus antibody against Il-1 (50 neutralizing units/ml), and 4) Medium 199 containing control medium only (control) in volume equal to that of preparation 1. Medium 199 was supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 2% FCS. Five microcuries of $^3$H-myoinositol per milliliter of medium was added to all medium preparations. Two 1.2 ml drops of each test medium were placed into a 100 mm petri dish, and two rings from one rat were placed into each drop (i.e., four rings from one rat into one type of medium). The rings were incubated for 16 hr in a humidified environment under 95% $O_2$–5% $CO_2$. After incubation, one ring from each medium preparation was used to assay the effects on ring contractile function; the remaining three rings from each medium preparation were used in measures of hydrolysis of PI.

An additional control experiment to test the effect of the polyvalent antibody against Il-1 was performed in which aortic rings were incubated with serum from a nonimmunized rabbit. Four aortic rings per rat were prepared, and the rings were distributed, one each, into one of four media preparations: 1) Medium 199 containing monocyte-conditioned medium sufficient to cause an approximately 50% decrease in aortic ring contractile performance, 2) Medium 199 containing monocyte-conditioned medium (50% suppression) and rabbit antibody against human Il-1 (50 neutralizing units/ml), 3) Medium 199 containing monocyte-conditioned medium (50% suppression) and rabbit preimmune serum in volume equal to that of preparation 2, and 4) Medium 199 only (control). The rings were incubated as described above, and then ring contractile function was assessed.

We also tested the direct effects of recombinant Il-1 on aortic ring contraction. Four aortic rings per rat were prepared and the rings distributed, one each, into Medium 199 containing 0 (control), 1, 5, or 10 units/ml recombinant Il-1. The rings were incubated as described above, and then ring contractile function was measured.

### Aortic Ring Contraction

Aortic ring contractile performance after incubation in conditioned medium was assessed by measures of isometric contraction in vitro. Each ring was mounted between two stainless steel hooks in 10 ml organ baths. Rings were bathed in Krebs-Ringer bicarbonate buffer ([KRB] millimolar composition: NaCl 118; KCl, 4.7; $CaCl_2$, 1.3; $MgSO_4 \cdot 7H_2O$, 1.2; $KH_2PO_4$, 1.2; $NaHCO_3$, 25.0; glucose, 11.7) at pH 7.4 while being continually bubbled with 95% $O_2$–5% $CO_2$. Contractions to NE were measured with a force transducer (Kulite Semiconductor, Ridgefield, NJ) attached to one of the two hooks, and responses were continuously recorded on a chart recorder. A resting tension of 2.5 g was applied to the rings, and, after 20 min equilibration, rings were contracted with NE ($10^{-7}$ M); acetylcholine ($10^{-6}$ M) was added to the baths at maximal contraction to confirm the functional integrity of endothelium-mediated vasodilation [13]. The rings were flushed with KRB until tension returned to resting values; ring contractions were then induced by stepwise cumulative additions of NE ($10^{-9}$ to $3 \times 10^{-5}$ M). In some experiments, phorbol 12, 13-dibutyrate ($10^{-6}$ M) was added to the baths after maximal contraction to $3 \times 10^{-5}$ M NE had been attained, and any additional increments in tension were recorded. The rings were blotted and weighed after experiments were completed.

### Inositol-1-Phosphate (IP) Accumulation

Receptor-mediated phosphoinositide breakdown was assessed by measuring aortic ring IP in the presence of lithium. Lithium inhibits the action of inositol-1-phosphatase, thereby disrupting the inositol phosphate cycle and causing an accumulation of IP [for review, see 14]. After incubation in the four media preparations, rings were treated (while in the petri dishes) with NE ($10^{-7}$ M, 10 min) followed by acetylcholine ($10^{-6}$ M, 5 min) to ensure that the rings were exposed to the same sequence of receptor agonists as were the sister rings utilized in measures of contraction. The rings were then washed in KRB (10 ml, ×2) for 20 min at 37°C while being continually gassed with 95% $O_2$–5% $CO_2$. After washing, rings were individually transferred to 12 × 75 mm culture tubes containing 0.3 ml KRB with 10 mM LiCl and incubated for 10 min at 37°C under a 95% $O_2$–5% $CO_2$ atmosphere. The three remaining rings from each rat (one was utilized in measures of contraction) were then treated with different stimuli: One ring received no stimulus and therefore provided a measure of basal IP accumulation, one ring was treated with $10^{-7}$ M NE, and the third ring received a maximally stimulatory dose of NE ($10^{-5}$ M). The rings were incubated for 30 min and then processed as described by Roth et al. [15]. Inositol phosphates were extracted from rings by sequential addition of 0.9 ml chloroform:methanol (1:2), 0.3 ml chloroform, and 0.3 ml $H_2O$. The tubes were vortexed vigorously between additions. After separation of the phases, 0.9 ml of the aqueous phase was placed on Dowex ion exchange columns (AG 1-X8, formate form, 0.8 × 2 cm) and sequentially eluted with 12 ml $H_2O$, 9 ml 0.06 M sodium formate/0.005 M sodium tetraborate, and 10 ml 0.2 M ammonium formate/0.1 M formic acid. Three milliliters of the final eluate were dispersed in 15 ml scintillation

fluid (Atomlight, New England Nuclear, Boston, MA) and counted by liquid scintillation β-spectrophotometry. The rings were blotted and weighed, and IP accumulation was expressed as counts per minute (cpm) per milligram of tissue after correcting for column blanks. The efficacy of separation of IP from other inositol phosphates was validated by tests with $^3$H-labeled inositol phosphate standards.

### Assay for Il-1

Mouse thymocyte comitogenic response to Il-1 was used to assay Il-1 activity in samples of medium conditioned by silica-stimulated human monocytes. Monocytes were cultured and the conditioned medium dialyzed as previously described. Thymuses were harvested from male 6–9-week-old C3H/HeJ mice (Jackson Laboratories, Bar Harbor, ME) and dispersed in RPMI 1640 medium supplemented with 10% v/v FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μM mercaptoethanol, and 1 μg/ml phytohemagglutinin. Thymocytes (final concentration, $10^6$ cells/200 μl well) were exposed to serially diluted samples of monocyte-conditioned medium and to recombinant Il-1 standard. The cells were incubated 72 hr under 95% air–5% $CO_2$ at 37°C. The cells were pulsed during the final 6 hr of incubation with 1 μCi/well $^3$H-thymidine and harvested onto glass fiber filters. The collected radioactivity was counted by liquid scintillation β-spectrophotometry. Sample values were compared with recombinant Il-1 as standard.

### Analysis of Data

Aortic ring contractile performance was characterized by integrating the tension developed by rings in response to sequential doses of NE: i.e., mg tension/mg tissue versus the natural log of the molar concentration of NE. $EC_{50}$ values ($EC_{50}$ = concentration of agonist causing a half-maximal contraction) were calculated by linear regression after logit-log transformation of dose responses. Tests for differences between $EC_{50}$ values were based on mean log values [16]. The influence of media on contractile performance or IP accumulation by incubated rings was examined by one-way analysis of variance (ANOVA) or paired t tests, depending on whether the experimental design utilized independent or paired samples; if significant differences by ANOVA were present then individual means were compared with a posteriori Student-Newman-Keuls tests [17]. Relationships between aortic ring contractile performance and IP accumulation were tested by correlation and regression analyses [17]. Differences with probabilities of 0.05 or less were accepted as significant. All data are expressed as the mean ± SE.

### Drugs and Reagents

NE bitartrate, L-glutamine, silica, and E-Toxate kits were purchased from Sigma (St. Louis, MO). Acetylcholine chloride was obtained from Calbiochem Behringer (La Jolla, CA). DMEM, HBSS, RPMI 1640, FCS, and penicillin-streptomycin were purchased from Gibco (Grand Island, NY). $^3$H-myoinositol (10–20 Ci/mmol) was obtained from New England Nuclear. $^3$H-thymidine (5 Ci/mmol) and $^3$H-inositol polyphosphate markers were purchased from Amersham (Arlington Heights, IL). Recombinant Il-1 (α form) was a gift from Alvin Stern and Peter Lomedico (Hoffman-La Roche, Nutley, NJ); 1 unit of activity by this preparation is defined as the amount that induces one-half of maximal thymocyte proliferation in the presence of 1 μg/ml phytohemagglutinin. Contamination of

recombinant Il-1, silica particles, or media by endotoxin was not detectable (<0.01 ng/ml, by *Limulus* amebocyte lysate test). Polyvalent rabbit antibody against human Il-1 was purchased from Genzyme (Boston, MA) and is stated by the supplier to bind specifically both PI 5.0 and PI 7.0 forms of human Il-1 and not to bind other cytokines, including tumor necrosis factor. One unit of neutralizing activity by this antibody against Il-1 neutralizes approximately 0.08 units of the recombinant Il-1 activity. Rabbit preimmune serum was a gift from Elizabeth Posillico (Genzyme) and was prepared in a manner similar to that of antibody against Il-1.

## RESULTS

### Aortic Ring Contraction

Incubation of isolated aortic rings in monocyte-conditioned medium (diluted from 1:4 to 1:12 on the basis of preliminary screening for suppressive activity) induced striking decreases in contractile performance in comparison to responses by rings incubated in control medium. Both sensitivity (reflected in $EC_{50}$ values; Table I) and maximum generated tension (Fig. 1) to NE were significantly compromised by the monocyte-conditioned medium. Integrated dose-response values, which are sensitive to changes in both measures of performance, were also greatly diminished (Table I).

Treatment of monocyte-conditioned medium with antibody against human Il-1 prior to incubation of rings prevented most of the decrease in contractile performance manifested by rings incubated in untreated, conditioned medium. Aortic rings incubated in antibody-treated, monocyte-conditioned medium were able to generate tension, at large doses of NE, similar to that of rings incubated in control medium and control medium treated with antibody; no significant differences in tension by rings incubated in the three media preparations were present during stimulation by $3 \times 10^{-7}$ to $3 \times 10^{-5}$ M NE (Fig. 1). The presence of anti-Il-1 antibody during incubation of rings in conditioned medium also partially reduced the alteration in $EC_{50}$ values for NE that resulted after incubation in untreated, conditioned medium; however, sensitivity to NE remained less than that of rings incubated in control or antibody-treated control media (Table I). The improvement in aortic ring response attributable to the presence of antibody during incubation was reflected in

**TABLE I. Contractile Reaction of Rat Aortic Rings to Stimulation by NE After Incubation in Control and Treated Media†**

| Medium | Mean integrated dose response | Mean log $EC_{50}$ (M) |
|---|---|---|
| Control | 4,319 ± 333 | −7.42 ± 0.11 |
| Control + anti-Il-1 antibody | 4,065 ± 325 | −7.38 ± 0.09 |
| Monocyte-conditioned medium + anti-Il-1 antibody | 3,298 ± 318* | −6.91 ± 0.13*** |
| Monocyte-conditioned medium | 798 ± 177***,*** | −5.91 ± 0.23***,*** |

†N = 9 to 11 per group; values are presented as mean ± SE.
*$P < 0.05$ versus control.
**$P < 0.05$ versus control + antibody.
***$P < 0.05$ versus conditioned + antibody.

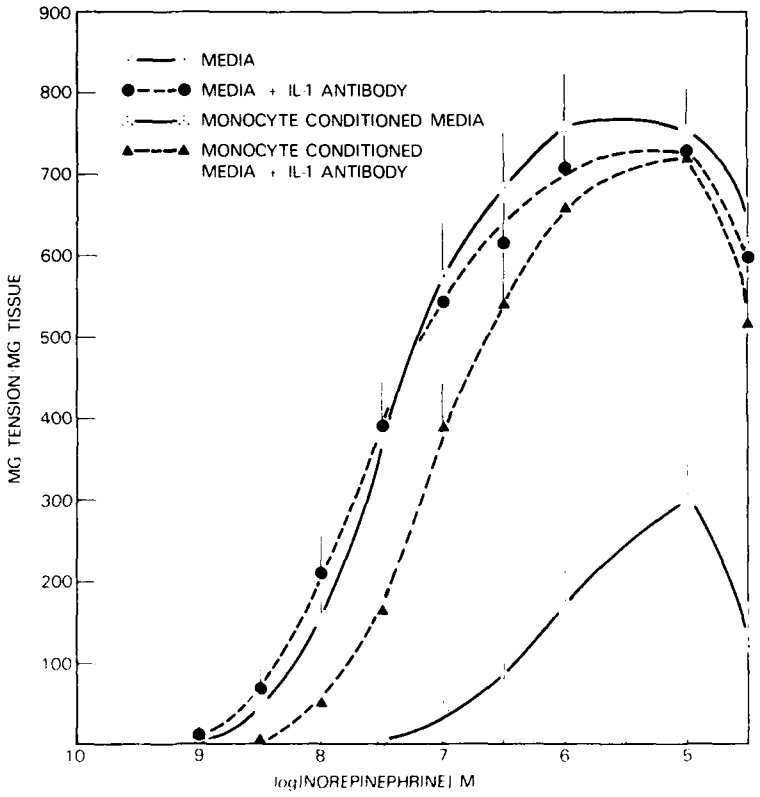


Fig. 1. Contractile responses to cumulative doses of NE by isolated rat aortic rings. Aortic rings isolated from separate rats were incubated 16 hr in control or monocyte-conditioned medium in the presence or absence of anti-Il-1 antibody (50 neutralizing U/ml). N = 11 rings per medium preparation. Each point plotted represents the mean ± SE. Some error bars are omitted for clarity but are similar to those shown.

significantly enhanced integrated dose responses to NE by rings incubated in antibody-treated versus untreated monocyte-conditioned medium (Table 1).

Addition of phorbol 12,13-dibutyrate (PDB) to the ring baths after the measurement of NE-induced contraction resulted in enhanced contraction by the suppressed rings. After treatment with PDB, the maximal contractions attained by rings incubated in control medium (774 ± 30 mg tension/mg tissue), control medium containing anti-Il-1 antibody (Ab) (777 ± 80), monocyte-conditioned medium (625 ± 82), and monocyte-conditioned medium containing anti-Il-1 Ab (630 ± 75) were not significantly different from each other (ANOVA, N = 9 per group).

A specific amelioration by anti-Il-1 antibody of the suppressive actions of monocyte-conditioned medium on aortic ring contractile function was apparent when aortic ring responses were compared in a paired manner. Rings incubated in monocyte-conditioned medium containing anti-Il-1 antibody expressed normal contractile performance whereas rings incubated in monocyte-conditioned medium containing preimmune rabbit serum were suppressed to the same extent as were rings

incubated in untreated, monocyte-conditioned medium (Fig. 2, Table II). Antibody to Il-1 also protected aortic ring sensitivity to NE; antibody-treated rings had significantly smaller $EC_{50}$ values than did those of the other treatment groups (Table II).

Incubation of aortic rings with recombinant human Il-1 resulted in decreased contraction that was indistinguishable from that observed after incubation of aortic rings in monocyte-conditioned medium. Rings incubated in as little as 1 U/ml Il-1 exhibited significant suppression of integrated contractile performance; after incubation with doses of 5 and 10 U/ml, rings also exhibited diminished sensitivity to NE (Fig. 3, Table III). Recombinant Il-1 was not as efficacious at suppressing ring contractile function as was medium containing monocyte-released Il-1. Samples of monocyte-conditioned medium contained an average of 2.7 ± 0.6 U/ml Il-1 activity (range 0.8 to 7.6 U/ml, N = 11); similarly prepared samples, utilized in experiments after at least a 1:4 dilution, produced a greater suppression of aortic ring contractile function on the basis of calculated Il-1 content than did the recombinant product (compare the degree of suppression of rings in Figs. 1 and 2 versus that shown in Fig. 3).

Exposure of aortic rings to a dose of NE of $3 \times 10^{-5}$ M always decreased ring tension to values smaller than those observed after a dose of $10^{-5}$ M, presumably via activation of $\beta_1$ receptors (Figs. 1–3). Vasodilatory responses to acetylcholine ($10^{-6}$

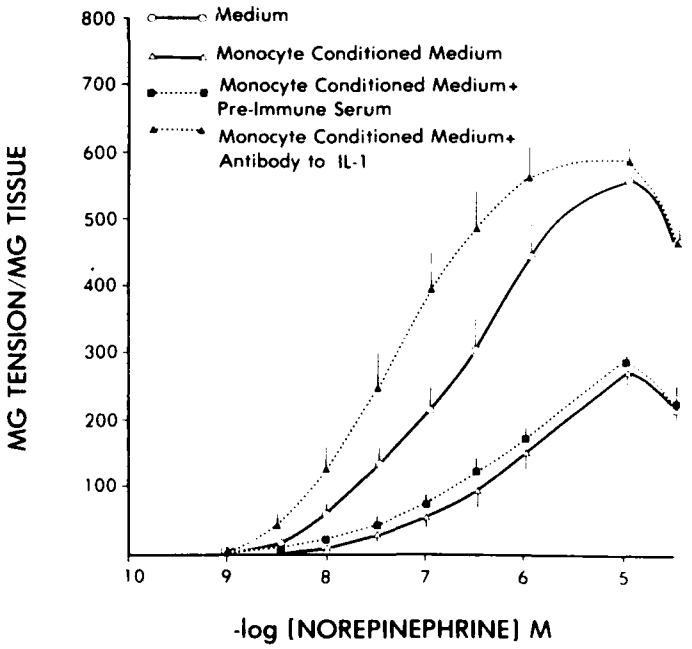


Fig. 2. Contractile responses to cumulative doses of NE by isolated rat aortic rings. Four rings were prepared from each rat and one ring allocated into each medium preparation. Rings were incubated 16 hr in control or monocyte-conditioned media or in monocyte-conditioned media containing anti-Il-1 antibody (50 neutralizing U/ml) or preimmune rabbit serum. N = 8 rats. Means and errors presented as in Figure 1.

**TABLE II. Contractile Reaction of Rat Aortic Rings to Stimulation by NE After Incubation in Control, Conditioned, and Supplemented Media†**

| Medium | Mean integrated dose response | Mean log $EC_{50}$ (M) |
|---|---|---|
| Control | 2,845 ± 280 | −6.72 ± 0.16 |
| Monocyte-conditioned medium + anti-Il-1 antibody | 3,736 ± 459 | 7.24 ± 0.13* |
| Monocyte-conditioned medium + preimmune serum | 1,231 ± 134*,** | 6.44 ± 0.19** |
| Monocyte-conditioned medium | 1,081 ± 83*,** | −6.19 ± 0.20** |

†N = 8 per group; values are presented as mean ± SE.
*$P < 0.05$ versus control.
**$P < 0.05$ versus conditioned + antibody.

**TABLE III. Contractile Reaction of Rat Aortic Rings to Stimulation by NE After Incubation in Medium Containing Recombinant Il-1†**

| Medium | Mean integrated dose response | Mean log $EC_{50}$ (M) |
|---|---|---|
| Control | 4,047 ± 273 | 7.33 ± 0.13 |
| + 1 U/ml Il-1 | 3,066 ± 284* | 7.03 ± 0.22 |
| + 5 U/ml Il-1 | 2,322 ± 448* | 6.93 ± 0.21* |
| + 10 U/ml Il-1 | 1,904 ± 380*,** | 6.92 ± 0.19* |

†N = 11 per group; values are presented as mean ± SE.
*$P < 0.05$ versus control.
**$P < 0.05$ versus 1 U/ml Il-1.

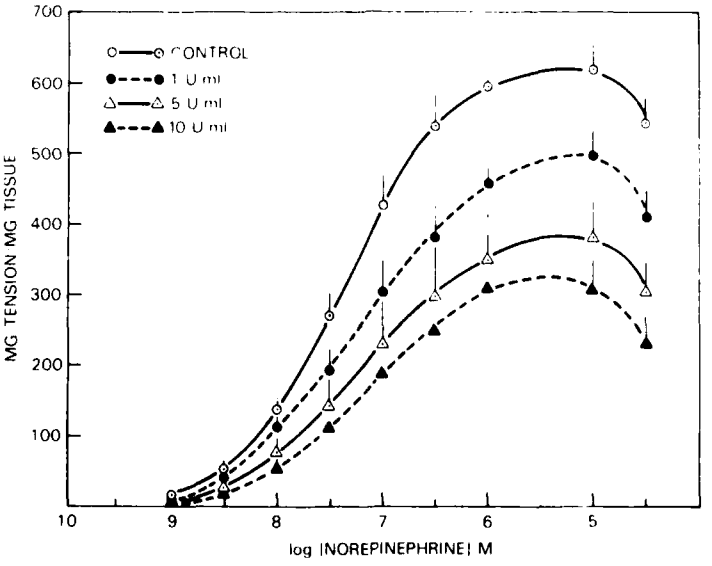


Fig. 3. Contractile responses to cumulative doses of NE by isolated rat aortic rings. Four rings were prepared from each rat and one ring allocated into each medium preparation. Rings were incubated 16 hr in control medium or in media containing 1, 5, or 10 U/ml recombinant human Il-1. N = 11 rats. Means and errors presented as in Figure 1.

M), indicative of the functional integrity of the endothelium, were present during the initial conditioning procedure in rings incubated in all media preparations (data not shown).

## Hydrolysis of Phosphoinositides

Ion exchange chromatography has been utilized by others [15,18] to separate the individual inositol phosphate products that result from receptor-mediated hydrolysis of PI in the rat aorta. We also found that this technique effectively separated $^3$H-labeled IP from other labeled inositol phosphate standards (Fig. 4). Recovery of IP standard from the Dowex column was 90%.

Stimulation of hydrolysis of PI by exposure of rings to NE at $10^{-7}$ M for 30 min generally resulted in little accumulation of IP, while maximal stimulation by NE at $10^{-5}$ M increased IP accumulation by five- to eightfold (Fig. 5). Accumulation of IP by aortic rings after stimulation by NE was variable, producing the large variances shown in Figure 5. This variability in IP accumulation is probably due to differences in responses to NE by vascular tissue isolated from individual animals, because the magnitudes of accumulation of IP by rings from the same rats after treatment with $10^{-7}$ or $10^{-5}$ M NE were significantly correlated in all media preparations except control (correlation coefficients ranged from 0.72 to 0.91; N = 11 for each medium

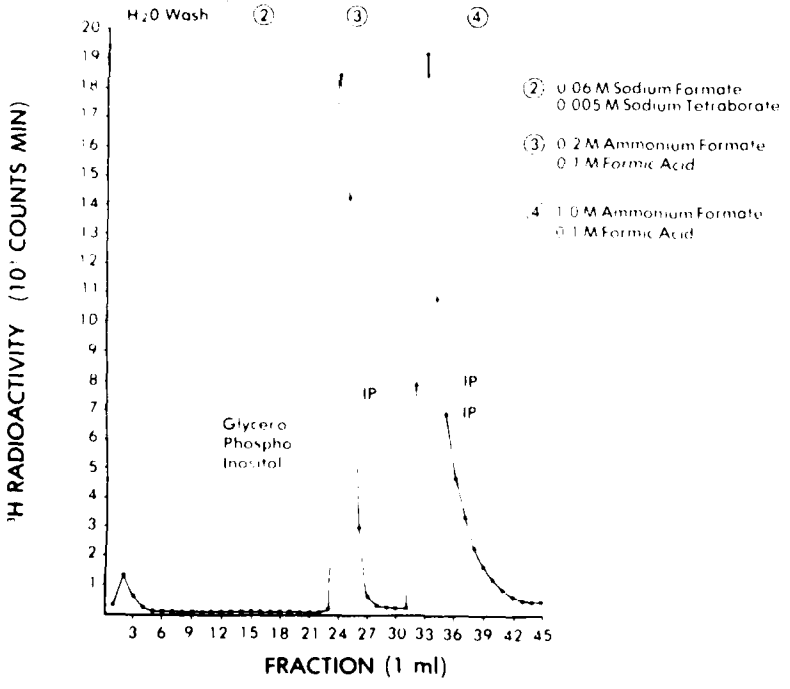


Fig. 4. Elution profile of $^3$H-labeled inositol phosphate standards. A standard solution containing 0.07 μCi of inositol 1-phosphate ($IP_1$), inositol-1,4-bisphosphate ($IP_2$), and inositol-1,4,5,-trisphosphate ($IP_3$) was dispersed in 1 ml of water, extracted, applied to a Dowex ion-exchange column, and eluted with stepwise gradients of formic acid/ammonium formate as detailed in Materials and Methods.

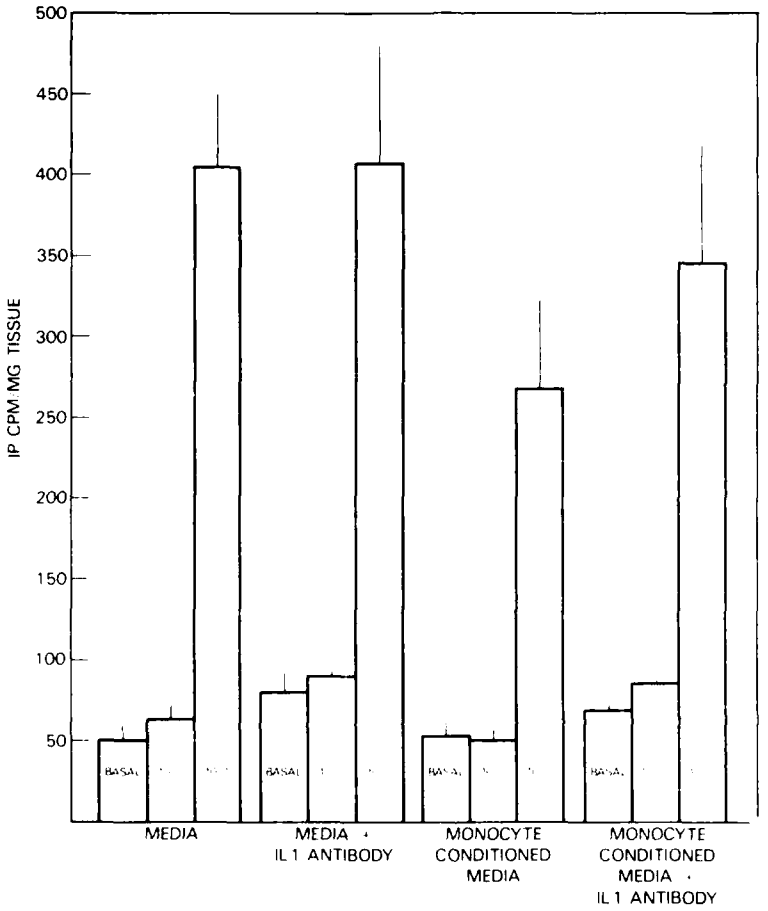


Fig. 5. Basal and NE-stimulated ($10^{-7}$ and $10^{-5}$ M) inositol-1-phosphate (IP) accumulation by aortic rings incubated 16 hr in control or monocyte-conditioned media in the presence or absence of anti-Il-1 antibody (50 neutralizing U/ml). N = 11 rings per each treatment (basal, $10^{-7}$ and $10^{-5}$ M NE) or 33 total for each medium preparation. Each bar represents the mean ± SE.

preparation; $P < 0.05$). No significant difference in accumulation of IP between rings incubated in each of the four media preparations could be demonstrated (by ANOVA) in comparisons of responses at basal levels of stimulation or in comparisons of responses after stimulation by $10^{-7}$ or $10^{-5}$ M NE. Possible influences of monocyte-conditioned medium and anti-Il-1 antibody on the accumulation of IP by aortic rings were present at low levels of tissue activity. Although rings did not exhibit a significant difference in accumulation of IP after stimulation by $10^{-7}$ M NE, the responses by these rings yielded an F value that approached significance ($P < 0.08$). Furthermore, treatment of control and monocyte-conditioned media with anti-Il-1 antibody augmented basal IP accumulation by incubated rings (Fig. 5); the pooled basal accumulation of IP by antibody-treated rings averaged 74.3 ± 8.3 cpm/mg

tissue, while the pooled basal accumulation of IP by rings incubated in equivalent nontreated media averaged 51.2 ± 6.5 ($P < 0.05$).

Regressing aortic ring tension on accumulation of IP after stimulation with $10^{-7}$ and $10^{-5}$ M NE yielded no significant relationship between the two variables by rings incubated in any medium preparation at either NE dose. However, when measures of ring tension and accumulation of IP after incubation in all media preparations were pooled, a significant positive relationship (slope = 0.348 ± 0.026, $P < 0.05$) between tension and IP accumulation was found at $10^{-5}$ M NE, but not at $10^{-7}$ M NE.

## DISCUSSION

The influence of Il-1 on the peripheral vasculature has primarily been characterized in terms of the effects of Il-1 treatment, in vitro, on vascular endothelial cell function. Endothelial cell responses to Il-1 include expression of cell surface proteins that are dependent on Il-1–induced protein biosynthesis [19]; these proteins may contribute to an Il-1–stimulated enhancement in tissue factor procoagulant activity [20], plasminogen activator inhibitor [21], and endothelial cell adhesiveness for lymphocytes [22] and neutrophils [23]. In addition, Il-1 influences endothelial cell arachidonic acid metabolism; treatment of endothelial cells with Il-1 increases biosynthesis and release of prostacyclin ($PGI_2$) and prostaglandin $E_2$ [24]. Endothelial cells themselves produce messenger RNA (mRNA) for Il-1 and express Il-1 biological activity when stimulated with endotoxin or tumor necrosis factor [25,26], suggesting the possibility of local regulation by the endothelium, via Il-1, of vascular cell function.

The effects of Il-1 on vascular smooth muscle cell function have not been investigated to the extent of Il-1 influence on endothelial cells. Il-1 enhances $PGI_2$ synthesis by smooth muscle cells [27]. Smooth muscle cells also have the capacity to synthesize mRNA for Il-1 and to release biologically active Il-1 in response to endotoxin [28]. Il-1 itself induces synthesis of mRNA for Il-1 by smooth muscle cells and the subsequent release of Il-1 biological activity [29], which again suggests the possibility of localized regulation of vascular function by an Il-1–mediated positive feedback mechanism.

Data from the present study support a significant role for Il-1 in modulating $\alpha_1$-adrenoceptor–stimulated vascular contraction. Our data show that exposure of aortic rings to Il-1 decreases contractile responses and that antibody against Il-1 is able to protect the contractile function of rings incubated in monocyte-conditioned medium. However, other monokines such as tumor necrosis factor ([TNF] cachectin) could contribute to the effects we observed on vascular function. TNF manifests significant vascular inflammatory effects when infused in vivo [30] and has also been shown to stimulate the production of mRNA for Il-1 by vascular endothelial cells [25]. We have previously demonstrated that incubation of aortic rings with TNF results in diminished contraction to NE; however, the concentration of TNF necessary to induce significant suppression of vascular contraction is larger than that required to induce similar defects by Il-1 [31]. Furthermore, the anti-Il-1 antibody protects the majority (Fig. 1) or entirety (Fig. 2) of vascular contractile function of rings incubated in monocyte-conditioned medium, suggesting that Il-1 and not TNF comprises the majority of vascular suppressive activity in the conditioned medium. These observations do not rule out a role for TNF in suppressing vascular contraction, because TNF could synergize with Il-1 to potentiate the action of Il-1. For instance,

microvascular injury via a local Schwartzman-like reaction occurs after injection of a combination of TNF and Il-1 into intradermal sites; injection of Il-1 or TNF alone results in much less inflammation [32]. The results from our experiments suggest that a similar relationship between the effects of a combination of Il-1 and TNF and the suppression of aortic rings may pertain; rings incubated in monocyte-conditioned medium, which presumably contained TNF in addition to Il-1, exhibited more impairment of contraction per unit of Il-1 activity in comparison to the suppression manifested by rings incubated with recombinant Il-1 only.

Phorbol 12,13-dibutyrate (PDB) can directly substitute for an intracellular second messenger (Diacylglycerol) that is normally released by $\alpha_1$-adrenergic receptor-mediated hydrolysis of PI. Treatment of vascular tissue by PDB activates phospholipid and calcium-dependent protein kinase C that in turn causes vascular contraction [for review, see 33]. The observed ability of aortas to respond to stimulation by PDB with contractions of normal magnitude after incubation in monocyte-conditioned medium suggests that diminished responses to NE are not simply due to the direct cytotoxic effects of agents released into the medium by activated monocytes, but rather reflect a discrete, induced lesion in the regulation of vascular cell function.

NE-stimulated IP accumulation, which can be utilized as an index for $\alpha_1$-adrenoceptor–mediated hydrolysis of phosphoinositides [for review, see 14], exhibits a tendency to decrease in aortic rings after incubation in monocyte-conditioned medium (Fig. 5). The decreases could not be demonstrated to be significant, however, and may reflect a minor influence of Il-1 directly on this NE-stimulated mechanism, a secondary response to Il-1 influence on other cellular activities, or a real decrease that is masked by the variability inherent in the experimental procedure. The lack of effect by Il-1 on NE-stimulated vascular function may be similar to that described by Abraham et al. [34] in which activation of LBRM-33 T cells by Il-1 is independent of modulation of hydrolysis of phosphoinositides.

The interpretation that monocyte-conditioned medium had no significant influence on NE-stimulated accumulation of IP in vascular tissue is limited by two assumptions that were made about the intracellular processing of $^3$H-inositol. These assumptions were that entry of the metabolically processed labeled compounds (i.e., PI) into a pool accessed by the $\alpha_1$-receptor–stimulated mechanism is equivalent between vascular tissue incubated in the different media preparations and that all rings possessed labeled pools of the same size. Deviations from these assumptions could mask, to a greater or lesser extent, differences in accumulation of IP that might exist in the incubated vascular tissue.

The small increase in IP accumulation measured after exposure of rings to $10^{-7}$ M NE (Fig. 5) probably exemplifies a rightward shifted dose response for vascular IP accumulation vis-à-vis vascular contraction to NE. This relationship has been described in the rat aorta by Rapoport [35]. Others have suggested that a large receptor reserve for $\alpha_1$-adrenoceptor–mediated vascular contraction exists in rat caudal arteries [18]; the presence of a similar $\alpha_1$-receptor reserve in the rat aorta could account for the disparity in the relationship between the little IP accumulation and the relatively large contraction observed after exposure of aortic rings to $10^{-7}$ M NE and the much greater IP accumulation that occurs without an equivalent increase in the magnitude of contraction after stimulation of the rings by $10^{-5}$ M NE.

Alternatively, subjecting vascular tissue to positive resting tension could alter NE-stimulated metabolism of PI and potentially could contribute to the nonequivalence between contractile responses and accumulation of IP measured in the rings. In our experimental design, all rings were incubated with $^{3}$H-labeled myoinositol while exposed to no resting tension. Thereafter, all rings were maintained in inositol-free buffer, and rings in which IP accumulation was measured were processed while exposed to no resting tension. In contrast, rings in which contractile responses were measured were subjected to 2.5 g resting tension prior to measures of contraction. The question of the influence of positive resting tension on NE-stimulated IP accumulation by the rat aorta was examined by Rapoport [35]; he concluded that aortic tissue under tension exhibited only small and nonsignificant enhancements in basal and NE-stimulated IP accumulation in comparison to responses by tissue not under tension. In view of these observations, it is unlikely that the measured accumulation of IP in this study is significantly skewed by the absence of positive resting tension in the aortic tissue.

Measures of vascular contraction and hydrolysis of PI were performed on rings with an intact endothelium, because previous studies demonstrated that the endothelium possesses a significant modulatory influence on vascular contractile function in tissue isolated from septic rats [2]. The measured accumulation of IP by NE-stimulated aortic rings therefore included the production of IP by both smooth muscle and endothelial cells.

An unexpected observation in this study is the evident augmentation of rat aortic contraction and IP accumulation that occurs after treatment with an antibody against human Il-1. The polyclonal antibody used in the experiments is stated by the supplier (Genzyme) to be species specific; we also have been unable to detect a significant neutralization of rat macrophage-derived Il-1 activity by this antibody in a mouse thymocyte proliferation assay in our laboratory (data not shown). Despite our inability to demonstrate a cross reaction between rat Il-1 and the antibody, the data presented in Figure 2 (monocyte-conditioned medium plus antibody) and Figure 5 (both media plus antibody and monocyte-conditioned media plus antibody) suggest that after treatment with this antibody vascular contraction and IP accumulation are both enhanced in comparison to that characteristic of untreated rings at rest or after stimulation with submaximal concentrations of NE. These observations lead to the speculation that if basal hydrolysis of PI influences vascular tone, or reflects activity by processes that influence cellular contractile state, then endogenously produced Il-1 may contribute to localized modulation of vascular tone.

Finally, the interaction between Il-1 and the aorta in vitro may recapitulate events that occur in the microvasculature after exposure to endotoxin in sepsis. In the rat, exposure of the microvasculature to endotoxin markedly augments the vasoconstrictor action of NE and epinephrine [36,37]. However, after exposure to endotoxin, the vasculature becomes progressively refractory to stimulation by NE. Rat microvessels dilate and do not respond to NE at 6 hr following an intravenous injection of endotoxin; in addition, tolerance to endotoxin that occurs after intravenous injection of the agent for 6 days is characterized by microvessel dilation and an insensitivity to NE [36]. Impaired vascular responses to NE, both in vivo [1] and in vitro [2,31], also occur during experimental sepsis. We suggest that the possibility exists that Il-1, released from mononuclear phagocytes and possibly from vascular endothelial and smooth muscle cells in response to sepsis-associated stimuli, diminishes vasocon-

striction that may occur during initial exposure to sepsis-associated endotoxin. Whether this action would ultimately be beneficial to the host in terms of preventing vasoconstriction-mediated tissue ischemia or, alternatively, whether the resulting modification in vascular function would cause changes in effective tissue perfusion that would be deleterious to the host remains to be determined.

## CONCLUSIONS

A product present in medium conditioned by activated human monocytes suppresses vascular contractile performance by isolated rat aortas after incubation in the conditioned medium. Antibody against human Il-1 shields the incubated tissue against suppression by the conditioned medium, whereas incubation of aortic tissue with recombinant human Il-1 induces defects in contractile performance that are similar to defects present after incubation in conditioned medium. The mechanism whereby monocyte-conditioned medium inhibits vascular contraction is unclear, since significant inhibition of NE-stimulated hydrolysis of phosphoinositides could not be demonstrated. These findings suggest that, regardless of the identity of the vascular intracellular processes engaged by exposure to Il-1, the monokine may have a significant role in modulating vascular contractile function in normal or pathophysiological states.

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## REFERENCES

1. Fink MP, Homer LD, Fletcher JR: Diminished pressor response to exogenous norepinephrine and angiotensin II in septic, unanesthetized rats: Evidence for a prostaglandin-mediated effect. J Surg Res 38:335–342, 1985.
2. McKenna TM, Martin FM, Chernow B, Briglia FA: Vascular endothelium contributes to decreased aortic contractility in experimental sepsis. Circ Shock 19:267–273, 1986.
3. Pomerantz K, Casey L, Fletcher JR, Ramwell PW: Vascular reactivity in endotoxin shock: Effect of lidocaine or indomethacin pretreatment. Adv Shock Res 7:191–198, 1982.
4. Schaller MD, Weaber B, Nussberger J, Brunner HR: Angiotensin II, vasopressin, and sympathetic activity in conscious rats with endotoxemia. Am J Physiol 249:H1086–H1092, 1985.
5. Soulsby ME, Ford GD, Davis JA, Wincklhofer K, Hess M: The effect of gram-negative endotoxin on calcium accumulation by subcellular fractions of vascular smooth muscle. Circ Shock 3:325–335, 1976.
6. Carcillo JA, Litten RZ, Suba EA, Roth BL: Alterations in rat aortic alpha$_1$-adrenoceptors and alpha$_1$-adrenergic stimulated phosphoinositide hydrolysis in intraperitoneal sepsis. Circ Shock 26:331–339, 1988.
7. Levin J, Poore TE, Zauber NP, Oser RS: Detection of endotoxin in the blood of patients with sepsis due to gram-negative bacteria. N Engl J Med 283:1313–1316, 1970.

8. Shenep JL, Barton RP, Morgan KA: Role of antibiotic class in the rate of liberation of endotoxin during therapy for experimental gram-negative bacterial sepsis. J Infect Dis 151:1012–1018, 1985.
9. Weiner R, Zweifach BW: Influence of *E. coli* endotoxin on serotonin contractions of the rabbit aortic strip. Proc Soc Exp Biol Med 123:937–939, 1966.
10. Wright RC, Winkelmann RK: The epinephrine response of isolated rabbit vascular strips after in vivo and in vitro endotoxin exposure. Angiology 22:495–500, 1971.
11. McKenna TM, Reusch DW, Simpkins CO: Macrophage-conditioned medium and interleukin 1 suppress vascular contractility. Circ Shock 25:187–196, 1988.
12. Fischer DG, Hubbard WJ, Koren HS: Tumor cell killing by freshly isolated peripheral blood monocytes. Cell Immunol 58:426–435, 1981.
13. Furchgott RF: Role of endothelium in responses of vascular smooth muscle. Circ Res 53:557–573, 1983.
14. Berridge MJ: Inositol trisphosphate and diacylglycerol as second messengers. Biochem J 220:346–360, 1984.
15. Roth BL, Nakaki T, Chuang D, Costa E: 5-Hydroxytryptamine$_2$ receptors coupled to phospholipase C in rat aorta: Modulation of phosphoinositide turnover by phorbol ester. J Pharmacol Exp Ther 238:480–485, 1986.
16. Fleming WW, Westfall DP, De La Lande IS, Jellett LB: Log-normal distribution of equieffective doses of norepinephrine and acetylcholine in several tissues. J Pharmacol Exp Ther 181:339–345, 1972.
17. Sokal RR, Rohlf FJ: "Biometry." San Francisco: WH Freeman and Co, 1969, pp 174–252.
18. Fox AW, Abel PW, Minneman KP: Activation of $\alpha_1$-adrenoceptors increases [$^3$H]inositol metabolism in rat vas deferens and caudal artery. Eur J Pharmacol 116:145–152, 1985.
19. Pober JS, Bevilacqua MP, Mendrick DL, LaPierre LA, Fiers W, Gimbrone MA: Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. J Immunol 136:1680–1687, 1986.
20. Nawroth PP, Handley DA, Esmon CT, Stern DM: Interleukin 1 induces endothelial cell procoagulant while suppressing cell-surface anticoagulant activity. Proc Natl Acad Sci USA 83:3460–3464, 1986.
21. Gramse M, Breviario F, Pintucci G, Millet I, Dejana I, van Damme J, Donati MB, Mussoni L: Enhancement by interleukin-1 (IL 1) of plasminogen activator inhibitor (PA-I) activity in cultured human endothelial cells. Biochem Biophys Res Commun 139:720–727, 1986.
22. Cavender DE, Haskard DO, Joseph B, Ziff M: Interleukin-1 increases the binding of human B and T lymphocytes to endothelial cell monolayers. J Immunol 136:203–207, 1986.
23. Schleimer RP, Rutledge BK: Cultured human vascular endothelial cells acquire adhesiveness for neutrophils after stimulation with interleukin 1, endotoxin, and tumor-promoting phorbol diesters. J Immunol 136:649–654, 1986.
24. Albrightson CR, Baenziger NL, Needleman P: Exaggerated human vascular cell prostaglandin biosynthesis mediated by monocytes: Role of monokines and interleukin 1. J Immunol 135: 1872–1877, 1985.
25. Libby P, Ordovas JM, Auger KR, Robbins AH, Birinyi LK, Dinarello CA: Endotoxin and tumor necrosis factor induce interleukin-1 gene expression in adult human vascular endothelial cells. Am J Pathol 124:179–185, 1986.
26. Stern DM, Bank I, Nawroth PP, Cassimeris J, Kisiel W, Fenton II JW, Dinarello CA, Chess L, Jaffe EA: Self-regulation of procoagulant events on the endothelial cell surface. J Exp Med 162:1223–1235, 1985.
27. Rossi V, Breviario F, Ghezzi P, Dejana E, Mantovani A: Prostacyclin synthesis induced in vascular cells by interleukin-1. Science 229:174–176, 1985.
28. Libby P, Ordovas JM, Birinyi LK, Dinarello CA: Inducible interleukin-1 gene expression in human vascular smooth muscle cells. J Clin Invest 78:1432–1438, 1986.
29. Warner SJ, Augur KR, Libby P: Human interleukin 1 induces interleukin 1 gene expression in human vascular smooth muscle cells. J Exp Med 165:1316–1331, 1987.
30. Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, Hariri RJ, Fahey III TJ, Zentella A, Albert JD, Shires GT, Cerami A: Shock and tissue injury induced by recombinant human cachectin. Science 234:470–474, 1986.
31. McKenna TM, Titius WAW: Role of monokines in altering receptor and non-receptor mediated vascular contraction in sepsis. In Roth BL, Nielsen T, McKee A (eds): "Molecular and Cellular Mechanisms of Septic Shock." Prog Clin Bio Res 286, New York: Alan R. Liss, 1989, pp 279–303.

32. Movat HZ: Tumor necrosis factor and interleukin-1: Role in acute inflammation and microvascular injury. J Lab Clin Med 110:668–681, 1987.
33. Rasmussen H, Takuwa Y, Park S: Protein kinase C in the regulation of smooth muscle contraction. FASEB J 1:177–185, 1987.
34. Abrahnam RT, Ho SN, Barna TJ, McKean DJ: Transmembrane signaling during interleukin 1-dependent T cell activation. J Biol Chem 262:2719–2728, 1987.
35. Rapoport RM: Effects of norepinephrine on contraction and hydrolysis of phosphatidylinositols in rat aorta. J Pharmacol Exp Ther 242:188–194, 1987.
36. Altura BM, Zweifach BW: Endogenous histamine formation and vascular reactivity. Am J Physiol 212(3):559–564, 1967.
37. Zweifach BW, Nagler AL, Thomas L: The role of epinephrine in the reactions produced by the endotoxins of gram-negative bacteria. II. The changes produced by endotoxin in the vascular reactivity to epinephrine in the rat mesoappendix and the isolated, perfused rabbit ear. J Exp Med 104:881–896, 1956.

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