American Journal of Pathology, Vol. 138, No. 4, April 1991

Interleukin-1 Gene Expression in Rabbit Vascular Tissue *In Vivo*

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Cultured human vascular endothelial and smooth muscle cells express interleukin-1 (IL-1) genes when exposed to bacterial lipopolysaccharides (LPS) and a variety of inflammatory mediators. Local production of IL-1 may contribute to the pathogenesis of various vascular diseases. Therefore the ability of intact vascular tissue to accumulate IL-1 mRNA and synthesize de novo ***biologically active IL-1 protein was examined.*** Escherichia coli ***LPS (10 μg/kg) was administered intravenously to adult rabbits and total RNA was isolated from aortic tissue at various times after LPS injection. In saline-injected rabbits, RNA extracted from the thoracic aorta contained little or no IL-1 message detected by Northern analysis using IL-1 α and β cDNA probes cloned from an LPS-stimulated rabbit splenic macrophage library. Lipopolysaccharide treatment promptly induced transient accumulation of mRNA for IL-1 α and IL-1 β within the aorta (maximal 1-hour after injection). Short-term organoid cultures of rabbit aorta exposed to LPS*** in vitro ***synthesized immunoprecipitable IL-1 α protein. Extracts of aortic tissue excised 1.5 to 3.0 hours after intravenous LPS administration contained immunoreactive and biologically active IL-1 α. Anti-rabbit IL-1 α antibody neutralized the biologic activity (more than 90%). Microscopic and immunohistochemical studies did not disclose adherent or infiltrating macrophages in rabbit aorta at the time of maximal IL-1 mRNA accumulation after LPS administration (1.5 hours), indicating that intrinsic vascular wall cells rather than mononuclear phagocytes probably account for the IL-1 activity induced by LPS. In addition, aortic tissue from rabbits fed an atherogenic diet showed an enhanced ability to accumulate IL-1 α and β mRNA and produce immunodetectible protein in response to LPS administration. These studies demonstrate inducible IL-1 gene expression in rabbit vascular tissue*** in vivo ***and support a local role for this cytokine in vascular pathophysiology. (Am J Pathol 1991, 138:1005–1014)***

Interleukin-1 (IL-1), integrated within a network of cytokines, mediates many local and systemic host responses to injury or infection.[1] IL-1 may participate in several of the generalized effects of bacterial toxins (eg, gram-negative lipopolysaccharide [LPS] or staphyloccal toxic shock protein), including fever, circulatory shock, intravascular thrombosis, and multiple-organ failure.[1] IL-1 alters many important functions of cultured vascular wall cells. Exposure to IL-1 alters the normally blood-compatible surface of cultured human endothelial cells in a manner that favors coagulation and thrombosis and retards fibrinolysis.[2–4] Treatment of cultured endothelial cells with IL-1 increases their adhesiveness for leukocytes, in some cases by inducing specific adhesion molecules.[5,6] This cytokine enhances synthesis of vasoactive substances, such as prostaglandins and platelet-activating factor, by cultured vascular endothelial and smooth muscle cells.[7]

This project was funded in part with federal funds from the U.S. Department of Agriculture, Agricultural Research Service under contract number 53-3K06-5-10. The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. Dr. Libby is an Established Investigator of the American Heart Association.

Dr. Fleet was a fellow of the American Heart Association, Massachusetts affiliate, during the course of this work.

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Accepted for publication December 14, 1990.

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Recombinant IL-1 can alter vascular reactivity to vasoactive agents[8,9] and produce hemodynamic changes in intact rabbits similar to those observed in gram-negative septic shock.[10] IL-1 also enhances the proliferation of vascular smooth muscle cells *in vitro*,[11] perhaps by stimulating autocrine production of platelet-derived growth factor.[12] Thus IL-1 profoundly alters vascular functions in pathophysiologically important ways.

Cultured vascular cells are not only targets for IL-1 but also can produce this cytokine. Human endothelial cells derived from both fetal and adult vessels elaborate IL-1–like biologic activity and accumulate IL-1 mRNA following stimulation with bacterial LPS or tumor necrosis factor α (TNFα)/cachectin.[13–16] We also have observed that IL-1 itself potently stimulates IL-1 gene expression in both endothelial and smooth muscle cells *in vitro*.[17,18] These findings suggest that activation of paracrine or autocrine feedback loops involving IL-1 within the vascular wall may enhance local inflammatory responses and promote the repair of damaged tissue. In addition to such desirable functions in host defense, IL-1 may mediate processes deleterious to the organism. For example, regional IL-1 release in blood vessels, acting in concert with other cytokines, probably contributes to the pathogenesis of a variety of acute and chronic diseases such as vasculitis, allograft rejection, fibrotic diseases of many organ systems, hypertension, and atherosclerosis.[19,20]

Previous investigations of vascular IL-1 expression have used cultured cells, an approach that allows defined observations on homogeneous and well-characterized cell types. However such *in vitro* observations do not establish that vascular cells may actually exhibit this function *in vivo*. The present studies characterized the induction of IL-1 gene expression in intact arterial tissue using intravenously administered bacterial LPS as a stimulus. We selected the rabbit as our experimental model because of the large body of experience with this species in studies of vascular inflammation[10,21] and diet-induced atherosclerosis.[22] We used cDNA probes for rabbit IL-1 α and IL-1 β[23] to evaluate mRNA expression. In addition, we used metabolic labeling with radioimmunoprecipitation to monitor IL-1 α synthesis in short-term aortic organoid cultures. Biologic assays and an IL-1 α radioimmunoassay (RIA) assessed IL-1 activity produced by rabbit vascular tissue in the basal state and following intravenous administration of LPS, a pathophysiologically relevant stimulus.

Materials and Methods

Northern Analysis of IL-1 Gene Expression

Female New Zealand white rabbits (certified *Pasturella*-free, Millbrook Farms, Amherst, MA) weighing 3 kg each were assigned randomly to their treatment. Five rabbits received an intravenous injection of LPS (10 μg/kg LPS, *Escherichia coli* serotype 055:B5, Sigma Chemical Co., St. Louis, MO) and were killed by intravenous injection of 120 mg/kg pentobarbital 1, 2, 3, 4, and 24 hours later. One rabbit was given a saline injection and served as a control. Two grams of spleen and the entire aorta from the aortic arch to the renal arteries (wet weight approximately 0.5 g) were removed from each rabbit, rinsed in sterile saline, trimmed of adventitial tissue, immediately homogenized in guanidinium isothiocyanate, and stored at −70°C. RNA was isolated by phenol extraction.[24] Northern blotting used 20 μg of total cellular RNA applied to each lane, separated on a 1.2% agarose-formaldehyde (2.2 mol/l [molar]) gel and transferred to a nylon membrane (Amersham Hybond-N, Arlington Heights, IL). The rabbit IL-1 α and β cDNA probes were cloned from a cDNA library constructed in lambda gt10 using polyadenylated RNA extracted from rabbit adherent splenic macrophages following LPS stimulation. Human IL-1 α and β cDNAs were used as hybridization probes to isolate the rabbit forms that subsequently were sequenced and used to produce recombinant rabbit IL-1 proteins.[23] The rabbit IL-1 α cDNA probe was labeled with ^{32}P-dCTP by the random priming (Pharmacia Inc., Piscataway, NJ) of an 830 base pair *Eco* RI to *Bgl* II fragment of the IL-1 α cDNA. The IL-1 β probe was an 830-base pair *Eco* RI to *Eco* RI fragment labeled by the same method. Hybridization and autoradiography used standard techniques.[25]

Characterization of Anti-rabbit IL-1 α Antibody

Goats received primary immunization by intradermal injection of recombinant rabbit IL-1 α[23] in complete Freund's adjuvant. The goats received subsequent injections in incomplete Freund's adjuvant at 2 and 4 weeks followed by 8-week intervals. Postimmunization sera were collected and immunoglobulins precipitated with 45% saturated ammonium sulfate and reconstituted in 0.9% NaCl, 0.5 mol/l Na_2HPO_4, pH 7.4, and dialyzed against the same solution. Immunoprecipitation studies examined the specificity of the polyclonal goat IgG isolated following immunization with recombinant rabbit IL-1 α (data not shown). Nonimmune goat serum showed no precipitating activity. The goat anti-rabbit IL-1 α antibody precipitated the corresponding radioiodinated recombinant rabbit protein without evidence of cross-reactivity with the IL-1 β protein. The molecular weight of the precipitated complex corresponded to the 17 kd expected for the recombinant IL-1 α[23]. The antibody then was used

in the following radioimmunoprecipitation studies and RIAs.

IL-1 Protein Production in Aortic Organoid Cultures

Male New Zealand White rabbits weighing 3 kg were killed with intravenous pentobarbital and the thoracic aorta and spleen were removed and rinsed in Roswell Park Memorial Institute (RPMI) 1640 media (without cysteine or methionine) containing 5% fetal calf serum (FCS), penicillin, streptomycin, and amphoterecin B. All culture materials were evaluated for endotoxin contamination with the chromogenic Limulus amebocyte lysate assay, which has a sensitivity of approximately 10 pg/ml. Aortic tissues were cut into pieces measuring 0.5 by 0.5 cm in surface area, and the spleens were sliced, followed by preincubation in media lacking cysteine or methionine at 37°C with 5% CO_2 for 30 minutes. The preincubation medium was removed and the tissue was placed in six-well, 16-mm culture dishes with fresh media containing ^{35}S-labeled cysteine and methionine (Tran^{35}S-label, ICN Biochemical, Lisle, IL; 100 μCi/ml, specific activity) and indomethacin (1×10^{-6} mol/l). Duplicate cultures of aorta and spleen were treated with LPS (10 μg/ml *E. coli* serotype 055:B5, Sigma Chemical Co.) or polymyxin B (10 μg/ml), and incubated with rocking for 24 hours at 37°C with 5% CO_2. Following culture, the tissues were separated from media and homogenized on ice in immunoprecipitation buffer followed by centrifugation to remove debris. Protease inhibitors were added to the culture medium, which then was centrifuged to remove debris. The supernatant was exchanged into immunoprecipitation media by centrifugation using a Centricon-10 filter unit (Amicon, Danvers, MA). Subsequent handling of tissue homogenates and conditioned media was identical. Samples were incubated at 4°C for 4 hours with nonimmune goat serum, followed by clearing with agarose bound recombinant protein-G for 1 hour at 4°C, and centrifuged. Supernatants were divided into aliquots, treated with control goat serum or goat anti-rabbit IL-1 α, and incubated for 24 hours at 4°C. Immune complexes were adsorbed to protein G-agarose for 1 hour at 4°C. Pellets were washed five times with buffer (50 mmol/l [millimolar] TRIS HCl, pH 7.4, 5 mmol/l ethylenedrinitrilo tetraacetic acid [EDTA], 0.5 NaCl, 0.02% sodium dodecyl sulfate [SDS], 0.1% Nonidet P-40, 0.1 mmol/l phenylmethylsulfonyl fluoride (PMSF), and protease inhibitors) and once with 62.5 mmol/l TRIS HCl, pH 6.8. The pellets were aspirated dry and heated to 95°C for 10 minutes with 50 μl Laemmli sample solvent followed by electrophoresis on SDS-polyacrylamide gels (12.5%). Gels were fixed (25% trichloroacetic acid [TCA], 25% glacial acetic acid, 50% ethanol), rinsed in deionized water, then treated with Fluorohance (RPI, Chicago, IL), dried, and fluorographed at −70°C.

Assay of IL-1 Biologic Activity

Male New Zealand White rabbits weighing 3 kg were given intravenous injections of sterile saline or LPS (10 μg/Kg), killed with intravenous pentobarbital 1.5 to 3 hours later, and the thoracic aortas were removed. The tissue (0.5 g) was rinsed in saline and homogenized in 10 ml of Dulbecco's Modified Eagle's Medium (DMEM) medium, centrifuged to remove debris, solubilized with 9 mmol/l Chaps for 20 minutes at 4°C, centrifuged, and the supernatants were dialyzed against DMEM with 300 volume exchanges over a 72-hour period at 4°C.

IL-1 bioactivity was examined by the D10S murine T-cell proliferation assay (D10S).[26,27] This assay used a subclone of the murine T helper cells D10.G4.1, which proliferate in response to femtomolar concentrations of IL-1 in the absence of other mitogens. D10S cells were propagated for 7 to 10 days in RPMI medium 1640 (MA Bioproducts) supplemented with antibiotics, L-glutamine, HEPES, 2-mercaptoethanol (5×10^{-5} mol/l), 10% FCS, and 10% rat spleen cell supernatant. For assay, the cells were washed gently three times, then 50 μl of the cell suspension (1×10^4 cells/ml) was added to the tissue extracts or standards. Test samples or recombinant standards were diluted in serial fourfold steps with identical medium, except for omission of the murine spleen cell supernatant. Tritiated thymidine (0.5 μCi/well, 6.7 Ci/mmol) was added during the last 4 hours of a 48-hour incubation and ^3H incorporation was measured. IL-1 activity was analyzed by probit analysis and expressed as picograms (pg) recombinant human IL-1 α/ml.

IL-1 bioactivity also was examined by a human dermal fibroblast proliferation assay.[28] In the fibroblast assay, cells were incubated for 24 hours in flat-bottom microtiter plates in 100-μl aliquots containing 5×10^3 cells in DMEM medium (10% FCS, L-glutamine, penicillin, and streptomycin). Medium was replaced with serial fourfold dilutions of tissue homogenates or recombinant standards. ^3H-thymidine (0.5 μCi/well at 6.7 Ci/mmol) was added during the last 24 hours of a 4-day incubation. The cells then were harvested and ^3H-thymidine uptake was measured by liquid scintillation spectroscopy.

Radioimmunoassay

The RIA procedures for rabbit IL-1 α are similar to those described for the human forms.[29,30] The assay has a

sensitivity of 0.1 ng/ml at the 95% confidence level. The assay does not cross-react with other cytokines tested and is unaffected by plant lectins, FCS, or endotoxin.

Immunohistochemistry

Sections of aortas were placed in optimal cutting temperature (OCT) tissue processing medium and frozen at −70°C. Frozen sections, 6 μ thick, were cut subsequently and immunohistochemistry performed using standard avidin-biotin techniques. A mouse monoclonal IgG capable of staining tissue macrophages (RAM 11) was diluted 1:100 in phosphate-buffered saline (PBS) and applied for 60 minutes.[31] The slides then were immersed in a solution of 1% hydrogen peroxide in methanol to block endogenous peroxidase activity. After immersion for 15 minutes in 1% normal horse serum diluted in PBS, a biotinylated horse anti-mouse IgG was applied for 45 minutes, followed by the application of ABC peroxidase complex (Vector Laboratories, Burlingame, CA). The slides were developed using aminoethylcarbazole as a chromogenic substrate and counterstained with hematoxylin. Positive controls consisted of frozen sections of rabbit lung previously shown to contain tissue macrophages that stain with RAM 11. Negative controls included the omission of the primary antibody (PBS control) or the use of an irrelevant IgG (UPC10) as the primary antibody.

IL-1 Gene Expression in Rabbits Fed an Atherogenic Diet

Male New Zealand White rabbits were fed Purina Certified Rabbit Chow (number 5322, Ralston-Purina, St. Louis, MO) or an atherogenic diet composed of 94% chow, 5% hydrogenated coconut oil, and 1% cholesterol for 10 weeks. The diets were prepared to our specifications by Research Diets Inc., New Brunswick, NJ. Rabbits were given intravenous injections of saline or LPS, as in the previous experiment, and the tissues harvested at 2 hours for Northern analysis and RIA. Three centimeters of thoracic aortic tissue was removed from each rabbit. Northern blotting and RIAs were completed as described above.

Results

IL-1 Gene Expression in Aortic and Spleen Tissue

Under baseline conditions, Northern analysis showed little or no IL-1 α and β mRNA in the rabbit aorta (Figure 1, time 0). Following LPS administration, steady state levels of IL-1 β mRNA increased promptly and returned to baseline by 3 hours. The spleen, a prototypic lymphoid organ selected as a positive control, also shows a rapid, although more pronounced and prolonged, accumulation of IL-1 β mRNA. Lipopolysaccharide-stimulated U937 cells (a human monocytoid cell line) and WI38 (human fetal lung fibroblasts) also served as positive controls and indicated cross-reactivity of the rabbit cDNA probe with human IL-1 β mRNA. Electrophoretic behavior on this and other northern blots (not shown) revealed that rabbit IL-1 β mRNA may be slightly smaller than the human 1.6-kb IL-1 β transcript that migrates near the 18s ribosomal RNA. A subsequent hybridization of the same membrane with rabbit IL-1 α cDNA revealed mRNA (migrating at approximately 24s) for this species of IL-1 following LPS administration, although at a lower intensity than seen with IL-1 β. Residual IL-1 β mRNA, which was incompletely stripped from the blot before hybridization with the IL-1 α probe, can be seen near the 18s marker. The exposure time for both autoradiographs was 24 hours. The LPS-induced increase in IL-1 was not due to a generalized effect on gene expression because the level in these samples of a constitutively expressed form of β-tubulin mRNA did not change appreciably (data not shown). The entire experiment has been replicated with nearly identical results.

Production of Immunoprecipitable IL-1 α by Aortic and Spleen Organoid Cultures

Metabolic labeling and immunoprecipitation showed detectable synthesis of IL-1 α precursors (approximately 30 kd) in the control, and to a greater extent, the LPS-treated aortic tissue in short-term organoid cultures (Figure 2). Following LPS stimulation, the aortic homogenates and the supernatants contained processed IL-1 α forms of approximately 15 to 18 kd. The unstimulated aortic tissue did not contain or release the 15 to 18 kd processed IL-1 α forms, which have greater biologic activity than the precursors. The splenic tissue showed similar results (Figure 3), although the untreated tissue showed no significant synthesis of IL-1 α precursors.

Production of IL-1 Biologic and Radioimmunologic Activity Within Rabbit Aortic Tissue

We also wished to determine whether exposure to LPS increased the production of biologically active IL-1 in the

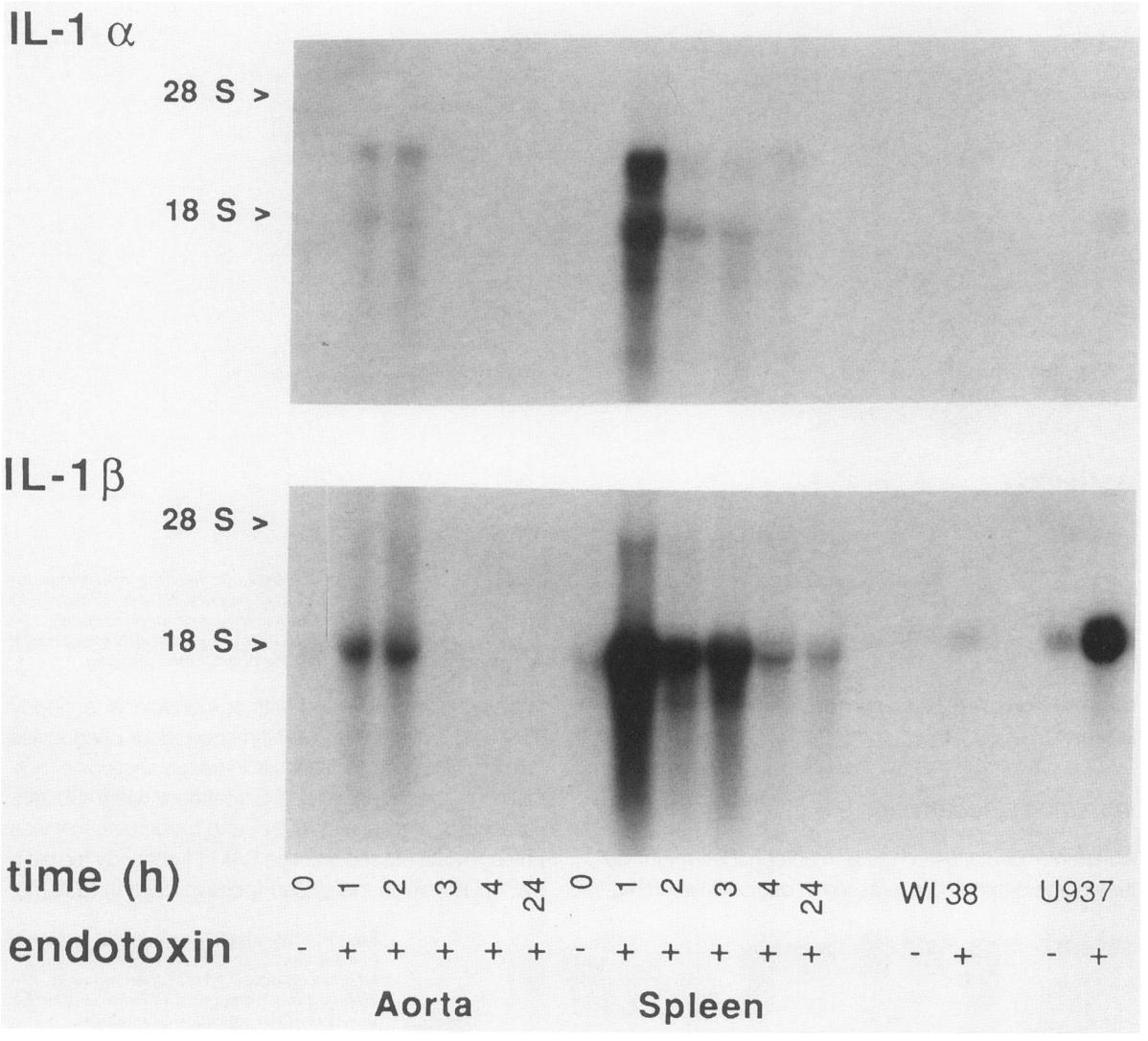

Figure 1. *Northern blot autoradiograms of RNA from the aorta and spleen of control rabbits and those treated with a single intravenous injection of 10 μg/kg of* E. coli *endotoxin (055:B5, Sigma Chemical Co.) at time 0. The blot initially was hybridized with a cDNA probe for rabbit IL-1β, washed to remove most of the IL-1 β signal (at approximately 18s), and rehybridized with rabbit IL-1 α cDNA (at approximately 24s).*

rabbit aorta. Extracts from the aortic tissue from four saline-injected control rabbits exhibited only scant IL-1 activity in the D10S assay (Table 1). Similar preparations taken from a rabbit 1.5 to 3 hours after injection with endotoxin contained significantly greater IL-1 activity in the D10S assay. Goat polyclonal antibody raised against recombinant IL-1 α neutralized this activity. Homogenates of LPS-stimulated spleens contained substantial IL-1 activity, which also was neutralized by the goat anti-rabbit IL-1 antibodies. These findings were confirmed by pooling the remaining tissue homogenates from each treatment group and evaluating IL-1 biologic activity in the human dermal fibroblast assay.[28] The unstimulated aorta and spleen showed less than 40 units of IL-1 bioactivity per milliliter of homogenate compared to 248 and 9763 units/ml, respectively, following LPS administration. Anti–IL-1 α antibody inhibited this activity by more than 90%. The augmentation of the mitogenic effect of certain lectins on murine thymocytes, known as the lymphocyte activation or thymocyte costimulation assay,[32] has been a standard method of evaluating IL-1 biologic activity. However this assay also responds to other cytokines such as IL-2 and IL-6. The D10S assay is more specific for IL-1, although these cells also respond to IL-2, IL-4, and IL-6, albeit to 10^3 higher molar concentrations.[27] The fibroblast proliferation assay does not measure IL-2, IL-4, or IL-6 under these conditions.[28] The RIA for IL-1 α showed a pattern similar to that observed for the biologic assays (Table 1). Little or no activity was noted in the uninduced aorta or spleen. Lipopolysaccharide administration sig-

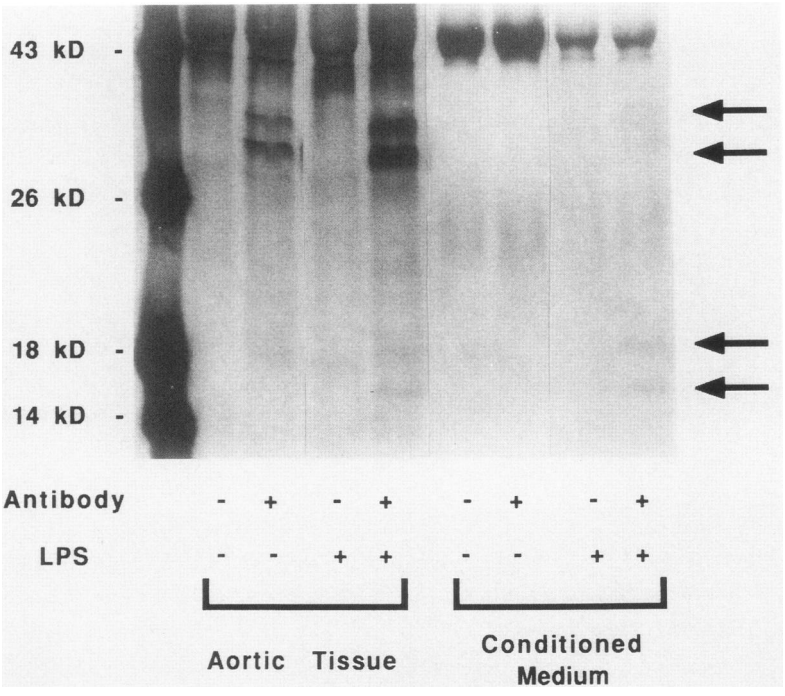

Figure 2. *Metabolic labeling and immunoprecipitation of precursors (approximately 30 kd) and processed forms (approximately 15–18 kd) of IL-1 α in organoid cultures of rabbit aorta and the conditioned medium.*

nificantly increased the immunoassayable IL-1 α in both tissues.

Immunohistochemistry

Adherent monocytes or tissue macrophages might be the source of the IL-1 expressed in aortic tissue. Immunohistochemical staining with a monoclonal antibody (RAM 11) that identifies rabbit mononuclear phagocytes failed to disclose reactive cells in frozen sections of normal aortic specimens after LPS treatment, when substantial accumulation of IL-1 mRNA and biologic activity was present (data not shown). The RAM 11 antibody did stain monocytes within the spleen (positive control) (data not

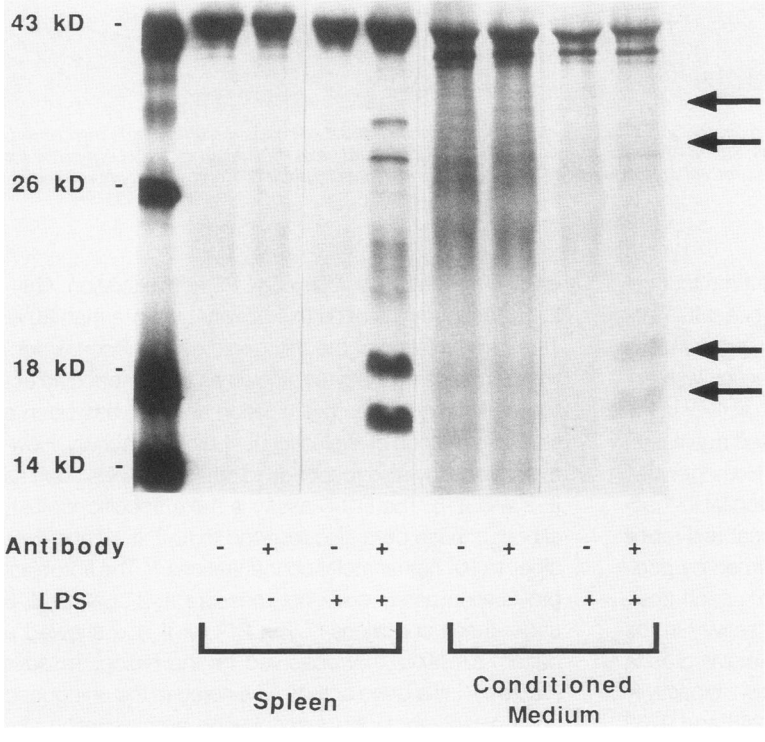

Figure 3. *Metabolic labeling and immunoprecipitation of precursors (approximately 30 kd) and processed forms (approximately 15–18 kd) of IL-1 α in organoid cultures of rabbit spleen and the conditioned medium.*

Table 1. *Biologic and Radioimmunoassayable IL-1 Activity in Homogenates of the Aorta and Spleen from Rabbits Treated with a Single Intravenous Dose of LPS 1.5 to 3.0 Hours Before Being Killed*

Tissue	D10S Bioassay* (pg rhIL-1β/mg)†	Radioimmunoassay* (pg rabbit IL-1α/mg)‡
Aorta		
−LPS	0 ± 0[a]	2.1 ± 0.7[a]
+LPS	330 ± 146[b]	25.1 ± 5.1[b]
Spleen		
−LPS	0 ± 0[a]	4.6 ± 1.7[a]
+LPS	798 ± 152[c]	161.2 ± 22.7[c]

* Data expressed as mean ± SE (n = 4). ANOVA shows significant effect of treatment at $P < 0.01$ for the bioassay and $P < 0.0001$ for the radioimmunoassay. Values with different superscripts are significantly different based on subsequent pairwise comparisons at $P < 0.05$.

† Biologic activity expressed as pg recombinant human IL-1β per mg of protein.

‡ Radioimmunoassay data expressed as pg of recombinant rabbit IL-1α per mg of protein.

shown). The immunohistochemical procedure also showed multiple reactive cells in sections of a region of the lung affected by *pneumonitis* of one rabbit from our colony that was not used for this study.

Induction of IL-1 Gene Expression in the Atherosclerotic Aorta

Three centimeters of thoracic aortic tissue from control rabbits weighed 0.54 ± 0.09 g (n = 4) and provided 58 ± 8 μg of RNA while a similar section from rabbits fed the atherogenic diet weighed 0.98 ± 0.11 g (n = 4) and provided 84 ± 9 μg of RNA. Twenty micrograms of total RNA was loaded into each lane for Northern analysis for rabbit Il-1 α and IL-1 β. Aortas from rabbits fed the atherogenic diet for 10 weeks showed an enhanced ability to accumulate mRNA for IL-1 α and β 2 hours after intravenous LPS administration (Figure 4). Similar results were obtained in three additional rabbits per treatment. We were unable to detect IL-1 mRNA in the control or atherosclerotic aorta under baseline conditions using Northern analysis. Approximately 25 mg of aortic tissue, from each of four rabbits per treatment, was homogenized and evaluated for IL-1 α by RIA. No IL-1 α activity was detected in the control or lipid-laden aortic tissue from rabbits not given LPS. Aortic tissue from the LPS-treated controls showed 40 ± 8 pg IL-1 α/mg protein (mean SEM) com-

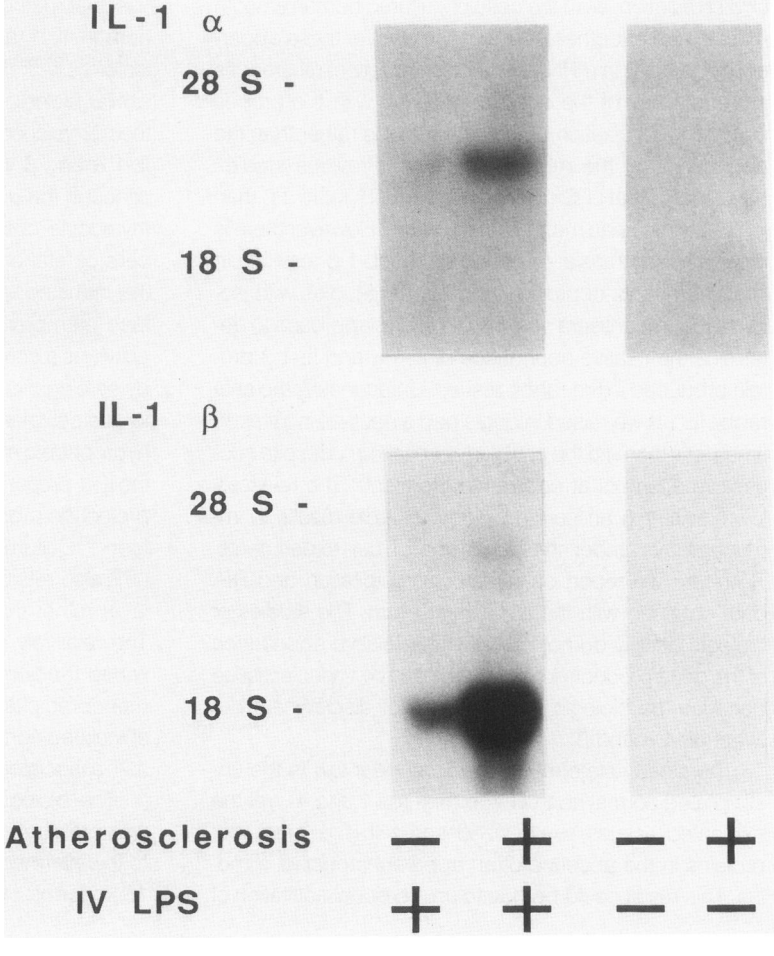

Figure 4. *Induction of IL-1α and β mRNA in aortic tissue 2 hours after intravenous injection of 10 μg/kg of* E. coli *endotoxin in rabbits fed control and atherogenic diets for 10 weeks. Three centimeters of thoracic aorta from control rabbits weighed 54 ± 9 g (n = 4) and provided 58 ± 8 μg of RNA, while a similar section from rabbits fed the atherogenic diet weighed 98 ± 11 g (n = 4) and provided 84 ± 9 μg of RNA. Twenty micrograms of total RNA was loaded into each lane for Northern analysis for rabbit IL-1α and IL-1β. The data presented represent one rabbit per treatment group. Similar results were observed for three additional rabbits per treatment.*

pared to 106 ± 42 pg IL-1 α/mg from rabbits fed the atherogeneic diet and given LPS.

Discussion

These results support the hypothesis that cells within the vascular wall in the intact rabbit can accumulate IL-1 mRNA and synthesize biologically active IL-1 protein *de novo* after stimulation by LPS. Our findings lend *in vivo* relevance to inducible IL-1 gene expression previously documented in cultured endothelial cells and smooth muscle cells.[14–16,33,34] The immunohistochemical studies indicate that intrinsic vessel wall cells rather than infiltrating leukocytes are the source of IL-1 in the LPS-stimulated nonatherosclerotic tissue, although we cannot completely exclude the presence of a small population of phagocytic leukocytes using this morphologic technique. The relative contribution of arterial smooth muscle and endothelial cells to IL-1 gene expression in the intact vessel will require further study, by use of *in situ* hybridization, for example.

The Northern analysis suggested that IL-1 β mRNA predominates over IL-1 α in the vascular wall following LPS stimulation, as in the case of cultured human smooth muscle and endothelial cells.[16,33] However these apparent differences in mRNA content could reflect differences in the stability of the duplexes formed with the probes used for hybridization of the two isoforms rather than the abundance of the mRNA species.[35] Previous studies have shown that LPS induces more mRNA for IL-1 β than for IL-1 α in human mononuclear cells.[36] However there is more efficient translation of IL-1 α than IL-1 β resulting in similar amounts of protein produced.[37] Studies with isoform-specific antisera could provide one approach to determine the relative abundance of IL-1 α and IL-1 β protein produced in the rabbit tissues. Unfortunately the anti-rabbit IL-1 β we raised in goats had a neutralizing titer 30 times less than did the anti–IL-1 α antiserum, despite successive attempts at boostering the titer.[23] The relatively low titer IL-1 β antiserum yielded variable results in immunoassays under the conditions of our experiments. Therefore we report only immunoprecipitation and RIA data obtained with the IL-1 α antiserum. The studies of biologic activity do not establish the relative abundance of the gene products for IL-1 produced by vascular tissue because the biologic assay could not discriminate between IL-1 α and β.

The steady state mRNA levels appear low in the unstimulated normal aorta *in vivo* (Figures 1 and 4), yet the explanted tissues synthesized some IL-1 α precursor proteins in the absence of an apparent inductive stimulus. This result could be due to unintentional activation of the endothelial or smooth muscle cells during harvesting or culture of the tissue. Despite our efforts to remove the tissue rapidly, some intravascular coagulation, with presumed activation of cytokine networks, may occur leading to activation of vascular cells. It is unlikely that inducers were present in the incubation media because the spleen showed no evidence of IL-1 α precursor formation under identical conditions. We did include the LPS antagonist polymyxin B in the incubations lacking LPS, and screened culture medium constituents for LPS contamination with a highly sensitive chromogenic Limulus amebocyte assay. Alternatively these results could indicate different regulation of IL-1 α gene expression in vascular tissue compared to the spleen *via* translational or post-translational mechanisms. Full elucidation of the molecular controls operating at each level of gene expression leading to the production of active forms of these cytokines will require further study. Our results do point to the complexity of biologic control of the formation of these potent mediators. They also illustrate why complete characterization of the regulation of the production of cytokines such as IL-1 must not rely solely on one index such as biologic activity or mRNA levels.

The relative importance of the α and β forms of IL-1 in vascular pathophysiology are unknown. Receptors for human IL-1 appear to recognize both the α and β isoforms.[37,38] These two species of IL-1 generally have similar biologic effects, despite substantial differences in their physicochemical properties.[39] Recombinant rabbit IL-1 α and β induced a similar febrile response *in vivo*, although the α form was less active than β in the murine thymocyte costimulation assay.[23] In human endothelial cells of fetal origin treated with tumor necrosis factor as the inducing stimulus, the IL-1 associated with the surface membrane is the α form, while the released IL-1 activity is a combination of the α and β species as judged by serologic criteria.[34] The targeting of the IL-1 α isoform for the cell membrane may result from the persistence of hydrophobic myristoyl moieties, added after translation to the IL-1 precursors (~31 kd), that are not removed during proteolytic processing of the IL-1 α species to the mature form.[40] Cultured human smooth muscle cells treated with LPS also retain most of the inducible IL-1 within the cell layer rather than secreting the cytokine into the media. The relatively small amount of IL-1 found in the conditioned medium of rabbit aortic tissue in the present immunoprecipitation experiments suggest that the LPS-stimulated aorta retains a large portion of the synthesized IL-1 associated with the cell.

The biologic activity of IL-1 increases progressively with proteolytic processing from the full-length precursor to the intermediate (~22 kd) and fully processed ~15 to 18 kd forms. However even lower molecular weight frag-

ments of IL-1 found in the circulation retain activity. This property may contribute to the substantial increases in biologic activity after LPS exposure *in vivo* (Table 1) with less striking increases in the quantity of immunoprecipitable precursor in the *in vitro* assay (Figure 2).

The advanced atherosclerotic lesion is the result of a prolonged, multifactorial process characterized by the accumulation of lipid, deposition of connective tissue matrix, and intimal proliferation of smooth muscle cells.[41] IL-1, acting in concert with other mediators, may contribute to the accumulation of morphologic and functional changes in the vessel wall during certain stages of atherogenesis. Rabbits fed a cholesterol-containing diet develop macrophage-rich lesions, similar to fatty streaks in humans, that may be precursors of complicated lesions occurring later in life. Using Northern (RNA) analysis, we failed to detect elevated IL-1 mRNA in the fatty streak of the rabbit aorta. However the addition of an *in vivo* stimulus, such as the LPS used in our study, produced a large increase in the IL-1 mRNA in the atherosclerotic vessel relative to the control. We postulate that the macrophage-derived foam cells account for the much greater inducibility of IL-1. However further studies using *in situ* hybridization and immunohistochemistry will be required to define the relative contributions of the various cell types. Ross et al[42] observed increased IL-1 β mRNA in the lesions of nonhuman primates maintained on hypercholesterolemic diets for 1 year. The primate lesions are more fibrous than the relatively early fatty streaks in the rabbits studied here. Thus IL-1 gene expression during atherogenesis may depend on the stage of the lesion and local factors such as endothelial disruption, platelet adherence, or the release of other cytokines within the developing lesion.

The capacity of the normal and atherosclerotic vessel wall to produce as well as to respond to IL-1 and other cytokines illustrates how vascular cells may play significant roles in regional tissue reactions to infection or injury and in the local immune response. Clinically significant sequellae of gram-negative sepsis, including adult respiratory distress syndrome and disseminated intravascular coagulation, may involve effects of LPS or LPS-induced cytokines, such as IL-1 or TNFα, on vascular endothelium. The inappropriate expression of IL-1 or response to IL-1 by cells of the vessel wall also may contribute to other disease processes involving vascular tissue, including immune-mediated vasculitis, tumor-induced angiogenesis, rejection of transplanted organs, and atherosclerosis. The present demonstration of inducible IL-1 gene expression in arterial tissue *in vivo* suggests that local IL-1 production might contribute to the pathologic findings in blood vessels in a variety of infectious or inflammatory states.

Acknowledgments

The authors thank Maria Muszynski, Nathan Margolis, and Elisa Simon for excellent technical assistance; Allen M. Gown for the RAM 11 antibody; and our colleagues Louis K. Birinyi, MD and the late Stephen J. C. Warner, M.B.B.Ch., Ph.D. for their inspiration and assistance.

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