Endothelial Cells Express the Interleukin-1 Receptor Type I

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Interleukin-1 (IL-1) profoundly affects a number of functions of vascular cells. Two distinct IL-1 receptors (IL-1R) are expressed on different cell types: the 80 Kd IL-1R on T cells and fibroblasts, and the 68 Kd IL-1R on B cells and myelomonocytic cells. The presence and functionality of IL-1R on vascular cells has been investigated by using polyoma-transformed mouse endothelial cell (EC) lines (sEnd.1 and tEnd.1). These cells expressed specific and saturable binding sites for IL-1 (1,273 sites per cell with kd 9.5 x 10^-10 mol/L for sEnd.1, and 771 sites per cell with kd 8.5 x 10^-11 mol/L for tEnd.1, with radiiodinated IL-1α as ligand). Binding of IL-1R

INTERLEUKIN-1 polypeptides are prototypic pleiotropic cytokines whose action encompasses various organs and tissues. Endothelial cells (EC) have emerged as one important target for IL-1.2-3 IL-1 and the functionally related cytokine tumor necrosis factor (TNF) activate EC functions mainly related to inflammation and thrombosis. These include production of procoagulant activity, prostaglandins,4-6 platelet-activating factor (PAF),7 and plasminogen activator inhibitor8; inhibition of the synthesis of plasminogen activator9 and alterations in the thrombomodulin/protein C anticoagulation pathway; expression of adhesion molecules10; and production of cytokines.11

The action of IL-1 on cells is mediated via specific receptors. An 80 Kd IL-1 binding protein (IL-1R) has been purified from a murine thymoma cell line12 and a cDNA that encodes this protein has been cloned.13 This 80 Kd IL-1R is expressed in mouse and human T cells, fibroblasts, keratinocytes, and epithelial cells.14-17 Affinity cross-linking suggested that the IL-1R on B cells would be a 60 to 68 Kd protein.18-20 Further evidence for the existence of a second distinct IL-1R (IL-1R2) has been provided recently.20-22 This receptor, identified using Epstein-Barr virus (EBV) transformed human B cell lines and the murine 70Z/3 pre-B cell line, is also expressed on mouse macrophages and bone marrow granulocytes.21 These cells do not express the IL-1R.

Little is known concerning the IL-1R present on EC. Thieme et al23 characterized the binding of IL-1α to human umbilical vein ECs. By immunoprecipitation and chemical cross-linking to the ligand they identified a 78 Kd IL-1-binding protein on human EC.24

Here we report that vascular cells (mouse EC; human EC, and smooth muscle cells [SMC]) express the 80 Kd IL-1R.

MATERIALS AND METHODS

Cells. The mouse endothelioma cell lines sEnd.1 and tEnd.1 were obtained through the courtesy of Dr E.F. Wagner (European Molecular Biology Laboratory, Heidelberg, FRG). These cell lines, derived from a subcutaneous (s) and thymic (t) hemangioma, have cobblestone endothelial-like morphology, express von Willebrand factor, and cause hemangiomata in vivo.25,26 Expression of von Willebrand factor was checked during this study. The cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 15% fetal bovine serum (FBS) and 750 pg/mL G418. Human ECs were isolated from umbilical veins and cultured in medium 199 (GIBCO, Paisley, Scotland) supplemented with 10% FBS. When cells were confluent, human recombinant IL-1α, radioiodinated with the chloramine T method, was purchased from Du Pont-NEN (Boston, MA) and had a specific activity of 2.2 x 10^6 dpm/μg. Endothelioma cells were grown to confluence on 8-chamber tissue culture chamber/slides (Lab-Tek Division, Miles Laboratories, Naperville, IL). Washed monolayers were incubated with approximately 0.3 nM IL-1α in 0.1 mL of DMEM with 15% FBS and 0.02% NaCl (binding medium) for 3 hours at room temperature with gentle agitation. At the end of the incubation, gaskets were removed, slides were extensively washed and

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Supported by a fellowship of Consiglio Mario Negri Sud, Eritrea 62, I-20157 Milano, Italy. Also supported by Ministero della Sanità—Instituto Superiore della Sanità—Istituto di Ricerche Farmacologiche Mario Negri, Via Eritrea 62, I-20157 Milano, Italy; and the Italian Association for Cancer Research—Division, Miles Laboratories, Naperville, IL. Washed monolayers were incubated with approximately 0.3 nM IL-1α in 0.1 mL of DMEM with 15% FBS and 0.02% NaCl (binding medium) for 3 hours at room temperature with gentle agitation. At the end of the incubation, gaskets were removed, slides were extensively washed and
by dipping in PBS and fixed for 30 seconds in acetone at room
temperature. Slides were stored at -20°C until autoradiography.
Slides were postfixed for 1 minute in cold acetone, then covered by
dipping with NTB3 Kodak photographic emulsion. After 2 days of
exposure, slides were developed with Kodak D19 developer and
Rapid Fixer and counterstained with hematoxylin. Results were
evaluated by the method of Schlessinger et al.27 The cut off for
positivity of IL-1 binding was calculated as the median number of
silver grains on cells incubated with cold IL-1 plus three times the
standard deviation (SD), multiplied for a field factor (ie, the
number of grains per field in cells without silver grains on the
slides incubated with labeled IL-1B divided by that in slides incubated with
cold IL-1B). 

IL-1 binding assay. Replicate confluent monolayers of 1 to 2 x
10⁶ cells per well of Cluster plates (Costar, Cambridge, MA) were
incubated with increasing doses of [³²P]IL-1 in 0.5 mL of binding
medium for 3 hours at room temperature under gentle agitation on
a rocking platform. Nonspecific binding was assessed in the
presence of a 500-fold molar excess of unlabeled IL-1. Wells were
then extensively washed in PBS, monolayers were dissolved in 0.5
mL 9.5 mol/L urea, and radioactivity was counted. Control monolayers
were trypsinized for exact determination of cell numbers. Calculations and Scatchard analysis were performed according to
Munson and Rodbard.18

Affinity cross-linking. [³²P]IL-1α was cross-linked to intact cells
with disuccinimidyl suberate (DSS) or with diethio bis (disuccini-
midyl propionate) (DSP) (Pierce, Rockville, MD) as previously described.19 Briefly, cell monolayers (3.0 x 10⁶ cells) in binding
medium were incubated with [³²P]IL-1α (0.5 mmol/L) for 3 hours at
room temperature in gentle agitation with or without a 500-fold
molar excess of unlabeled IL-1. After washing the unbound IL-1,
DSS or DSP (50 mg/mL in DMSO) were added to a final
centration of 2 mg/mL, and the reaction was allowed to proceed for
45 minutes at room temperature with vigorous shaking, then
centrifuged in Eppendorf for 5 minutes. The supernatant was
denatured with Laemmli sample buffer.20 Sodium dodecyl sulfate-
polyacrylamide gel electrophoresis (SDS-PAGE) was run on 10%
gel using colored molecular weight markers (Amersham, Little
Chalfont, UK). Dried gels were exposed at -40°C using Kodak
XAR-5 autoradiographic film.

Northern blot analysis. Northern blot analysis was performed
according to standard procedures.19 Total RNA was isolated from
confluent cell cultures by guanidine isothiocyanate method.21 In
some instances, polyadenylated RNA was prepared by chromatogra-
phy on oligo dT cellu-lose columns (Pharmacia, Uppsala, Swe-
eden). Aliquots of 10 μg total RNA were analyzed by electrophore-
sis through 1% agarose formaldehyde gels followed by Northern
blot transfer to Gene Screen Plus membrane (Du Pont-NEN). A human IL-1 receptor cDNA (HindIII-EcoRI fragment of 477 bp)22
was obtained through the courtesy of Dr S.K. Dower (Immunex
Corp, Seattle, WA). The mouse IL-1 receptor cDNA (SalI fragment of 1,800 bp) was cloned in this laboratory by polymerase chain reaction according to the sequence cloned by Sims et al.23 The mouse IL-6 cDNA probe (EcoRI-BglII fragment of 650 bp) was a kind gift of Dr J. Van Snick (Ludwig Institute, Bruxelles,
Belgium). These fragments were labeled to a specific activity of 10⁷
cpm/μg by using hexanucleotide primers and α³²P-CTP. Mem-
branes were pretreated and hybridized in 50% formamide (Merck,
Rahway, NJ) with 10% dextran sulfate (Sigma) and washed twice
with 2x SSC (1x SSC: 0.15 mol/L sodium chloride, 0.015 mol/L
sodium citrate) then twice with 2x SSC plus 1% SDS (Merck) at
60°C for 30 minutes and finally twice with 0.1x SSC at room
temperature for 30 minutes.

The membranes were exposed for 12 to 24 hours at -80°C with
intensifying screens. RNA loading and transfer to membrane was
checked by examination under UV light and hybridization of the
blot with an α-actin probe.

IL-6 bioactivity assay. IL-6 was measured as hybridoma growth
factor (HGF) activity using the 7TD1 cell line, obtained through
the courtesy of Dr J. Van Snick. Briefly, 2 x 10⁶ cells in 200 μL
(four replicates per experimental point) were cultured for 72 hours
with different dilutions of the supernatants to be tested or of the
appropriate control medium. Routinely, cell proliferation was
assessed by the [3H]dThd (sp act 185 GBq/mmol; Amersham) for
6 hours. HGF activity resulting in half maximal stimulation of target cell growth was arbitrarily defined as 1 U. Reference standards used in these experiments consisted of human recombinant IL-6 or IL-6-rich supernatant of a mixed lymphocyte
reaction.

RESULTS AND DISCUSSION

Vascular cells are one important target for IL-1. On interaction with IL-1, ECs undergo a complex reprogram-
mind of function, which favors thrombosis, leukocyte recruit-
ment, and inflammation.3 A limitation to the detailed
analysis of modulation of EC functions has been the
unavailability of continuous EC lines. Recently, murine
endotheloma cells lines have been established from heman-
giomas developed in mice on infection with a retroviral vector carrying the polyoma virus middle T oncogene.25,26
These lines have endothelial morphology and express EC
markers,25 and might thus be suitable for study of EC
modulation in vitro, if they also maintain EC functional
characteristics. In order to evaluate the ability of polyoma-
transformed cells to respond to IL-1 modulation, two of
these lines, sEnd.1 and tEnd.1, have been analyzed for
expression of IL-1R and functional response to IL-1 stimu-
lation. We focused on mouse EC initially because most
available information concerning the existence of differen-
tial distribution of distinct IL-1R has been obtained in the
mouse.21,22

Specific binding of radiiodinated IL-1 to sEnd.1 cells
was first evaluated at the single cell level by autoradiogra-
phy on cells cultured on glass slides. As shown in Fig 1,
sEnd.1 cells could efficiently bind radiiodinated IL-1α.
Binding capacity was, however, not homogeneous, with
approximately 30% of cells showing significant binding of
IL-1α (8% had >100 grains per cell, 9% between 50
and 100 grains per cell; and 13% between 30 and 50 grains
per cell), and the other 70% showing only few associated silver
gains (< 30 per cell). Cells showing the highest density of
associated grains were highly adherent and with a large
cytoplasm (Fig 1). These data indicate that endotheloma
cells possess receptors for IL-1.

Two structurally distinct IL-1R have been identified, with
differential cellular distribution.14,21,22 Only the 80 Kd IL-
1Rα, expressed on T cells and fibroblasts, has been molecu-
larly cloned and sequenced.14,15 A second type of IL-1R has
been found on B cells and on myelomonocytic cells.19,22,23
The IL-1Rα has a molecular weight of approximately 68 Kd and,
at variance with IL-1Rα, apparently binds IL-1β more
abundantly than IL-1α.30,32 The role of the two types of
IL-1R in the mechanisms of cellular activation by IL-1 is

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Fig 1. Binding of IL-1 to murine endothelioma cells. Monolayers of sEnd.1 cells on glass slides were incubated with \(^3^2\)P IL-1α for 3 hours then examined for IL-1 binding by autoradiography. Original magnification: \(\times 100\) (A), \(\times 500\) (B).

not clear. Presumably, the two IL-1R types are coupled to distinct intracellular signal transduction pathways\(^3^9\) and are associated with different rates of ligand internalization and intracellular degradation.\(^3^4\)

The characteristics of the IL-1R present on endothelioma lines have been assessed by binding studies. As shown in Fig 2 (A, B), the two endothelioma lines tEnd.1 and sEnd.1 showed specific and saturable binding of radiiodinated human recombinant IL-1α. The number of IL-1 binding sites per cell and the affinity of binding were similar between the two lines (1,273 sites per cell with \(K_d = 9.5 \times 10^{-11}\) mol/L for sEnd.1, and 771 sites per cell with \(K_d = 8.5 \times 10^{-11}\) mol/L for tEnd.1, respectively). IL-1 receptors on human EC showed similar characteristics (ref. 23 and 35; 2 experiments not shown), with a somewhat lower number of binding sites (100 to 500 sites per cell). By chemical cross-linking of radiolabeled IL-1α to sEnd.1 membrane proteins with DSS, a single cross-linking product could be seen, of molecular weight of approximately 98 Kd (Fig 2, C). Accordingly, the presence of a 98 Kd product was evident also when DSP was used as a cross-linking agent (data not shown). By subtracting the molecular weight of bound IL-1α (17.5 Kd), this corresponded to an IL-1 binding protein of approximately 80 Kd. This is in agreement with the molecular weight of the IL-1R described on human EC (ie, 78 Kd)\(^2^4\) and suggests that the IL-1R expressed by EC is the IL-1R\(_\alpha\). To clarify this point, the expression of mRNA encoding the IL-1R\(_\alpha\) has been examined. As shown in Fig 3, both sEnd.1 and tEnd.1 had appreciable levels of IL-1R\(_\alpha\) transcripts, though these were substantially lower than those detected in 3T3 murine fibroblasts used as positive controls (these express over 5,000 IL-1R per cell).\(^2^1\)\(^,^3^6\)

Having established that the mouse endothelial lines express IL-1R\(_\alpha\), it was important to evaluate whether these cells indeed respond to IL-1 stimulation, ie, whether their IL-1R was functional. As shown in Fig 4, IL-1 was able to induce high levels of IL-6 mRNA in both the sEnd.1 and the tEnd.1 lines. In parallel to detection of message transcription for IL-6, an increase of IL-6 biologic activity could be detected in the culture supernatant. In fact, IL-1-stimulated sEnd.1 and tEnd.1 cells produced 568 U/mL and 1,250 U/mL of IL-6, respectively, whereas only 82 U/mL and 83 U/mL of IL-6 bioactivity could be detected...
in the absence of IL-1. The ability of IL-1 to induce IL-6 synthesis in murine endothelioma cells is in full agreement with previous observations in human EC. It is of interest that the recently identified IL-1 receptor antagonist, reportedly interacting with type I receptors, inhibits the action of IL-1 on human and murine endothelium (data not shown).

Thus, murine endothelioma cell lines sEnd.1 and tEnd.1 express functional IL-1R, similar to that observed on human EC. However, that the IL-1R present on human vascular cells is in fact the IL-1R, was inferred only by the size of the IL-1 binding protein observed in cross-linking studies, i.e., approximately 78 kDa. To ascertain that human vascular cells possess the IL-1R, we have examined human

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**Fig 2.** Equilibrium binding and cross-linking of ^125^I IL-1α to murine endothelioma cells. For equilibrium binding analysis, specific binding of increasing amounts of ^125^I IL-1α (free) was determined in the presence of 500-fold molar excess of unlabeled IL-1. Scatchard analyses are reported in the insets: B, bound; F, free. In abscissa are bound fmol/well. (A) tEnd.1 cells. (B) sEnd.1 cells. (D) Cross-linking of ^125^I IL-1α on sEnd.1 cells, performed with DSS in the absence (−) or in the presence (+) of unlabeled IL-1.
EC and SMC for IL-1R mRNA expression. As shown in Fig 5, both EC and SMC express mRNA for IL-1R. Exposure of human vascular cells, as well as murine endothelioma cells, to IL-1 did not appreciably affect IL-1R mRNA expression (Fig 5, data not shown).

The results reported here demonstrate that murine EC and human EC and SMC express IL-1R, though they do not formally exclude that these cells may at the same time have other IL-1R types on their membrane.

The availability of the recently cloned soluble form of IL-1R and that of a neutralizing monoclonal antibodies for the murine IL-1R will help to finely evaluate in vitro and in vivo the precise role of IL-1 in vascular cell activation in pathologic conditions.

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