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Endogenous Interleukin 1 Alpha Must Be Transported to the Nucleus To Exert Its Activity in Human Endothelial Cells

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We have previously shown that the signal peptideless cytokine interleukin 1α (IL-1α) may play a role as an intracellular regulator of human endothelial cell senescence (J. A. M. Maier, P. Voulalas, D. Roeder, and T. Maciag, Science 249:1570–1574, 1990). To investigate the potential intracellular function of IL-1α, transformed endothelial cells were transfected with the human cDNAs that code for the two forms of IL-1α, the precursor molecule IL-1_{1-271} and the mature protein IL-$1_{113-271}$. The subcellular localization of the two different polypeptides was investigated directly or by using chimeric genes constructed by fusion of different fragments of the IL-1α gene and the β-galactosidase open reading frames. The IL-$1_{113-271}$ protein was cytoplasmic, while IL-1_{1-271} was nuclear. The basic cluster at the NH_2 terminus of IL-1, KVLKKRR, has been shown to mediate IL-1α nuclear targeting. Moreover, nuclear localization of IL-1α correlates with impaired cell growth and expression of some IL-1α-inducible genes. These results suggest that transport of endogenous IL-1_{1-271} into the nucleus is required for it to modulate endothelial cell function.

Endothelial cells are important producers of cytokines that regulate the hematopoietic system, the differentiation of T and B cells, and the extravasation of leukocytes at inflammatory sites (27). Moreover, endothelial cells express receptors for several of these cytokines (3, 5) which profoundly affect their behavior (36). Among others, endothelial cells produce interleukin 1α (IL-1α) (31). IL-1α is synthesized and accumulates as an intracellular precursor molecule with a molecular mass of 31 kDa (IL-1_{1-271}), which can be subsequently processed as an 18-kDa carboxy-terminal fragment (IL-$1_{113-271}$) (30) by a calpain-like protease (4, 20). Both IL-1_{1-271} and IL-$1_{113-271}$ are biologically active as exogenous cytokines in vitro (33). However, the sequence of the 31-kDa precursor does not appear to contain a typical signal sequence for secretion, either N terminal or internal (28). In accordance with this, immunoelectron microscopy studies failed to demonstrate IL-1α molecules in the endoplasmic reticulum and in the Golgi apparatus (42).

It is noteworthy that in vitro passage of human endothelial cells results in spontaneous induction of IL-1α synthesis (25). Because IL-1α is not secreted and an antisense oligomer targeted against IL-1α extends the endothelial life span, an intracellular role for IL-1α has been postulated (25). Interestingly, IL-1α has been observed to exist as a nucleus-associated protein in cultured human glomerular mesangial cells (45), as well as in keratinocytes, dermal fibroblasts, and endothelial cells after topical exposure of the murine epidermis to phorbol esters (35), supporting the concept that proliferation and differentiation in at least some cell types is modulated by IL-1α intracellularly. Moreover, intranuclear IL-1α has been found in cells which were stimulated with exogenous IL-1α. Several groups have found that fibroblasts and lymphocytes demonstrate receptor-mediated endocytosis and nuclear translocation of receptor-bound IL-1α (16, 32), suggesting that the IL-1α receptor is responsible for the nuclear localization of exogenous IL-$1_{113-271}$ (16). Further, the potential function of intracellular IL-1α has been highlighted by the discovery of an intracellular IL-1 receptor antagonist which is expressed in keratinocytes (17). This protein could function as an intracellular competitor of either internalized or endogenously produced IL-1α.

To determine whether IL-1α represents an intracellular regulatory molecule in endothelial cells, we transfected transformed endothelial cells (tEC) (46) with plasmids containing the cDNAs that code for IL-1_{1-271} and IL-$1_{113-271}$. The subcellular localization of the two forms was investigated directly or by using chimeric genes constructed by fusion of the IL-1α and β-galactosidase (β-Gal) open reading frames. IL-1_{1-271} was nuclear, while IL-$1_{113-271}$ was cytoplasmic. The sequence responsible for the nuclear targeting of IL-1 is contained within seven amino acid residues at positions 79 to 85. We also determined whether or not the activity of endogenous IL-1α correlates with its ability to localize to the nucleus. The following data suggest that transport of IL-1α into the nucleus is required for it to modulate endothelial cell properties.

MATERIALS AND METHODS

Plasmid construction. Most plasmids were constructed by PCR amplification of the IL-1α cDNA with primers bearing unique sequence and restriction enzyme sites. Plasmid Cfla, containing the full-length cDNA for IL-1α, was kindly provided by R. Gayle (Immunex, Seattle, Wash.).

The primers used to clone IL-1α in pMEXneo (29) were IL-1_{1-271} sense (5′-CCG TCG ACC CAC CAT GGC-3′), IL-$1_{113-271}$ sense (5′-CCG TCG ACC CAC CAT GGCCGCA CCT TTT AGC TTC CTG CTG AGC-3′), and IL-1_{1-271} and IL-$1_{113-271}$ antisense (5′-GAG GAT CCT CTA GAG TCA ACA CTG CAC A-3′). To generate the mature form of IL-1α, the Kozak sequence and the ATG site were added at the 5′ end.

We generated plasmids with the various IL-1α inserts in frame with the β-Gal-encoding gene by obtaining the corresponding fragments by PCR and ligating them into the *Sma*I site of pMC1871 (Pharmacia), which contains the β-Gal re-

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porter gene. The primers used were IL-1_{1-271} sense (5′-CTC CCC GGG CCA CCA TGG CCA AAG TTC CAG ACA G-3′), IL-$1_{113-271}$ sense (5′-CTC CCC GGG CCA CCA TGG CCG CAC CTT TTA G-3′), and IL-1_{1-271} and IL-$1_{113-271}$ antisense (5′-CTT CCC GGG CGC CTG GTT TTC CAG TAT CTG-3′). In construction of the IL-1α nuclear translocation sequence (NTS) fused to the β-Gal sequence, the pairs of primers used were 5′-CCG GAT CCG TCG ACC ACC ATG GCT AAG GTT CTG AAG AAG AGA CGG CCC GTC GTT TTA CAA CGT CGT-3′ and 5′-GGT CGA CGG ATC CCC CCT GCC-3′. The template used in the PCR construction was pMC1871. PCR was carried out for 30 cycles under the following conditions: denaturation for 1 min at 95°C, primer annealing for 2 min at 55°C, and extension for 3 min at 72°C. The PCR products were purified (Qiagen), digested with *Sma*I, and inserted into the *Sma*I site of pMC1871 (Pharmacia). Subsequently, the IL-1α–β-Gal fragments were digested with *Bam*HI and cloned into the *Bam*HI site of eukaryotic expression vector pSVL (Pharmacia). The correct sequence and orientation were confirmed by sequencing with the Sequenase kit.

Cell culture, transfection, and proliferation assay. tEC were kindly provided by Y. Mitsui and T. Imamura (46) and cloned to obtain a monoclonal population. The cells are characterized by a cobblestone monolayer growth pattern, contact inhibition, and high proliferative potential without any specific growth factor requirement. The cells have Weibel-Palade bodies and angiotensin-converting enzyme activity. tEC were maintained by serial passage in medium M199 supplemented with 10% (vol/vol) fetal calf serum and antibiotics. pMEXneo-IL-1_{1-271} (10 μg) and pMEXneo-IL-$1_{113-271}$ (10 μg) were transfected into 2×10^5 tEC via the calcium phosphate coprecipitation technique (48). Transfectants were selected by growth in M199 supplemented with serum and 1 mg of G418 (GIBCO) per ml. To perform proliferation assays, tEC were seeded at a low density (5,000/cm^2) in M199 containing 10% fetal calf serum and counted with a hemocytometer. In some experiments, cells were incubated in the presence of oligomers based on the IL-1α-encoding gene (40 μM) (24) or treated with the IL-1 receptor antagonist (ILra; 100 ng/ml) (kindly provided by R. C. Thompson of Synergen) (10).

Cos cells were grown in Dulbecco's modified Eagle's medium containing 10% (vol/vol) fetal calf serum and transfected with IL-1α–β-Gal cDNA-containing plasmids by the DEAE-dextran method (15). After 60 h, immunofluorescence analysis was performed.

Immunofluorescence and immunoprecipitation analyses. IL-1_{1-271}- and IL-$1_{113-271}$-transfected tEC, as well as Cos cells transfected as described above, were fixed in 3% paraformaldehyde and permeabilized with phosphate-buffered saline containing 0.5% Nonidet P-40. After 1 h of incubation with an anti-β-Gal antibody (5 Prime 3 Prime, Inc.), Cos cells were incubated with fluorescently labeled anti-rabbit antibody and visualized by fluorescence microscopy. tEC were incubated with an immunopurified serum raised against human IL-1α (kindly provided by R. Chizzonite, Hoffmann-La Roche, Nutley, N.J.) before exposure to fluorescent anti-goat antibody.

The presence of IL-1α in IL-1α-transfected tEC was confirmed by immunoprecipitation analysis with an immunopurified serum against human IL-1α as previously described (49).

Purification of RNA and Northern (RNA) blot analysis. Total RNA was obtained from confluent cultures. The cells were rinsed with phosphate-buffered saline and lysed in 4 M guanidium isothiocyanate, and total RNA was purified as previously described (24). RNA was electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde, capillary blotted onto a Zeta-probe membrane (Bio-Rad), and UV cross-linked (26). The cDNA fragments for human collagenase, plasminogen activator inhibitor 1 (PAI-1), and glyceraldehyde-3-phosphate dehydrogenase were labeled with a random-primer labeling kit (Bethesda Research Laboratories). Filters were hybridized in 0.5 M sodium phosphate (pH 7.2) containing 7% sodium dodecyl sulfate, 1% bovine serum albumin, 1 mM EDTA, and 20% formamide at 65°C for 20 h and extensively washed at high stringency (0.1× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 65°C) (26). mRNAs were visualized by autoradiography.

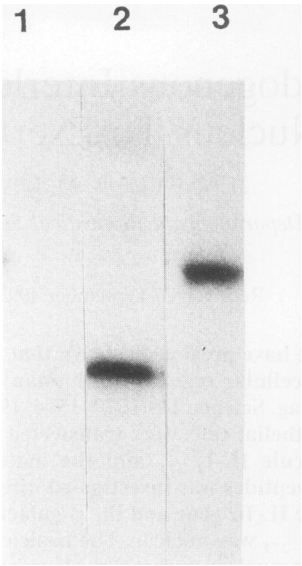

FIG. 1. Immunoprecipitation of IL-1_{1-271}- and IL-$1_{113-271}$-transfected tEC. Transfected tEC were labeled with [^{35}S]methionine, lysed, and immunoprecipitated with an anti-human IL-1α immunopurified serum. Immunoprecipitates were resolved by sodium dodecyl sulfate–15% polyacrylamide gel electrophoresis, and proteins were visualized by autoradiography. Lanes: 1, pMEXneo-transfected tEC; 2, pMEXneo-IL-$1_{113-271}$-transfected tEC; 3, pMEXneo-IL-1_{1-271}-transfected tEC.

RESULTS

Transfection and characterization of cloned cells. To study the potential intracellular role of IL-1α in endothelial cells, we used tEC (46). tEC represent a particularly convenient experimental model for this purpose because they do not express detectable levels of IL-1α (25). Since IL-1α exists as a precursor (IL-1_{1-271}) and as a truncated form (IL-$1_{113-271}$) (8), we generated the corresponding fragments by PCR and inserted them into eukaryotic expression vector pMEXneo (29) to attain high-level constitutive expression of the two forms of IL-1α. pMEXneo-IL-1_{1-271} and pMEXneo-IL-$1_{113-271}$ were transfected into tEC via calcium phosphate precipitation (48). Comparative analysis of IL-1_{1-271}- and IL-$1_{113-271}$-transfected cells demonstrated that similar steady-state levels of IL-1_{1-271} and IL-$1_{113-271}$ were expressed (Fig. 1). No IL-1α was detected in the lysate of control tEC which had been transfected with the vector alone (Fig. 1). There is no evidence for the presence in cell lysates of IL-1_{1-271}-transfected tEC of processed forms of the IL-1 precursor, in accordance with the observation that the ability to process IL-1_{1-271} is not a property of all mammalian cells (4, 30). Further, we did not detect either the precursor or the truncated form of IL-1 in the membrane of

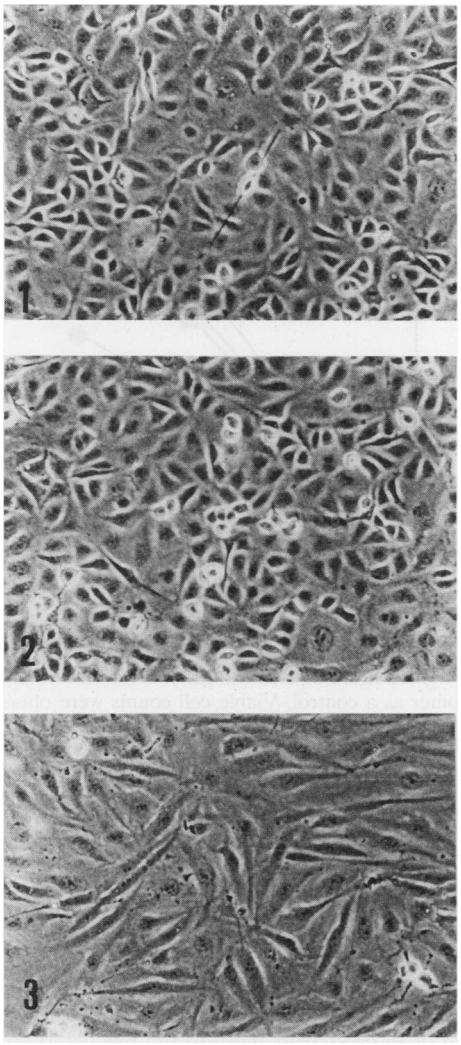

FIG. 2. Morphology of tEC transfectants. The morphology of tEC transfected with the pMEXneo vector alone (panel 1), IL-$1_{113-271}$ (panel 2), and IL-1_{1-271} (panel 3) was examined by phase-contrast microscopy (magnification, ×20).

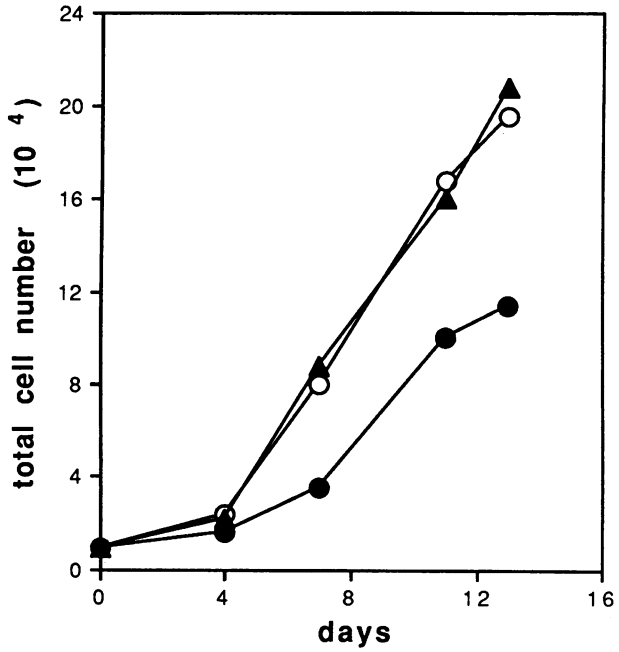

FIG. 3. Cell proliferation assays. Transfected tEC were grown in triplicate as described in Materials and Methods. At the indicated time intervals, the cells were harvested by digestion with trypsin and the viable cell number was determined as described in the text. Symbols: ○, pMEXneo-transfected tEC; ▲, IL-$1_{113-271}$-transfected tEC; ●, IL-1_{1-271}-transfected tEC.

transfected cells or extracellularly, even after 50-fold concentration (data not shown). The endogenous expression of IL-1_{1-271} promoted the transition from a cobblestone monolayer phenotype to a spindle-shaped, fibroblast-like phenotype (Fig. 2). This phenotype is very similar to the morphology of endothelial cells during the early phases of the endothelial cell differentiation pathway in vitro (23).

Proliferation rate of transfected cells. Because an antisense oligonucleotide against IL-1α extends the in vitro life span of endothelial cells, it has been argued that IL-1α may represent an endogenous brake for endothelial proliferation and life span (25). To evaluate this possibility, we studied the growth rate of the transfected clones. Cells were seeded at a low density and counted twice a week. IL-1_{1-271}-transfected tEC showed a much slower proliferation rate than the control cells (Fig. 3). In contrast, no growth rate modification was observed in IL-$1_{113-271}$ transfected cells in comparison with the control (Fig. 3). Similar results were obtained with several transfected clones isolated after G418 selection which had been shown to express either IL-1_{1-271} or IL-$1_{113-271}$ (data not shown). It is noteworthy that IL-$1_{113-271}$ purified from IL-$1_{113-271}$-transfected-cell extracts was biologically active when added to the culture medium of human endothelial cells and compared with recombinant IL-1α (data not shown). To rule out the possibility that the inhibitory effect on the proliferation of IL-1_{1-271}-transfected tEC was due to the presence of small amounts of IL-1α that leaked into the medium and were undetectable with the aforementioned techniques, we incubated these cells with the ILra (100 ng/ml) (10). The ILra binds to the IL-1α receptor but does not activate it (8). Lacking biological activity, it can be added to the culture medium in doses sufficient to occupy IL-1α receptors and therefore prevent the binding of IL-1α eventually present extracellularly. Treatment with the ILra did not alter the proliferation rate of the IL-1_{1-271}-transfected cells (Fig. 4), a result which is consistent with the lack of extracellular IL-1α.

To confirm the role of IL-1_{1-271} in braking cell proliferation, we treated IL-1_{1-271}-transfected tEC with the antisense oligomer that blocks IL-1α translation (25). Figure 5 shows that daily exposure to the oligomer (40 μM) restored the normal proliferation rate. As a control, we used the same amount of the complementary-sense oligomer and no modification of growth was detected. These data confirm the direct role of intracellular IL-1_{1-271} in modulating the proliferation rate of IL-1_{1-271}-transfected cells.

Gene expression in transfected clones. Collagenase and PAI-1 have been shown to be induced by treatment with recombinant IL-1α (27, 36). Because it has been speculated that the overexpression of IL-1α in senescent endothelial cell (25) and fibroblasts (21) may explain the upregulation of several IL-1α-inducible genes, we studied the expression of collagenase and PAI-1 mRNA in IL-1_{1-271}- and IL-$1_{113-271}$-transfected cells. As shown in Fig. 6, the level of expression of

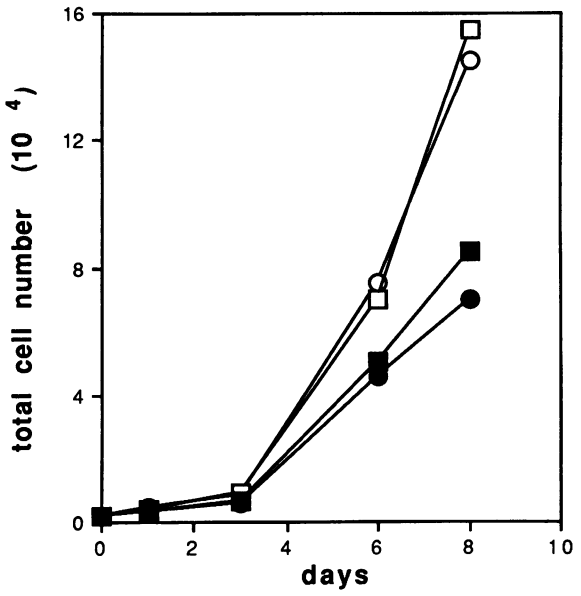

FIG. 4. Effect of ILra on cell growth. IL-1_{1-271}-transfected tEC were treated with ILra (100 ng/ml). At the indicated time intervals, cells were trypsinized and counted with a hemocytometer as described in the text. Results are shown for IL-1_{1-271}-transfected control (●) and ILra-treated (■) tEC and pMEXneo-transfected control (○) and ILra-treated (□) tEC.

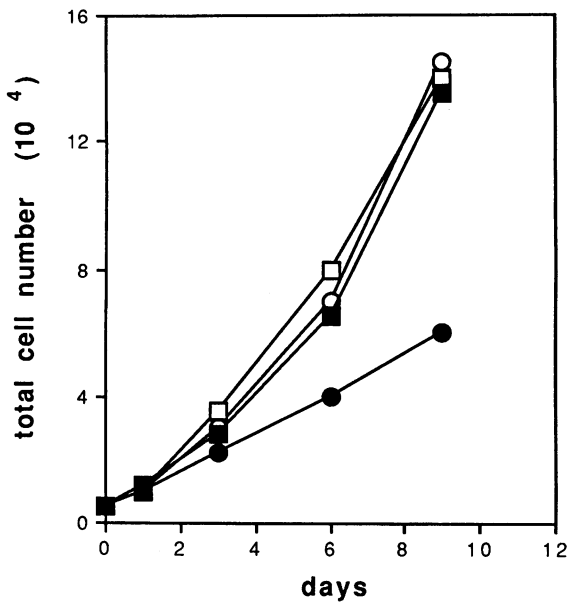

FIG. 5. IL-1_{1-271}- and control pMEXneo-transfected tEC were treated daily for the duration of the experiment with an antisense oligomer targeted against IL-1α (40 μM) or with the complementary-sense oligomer as a control. Viable cell counts were obtained at the indicated times. The experiment was performed in triplicate. Symbols: ■, antisense oligomer-treated IL-1_{1-271}-transfected cells; □, antisense oligomer-treated pMEXneo-transfected cells; ●, sense oligomer-treated IL-1_{1-271}-transfected cells; ○, sense oligomer-treated pMEXneo-transfected cells.

these genes is very low in control cells. Similarly, tEC overexpressing IL-$1_{113-271}$ did not show any modification of gene expression. In contrast, IL-1_{1-271}-transfected tEC upregulated the mRNAs for collagenase and PAI-1. This effect was not inhibited by incubation in the presence of ILra, thus ruling out the possibility that small amounts of protein could leak into the medium and interact with the receptor (data not shown).

Cellular localization of IL-1α in transfected tEC. Because cells transfected with IL-1_{1-271} showed impaired growth and upregulation of IL-1α inducible genes, whereas no major modifications occurred in cells transfected with IL-$1_{113-271}$, we questioned whether a different intracellular location could be somehow responsible for the differences observed. We examined the intracellular distribution of IL-1α in fixed, permeabilized IL-1_{1-271}- and IL-$1_{113-271}$-transfected tEC via indirect immunofluorescence with a goat immunopurified antibody to IL-1α, followed by a fluorescein-conjugated anti-goat antibody. As shown in Fig. 7, IL-1_{1-271}-transfected cells showed intense nuclear staining whereas IL-$1_{113-271}$-transfected cells stained uniformly throughout the cytoplasm, with evident nuclear exclusion. Preincubation of the IL-1α antibody with recombinant IL-1α reduced the staining to background levels, demonstrating that the increased immunofluorescence was specifically due to IL-1α (data not shown). These experiments establish a correlation between the nuclear localization of endogenous IL-1_{1-271} and the modulation of endothelial function.

Nuclear localization studies. The ultimate destination of endogenous IL-1α within the cell was investigated by searching for an NTS (9). Indeed, IL-1α has a basic amino acid cluster (positions 79 to 85) which is homologous to the nuclear targeting signal of the simian virus 40-encoded large tumor antigen (18, 22). To determine the role of this sequence, fusion proteins containing all or part of the coding region of IL-1α cDNA inserted in frame upstream of the β-Gal-encoding gene in pSVL were constructed. The chimeric genes were composed of the following sequences coupled amino terminally to the β-Gal-encoding gene: (i) IL-1_{1-271}, (ii) IL-$1_{113-271}$, and (iii) the sequence KVLKKRR, a potential NTS localized at positions 79 to 85. In every case, the fusion proteins produced upon expression of the construct were of the predicted size and the molecular mass was higher than 110 kDa (23a). A protein of this size cannot traverse the nuclear pore without a legitimate signal for entry (9). Each of these constructs was transfected into Cos cells by the DEAE-dextran method (15), and the location of the translated proteins was determined by immunofluorescence. The results are shown in Fig. 8. IL-1_{1-271}–β-Gal, but not IL-$1_{113-271}$–β-Gal, is associated with the nucleus. Cells transfected with the putative NTS fused to β-Gal also showed nuclear staining, demonstrating that residues 79 to 85 are involved in the targeting of IL-1α to the nucleus.

DISCUSSION

The function of intracellular IL-1α in human endothelial cells is of general interest, since (i) IL-1α has been shown to represent an endogenous brake for endothelial cell proliferation (14) and life span (25) and (ii) IL-1α has been demonstrated to inhibit angiogenesis in vivo (7) and the formation of new blood vessels is a requisite for solid tumor growth and metastasis (12). Moreover, Kaposi's sarcoma cells (6), whose relationship to vascular elements remains unclear, produce large amounts of IL-1α (11) and it is of interest to get insights into a potential intracellular role of this cytokine in modulating Kaposi's sarcoma cell phenotype and functions.

The experiments described here demonstrate that a relationship exists between the nuclear localization of endogenous

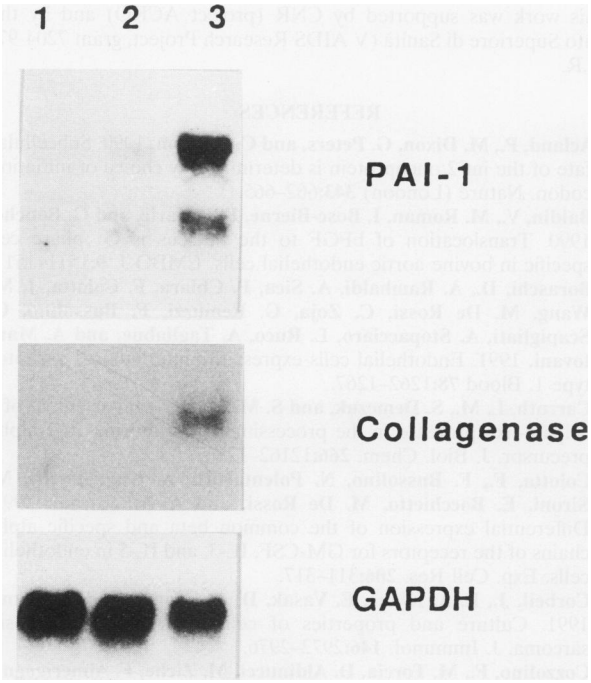

FIG. 6. Expression of collagenase and PAI-1 mRNAs by transfected tEC. Total RNA was prepared from confluent transfected tEC and probed with human collagenase and PAI-1 cDNAs. The blot was rehybridized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to verify equal RNA loading among the lanes. Lanes: 1, pMEXneo-transfected tEC; 2, IL-$1_{113-271}$-transfected tEC; 3, IL-1_{1-271} transfected tEC.

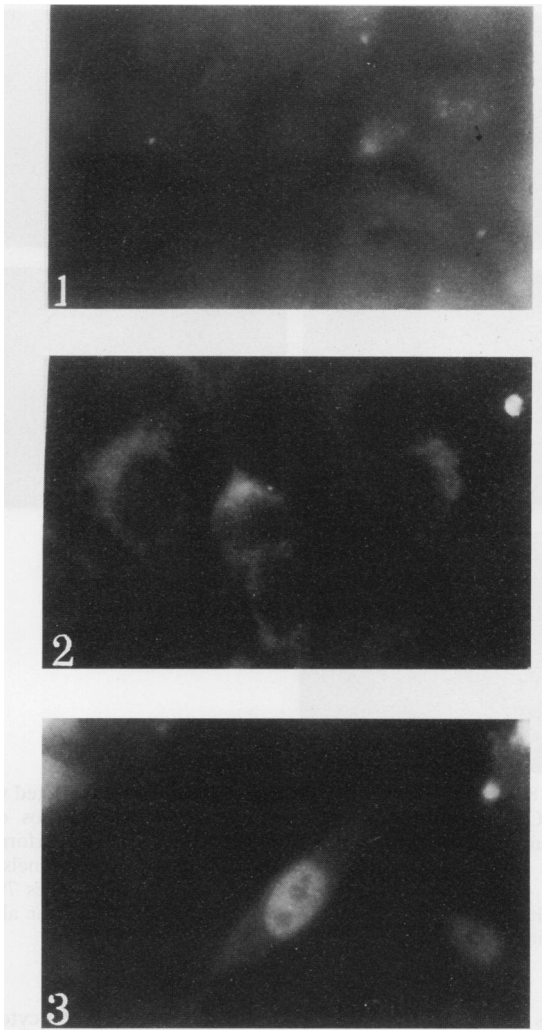

FIG. 7. Immunofluorescence analysis of transfected tEC. Immunofluorescence staining of tEC expressing the following proteins was performed with an immunopurified serum against human IL-1α as described in Materials and Methods. Panels: 1, control pMEXneo-transfected tEC; 2, IL-$1_{113-271}$-transfected tEC; 3, IL-1_{1-271}-transfected tEC (magnification, ×100).

IL-1α and its ability to alter endothelial cell behavior. Indeed endothelial cells overexpressing IL-1_{1-271}, which localizes in the nuclei, showed impaired proliferation and upregulation of IL-1α-inducible genes, whereas cells synthesizing IL-$1_{113-271}$, which remains in the cytoplasm, behaved like the control. The failure of intracellular IL-$1_{113-271}$ to modulate endothelial cell growth is in agreement with a report showing that while an intracellular role for gamma interferon (43) and tumor necrosis factor α (44) was observed by microinjection technology, no effect of microinjected IL-$1_{113-271}$ was detected (43).

Recently, examples of growth factors exerting their effect in the nucleus have emerged. Exogenously applied platelet-derived growth factor (38), basic fibroblast growth factor (2), and schwannoma-derived growth factor (19) accumulate in the nuclei of target cells, where they may exert their activity. Also, exogenously added IL-$1_{113-271}$ has been reported to translocate to the cell nucleus in a receptor-dependent manner (16, 32). We have examined the fate of endogenously synthesized IL-1_{1-271} and IL-$1_{113-271}$. Since endogenous IL-$1_{113-271}$ fails to localize in the nucleus, we speculate that two different pathways exist for transport to the nucleus, one that recognizes protein binding through a plasma membrane receptor and one that recognizes protein in the cytoplasm. Nuclear entry of proteins results from a specific translocation process (34, 41). The protein to be transported contains a short stretch of basic amino acid acting as a specific NTS (9). This sequence is recognized by NTS-binding proteins that appear to function as adaptor molecules of the nuclear transport machinery (34, 41). The NTS we have identified in IL-1_{1-271} (residues 79 to 85) is similar to the prototypical one of the simian virus 40 large tumor antigen in its high content of basic amino acids (18, 22). It is noteworthy that not only residues 79 to 85 but also the IL-1α precursor can translocate β-Gal to the nucleus. Indeed, it has been reported that sequences distant from the NTS can affect its ability to function (13). Moreover, it has been shown that acidic fibroblast growth factor (aFGF) contains a putative NTS which is able to direct the expression of β-Gal to the nucleus; however, this NTS is unable to target either aFGF itself or an aFGF–β-Gal fusion protein to the nucleus, suggesting that aFGF contains an additional sequence which prevents endogenously expressed aFGF from being translocated into the nucleus (50).

It is tempting to speculate about the function of IL-1α. Owing to the acidity of this polypeptide, it is difficult to cast it in the role of a DNA-binding protein. However, IL-1α could function as a carrier protein designed to convey nontargeted DNA-binding proteins into the nucleus. It may also serve as a protein which loosens histones from DNA to modulate transcription (40). It is noteworthy that IL-1β, which has the same three-dimensional structure and biological activities as IL-1α

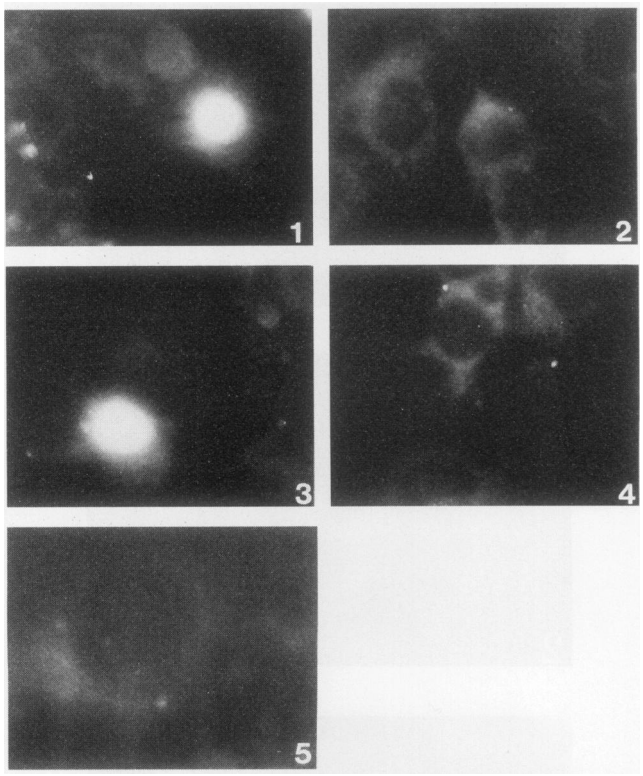

FIG. 8. Immunofluorescence analysis of Cos cells transfected with IL-1–β-Gal constructs. Immunofluorescence staining of Cos cells expressing the following IL-1–β-Gal fusion proteins was performed with an anti-β-Gal antibody as described in the text. Panels: 1, IL-1_{1-271}–β-Gal; 2, IL-$1_{113-271}$–β-Gal; 3, IL-1α NTS (residues 79 to 85)–β-Gal; 4, β-Gal; 5, Cos cells transfected with the vector alone (magnification, ×100).

(8), has been reported to be located exclusively in the cytosol of IL-1β-transfected hamster fibroblasts (49). Interestingly, the NTS we have described at positions 79 to 85 of IL-1_{1-271} is not present in the corresponding region of IL-1β (28). Moreover, unlike IL-1α, IL-1β can be secreted through an alternative pathway (39) and may function as an autocrine regulator of human endothelial cell growth (7). These results argue that different pathways for intracellular localization exist for the IL-1s, as described for other polypeptide hormones, such as the fibroblast growth factors (1, 37).

Furthermore, since the submission of this report for review, the domain from positions 79 to 86 of the IL-1α precursor has been shown to function as an NTS (47). An IL-1α precursor point mutant in which Lys-82 was replaced by Glu was synthesized. Transfection of NIH 3T3 cells with the IL-1α precursor point mutant resulted in a dramatic reduction in the ability of the IL-1α precursor to associate with the nucleus (47).

Overall, our results and those of others provide direct evidence that IL-1α nuclear localization is critical to its mode of action and, more generally, demonstrate that cytokines and peptide hormones with pleiotropic functions may exert their effects through interaction with intracellular targets distinct from surface receptors.

ACKNOWLEDGMENTS

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