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Expression of interleukin 1-inducible genes and production of interleukin 1 by aging human fibroblasts

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ABSTRACT **The interleukin 1 (IL-1)-inducible mRNAs for plasminogen activator inhibitor type 2, manganese superoxide dismutase, and urokinase are overexpressed in old (>70% of life-span completed) but not in young (<40% of life-span completed) human foreskin fibroblasts. Furthermore, the activity of this superoxide dismutase is greater in old than in young fibroblasts. IL-1β mRNA is detected by Northern blot analysis in old fibroblasts and its expression is further enhanced by a treatment with IL-1α. IL-1α and IL-1β mRNAs are detected in old foreskin and lung fibroblasts by a sensitive reverse transcription-PCR assay. IL-1 mRNA is consistently expressed after fibroblasts have completed 85% of their *in vitro* life-span; an assay with specific antibodies shows that IL-1α is present in these fibroblasts. Prolonged treatment with IL-1 receptor antagonist decreases the levels of IL-1α and of IL-1α and IL-1β mRNAs. This observation suggests that IL-1 receptor antagonist inhibits an autocrine loop responsible for IL-1 expression. IL-1 mRNA accumulates in young fibroblasts treated with cycloheximide, suggesting that it is transcribed but unstable in these cells; accumulation of IL-1 mRNA in old fibroblasts may be due at least in part to increased stability. IL-1α stimulates DNA synthesis in young fibroblasts but has progressively less effect as the cells age in culture. These data indicate that IL-1 is "constitutively" produced by aging fibroblasts and that IL-1 induces the expression of specific proteins in these cells. The mechanism for this constitutive production of IL-1 is explored in this paper.**

Human diploid fibroblasts (HDFs) have a limited capacity to proliferate in culture (1, 2). After ≈20–60 population doublings, HDFs undergo a process called cellular senescence. Senescent HDFs remain viable and continue to synthesize RNA and protein (3) but become refractory to mitogenic stimuli (4). Senescence is irreversible and quite distinct from quiescence produced by the lack of serum or growth factors; cell division resumes when appropriate growth factors are provided to quiescent HDFs (5). The number of cell divisions before the onset of senescence is inversely correlated with the age of tissue donors, suggesting that cellular senescence *in vitro* reflects aging *in vivo* (1, 2).

Because of their limited proliferation, HDFs are used as a model for studying *in vitro* aging (4). Senescent HDFs contain mRNAs that inhibit DNA synthesis when injected into proliferating cells and suppress DNA synthesis after fusion with proliferating cells (3, 6). These findings suggest that specific proteins regulate cellular senescence. HDF aging may also be regulated by tumor suppressor genes (7). Recently, Stein *et al.* (8) showed that senescence is associated with the absence of the overphosphorylated forms of retinoblastoma protein that normally accumulate as cells emerge from quiescence and approach the G_1/S boundary. Proliferating HDFs also differ from senescent cells in the expression of the c-*fos* gene that is repressed in senescent HDFs (9). However, expression of c-*myc* and c-Ha-*ras* protooncogenes is similar in senescent and proliferating HDFs (10). Some mRNAs (11) and proteins are overexpressed in aging HDFs: collagenase (12), β-amyloid precursor protein (13), and fibronectin (14). In contrast, a post-transcriptional block inhibits the expression of the proliferating cell nuclear antigen (15).

During a screening of different cell lines, we observed that aging foreskin HDFs expressed much greater levels of plasminogen activator inhibitor type 2 (PAI-2) mRNA than early passage HDFs. PAI-2 expression is induced by tumor necrosis factor (TNF) and interleukin 1 (IL-1) (16, 17). The goal of the present investigation was to find an explanation for the overexpression of PAI-2 and other mRNAs in aging HDFs.

MATERIALS AND METHODS

Materials. Human recombinant IL-1α and IL-1β cDNAs were kindly provided by Peter T. Lomedico (Hoffmann–La Roche). Human recombinant IL-1 receptor antagonist (IL-1ra) was kindly provided by Robert C. Thompson (Synergen, Boulder, CO). The plasmids containing PAI-2 and β-actin cDNA were previously described (16); urokinase-type plasminogen activator (u-PA) cDNA was purchased from the American Type Culture Collection; and manganese superoxide dismutase (MnSOD) cDNA was a gift from David Goeddel (Genentech). Reverse transcriptase was purchased from Molecular Genetic Resources (Tampa, FL); AmpliTaq DNA polymerase was from Perkin–Elmer/Cetus; RNasin was from Promega; $(dT)_{12-18}$ was from Pharmacia LKB; and *Escherichia coli* 055:B5 lipopolysaccharide (LPS) was from Sigma.

Cell Culture and Treatment. HDFs were cultured in M199 medium supplemented with 10% fetal bovine serum as described (18). The strain RIG was derived from newborn foreskin; IMR-90 lung fibroblasts were purchased from the National Institute of Aging Cell Repository (Camden, NJ). Cultures were seeded at 6.67×10^3 cells per cm^2 and the cell number at each passage was used to calculate population doublings. To calculate the *in vitro* life-span, HDFs were serially cultivated at weekly intervals until there was no further increase in cell number. Cells were counted at each passage to compute the percentage of life-span completed.

RNA Extraction and Analysis. RNA extraction and Northern blot analysis were performed as described (19). For reverse transcription-polymerase chain reaction (RT-PCR), 2 μg of RNA in 16.5 μl of water was heat-denatured at 65°C for 5 min and then added to 13.5 μl of solution containing 6 μl of 250 mM Tris·HCl, pH 8.3/300 mM KCl/50 mM $MgCl_2$/50 mM dithiothreitol, 1.5 μg of $(dT)_{12-18}$, 1.5 μg of acetylated bovine serum albumin (BSA), 1.5 μl of 10 mM dNTPs, 20

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Abbreviations: HDF, human diploid fibroblast; IL-1, interleukin 1; PAI-2, plasminogen activator inhibitor type 2; SOD, superoxide dismutase; u-PA, urokinase-type plasminogen activator; IL-1ra, IL-1 receptor antagonist; ECM, extracellular matrix; RT-PCR, reverse transcription-polymerase chain reaction; LPS, lipopolysaccharide; BSA, bovine serum albumin; TNF, tumor necrosis factor.

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units of RNasin, and 20 units of reverse transcriptase. The reaction was carried out at 42°C for 1 hr; 20 μl of water was then added and 5 μl (2.5 μl for actin) was used for each PCR. The PCR was performed for 30 cycles in a 50-μl reaction mixture containing 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, 1 μM primers, and 1.25 units of AmpliTaq DNA polymerase. The samples were overlaid with 50 μl of mineral oil and cycling was performed on a thermal cycler (Coy Laboratory Products, Ann Arbor, MI) with the following parameters: denaturation at 94°C for 1 min, annealing at 60°C for 2 min, and extension at 72°C for 3 min followed by a final incubation at 72°C for 7 min. The PCR was originally performed for 20, 30, and 40 cycles, and 30 cycles of PCR were found to be optimal for most RNAs as the amplification was still linear. Eight microliters of each RT-PCR sample was fractionated by 1.5% agarose gel electrophoresis and visualized with ethidium bromide. The gels were vacuum-blotted onto GeneScreen membranes (New England Nuclear), hybridized to labeled probes, washed according to the manufacturer's protocol, and exposed to Kodak XAR-5 film. The radioactivity was measured in a Betascope 603 (Betagen, Waltham, MA).

Primer Design and Sequences. Primers were designed from known sequences (GenBank) using OLIGO, a primer analysis software for optimal amplification (National Biosciences, Hamel, MN). All primers were 18–24 nucleotides long and were synthesized in an automated Applied Biosystems DNA synthesizer (Foster City, CA). The following primer sequences with the size of the amplified fragment in parentheses were used: IL-1α [816 base pairs (bp)], 5′-ATGGCCAAAGTTCCAGACATGTTTG and 3′-GGTTTTCCAGTATCTGAAAGTCAGT; IL-1β (811 bp), 5′-ATGGCAGAAGTACCTAAGCTCGC and 3′-ACACAAATTGCATGGTGAAGTCAGTT; and β-actin (548 bp), 5′-GTGGGGCGCCCCAGGCACCA and 3′-CTCCTTAATGTCACGCACGATTTC.

DNA Synthesis. Cells (5×10^4) were seeded in 24-well cluster plates and grown in complete medium for 24 hr prior to addition of 4 μCi of [^{3}H]thymidine (1 Ci = 37 GBq) per well for an additional 16 hr. The cells were then dissolved in 0.5 M NaOH and 10% trichloroacetic acid was added; the precipitate was collected on glass fiber filters for assay of radioactivity. The radioactivity was normalized to the cell number.

IL-1α Assay. Cells were harvested in saline, sonicated for 45 sec, and centrifuged for 5 min at 10,000 × *g*. The supernatant was assayed for IL-1α with an ELISA kit purchased from R & D Systems (Minneapolis). The culture medium was concentrated ≈50-fold in a cell flow concentrator (Amicon) with a PM10 membrane.

RESULTS

Expression of Genes Inducible by TNF or IL-1 in Old Fibroblasts. TNF induces PAI-2 mRNA in melanoma and fibrosarcoma cells (16, 19). To extend this study of TNF activity, we examined untreated HDFs of different *in vitro* ages by Northern blot analysis. HDFs that had completed <40% or >70% of their *in vitro* life-span are defined as "young" or "old," respectively (20). A Northern blot containing RNA from TNF-treated or untreated young and old HDFs was sequentially probed with PAI-2, MnSOD, u-PA, and IL-1β cDNA. The corresponding mRNAs were overexpressed in untreated old HDFs in comparison to untreated young HDFs (Fig. 1, lanes 1 and 3). MnSOD is coded for by mRNAs of 4 and 1 kb that have an identical coding region but differ in the 3′ untranslated sequence (21); both mRNAs were overexpressed. However, the 1-kb mRNA is difficult to visualize in Fig. 1 due to brief exposure of the blot. To determine if the translation product of these mRNAs was also

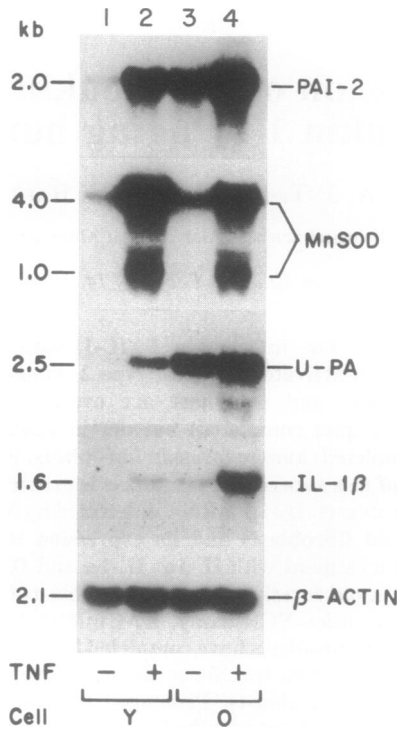

FIG. 1. Northern blot analysis of RNA from young (Y) and old (O) HDFs untreated (lanes 1 and 3) or treated (lanes 2 and 4) with 10 ng of TNF per ml for 18 hr. Ten micrograms of RNA was analyzed in each lane. The same blot was stripped and rehybridized to different probes as indicated. kb, Kilobases.

overexpressed in old HDFs, we assayed the MnSOD activity in a zymogram (22). Old HDFs showed greater MnSOD activity than young HDFs but showed similar CuZnSOD activity (Fig. 2). CuZnSOD is not a TNF-inducible enzyme (23). In addition, a novel isozyme of MnSOD was expressed in old HDFs (see Fig. 2 legend).

To confirm that the mRNAs examined are inducible by TNF in these cells, we treated young and old HDFs with this cytokine. PAI-2, MnSOD, and u-PA mRNAs were induced to a similar extent in young and old HDFs (Fig. 1, lanes 2 and 4). However, the induction in old HDFs appears lower than in young HDFs because of the high basal level of these mRNAs in untreated old cells. An explanation for the presence of TNF-inducible mRNAs in untreated old HDFs is an autocrine stimulation by secreted TNF. However, TNF was

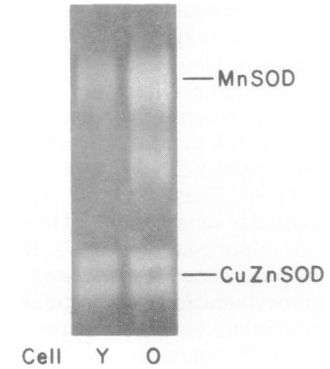

FIG. 2. Analysis of SOD activity in young (Y) and old (O) HDFs. Cytoplasmic extracts were prepared to assay MnSOD activity in a zymogram. Twenty-five micrograms of protein was analyzed in each lane. Bands corresponding to the two types of SOD are indicated. The additional band in old HDFs is presumably a MnSOD isozyme since it is cyanide insensitive (22).

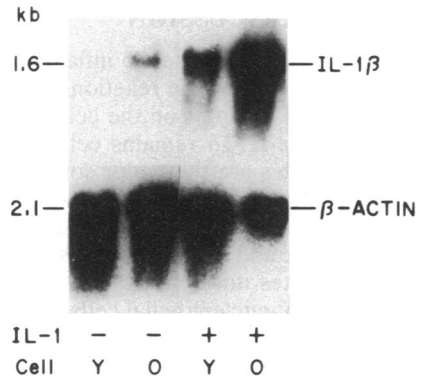

FIG. 3. Northern blot analysis of IL-1β mRNA in young (Y) and old (O) HDFs untreated or treated for 18 hr with 10 ng of IL-1α per ml. The untreated samples (− lanes) were exposed for 4 days to increase sensitivity, whereas the IL-1-treated samples (+ lanes) were exposed for 1 day. The blots were also hybridized to a β-actin probe and exposed for 1 day.

not detected in the old HDF culture medium nor was TNF mRNA detected by Northern blot or RT-PCR analysis (data not shown).

Expression of IL-1 mRNA in Old Fibroblasts. The overexpressed mRNAs are also inducible by IL-1 (17), and the presence of small amounts of IL-1β mRNA in old HDFs (Fig. 1, lane 3) suggested that autocrine stimulation by IL-1 could result in their induction. To explore this possibility fibroblasts were treated with IL-1α. The IL-1β mRNA was induced by IL-1α in young and old HDFs (Fig. 3).

IL-1 is a pleiotropic mediator of inflammation that shows different effects on various cells (24) and is mitogenic for fibroblasts (25). It was thus surprising to detect IL-1 mRNA specifically in old HDFs that divide slowly. Because of the relatively low level of expression of IL-1 mRNA (Figs. 1 and 3), in subsequent experiments we used a RT-PCR assay that is at least 100 times more sensitive than Northern blot analysis. With this technique, we examined RNA prepared from IMR-90 human lung fibroblasts at different passages. IL-1α and IL-1β mRNAs were detected only in old IMR-90 cells (Fig. 4*A*). These results suggested that IL-1 mRNA was expressed during aging in different HDF lines. To determine when IL-1 mRNA was expressed during the HDF life-span, RT-PCR analysis was carried out on RNA made from successive cell passages (Fig. 4*B*). IL-1α and IL-1β mRNAs were first detected at passage 34 (corresponding to 85% of life-span completed).

Table 1. IL-1α in fibroblasts

Cells	% life-span completed	Treatment	IL-1α, pg per 10^6 cells
Young	10	—	<6
Intermediate	69	—	<6
Old	90	—	11
Old	90	IL-1ra	<6
Old	90	IL-1α	60
Old	95	—	26
Old	95	IL-1ra	16

The cells were treated with 50 ng of IL-1α per ml or 100 ng of IL-1ra per ml for 8 weeks. The IL-1α was measured in cell extracts with an ELISA. The % life-span completed at the end of the treatment is shown. The sensitivity of the assay is ≥6 pg of IL-1α.

Since old HDFs were predominantly in G_1, it seemed possible that IL-1 was expressed during this phase of the cell cycle. However, IL-1α and IL-1β mRNAs were expressed only in old HDFs but not in young HDFs arrested in G_1 by serum starvation (data not shown).

IL-1α in Old Fibroblasts. Expression of IL-1 mRNA is relevant only if this mRNA is translated. Since IL-1α is not secreted (26), cell extracts from young and old HDFs were assayed for IL-1α with an ELISA. The results correlated well with those from RT-PCR analysis (Table 1). IL-1α was undetectable in young HDFs but was present at 11 and 26 pg per 10^6 cells in fibroblasts that had completed 90% and 95%, respectively, of their *in vitro* life-span. IL-1α could not be detected in the cell medium even after 50-fold concentration, indicating that it was not secreted, in agreement with earlier reports (26).

IL-1α was also measured in HDFs treated with this cytokine or with a receptor antagonist (IL-1ra) to probe the mechanism of autocrine stimulation. IL-1α induced its own production about 5-fold, whereas IL-1ra reduced its production to about 50% but could not completely eliminate it (Table 1). These results provided further support for an autocrine mechanism of IL-1 production. To investigate the mechanism of action of IL-1ra, RNA was prepared from old HDFs after 8 weeks of treatment with 100 ng of IL-1ra per ml; expression of IL-1α and IL-1β mRNAs was drastically reduced (Fig. 4*C*). In HDFs treated with 50 ng of IL-1α per ml as a further control, the expression of these mRNAs was increased (Fig. 4*C*). Similar results were obtained after 6 or 10 weeks of

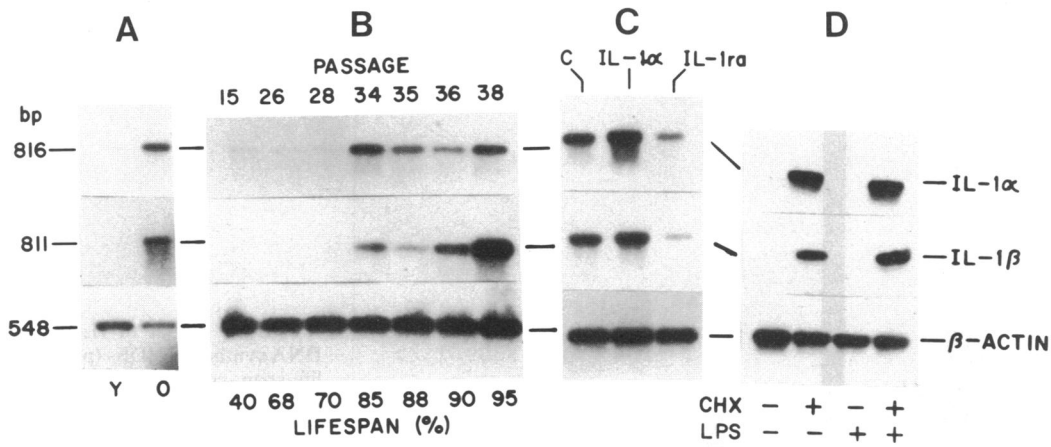

FIG. 4. RT-PCR analysis of HDF RNA. (*A*) Early and late passage (Y and O) IMR-90 lung fibroblasts. In these experiments, 2 μg of total cellular RNA was reverse-transcribed; 1/10 of the cDNA obtained was amplified for each mRNA (1/20 for β-actin) to obtain DNA fragments of the size indicated. (*B*) HDFs at different passages covering their life-span. (*C*) HDFs (56% of life-span completed) treated for 8 weeks with 50 ng of IL-1α per ml or 100 ng of IL-1ra per ml; C, untreated cells. (*D*) Young HDFs treated for 4 hr with 10 μg of cycloheximide (CHX) per ml and/or 1 μg of LPS per ml. Control cells were untreated.

treatment, but a smaller reduction in IL-1 mRNA expression was observed after 4 weeks (data not shown). This suggested that a long exposure to IL-1ra was required to inhibit the proposed autocrine loop. Northern blot analysis of RNA from IL-1ra-treated HDFs also showed reduced expression of MnSOD, stromelysin (an IL-1α-inducible gene overexpressed in old HDFs), and IL-1β mRNA (data not shown). These results indicated that prolonged treatment with IL-1ra greatly reduced but did not completely suppress IL-1 mRNA expression.

Stabilization of IL-1 mRNA. IL-1 is produced by aging HDFs in response to unknown stimuli. In experiments with vascular smooth muscle cells, endotoxin induces expression of IL-1β mRNA and endotoxin plus cycloheximide induce IL-1α mRNA (27). These findings suggest that post-transcriptional regulatory mechanisms may play some role in the expression of IL-1 mRNA. Therefore, we examined the effect of LPS on the level of IL-1 mRNA in young HDFs but did not detect any effect (Fig. 4*D*). An assay for mechanisms regulating mRNA stability is based on the inhibition of protein synthesis by cycloheximide that promotes the accumulation of short-lived mRNAs (28). IL-1α and IL-1β mRNAs accumulated in young HDFs treated with cycloheximide (Fig. 4*D*). LPS added together with cycloheximide had little or no further effect. These results suggested that IL-1 mRNA was transcribed by young and old HDFs; increased stability could possibly explain the accumulation of these mRNAs in old HDF.

Effect of IL-1α and IL-1ra on DNA Synthesis. To examine the effect of IL-1 on DNA synthesis, we treated passage 22 HDFs (≈50% life-span completed) with 50 ng of IL-1α per ml or 100 ng of IL-1ra per ml for 10 weeks. The HDFs were fed twice a week with fresh medium containing IL-1α or IL-1ra and subcultured every 2 weeks. At this time, the HDFs were assayed for [^{3}H]thymidine incorporation. Control HDFs showed a decreasing rate of DNA synthesis. IL-1α increased DNA synthesis during the first 4 weeks of cell culture but was less effective afterward. IL-1ra did not have any effect on DNA synthesis, since [^{3}H]thymidine incorporation was comparable to that of control HDFs (Fig. 5). Treatment with IL-1α and IL-1ra for 12–72 hr gave results similar to those for long-term treatment (data not shown). These data indicated that IL-1ra had no effect on the progressive loss of proliferative potential of HDFs aging *in vitro*.

DISCUSSION

IL-1 is a key mediator in responses to inflammation, injury, microbial infections, and immune reactions (24). IL-1α and IL-1β bind to the same receptor on the cell surface (29) but IL-1β is secreted while IL-1α remains cell-associated (26). Monocytes/macrophages are the major source of IL-1, but other cells are also capable of producing IL-1. These include fibroblasts, keratinocytes, and Langerhans cells in the skin, vascular smooth muscle and endothelial cells, mesangial cells in the kidney, astrocytes and microglial cells in the brain, corneal, gingival and thymic epithelial cells, and some T-cell lines (24). In most cells, IL-1 is produced in response to external stimuli that regulate its synthesis.

HDFs produce various cytokines, including IL-1, in response to specific stimuli (30). In this report, we have demonstrated "constitutive" IL-1 production in aging HDFs. Surprisingly, IL-1 mRNA appears at a characteristic cell age between passages 28 and 34. HDFs show interclonal variation in their proliferative potential (31); however, we have not localized IL-1 production to individual cells. The striking appearance of IL-1 mRNA that occurs after 70–85% of the *in vitro* life-span of HDFs is completed may result from an autocrine stimulation and accumulation of IL-1 mRNA once a threshold level of IL-1 is reached. In agreement with this hypothesis, we detected IL-1α in HDFs only after passage 35 (≈88% of life-span completed). Furthermore, it is unlikely that IL-1 production is stimulated by endotoxin in the culture medium since treatment with LPS has no effect on IL-1 expression in young HDFs (Fig. 4*D*).

The mechanism that increases the level of IL-1 mRNA in aging HDFs is presently unknown. Experiments with cycloheximide-treated young HDFs suggest that IL-1 mRNA is transcribed in these cells but that it does not accumulate. Stabilization of IL-1 mRNA by cycloheximide has been described in monocytes (32), myeloid cells (33), and fibroblasts (30). A combined treatment with cycloheximide and endotoxin superinduces IL-1 mRNA in vascular smooth muscle cells and monocytes (27, 32). However, LPS does not increase IL-1 mRNA accumulation in HDFs treated with cycloheximide (Fig. 4*D*).

IL-1 belongs to a group of transiently expressed cytokine genes that share (A+U)-rich sequence in the 3′ untranslated region of their mRNAs (34, 35). These sequences are thought to determine rapid mRNA turnover. It seems likely that IL-1

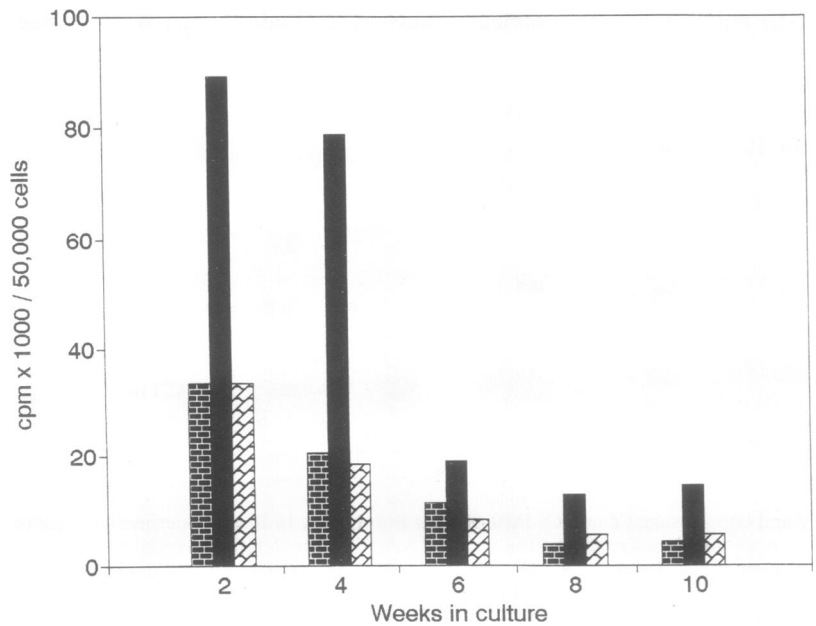

FIG. 5. Effect of IL-1α and IL-1ra on DNA synthesis. HDFs (passage 22; ≈50% of life-span completed) were fed with fresh medium or medium supplemented with 50 ng of IL-1α per ml or 100 ng of IL-1ra per ml twice a week. Every 2 weeks, [^{3}H]thymidine incorporation was measured. Filled bars, IL-1α-treated HDFs; brick bars, untreated HDFs; cobblestone bars, IL-1ra-treated HDFs. The mean values of two independent assays are shown.

mRNA is transcribed by young HDFs but that it does not accumulate because of instability; cycloheximide stabilizes IL-1 mRNA in these cells (Fig. 4*D*). The accumulation of IL-1 mRNA in old HDFs may result from its interaction with proteins that bind to (A+U)-rich sequences (36) or to other specific sequences in the 3′ untranslated region. Recently, Random *et al.* (37) have proposed that a specific protein regulates post-transcriptionally the stability of erythropoietin mRNA in response to hypoxia. This observation provides a model to explain the accumulation of specific mRNAs.

IL-1α is growth inhibitory for human endothelial cells (38). Moreover, treatment of these cells with an antisense oligonucleotide complementary to IL-1α mRNA extends their life-span (39). In our experiments, however, IL-1α acted as a mitogenic factor for HDFs (Fig. 5), in agreement with previous studies (25). IL-1 is also an autocrine growth factor for acute myeloid leukemia cells (40). These data indicate that IL-1 may be either growth stimulatory or inhibitory in different cells.

Prolonged treatment with IL-1ra does not have any effect on DNA synthesis in HDFs despite a marked reduction in the expression of IL-1α and IL-1β mRNAs (Figs. 4*C* and 5). Since its discovery and cloning (41), IL-1ra has been shown to inhibit IL-1 activity by acting as a true antagonist (42). However, IL-1ra treatment did not eliminate IL-1 expression in old HDFs, suggesting that the constitutive expression of IL-1α may be regulated by a mechanism in addition to stimulation by secreted IL-1. Since little if any IL-1α is secreted by HDFs and IL-1ra acts at the level of cell surface receptors, it is likely that IL-1ra inhibits only IL-1β activity. A prolonged treatment of old HDFs with IL-1ra may be required to block the autocrine stimulation by secreted IL-1β and gradually reduce the production of cell-associated IL-1α.

There are indications that cytoplasmic IL-1α has some function, since receptor-bound IL-1α translocates to the nucleus in murine T cells (43) and an intracellular form of IL-1ra has recently been identified in epithelial cells (44). The stringent regulation of IL-1 expression strongly suggests that IL-1 has a wide biological role in addition to its function as a mediator of inflammation. A considerable amount of extracellular (ECM) components is synthesized by HDFs. Cell–ECM interaction, diffusion of soluble factors, and cell–cell contact are known to influence morphogenesis of epithelial tissues (45). Because of its ability to stimulate cell proliferation and induce ECM proteases, IL-1 may have some role in the morphogenesis of epithelial tissues. In addition, constitutive production of IL-1 by HDFs may have profound effects on connective tissue, since IL-1 modulates the activity of various ECM components and of their degradative enzymes (46). Involvement of IL-1 in the regulation of metalloproteinases and in the etiology of cartilage degeneration has been suggested (47).

We do not presently understand the biological role of IL-1 expression in old HDFs. However, production of this cytokine accounts for the phenotype of these cells that is characterized by overexpression of IL-1-inducible proteins. It is tempting to speculate that IL-1 may be produced in an attempt to stimulate mitogenesis in late-passage HDFs, whereas young HDFs may be adequately stimulated by serum growth factors. As HDFs age, the requirement for additional growth factors may lead to the establishment of an IL-1 autocrine loop. It is also possible that production of IL-1 makes HDFs proliferate faster and reach senescence sooner. In both hypotheses, IL-1 would function as a mitogen for fibroblasts. Further experiments in which IL-1 expression is suppressed may elucidate the role of this cytokine in cellular aging.

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