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Lens Epithelium-Derived Growth Factor: Effects on Growth and Survival of Lens Epithelial Cells, Keratinocytes, and Fibroblasts

Dhirendra P. Singh,* Nobuyuki Ohguro,* Takanobu Kikuchi,* Toshiharu Sueno,* Venkat N. Reddy,† Kenshi Yuge,† Leo T. Chylack, Jr.,* and Toshimichi Shinohara*,[1]

**Center for Ophthalmic Research, Brigham and Women's Hospital; Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts 02115; and †Eye Research Institute, Oakland University, Rochester, Michigan*

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We isolated a clone encoding a protein from a human lens epithelial cell (LEC) cDNA library with antibody (Ab) from a cataract patient and named it "lens epithelium-derived growth factor" (LEDGF). LEDGF is found to be identical to p75, a coactivator of both transcription (1) and pre-mRNA splicing (2). In serum-free medium LEDGF stimulated growth of LECs, cos7 cells, skin fibroblasts, and keratinocytes, and prolonged cell survival. Without LEDGF, the aforementioned cells did not survive. Also in serum-free medium, Ab to LEDGF neutralizing LEDGF blocked cell growth and caused cell death. Thus, LEDGF, a regulatory factor, may play an important role for growth and survival of a wide range of cell types.

Key Words: **autoantibody; autocrine; age-related cataract; lens epithelium-derived growth factor (LEDGF); survival factor; trafficking.**

Although there has been considerable research on growth, development, and differentiation of lens epithelial cells (LEC), there has been little research on the mechanisms of LEC survival. The lens contains three major cell types: (1) central epithelial cells which survive throughout life, but rarely divide (3), (2) preequatorial or "germinative" epithelial cells which divide throughout life and differentiate into nucleated, equatorial fiber cells, and (3) terminally differentiated deep cortical or nuclear, organelle-poor, fiber cells. The mechanisms regulating quiescence, growth, and prolonged survival of LECs are poorly understood. In 1992–1993 investigators were able to demonstrate that LECs secrete survival factor(s), but they were not able to identify them (4, 5). Subsequent studies demonstrated that growth factors; TGF-β (6), FGF, PDGF, EGF, IGF-I and insulin (7, 8) influenced proliferation and differentiation of LECs, but most did not specify if the growth factor was also a survival factor. Renaud *et al.* (9), however, showed that endogenous acidic FGF (aFGF) expression was higher in non-dividing LEC than in exponentially dividing cells and suggested that aFGF was not a mitogen, but a survival factor. Also, Stolen *et al.* (10) demonstrated that FGF-2 modulated not only the later stages of differentiation, but also the survival of lens fiber cells.

Cohen (11) demonstrated that nerve growth factor (NGF) stimulated growth and prolonged survival of neuronal cells, and that anti-NGF Abs killed these same cells. Recently we explored the possibility that an Ab to a LEC survival factor could increase the risk of cell death. First we demonstrated that anti-β crystallin Abs injected into a naive mouse caused LEC damage and cataract (12). Second we found that serum from most (>96%) patients with age-related cataract (ARC) had auto-antibodies (auto-Abs) cytocidal for LECs in culture (13, 14), and most recently we showed that Abs cytotoxic to LEC could be neutralized with whole lens total soluble proteins (13). These results encouraged us to search for LEC survival factors and Abs to them.

In this report we describe a factor which originally we cloned from a LEC cDNA library and named "lens-epithelium-derived growth factor" (LEDGF). We characterized further the growth- and survival-enhancing effects of LEDGF, and we present evidence that anti-

Abbreviations used: ARC, age-related cataract; aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor, CFA, complete Freund's adjuvant; DMEM, Dulbecco's modified Eagle medium; FCS, fetal calf serum; GST, glutathione-*S*-transferase; HATH, homologous to the amino acid terminus of HDGF; HDGF, hepatoma-derived growth factor; IFA, incomplete Freund's adjuvant; LDH, lactate dehydrogenase; LECs, lens epithelial cells; LEDGF, lens epithelium-derived growth factor; HRP-1 and HRP-2, HDGF-related proteins-1 and -2; NGF, nerve growth factor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PBS, phosphate-buffered-saline.

[1] To whom correspondence should be addressed. Fax: (617) 277-6717. E-mail: tshinohara@rics.bwh.harvard.edu.

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LEDGF Abs can killed LECs, cos7 cells, fibroblasts, and keratinocytes.

MATERIALS AND METHODS

Isolation of cDNA. A human LEC cDNA library was generated with the ZAP-cDNA Synthesis Kit (Stratagene, La Jolla, CA) from mRNAs isolated from 100 capsulotomy specimens obtained at routine cataract surgery (14). The detailed protocol for isolating the cDNAs is described in the instruction manual for the ZAP-cDNA Synthesis Kit protocol. Several clones were identified and isolated from the library by immunoscreening it with serum (diluted 1/100 in phosphate-buffered saline (PBS)) from a patient with ARC following the Pico Blue antibody immunoscreening protocol (Stratagene, La Jolla, CA). The second Ab, anti-human IgG (Kirkegaard & Perry Lab. Inc., Gaithersburg, MD), was diluted 1/5000 in PBS.

DNA sequence determination. The DNA sequence was determined in the Brigham and Women's Hospital's Automatic Sequencing and Genotyping Facility. Restriction endonucleases and modifying enzymes were purchased from Gibco-BRL (Bethesda, MD). Oligonucleotide probes were purchased from Lofstrand Labs, Limited (Gaithersburg, MD). The methods for restriction enzyme treatment, subcloning, DNA extraction, bacterial culture, agarose gel electrophoresis, ethidium bromide staining, and purification of DNA fragments are described elsewhere (16, 17). Computer analyses of protein and DNA sequences were done using the GCG "Idea" program (18).

Expression of LEDGF in prokaryotic and eukaryotic expression systems: Construction of GST-LEDGF. Two cDNA constructs were generated; one coding sequence for an N-terminal segment of LEDGF, and a second for full-size LEDGF. A fusion protein, LEDGF-glutathione-*S*-transferase (GST), (GST-LEDGF) was generated by inserting the entire coding sequence of the LEDGF cDNA between the *Bam*H1 and *Eco*R1 sites of a plasmid pGEX-2T vector (Pharmacia Biotech, Piscataway, NJ). Since the LEDGF cDNA lacked a *Bam*H1 site, we created one in the 5′-noncoding region just upstream of the initiation site ATG (nucleotide position; 311) using a synthetic primer, 5′-ccccggatccatgactcgcgatttcaaacct-3′, which contained a *Bam*H1 site. Although the LEDGF cDNA contained an *Eco*R1 site (nucleotide 2531), we created a second *Eco*R1 site at nucleotide position 875 to enable us to generate a smaller fragment of LEDGF cDNA which encoded the N-terminal region of LEDGF. This was accomplished by inserting another synthetic primer: 5′-tcttgaattctgtagctgcaggtcgtcctct-3′ in the 3′-prime end of the LEDGF cDNA. Using two primers, we generated a fragment of cDNA by a PCR amplification. The resulting *Bam*H1-*Eco*R1(added) fragment was 576 bp long; it was ligated into the pGEX-2T vector between the *Bam*H1 and *Eco*R1 sites; this was used to generate an N-terminal fragment of LEDGF (1–116 residues). To generate full size LEDGF, we prepared a *Xho*1 and *Eco*R1 fragment of 2153 bp from the original LEDGF cDNA ligated it into the *Eco*R1 and *Xho*1 sites of the previous construct. *E. coli* (BL21) was transfected with the construct and cultured in 500 ml of LB-ampicillin medium at 37°C with shaking until the optical density of the culture reached 0.6 OD_{600}. Then isopropyl β-D-thiogalactopyranoside (IPTG) was added at a final concentration of 100 μM, and the incubation continued for 6 more hours. To purify the fusion protein we followed a method provided by Pharmacia Biotech. The protein concentration was determined with the Bradford method (19).

Construction of pcDNA3-LEDGF. To enable the overexpression of LEDGF, we subcloned the entire coding sequence of LEDGF cDNA into a mammalian expression vector, pcDNA3 (Invitrogen Corp., Carlsbad, CA), at the *Eco*R1 site. Expression of LEDGF in this plasmid was under the control of a cytomegalovirus promoter. Mouse LECs and cos7 cells (5×10^5 cells per 60 mm dish) were transfected with each of the above constructs using a calcium phosphate protocol (Invitrogen Corp.). Cells transfected with plasmid pcDNA3 served as controls. The transfected cells were further incubated in DMEM with geneticin (G418) (Sigma Chemical Co., St. Louis, MO) at 500 μg/ml for up to 30 days to generate stably transfected cells. In this paper stably transfected LECs or cos7 cells over expressing LEDGF are called "LEC-LEDGF" or "cos7-LEDGF," and cells transfected with the LEDGF-free vector are called "LEC-vector" or "cos7-vector." Stably transfected cells were then aliquoted (1×10^5 cells/tube), frozen, and stored at −70°C. Photomicrographs were taken with a Nikon Eclipse E600 microscope.

Cell culture. LECs were obtained from 6–7 week-old mouse lenses. Human foreskin fibroblasts and monkey cos7 cells were obtained from the American Tissue Culture Collection (Bethesda, MD). Mouse keratinocytes (PAM 212) were a gift from the Harvard Skin Disease Research Center, BWH (Boston, MA). Cells were trypsinized (0.25% trypsin and 1 mM EDTA-Na in PBS) for 5–10 min at room temperature. After washing with PBS, the cells were cultured at 37°C in a 5% CO_2 atmosphere in Dulbecco's Modified Eagle Medium (DMEM) (Gibco-BRL, Bethesda, MD) supplemented with heat-inactivated fetal calf serum (FCS) (20% for human LECs and 10% for other cell types). The DMEM also contained 1 mM L-glutamine, 25 mM Hepes, 100 unit/ml penicillin, and 100 μg/ml streptomycin. Every day or every third day, we replaced the medium. Detailed protocols for human LEC culture are given elsewhere (20, 21). The mouse keratinocytes were cultured in RPMI medium supplemented with 10% calf serum (Gibco-BRL).

Protein blot analysis. Proteins from various tissues dissolved in SDS–PAGE sample buffer were separated on 8–12% SDS–PAGE and blotted onto Immobilon-P filters (Millipore Corp., Bedford, MA) (22). The filters were incubated overnight with affinity-purified Abs diluted 1/10–1/10^4. Secondary Abs were goat anti-rabbit or anti-human IgG labeled with horseradish peroxidase (1/5000) (Kirkegaard & Perry Lab., Inc.). The color was developed with 0.01% hydrogen peroxide and 0.05% 3,3-diamino-benzidine-tetrahydrochloride (DAB) (Bio-Rad Lab., Hercules, CA). Protein size markers were purchased from Bio-Rad Laboratories.

Immunohistochemistry. Human LECs were cultured on glass cover slips for two days in DMEM as described (20). The cells were fixed in cold ethanol and acetone (1:1) (−20°C) for 5 min. After air drying, the cells were blocked with 10% goat serum for 1 h. Then the cells were incubated with anti-(C-terminal)LEDGF Ab (1:200 dilution) at 37°C for 30 min. The cells were washed with PBS and further incubated at 37°C for 30 min with secondary goat anti-rabbit Abs conjugated to horseradish peroxidase (Bio-Rad, Hercules, CA). The cover slips were mounted and photographed with a camera attached to a fluorescence microscope.

Rapid colorimetric assay for cellular survival (MTT assay). The colorimetric assay using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) salt detects only living cells (23). At the indicated time, 10 μl of stock MTT solution were added to each well of the microtiter plates. After 4 h of incubation, the medium was aspirated from the well without disturbing the formazan crystals. One hundred microliters of DMSO were added to each well, the plates were shaken for 5 min on the plate shaker, and the OD_{570} was measured by an ELISA reader (Microplate Reader MR 600, Dynatech Product, Alexandria, VA). We validated the MTT-derived estimates of live LEC and cos7 cells by direct counting of live cells under the microscope.

Immunization of animals. In conducting the research described in this report, the investigators adhered to the NIH guide for the Care and Use of Laboratory Animals. Three times we injected subcutaneously into the back of an albino rabbit (Harlan Sprague–Dawley) 5.0 mg/500 μl of each peptide emulsified with complete Freund's adjuvant (CFA) for the initial injection or incomplete Freund's adjuvant (IFA) (Gibco-BRL, Bethesda, MD) for the subsequent two injections. The peptides had been conjugated with

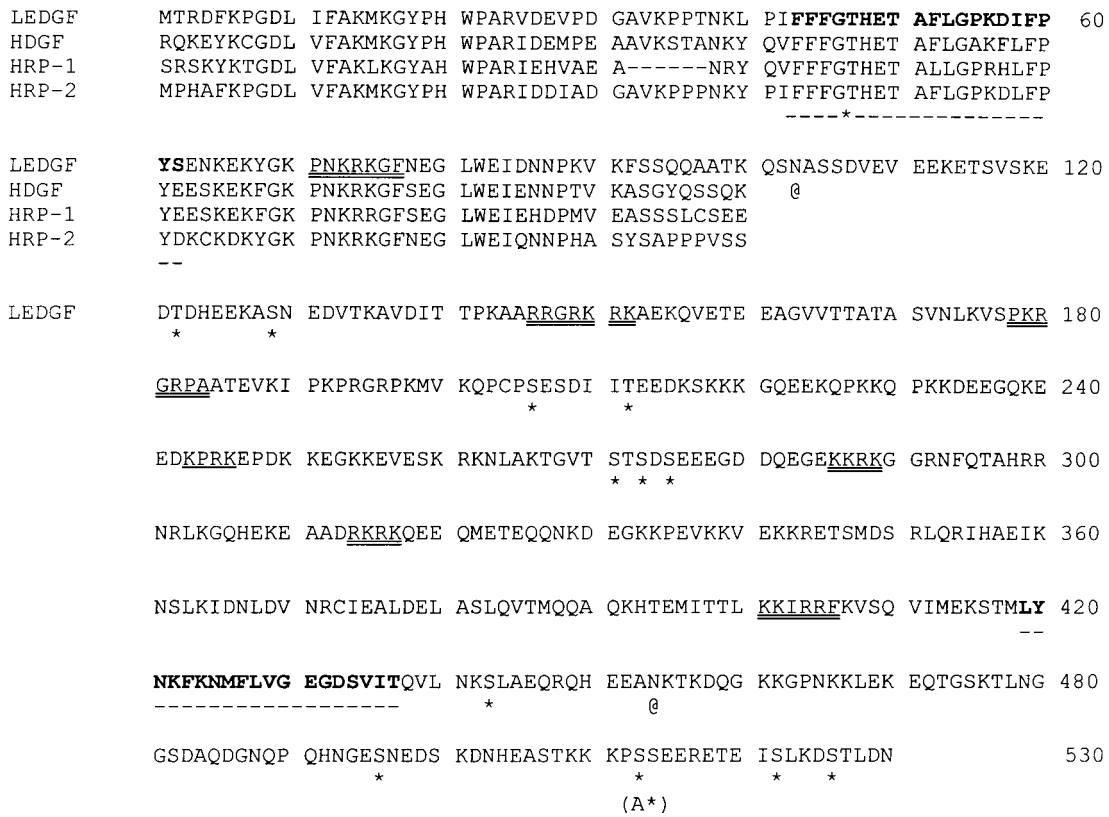

FIG. 1. Deduced amino acid sequence of LEDGF produced by clone HLC10 and published partial sequences of **H**omologous to the **A**mino acid **T**erminus of **H**DGF, "**HATH,**" regions of HDGF, HRP-1, and HRP-2 (29). The whole sequence of LEDGF is shown. The HATH region sequences are HDGF (6–96), HRP-1 (5–89), and HRP-2 (10–100). Amino acid sequences of functional interest: putative nuclear localization sequences (solid double underlines); putative casein kinase II sites (*); an A-kinase sites (A* at 523); putative glycation sites (@). Synthetic oligo-peptides used in production of rabbit Abs to LEDGF (bold letters with dotted underlines); one was an Ab to an N-terminal peptide (43–62), and the other was a Ab to a C-terminal peptide (419–437).

keyhole-limpet hemocyanin using the formaldehyde method of Harlow and Lane 1988 (24). Five weeks after the first injection, we collected immune serum. The serum was heated at 56°C for 30 minutes to inactivate complement. The anti-LEDGF Abs were purified on an immobilized protein-A Sepharose column following the Pierce protocol (Pierce, Rockford, IL). Rabbit serum containing anti-LEDGF Abs was diluted 1/100–1/200 and used in immunocytochemical experiments and in assays of the cytotoxicity of the Ab. Anti-LEDGF-Abs were neutralized with purified GST-LEDGF by adding an equal volume of GST-LEDGF (100 μg/ml) and incubating the mixture overnight at 4°C.

RESULTS AND DISCUSSION

Sequence of LEDGF. We have isolated a clone (HLC 10) from a human LEC cDNA library with serum from a 63 year old female patient with cortical ARC. The nucleotide sequence of HLC 10 was determined and deposited in the GenBank (Accession No. AF063020). A long open reading frame encoded a polypeptide with 530 amino acid residues and a calculated molecular weight of 60,103 Daltons. A search for homologous proteins revealed that the amino acid sequence of LEDGF was virtually identical to that of the transcriptional co-activator, p75 (1). Three amino acid residues in LEDGF differed from those in p75; the residue at 224 was E (instead of G); the residue at 301 was R (instead of N), and the residue at 420 was Y (instead of F) (1). Other proteins homologous to LEDGF were (1) an unknown protein (25), (2) hepatoma-derived growth factor (HDGF) (15), (3) and (4) HDGF-related-proteins-1 and -2 (HRP-1 and HRP-2) (26). The highest homologies were found in the C-terminal "HATH" **(H**omologous to the **A**mino acid **T**erminus of **H**DGF) region (26) (Fig. 1). In the 98 N-terminal amino acid residues of the "HATH" regions of HDGF, HRP-1, and HRP-2, identity was 49% and similarity was 80–90%. LEDGF contained six nuclear localization consensus sequences, several casein kinase II sites, an A-kinase site, and two putative glycation sites (N:103 and 454). The predicted secondary conformation of LEDGF was predominantly β-sheets and turns (27).

The deduced amino acid sequence was validated by Ab binding studies. Two oligopeptides were synthesized; one corresponded to the highly conserved site in the N-terminus (FFFGTHETAFLGPKDIFPYS; 43–62)

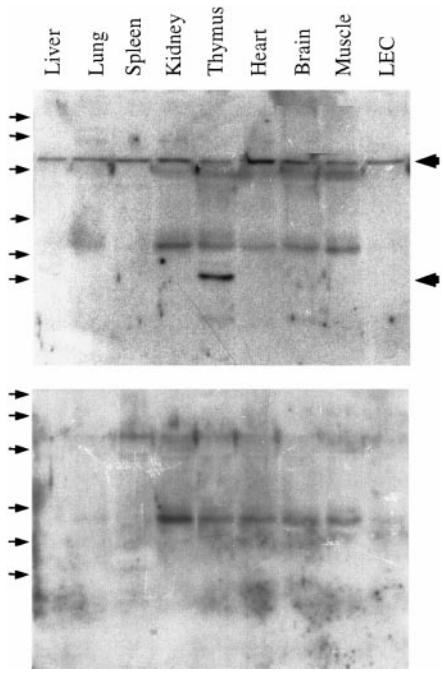

FIG. 2. Protein blot immunostained with the affinity-purified anti-(C-terminal)LEDGF Ab neutralized with GST-LEDGF. Molecular size markers are indicated by solid arrows in the left margin (from top: 108, 80, 51, 34, 27, and 16 kDa).

and the second to a specific site in the C-terminus (LYNKFKNMFLVGEGDSVIT; 419–437). Abs to each peptide were raised in rabbits. Both anti-LEDGF Abs bound to a protein with a molecular weight of 60 kDa in human and mouse LECs. In addition, both Abs bound to LEDGF expressed in *E. coli* (see below).

Tissue specificity of LEDGF. We studied protein expression by immunoblot analysis using an affinity-purified anti-(C-terminal)LEDGF Ab (Fig. 2). A 60 kDa protein (upper arrowhead) was variably expressed in all tissues. In addition a 17 kDa protein (lower arrowhead) was seen in thymus. That the 60 kDa band was LEDGF was demonstrated with duplicate blots immunostained with Ab absorbed with either purified GST-LEDGF (lower panel) or with synthetic C-terminal LEDGF oligo peptides (data not shown).

LEDGF expression in normal mouse LECs was investigated immunohistochemically with Abs to the C-terminus of LEDGF. The nuclei stained strongly; the cell surface and cytoplasm stained weakly (Fig. 3A). Nuclear staining was absent with preimmune rabbit serum (data not shown), and with Abs neutralized with purified GST-LEDGF (Fig. 3B). The results suggested that LEDGF, albeit present in the cytoplasm, was greatest in the nucleus of mouse LECs.

LEDGF stimulated attachment and shortened lag time of LECs, fibroblasts, and keratinocytes in culture. Based upon its homology to HDGF, we expected LEDGF to be a growth factor. To confirm this, we studied growth stimulation using LEC-LEDGF and LEC-vector in serum-free medium. Mouse LECs were cultured for 1 day in DMEM containing 10% FCS, then trypsinized and seeded in culture plates containing serum-free medium. Most LEC-LEDGF cells attached to the culture plate within 1 h (Fig. 4A), but LEC-

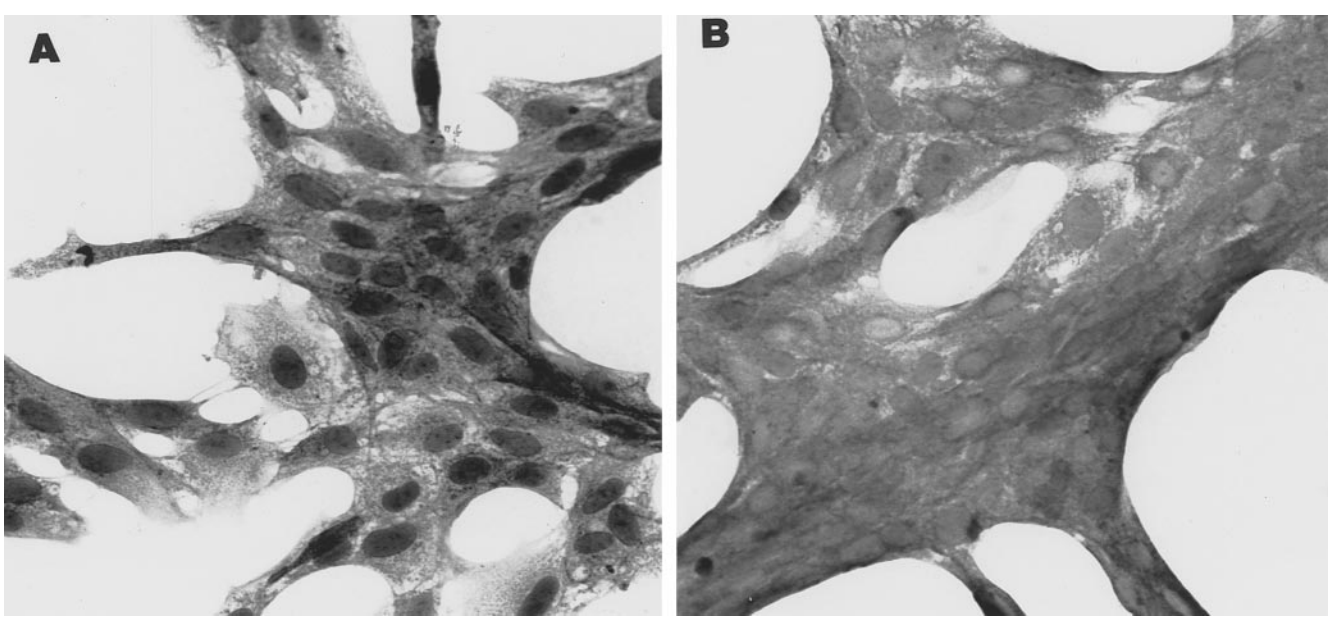

FIG. 3. Immunocytochemistry of cultured mouse LECs. Cells were immunostained with anti-(C-terminal)LEDGF Ab and visualized with a second horseradish-peroxidase-labeled Ab. Note strong nuclear staining in A. In B, mouse LECs were stained with anti-(C-terminal)LEDGF Abs neutralized with purified GST-LEDGF; note negligible staining.

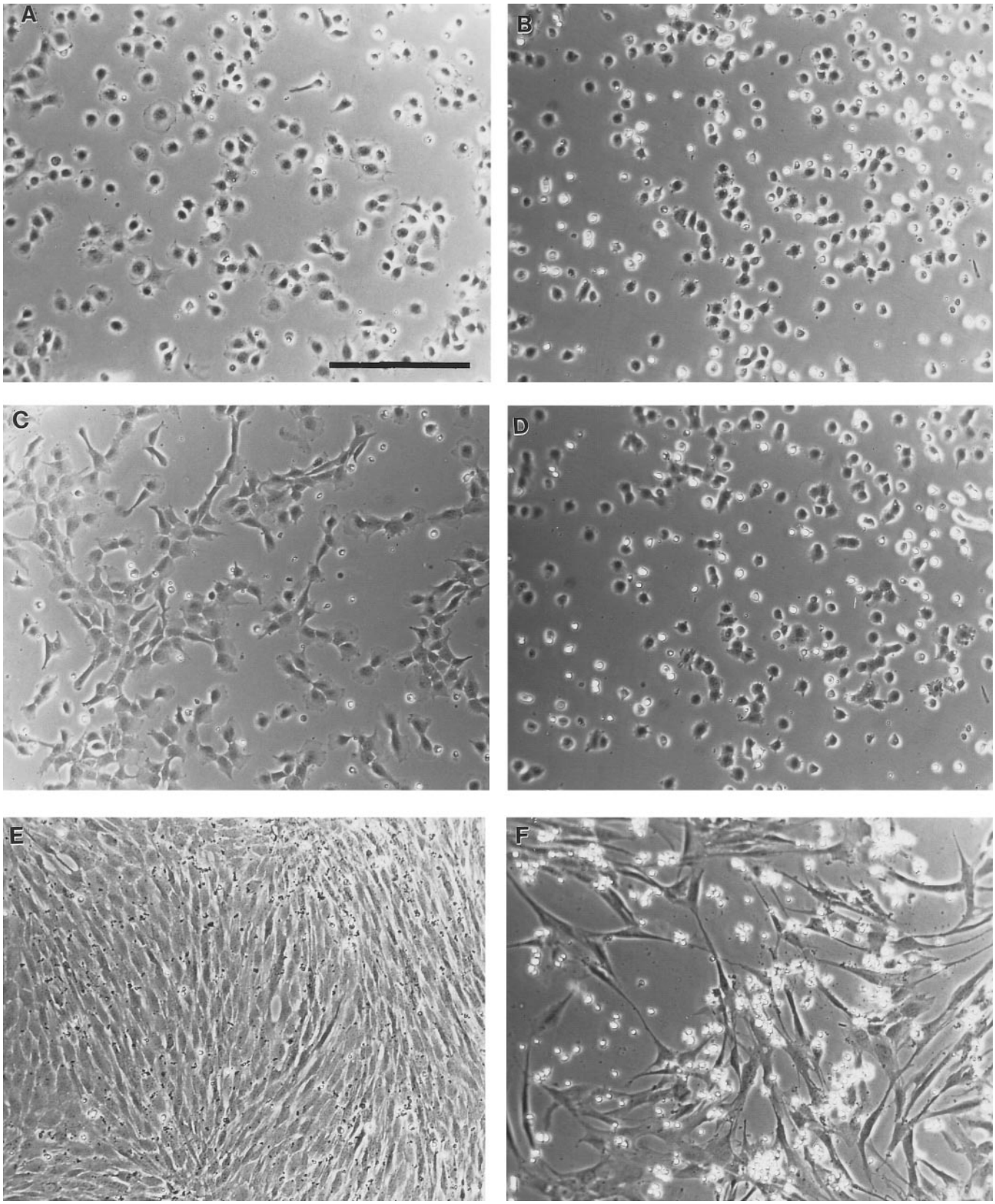

FIG. 4. Effect of overexpression of LEDGF on survival and growth of mouse LECs. Stably transfected mouse LEC-LEDGF cells (A, C, and E) and LEC-vector cells (B, D, and F) were cultured in serum-free DMEM for 3 days. The cell attachment to plastic dishes, enhancement of survival, and growth stimulation were observed in the transfected LEC-LEDGF cells 1 h (A and B), 6 h (C and D), and 3 days after inoculation (E and F). The photographs were taken under identical conditions.

vector cells took more than 6 h to manifest the same level of attachment (Fig. 4D). The growth of LEC-LEDGF cells was obvious by 6 h (Fig. 4C); LEC-vector cells took nearly 24 h manifest the same growth. By 3 days, LEC-LEDGF cells reached confluence (Fig. 4E); the LEC-vector cells showed many fewer growing cells and more dead cells (Fig. 4F). These results indicated that over-expression of LEDGF in LECs stimulated cell attachment, shortened the initial lag in growth, and enhanced the rate of growth. In a parallel study of cos7-LEDGF cells, attachment and growth were faster than in cos7-vector cells (data not shown).

LEDGF as a survival Factor for LECs, fibroblasts, and keratinocytes in culture. To ascertain the effect of cell density of the initial inoculum on cell survival, LECs were inoculated at densities of 10,000, 5000, 2500, 1250, and 600 cells/microtiter well in DMEM containing 10% FCS. Most cells attached to the bottom of the well during the first 24 h. Then each well was gently washed twice with 100 μl of serum-free DMEM, and the culture continued in serum-free DMEM. The culture medium was changed daily to remove endogenously secreted LEDGF, and the numbers of live cells were counted under the microscope. By the 4th day, cells incubated without LEDGF at a density of 600 cells/well were dead; in wells with 1200, 2500, and 5000 cells/well 36%, 84%, and 101% of the cells survived, respectively. The number of cells in the well containing 10,000 cells/well actually increased 22%. Thus, we selected 600 cells/well as the optimum cell density to study the survival effects of LEDGF.

In a separate set of experiments, all conducted at a density of 600 cells/well, mouse LECs cultured without LEDGF or GST-LEDGF or with 0.01 ng/ml of LEDGF or GST-LEDGF died; cells cultured with LEDGF or GST-LEDGF at concentrations of $\geq$0.1 ng/ml survived. Approximately 100% of cells cultured with 1 ng LEDGF/ml or 1 ng GST-LEDGF/ml survived, but 100% of cells cultured with 10–100 ng LEDGF or GST-LEDGF/ml survived and grew slightly (Fig. 5A). These results indicated that LEDGF or GST-LEDGF at concentrations of 1.0 ng/ml prolonged the survival of mouse LECs in serum-free medium.

Next, we investigated if exogenous LEDGF prolonged survival of non-lenticular cells. In experiments similar to those in the paragraph above, we studied the survival of fibroblasts and keratinocytes. Keratinocytes and fibroblasts were cultured with or without 10 ng GST-LEDGF or GST/ml (Figs. 5B and 5C). At a cell density of 5,000 cells/well and a GST-LEDGF concentration of 10 ng/ml, all cells survived and grew for 6 days in serum-free medium. In contrast, the majority of cells incubated without GST-LEDGF did not survive (Figs. 5B and 5C). These results indicated that exogenous GST-LEDGF promoted survival of LECs, fibroblasts, and keratinocytes in serum-free medium.

Rabbit anti-LEDGF Abs killed LECs, fibroblasts, and keratinocytes in culture. To confirm the importance of GST-LEDGF as a survival factor, we blocked the GST-LEDGF effect with serum containing an anti-(C-terminal)LEDGF Ab. First, human LECs were cultured for 14 days in DMEM with 20% FCS to which we added pre-immune rabbit serum (1/100 dilution), or serum from a rabbit immunized with the C-terminal peptide of LEDGF. For 6 days LECs incubated with and without the anti-LEDGF Abs grew well, but on the 7th day, a large number of cells incubated with this serum manifested damage. By the 14th day, most cells incubated with this serum were dead. Control cells incubated with added pre-immune rabbit serum grew normally throughout the 14-day period (Fig. 6).

Human fibroblasts and mouse keratinocytes were cultured in medium containing 10% FCS and either pre-immune serum or serum containing anti-(C-terminal)LEDGF Abs (1/100 dilution). By 6–8 days, in the presence of the anti-LEDGF Abs, most fibroblasts and keratinocytes were dead. Cells cultured with pre-immune rabbit serum grew continuously for 6 days (Figs. 7A and 7B). Rabbit serum neutralized with 100 μg purified GST-LEDGF/ml, not only lost its cytotoxicity, but also stimulated cell growth (data not shown).

We reported the discovery of LEDGF in May 1998 at the Annual Meeting of the Association for Research in Vision and Ophthalmology (13). Although we knew then that LEDGF was homologous to HDGF family of proteins, we did not know until January 1999 that the sequence of LEDGF was nearly identical to that of a transcriptional co-activator and pre-mRNA splicing factor, p75. p75 and p52 stimulated transcriptional activity of the ubiquitous genes in Hela cells (1) and pre-mRNA splicing (2). Our results taken together with those of Ge *et al.* (1, 2) indicate that LEDGF is a pluripotential protein essential for cellular survival.

Recently we reported that LECs overexpressing LEDGF produced more heat shock protein 27 and αB-crystallin (14) and manifested increased resistance to a variety of stresses (serum starvation, heat, and oxidation). Prolonged survival of these cells may be due to the higher expressed levels of these heat shock proteins. We have shown also that heparin potentiates the growth-enhancing properties of LEDGF, protects it from proteolytic degradation, and facilitates its uptake into the cytoplasm and its transport into the nucleoplasm (26).

LEDGF belongs to a new family of growth factors. LEDGF (p75 protein), p52 protein, HDGF, HRP-1 and HRP-2 share a homologous sequence in the HATH region, but the remaining parts of the sequences are not homologous (27). HDGF was first isolated from a human hepatoma-derived cell, Hu-7. It, like LEDGF, stimulated growth of most cell types (15). HDGF is present in the cytoplasm, but not in the nucleus (15). LEDGF in contrast is present in the cytoplasm and

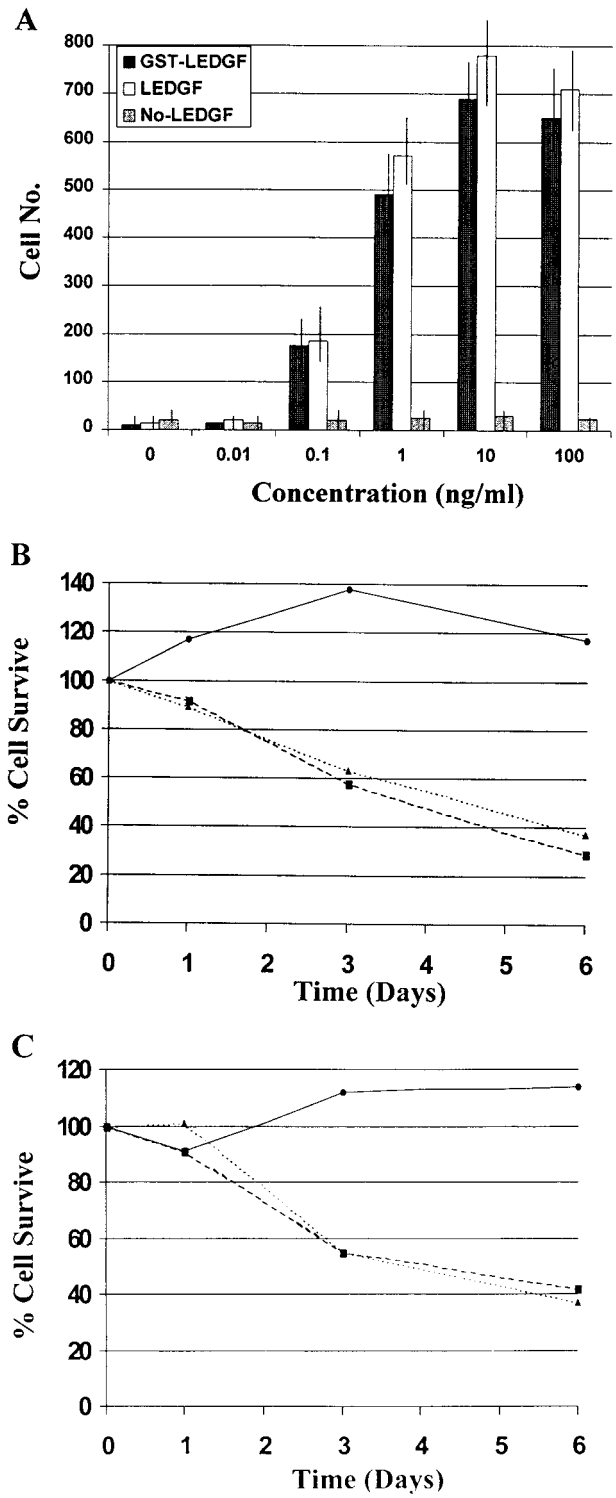

FIG. 5. Effect of LEDGF and GST-LEDGF on the survival of (A) mouse LECs, (B) human fibroblasts, and (C) mouse keratinocytes. LECs (A) (600 cells/well in a 96 well plate) were cultured for 4 days in serum-free DMEM with various amounts of GST-LEDGF or LEDGF. LECs survived well in concentrations of 0.1–100 ng GST-LEDGF or LEDGF/ml. LECs in cultures lacking LEDGF or having less than 0.01 ng GST-LEDGF or LEDGF/ml died. Fibroblasts (B) (5000 cells/well) and keratinocytes (C) (5000 cells/well) were cultured for 6 days in serum-free medium with 10 ng GST-LEDGF/ml (●), 10 ng GST/ml (■), or medium alone (▲). Ten ng GST-LEDGF/ml or 1.0 ng GST/ml were added at the 0 and 3rd day in culture media. Without LEDGF, these cells died or did not grow. The standard deviation was less than 10%. Cell survival was evaluated by the MTT method.

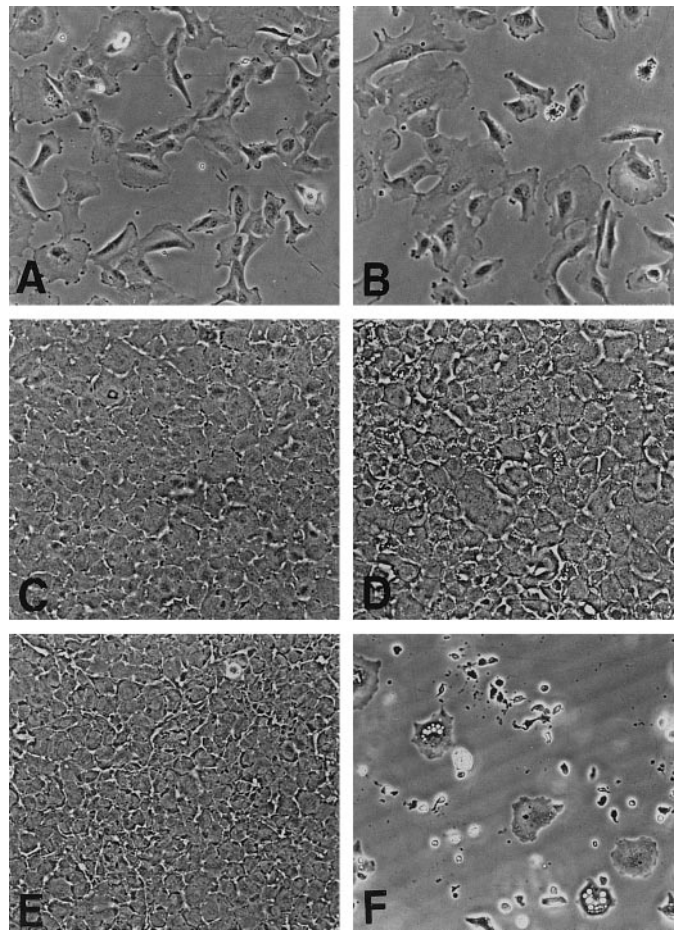

FIG. 6. Effects of the Abs to the C-terminus of LEDGF on human LEC survival in culture. Human LECs were cultured with anti-(C-terminal)LEDGF Abs (B, D, and F) or with a control preimmune rabbit serum (A, C, and E). An equal number of LECs was inoculated (A and B) and the cells grew well for the first 4–5 days (C and D). After 7 days in culture, the cells began to die, and by the 14th day (F), almost all LECs in cultures with Abs to LEDGF had died. In LEC cultures with preimmune serum, the cells grew normally during the experimental period of 14 days (E).

nucleoplasm of most cell types. HRP-1 (248 residues) is a testis-specific protein; HRP-2 (670 residues) is a ubiquitous protein, and both HRP-1 and -2 have nuclear localization consensus sequences (28). p75 and p52 proteins are thought to be products of alternative splicing of a single gene (1). All members of this family appear to have affinity to heparins (26). Thus these homologous proteins may have functions similar to those of LEDGF.

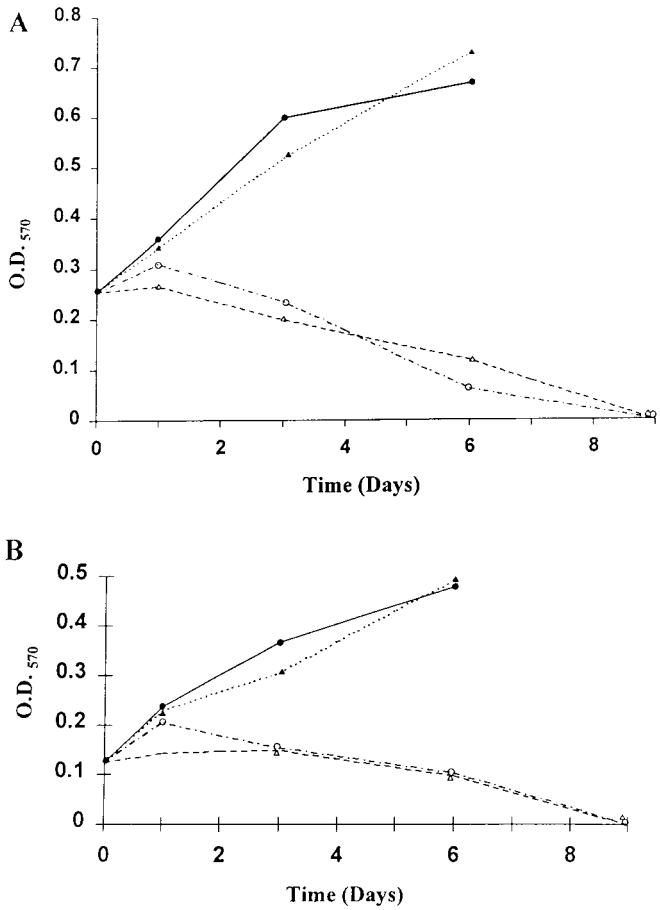

FIG. 7. Effects of the Abs to LEDGF on (A) fibroblasts and (B) keratinocytes. Cells were cultured in media having 10% FCS with (○, △) and without (▲, ●) one of two Abs to LEDGF: (anti-(N-terminal)LEDGF (○ or anti-(C-terminal)LEDGF) (△)). Medium without anti-LEDGF Abs had rabbit anti-GST Abs (●, solid line) or no Abs (▲, dotted line).

Cell death can be prevented or delayed *in vivo* by experimentally increasing the level of survival factors (29, 30). Many growth factors have been found to be survival factors. Among them, basic FGF, interleukin, gamma interferon, and IGF, are transported into the nucleus where they exert a functional role. In addition, acidic FGF (9), and basic FGF-2 (10) are known to be survival factors for LECs. Transferrin is also a survival factor in LECs (31) and in Hela cells (32). How LEDGF enhances survival of a wide range of cell types is under investigation in our laboratory.

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