MOLECULAR AND CELLULAR BIOLOGY, Nov. 1989, p. 4587–4595
0270-7306/89/114587-09$02.00/0
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Vol. 57, No. 11

Response of Bone Marrow Stromal Cells to Adipogenic Antagonists

JEFFREY MARTIN GIMBLE,[1]* MARIE-ANNETTE DORHEIM,[1] QI CHENG,[2] PHILLIP PEKALA,[3] SVEN ENERBACK,[4] LARRY ELLINGSWORTH,[5] PAUL W. KINCADE,[1] AND CHI-SUN WANG[2]

Immunobiology and Cancer Program[1] and Lipoprotein/Atherosclerosis Research Program,[2] Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104; Department of Biochemistry, East Carolina School of Medicine, Greenville, North Carolina 27858[3]; Department of Physiological Chemistry, University of Gothenburg, Gothenburg, Sweden[4]; and Celtrix Laboratories, Collagen Corporation, Palo Alto, California[5]

Received 6 June 1989/Accepted 21 July 1989

Adipocytes constitute a major part of the bone marrow stroma in vivo and may play an active role in lymphohematopoiesis. Earlier studies had shown that the bone marrow stromal cell clone BMS2 was capable of adipocyte differentiation in vitro, in addition to its well-defined ability to support B lymphopoiesis. We now demonstrate that the process of adipogenesis in this functional bone marrow stromal cell clone can be inhibited by the cytokines interleukin-1α, tumor necrosis factor, and transforming growth factor β. Exposure of preadipocyte BMS2 cells to these agents blocked the induction of adipocyte differentiation as assessed by morphologic criteria and analysis of the neutral lipid content. Both interleukin-1α and tumor necrosis factor elicited a rapid transient elevation in the steady-state mRNA levels of c-*fos*, c-*jun*, and JE. When added to differentiated adipocytes, the three cytokines continued to act as adipogenic antagonists. This was indicated by concentration- and time-dependent decreases in the activity of an adipocyte-specific enzyme, lipoprotein lipase. These changes in enzyme activity correlated directly with a decrease in steady-state levels of lipoprotein lipase mRNA. Another RNA marker of adipocyte differentiation (adipsin) was less influenced by the adipogenic antagonists. This may reflect the longer half-life of this mRNA transcript compared with those of lipoprotein lipase. Our results dramatically demonstrate that the differentiation state of bone marrow stromal cells can be modulated by exogenous factors in vitro. It is also the first report that transforming growth factor β regulates the activity of lipoprotein lipase. These data suggest potential physiologic actions for these cytokines in vivo within the overall context of lymphohematopoiesis.

Bone marrow stromal cells are necessary for the support of lymphohematopoiesis, providing the appropriate cell-cell interactions and cytokines required for the differentiation and proliferation of the blood lineage progenitor cells (13, 25, 28, 58, 59). Although stromal cells serve in a regulatory capacity, they are themselves subject to control by exogenous factors (19, 30, 36, 40, 46, 61). Both autocrine and paracrine pathways may modulate stromal cell function in vivo. By using long-term bone marrow culture techniques, it has been possible to clone stromal cell lines capable of supporting B-lymphocyte proliferation and development in vitro (40; J. M. Gimble et al., submitted for publication); the BMS2 cell line derived in our laboratory is one example (40). In common with some stromal cell clones, BMS2 cells exhibited a fibroblastoid morphology when passaged frequently, yet were capable of spontaneous differentiation into adipocytes when left for several weeks in culture. Adipocytes make up a significant percentage of the bone marrow stroma in adult mammals, increasing with the age of the organism (12, 24, 54). Unlike extramedullary adipocytes, bone marrow adipocytes do not respond to starvation with increased lipolysis (2). However, lipolysis did increase in bone marrow adipocytes during periods of anemia and increased erythropoiesis (1). For these and other reasons, the process of adipogenesis has been postulated to differ in bone marrow and extramedullary sites (20, 21). We have recently characterized the initiation of adipogenesis at the biochemical and molecular biologic levels in the BMS2 cell line (40; Gimble et al., submitted). The addition of hydrocortisone and a xanthine derivative accelerated adipocyte differentiation and induced a number of specific enzymes and mRNAs, including lipoprotein lipase (LPL), glycerol phosphate dehydrogenase, adipsin, and adipocyte P2 (Gimble et al., submitted). These results demonstrated that lipid metabolism in bone marrow adipocytes was similar in most respects to that in extramedullary adipocytes.

The cytokines interleukin-1α (IL-1), tumor necrosis factor (TNF), and transforming growth factor β (TGF β) have all been reported to act as adipogenic antagonists in extramedullary adipocyte models (3, 4, 10, 26, 34, 38, 41, 42, 56, 57, 62). We now show that IL-1, TNF, and TGF β act as antagonists to adipogenesis in a bone marrow stromal cell line capable of supporting B lymphopoiesis in vitro. The effects of these agents were examined on cells at the preadipocyte and adipocyte stages of development. The responses of early active genes (c-*fos*, c-*jun*, and JE) as well as genes reflecting commitment to adipocyte differentiation (LPL and adipsin) were determined with respect to time and dose of each cytokine. These findings have important implications regarding the physiologic roles of IL-1, TNF, and TGF β in the bone marrow microenvironment.

MATERIALS AND METHODS

Cell culture and induction studies. BMS2 cells (40) were cultured in Dulbecco modified Eagle medium (4,500 mg of sucrose per liter) supplemented with 10% fetal bovine serum (no. 240-6000, lot 32N0079 (GIBCO Laboratories, Grand Island, N.Y.; or no. A-1110-L, lot 1111794; Hyclone, Logan, Utah), 1 mM pyruvate, 100 mg of streptomycin per ml, and 100 U of penicillin per ml. Cells were maintained in 35-mm plastic culture dishes (Lux 5214; Nunc, Napierville, Il.) at 37°C and 7% CO_2 in a humidified atmosphere. Cells were initially passaged at 3×10^3 cells per cm^2.

* Corresponding author.

Preadipocyte BMS2 induction studies were performed on cultures which had reached confluence and had been allowed to reach a quiescent state (no medium change for 72 to 96 h prior to the experiment). Cytokines were added in a volume comprising 1% of the medium in the culture; no fresh serum was introduced at this time. The following concentrations were introduced: IL-1, 1,000 U/ml (courtesy of S. Gillis, Immunex, Seattle, Wash.); TNF, 20 U/ml (Endogen, Boston, Mass.); and TGF β, 10 ng/ml (Collagen Corp., Palo Alto, Calif.) (48). At various times from 0 to 240 min, the cultures were harvested for total RNA preparation (see below).

Adipocyte differentiation was induced by treatment of confluent monolayers of cells with 0.5 mM methylisobutyxanthine (MIBX; Sigma Chemical Co., St. Louis, Mo.)–0.1 μM hydrocortisone (Elkin-Sinn, Cherry Hill, N.J.) (Gimble et al., submitted) or by passage of the cells in medium containing 0.5 mM MIBX, 0.5 μM hydrocortisone, and 5 μg of insulin per ml and supplemented with 5% calf serum. After a 48-h induction period, the medium was replaced with fresh medium without added factors. Adipogenic antagonists were added during this initial 48-h period at the following concentrations: IL-1, 1,000 U/ml (provided courtesy of S. Gillis, Immunex); TNF, 20 U/ml (Endogen); TGF β, 1 μg/ml (Collagen Corp.). Both IL-1 and TNF were maintained at these concentrations throughout the experiment, while TGF was present only during the initial 48-h induction period. After an additional 2 weeks or more in culture, a significant proportion of the cells had attained an identifiable adipocyte morphology in the absence of antagonists.

Treatment of mature adipocytes with adipogenic antagonists. Two weeks following adipocyte induction, cultures were treated for various lengths of time with adipogenic antagonists at the following concentrations: IL-1 (courtesy of S. Gillis; stock concentration, 10^6 U/ml), 1, 10, 100, or 1,000 U/ml; TGF β (courtesy of Larry Ellingsworth, Collagen corp.; stock concentration, 1 μg/ml), 0.01, 0.1, 1, or 10 ng/ml; TNF (Endogen; stock concentration, 4,000 U/ml), 1, 5, 10, 20, or 50 U/ml. After various periods of time (2 to 24 h) in the presence of these agents, the medium was removed and replaced with 1 ml of medium supplemented with 10 U of heparin per ml (Sigma), and the cultures were incubated for an additional hour at 37°C. Each data point was performed in triplicate. The supernatant was harvested and stored at −70°C immediately for the subsequent determination of LPL activity. The adherent cells were suspended in 0.5 ml of 4 M guanidium isothiocyanate prior to purification of the total RNA.

LPL assay. Enzyme activity was determined according to a modification of the method of Nihlsson-Ehle and Schotz (37). The substrate used was lecithin (dioleoylglycerol)-emulsified glycerol tri[9,10-^{3}H]oleate with a lecithin/trioleoylglycerol molar ratio of 1:10 and a specific activity of 1.4 μCi/μmol. In a final volume of 200 μl, the reaction consisted of 50 mM NH_4OH-HCl, pH 8.5, 1.4 mM trioleoylglycerol, 60 mg of bovine serum albumin (as a fatty acid acceptor) per ml, 2% human serum (as a source of lipase activator), and a 10-μl sample of heparin-released LPL. Following a 1-h incubation at 37°C with agitation, the addition of 3.2 ml of chloroform-heptane-methanol (5:4:5.6, vol/vol/vol) and 1 ml of 0.2 M NaOH terminated the reaction. Samples were centrifuged, 1.2 ml of supernatant was removed and mixed with 10 ml of Instagel (Packard Instrument Co., Inc., Rockville, Md.), and the radioactivity was determined in a Packard liquid scintillation counter. One milliunit of activity represented the release of 1 nmol of fatty acid per min at 37°C. Each reaction was performed in triplicate. Activities were expressed as a percentage relative to control samples incubated in the absence of adipogenic antagonists. Control values were defined as 100%.

RNA purification and Northern (RNA) blot analysis. The total RNA was purified by a modification of the method of Chomczynski and Sacchi (6). The guanidium isothiocyanate cell suspension was acidified with sodium acetate, extracted with phenol-chloroform, and twice precipitated with 2-propanol. The purified total RNA from three 35-mm plates was suspended in 100 μl of diethylpyrocarbonate (Sigma)-treated water. Approximately 30 μg of total RNA was obtained, based on UV A_{260}/A_{280}. Twenty-microliter volumes containing approximately 6 μg of total RNA were mixed with 10 μl of 5.5% formaldehyde–50% formamide, heated at 65°C for 5 min, and loaded onto a 1% agarose–0.55% formaldehyde gel (55). The gel was electrophoresed at 40 V for a period of 6 to 7 h and transferred by capillary action to a Nytran (Schleicher & Schuell, Keene, N.H.) nylon membrane, and the RNA was cross-linked by UV irradiation (7). Prior to hybridization, the blots were washed for 1 h at 65°C in 0.1% sodium dodecyl sulfate (SDS)–1× sodium chloride-sodium citrate buffer (SSC; 150 mM NaCl plus 15 mM sodium citrate, pH 7.0).

Random priming and hybridization. The following cDNA probes were used: hamster CHO-B (22), courtesy of Randolph Wall, UCLA, Los Angeles, Calif.; guinea pig LPL (10, 16); murine adipsin, courtesy of Howard Green, Harvard University, Cambridge, Mass. (50); murine c-*fos* (pc-*fos*, m3), courtesy of John Sisson and Inder Verma, Salk Institute, La Jolla, Calif. (33); murine c-*jun* (AH119), courtesy of Rodrigo Bravo, EMBL, Heidelberg, Federal Republic of Germany (47); murine JE, courtesy of Charles D. Stiles, Dana Farber Cancer Institute, Boston, Mass. (8). The probes were radiolabeled with [α-^{32}-P]dCTP, 3,000 Ci/mmol (ICN, Irvine, Calif.), to a specific activity of >10^8 cpm/μg by the random primer method of Feingold and Vogelstein (18),

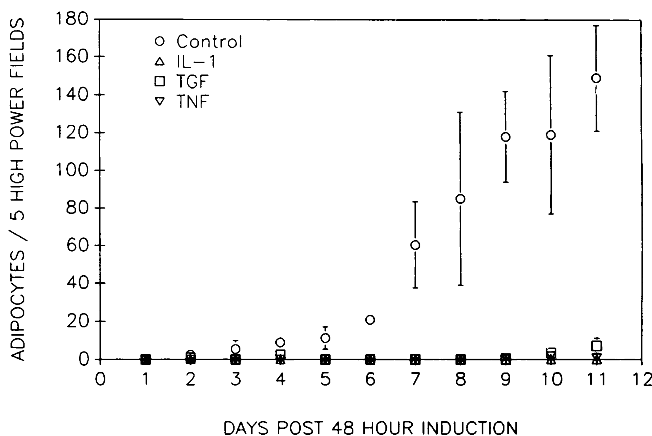

FIG. 1. Adipogenesis in BMS2 stromal cells: response to antagonists. Confluent preadipocyte BMS2 cultures were induced with 0.5 mM MIBX and 0.1 μM hydrocortisone alone (control) or in the presence of adipogenic antagonists at the following concentrations: IL-1 (Immunex), 1,000 U/ml; TGFβ (Collagen Corp.), 1 ng/ml; TNF (Endogen), 20 U/ml. The cells were incubated for 48 h, and the medium was replaced. The concentrations of IL-1 and TNF were maintained throughout the length of the study, while TGFβ was present for only the 48-h induction period. The cells were examined daily under phase-contrast microscopy, and the number of adipocytes per five fields (×32 magnification) was determined. Each value represents the mean of three experiments conducted in parallel.

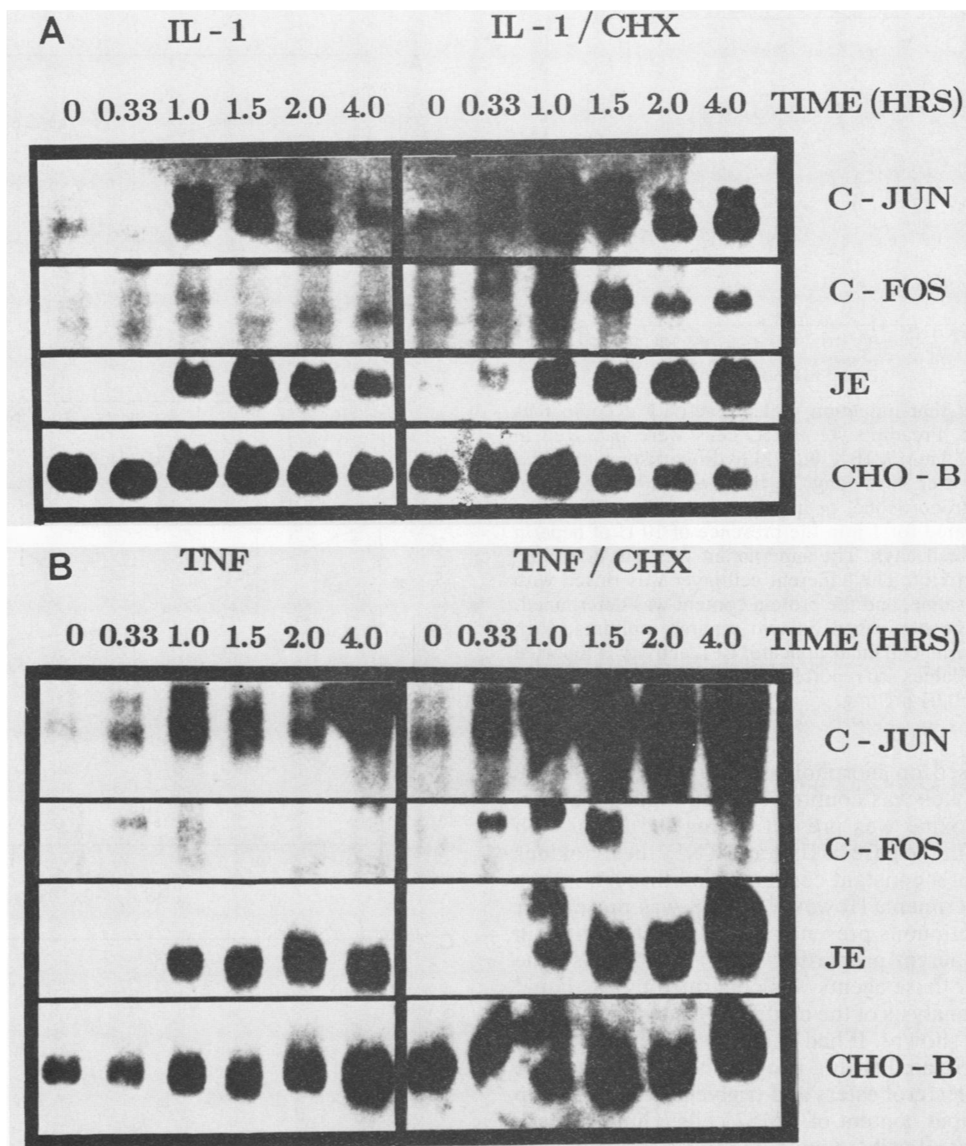

FIG. 2. Early active gene response in BMS2 preadipocytes exposed to IL-1 and TNF. Confluent cultures of preadipocyte BMS2 cells were maintained in culture for 72 to 96 h without the addition of fresh medium. At time zero, either 1,000 U of IL-1 (A) or 20 U of TNF (B) per ml was added in a volume no greater than 1% of the culture medium. Experiments were performed in the absence or presence of cycloheximide (CHX) at 20 μg/ml. Cells were harvested for total RNA in guanidium isothiocyanate (4 M) at 20 to 240 min after the addition to the cytokine. Northern blots were prepared with approximately 7 μg of total RNA per lane and successively hybridized with radiolabeled probes for c-*fos*, c-*jun*, JE, and CHO-B. The c-*fos*-specific band is the uppermost band observed in each lane. The apparently high signal intensity after 4 h of exposure to TNF alone is an artifact appearing on this particular autoradiograph.

using a commercial kit (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Northern blots were prehybridized for 4 h at 42°C in 10 ml containing 50% formamide, 5× SSC, 5× Denhardt solution, 0.1% SDS, 5 mM EDTA, 100 μg of yeast RNA per ml, 100 μg of sheared salmon sperm DNA per ml, and 10 μg of polyadenylic acid per ml. The radiolabeled probes were heated at 90°C for 10 min and suspended in 10 ml of 40% formamide–10% dextran sulfate–5× SSC–1× Denhardt solution–20 mM sodium phosphate, pH 6.8–0.2% SDS–5 mM EDTA, 100 μg of yeast RNA per ml–100 μg of salmon sperm DNA per ml–10 μg of polyadenylic acid per ml. The prehybridization solution was drained from the blot and replaced with the radiolabeled probe in hybridization solution, and the blot was incubated at 42°C for 14 h. The blot was then washed at low stringency (2× SSC–0.1% SDS, 50°C, 30 min) and high stringency (0.1× SSC–0.1% SDS, 50°C, 30 min), wrapped in cellophane while wet, and exposed with an intensifier screen for 1 to 6 days at −70°C. Autoradiographs were developed and scanned with an LKB model 2202 Ultrascan Densitometer (Pharmacia, Inc., Piscataway, N.J.) to determine the relative signal intensities.

RESULTS

Response of preadipocyte BMS2 cells. It was found previously that the process of adipogenesis in BMS2 cells can be condensed into a 2- to 3-week period following a 48-h stimulation with 0.5 mM MIBX–0.1 μM hydrocortisone (Gimble et al., submitted). The addition of the antagonists IL-1, TNF, and TGFβ blocked the induction of adipocyte

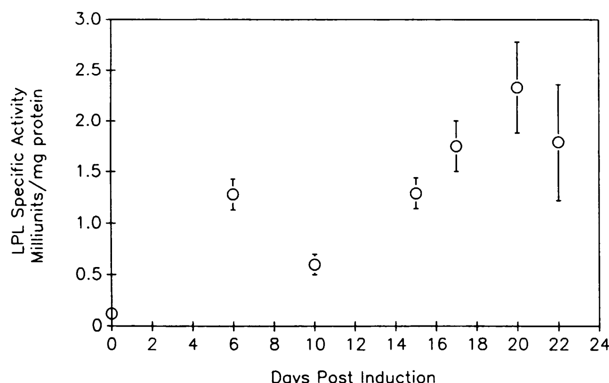

FIG. 3. Evidence that induction of LPL activity accompanies BMS2 adipogenesis. Preadipocyte BMS2 cells were passaged in medium containing 0.5 mM MIBX, 0.5 μM hydrocortisone, and 5 μg of insulin per ml. After 48 h (day 3), the medium was replaced without MIBX, hydrocortisone, or insulin supplements. Duplicate cultures were incubated for 1 h in the presence of 10 U of heparin per ml on the indicated days. The supernatant was harvested and assayed for LPL activity. The adherent cell layer was rinsed with phosphate-buffered saline, and the protein content was determined. All experiments were performed on two separate cultures; each LPL assay was performed in quadruplicate. LPL activity is reported as specific activity. Values are reported with the standard deviation; at day 0, this was ±0.03 mU/mg.

differentiation based on morphologic criteria (Fig. 1). The number of adipocytes was counted daily in replicate experiments. Each cytokine was present during the initial 48-h induction period. In the case of IL-1 and TNF, the cytokines were maintained at a constant concentration throughout the course of the experiment. However, TGFβ was present for 48 h only; the continuous presence of this agent resulted in the loss of the adherent properties of the BMS2 cells. The inhibitory effect of these agents was confirmed by gas-liquid chromatographic analysis of the neutral lipids extracted from the cells (data not shown). It had been shown that adipocyte differentiation correlated with a progressive increase in the percentage of cholesterol esters and triglycerides making up the total neutral lipid content of BMS2 cells (Gimble et al., submitted). In control cells, adipocyte induction resulted in a shift of the neutral lipids to >80% cholesterol esters and triglycerides. However, in the presence of the antagonists, cholesterol accounted for >70% of the neutral lipids, which indicates that there was no induction of cholesterol ester or triglyceride synthesis.

The exposure of BMS2 preadipocytes to the cytokines resulted in rapid intracellular changes at the genomic level (Fig. 2). Preadipocyte cells were allowed to reach confluency and to achieve a quiescent state. After cytokines were added to the cultures, they were harvested for total RNA at different time points. The steady-state levels of mRNA for the oncogenes c-*fos* and c-*jun* and the early active gene JE were examined based on Northern blot analysis. As a control for equal loading between lanes, the blots were hybridized with a probe for CHO-B, a housekeeping gene with relatively constant expression (22). In the case of IL-1 and TNF, the 2.2-kilobase transcript of c-*fos* and the 3.4- and 3.6-kilobase transcripts of c-*jun* were induced within 20 to 60 min following the addition of the cytokines. This increase in steady-state mRNA levels was transient, returning to baseline levels within 2 to 4 h. If the experiments were performed in the presence of the protein synthesis inhibitor cycloheximide, the increases in mRNA levels were enhanced and

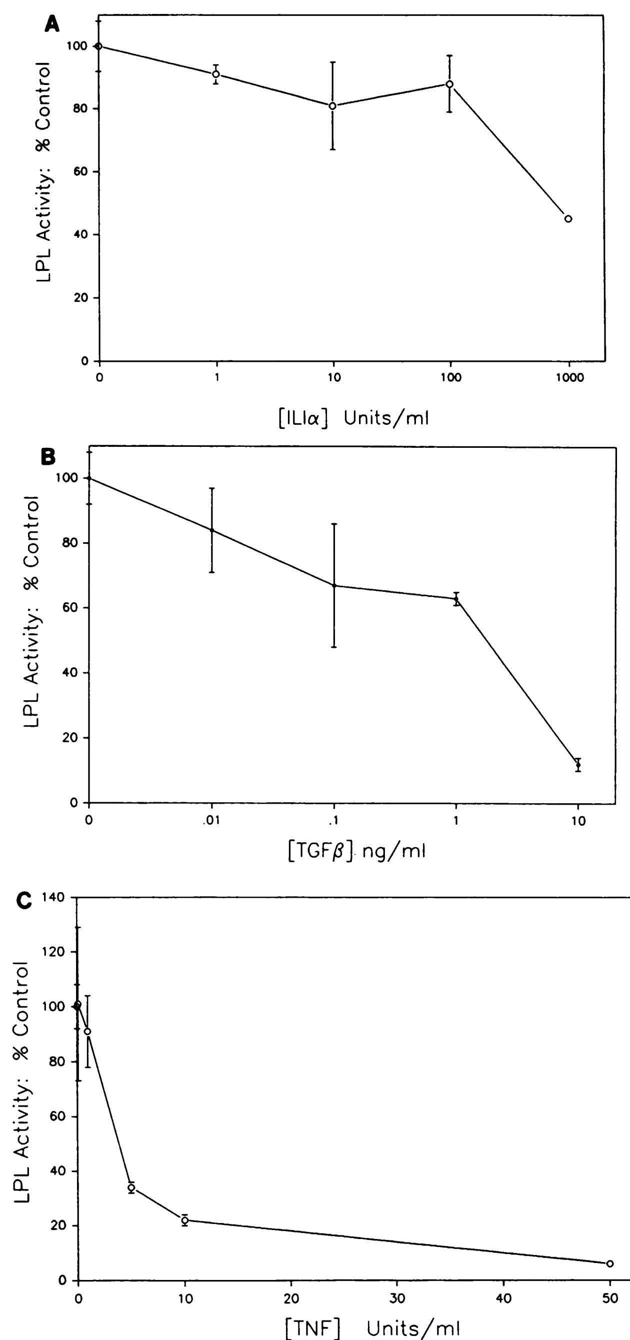

FIG. 4. Dose-dependent inhibition of LPL activity by adipogenic antagonists. Cultures of BMS2 adipocytes (2 to 3 weeks post-MIBX-hydrocortisone induction) were incubated in the presence of cytokines at the indicated concentrations for 18 h. The medium was then removed, replaced with medium containing 10 U of heparin per ml, and incubated for 1 h at 37°C. The conditioned medium was then harvested, and the LPL activity was assayed as described in Materials and Methods. Values were expressed relative to control or untreated cultures, defined as 100%; the absolute value of the control LPL activity was 5.5 ± 0.5 mU/ml. All values represent the mean of two separate experiments in which the LPL assays were performed in triplicate. Error bars indicate the standard deviation. (A) Recombinant IL-1; (B) purified TGFβ; (C) recombinant TNF.

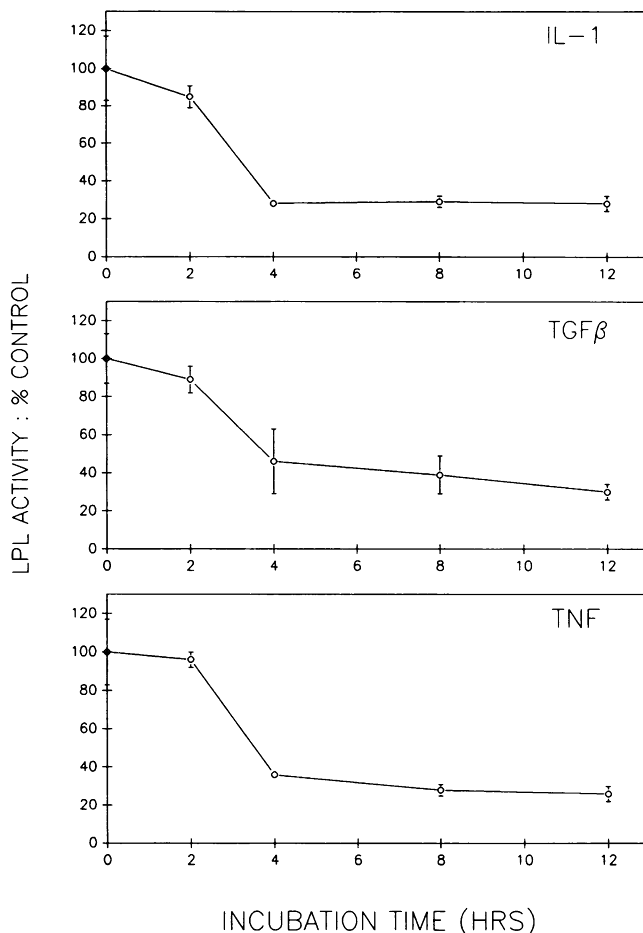

FIG. 5. Time-dependent inhibition of LPL enzyme activity by adipogenic antagonists. Confluent cultures of BMS2 adipocytes (2 to 3 weeks post-MIBX-hydrocortisone induction) were exposed for various lengths of time (0 to 12 h) to adipogenic antagonists at the following concentrations: IL-1, 1,000 U/ml; TGFβ, 10 ng/ml; TNF, 50 U/ml. At each time, the medium was replaced with medium containing 10 U of heparin per ml and incubated for 1 h at 37°C, and the conditioned medium was harvested and assayed for LPL activity. Each time point was conducted in duplicate, and LPL assays were performed in triplicate. Error bars indicate the standard deviation.

prolonged up to 4 h or more. The elevation in JE mRNA (approximately 1 kilobase in size) occurred 1 h after the addition of IL-1 or TNF and persisted for up to 2 to 4 h. The addition of cycloheximide prolonged but did not markedly enhance this increase.

The exposure of BMS2 preadipocytes to cycloheximide alone also increased steady-state levels of c-*fos* and c-*jun* mRNA. The onset of these increases was observed between 20 min and 2 h following exposure (data not shown). In contrast, the addition of TGFβ alone did not reproducibly increase the mRNA level of c-*fos* or c-*jun* (data not shown). These results demonstrate the ability of the preadipocyte BMS2 cells to respond directly to the antagonists IL-1 and TNF as well as potential differences existing among the adipogenic antagonists.

Response of adipocyte BMS2 cells. Comparison of preadipocyte and adipocyte BMS2 cells has shown that differentiation correlated with an increase in LPL expression based on mRNA analysis and enzyme activity. Following induction of BMS2 cells with 0.5 mM MIBX, 0.5 μM hydrocortisone, and 5 μg of insulin, the specific enzyme activity of LPL increased in a time-dependent fashion up to 20-fold above the preadipocyte levels (Fig. 3). Differentiated adipocytes were treated with each antagonist over a range of concentrations for 18 h (Fig. 4). Each cytokine exhibited a dose-dependent inhibition of heparin-releasable LPL activity. At the maximal effective concentrations, LPL enzyme levels had dropped to <15% of control levels. These corresponded to 10 ng of TGFβ per ml, 1,000 U of IL-1 per ml, and 50 U of TNF per ml. The inhibition process was time dependent as well (Fig. 5). Within 2 to 4 h of exposure, enzyme activity levels had fallen to 40 to 50% of pretreatment values. By 12 h of exposure, enzyme activity had dropped to 30% of control levels. The pattern of enzyme inhibition was similar for each cytokine tested.

The decrease in LPL enzyme activity correlated with a decline in steady-state LPL mRNA levels (Fig. 6). A guinea pig LPL cDNA probe was used to probe BMS2 total RNA. A comparison of mRNA from BMS2 and 3T3-L1 adipocytes, using this probe, revealed that both cells expressed two LPL transcripts of approximately 3.7 and 3.9 kilobases in size (data not shown). A Northern blot was prepared with total RNA prepared from BMS2 adipocytes exposed for various lengths of time to the antagonist cytokines IL-1, TNF, and TGFβ (Fig. 6A). As a control for equal loading in each lane, the same blot was hybridized with a probe for CHO-B. The LPL mRNA signals were compared between lanes based on densitometry normalized relative to the CHO-B signal (Fig. 6B). There was a time-dependent decrease in LPL mRNA following exposure to all cytokines. In the case of IL-1 and TNF, LPL mRNA levels fell to 30 to 40% of pretreatment values. A 24-h exposure to TGFβ resulted in a fall to 10% of control levels. In general, the decline in LPL mRNA levels correlated with the observed decrease in enzyme activity, exhibiting a similar time course and magnitude.

The mRNA level of adipsin, another marker of adipocyte differentiation, was examined on a similar Northern blot (Fig. 7). Here, treatment with TGFβ resulted in a temporal decline in adipsin mRNA levels. The magnitude of the decrease was less than that of LPL. However, neither IL-1 nor TNF consistently affected the adipsin mRNA expression over the 24 h of treatment. Prolonged exposure (4 days) to IL-1 but not TNF did decrease the adipsin mRNA levels. The difference between LPL and adipsin mRNA steady-state levels may be related to the mRNA half-lives, which vary from estimates of 1 h (LPL) to >30 (adipsin) h (34, 60). Consequently, changes in the rates of transcription may not be immediately reflected at the steady-state mRNA level.

DISCUSSION

The BMS2 stromal cell line, which is capable of supporting B-lymphocyte growth and proliferation in vitro, is subject to regulation by the adipogenic antagonists IL-1, TNF, and TGFβ. The effects of these agents were demonstrated on both the preadipocyte and adipocyte forms of these cells. At appropriate concentrations, each cytokine completely blocked the onset of adipogenesis as stimulated by hydrocortisone and MIBX. Both IL-1 and TNF elicited a transient elevation in the steady-state levels of c-*fos* and c-*jun* mRNA in preadipocyte cells, which was prolonged and enhanced by the addition of cycloheximide. These agents also increased the steady-state level of JE, an early active gene with extensive homology to the cytokines macrophage-colony-stimulating factor (M-CSF), IL-2, and IL-6 (45). In contrast,

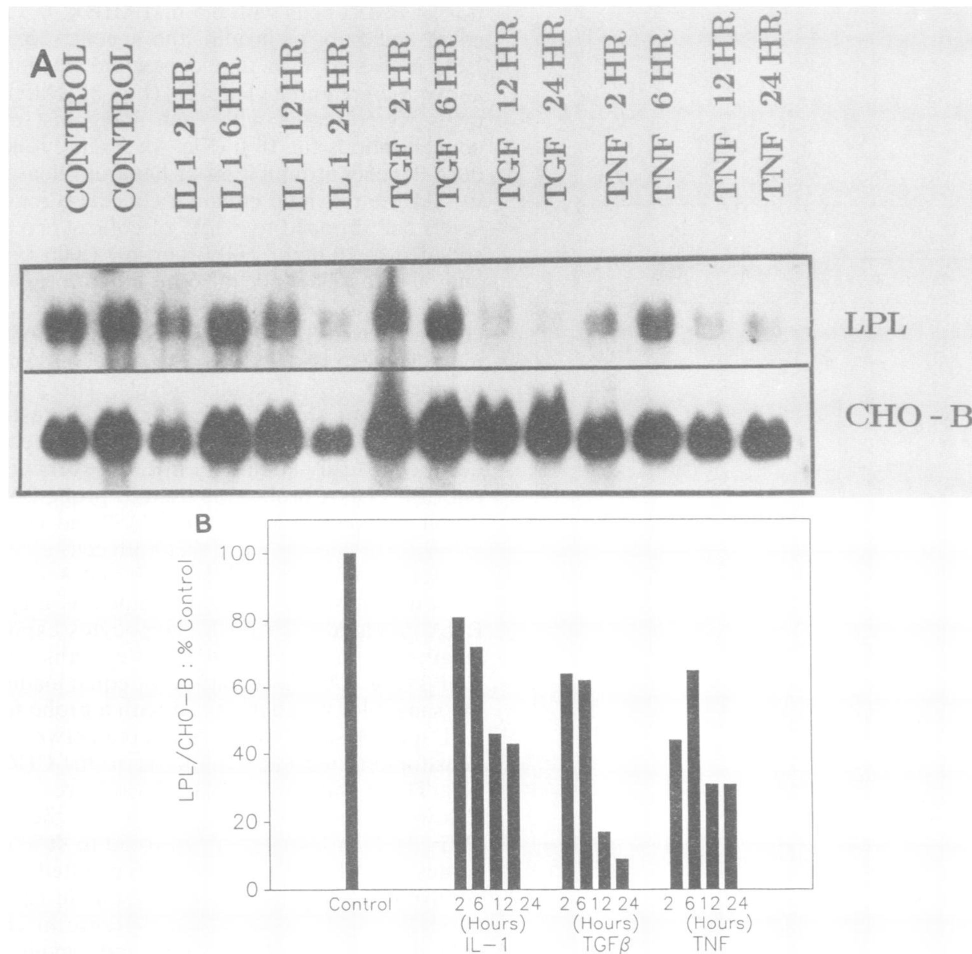

FIG. 6. Time-dependent decrease in steady-state levels of LPL mRNA following exposure to adipogenic antagonists. The total RNA from BMS2 adipocytes exposed for 0 to 24 h to IL-1 (1,000 U/ml), TGFβ (10 ng/ml), or TNF (50 U/ml) was isolated. A Northern blot was prepared with approximately 7 μg of RNA per lane. The blot was successively hybridized to a cDNA probe for guinea pig LPL and CHO-B, a housekeeping gene (A). The signal intensity was determined by laser densitometry, and the LPL signal was normalized relative to that of CHO-B (B). The LPL signal was then compared with that of control or untreated cells, defined as 100%.

TGFβ did not reproducibly elevate the mRNA levels of the oncogenes c-*fos* and c-*jun*. Adipocyte differentiation of BMS2 cells correlated directly with increased LPL enzyme activity. Treatment of fully differentiated adipocytes with all three cytokines resulted in a concentration- and time-dependent inhibition of heparin-releasable LPL activity. This was paralleled by a corresponding decrease in the steady-state level of LPL mRNA. However, the mRNA levels of another adipocyte-specific gene, adipsin, did not respond rapidly to cytokine exposure. This may reflect differences in the stability of these mRNAs rather than their transcriptional rates. Together, these data demonstrate the ability of bone marrow stromal cells to alter their differentiation state in response to monokines and other exogenous factors.

The cytokines IL-1, TNF, and TGFβ have each been reported to act as adipogenic antagonists in extramedullary adipocyte models (3, 4, 10, 17, 26, 34, 38, 41, 42, 56, 57, 62). They not only inhibit the morphologic differentiation of cells following adipocyte induction, but also return cells to their preadipocyte phenotype once differentiation has occurred. The effects of these cytokines have been followed at the level of specific genes expressed by adipocytes. In the cell line TA1, both TNF and TGFβ reduced the levels of a number of mRNAs which, although unidentified, are specific to mature adipocytes (57). In 3T3-F442A adipocytes, a 24-h exposure to 2,500 U of TNF per ml reduced the mRNA levels of adipsin and adipocyte P2 to 44 and 55% of the control, respectively; after 120 h, the levels had fallen to <10% of control (34). A number of investigators have examined the inhibitory effects of TNF and IL-1 on LPL enzyme and mRNA levels in 3T3-L1 cells (4, 10, 38, 41, 42, 62). In one study, levels of enzyme and mRNA did not necessarily correlate (62). While TNF treatment of 3T3-L1 adipocytes decreased both LPL enzyme activity and steady-state mRNA levels to 10 to 20% of control levels, IL-1 treatment only reduced enzyme activity (62). The steady-state mRNA levels remained at 70 to 80% of control levels, even at IL-1 concentrations as high as 1,000 U/ml (62). Based on their results, these authors concluded the TNF, but not IL-1, regulated LPL at the transcriptional level. IL-1 regulation was believed to occur at a later step (62). In our work, all three cytokines elicited a reduction of LPL mRNA levels to 50% or less within 12 h of treatment. Compared with adipsin, the LPL response was far more sensitive to cytokine exposure. In BMS2 adipocytes, only TGFβ appeared to reduce adipsin mRNA levels significantly. Neither TNF nor IL-1 consistently decreased the adipsin signal over a 24-h period.

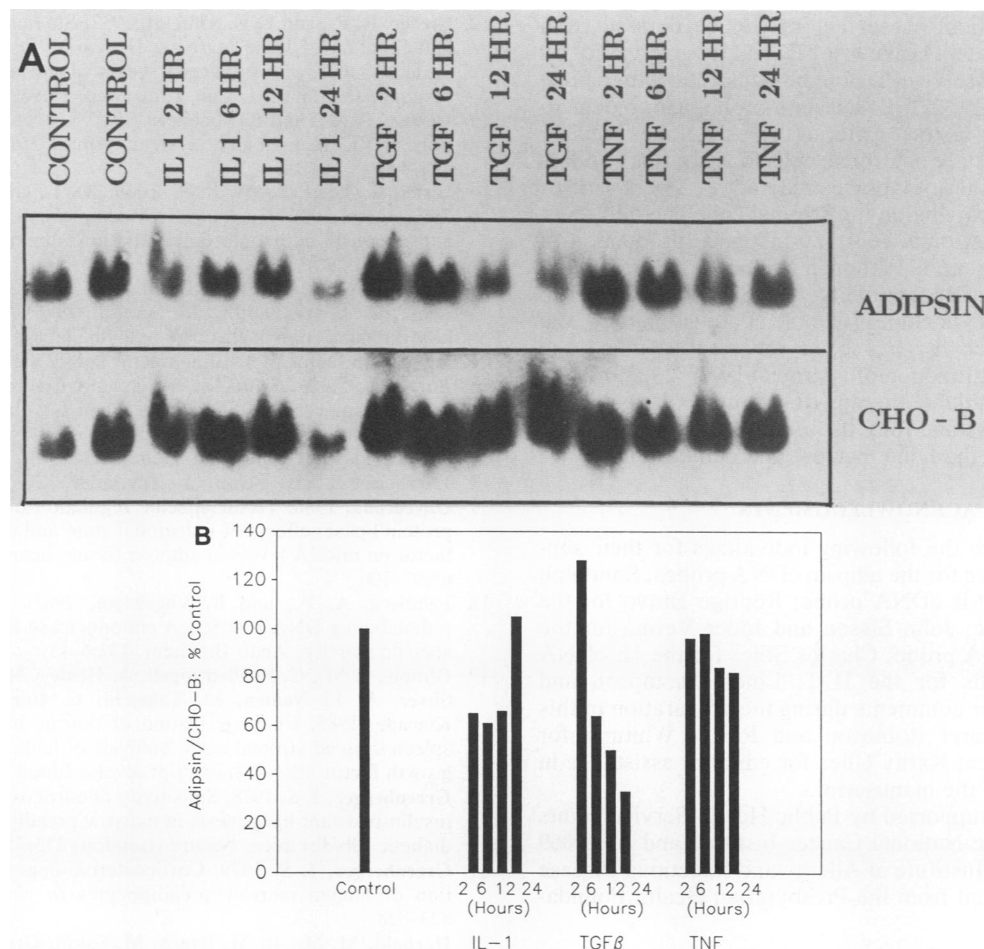

FIG. 7. Time-dependent changes in steady-state levels of adipsin mRNA following exposure to adipogenic antagonists. A Northern blot identical to that used in Fig. 6 was prepared with total RNA isolated from BMS2 adipocytes exposed to adipogenic antagonists for various lengths of time. The blot was successively hybridized with radiolabeled probes to murine adipsin and CHO-B (A). The signal intensity was determined by laser densitometry, and the adipsin signal was normalized relative to CHO-B to account for any uneven loading between lanes (B). The adipsin signal was then compared with control or untreated cells, defined as 100%.

The regulation of adipocyte-specific genes has been proposed to involve the binding of a c-*fos*/c-*jun* protein complex to specific sequence motifs within their 5′-flanking region (14, 44). The evidence for this model is most compelling for the gene adipocyte P2, for which gel retardation experiments have demonstrated that adipocyte differentiation corresponds to an alteration in the protein-DNA migration pattern. The radiolabeled DNA complex could be immunoprecipitated with antibodies directed against c-*fos* or c-*jun* protein products (14, 44). It is of interest that cytokines which clearly act as antagonists to adipocyte differentiation at both the preadipocyte and differentiated cell levels were able to induce a transient elevation in the steady-state mRNA levels of both c-*fos* and c-*jun*. Recent findings suggest that transcriptional regulation involves a number of proteins acting in concert and that a single factor may not be sufficient to control a single gene (11). Similarly, a number of cytokines have been reported to elevate the expression of both c-*fos* and c-*jun* mRNAs. These include TNF, TGFβ, insulin, and epidermal growth factor, among others (5, 9, 31, 32, 35, 39, 43, 47, 49, 51). Fluxes in the expression of these oncogenes may reflect the activation state of a cell rather than the final differentiation pathway which the stimulus elicits. Consequently, although TNF and IL-1 exposure increased c-*fos* and c-*jun* mRNA levels, this does not necessarily correlate with their activity as adipogenic antagonists.

Both TNF and IL-1 induced the steady-state mRNA level of JE, an early active gene derived from platelet-derived growth factor-induced 3T3 fibroblasts (8). This is consistent with earlier observations that IL-1 induced JE expression in 3T3 cells (45). It is of interest that the JE nucleic acid sequence contains regions of up to 60% homology with the cytokines M-CSF, IL-2, IL-6, and alpha interferon (45). Although JE has not been demonstrated to act as a cytokine (45), its induction in stromal cells may provide a model for defining its activity.

The significance of bone marrow preadipocyte stromal cell clones lies in their functional capacity to support B-lymphocyte and myelocyte proliferation in vitro, thus providing a model for events in vivo. Theoretically, by controlling the production of the various blood lineage cells, stromal cells maintain homeostasis within the circulation (28, 58, 59). Information must feed back to the stromal cell to provide coordinate regulation. Each of the cytokines examined in this work is produced by elements within the bone marrow or the circulation. Macrophages, which are considered a component of the "stroma" in vivo, are a source of IL-1 and

TNF (3). In addition, these factors can be derived from lymphocytes (52, 53). Likewise, TGFβ is expressed by stromal cells themselves, T lymphocytes, and other bone marrow cells (15, 19, 27). Based on morphologic, biochemical, and molecular biologic criteria, these cytokines regulate the differentiation state of stromal cells in vitro. It is possible that one or all of these cytokines may act in a paracrine or autocrine loop impinging on the stromal cell. The differentiation state of the stromal cell may influence production of the hematologic lineages. Although adipocyte differentiation did not appear to alter the ability of BMS2 stromal cells to support B-lymphocyte clones (Gimble et al., submitted), the presence of adipocytes has been reported to affect the myelocyte support function of a stromal layer (23, 29). With the advent of cytokine therapy for human disease, it is important to understand fully the effects of these factors on all components of the bone marrow microenvironment.

ACKNOWLEDGMENTS

We acknowledge the following individuals for their support: Howard Green for the adipsin cDNA probes; Randolph Wall for the CHO-B cDNA probe; Rodrigo Bravo for the *c-jun* cDNA probe; John Sisson and Inder Verma for the *c-fos* genomic DNA probe; Charles Stiles for the JE cDNA probe; Steve Gillis for the IL-1; Linda Thompson and Rodger McEver for comments during the preparation of this manuscript; Margaret Robinson and Randy Whitmer for technical assistance; Kathy Giles for editorial assistance in the preparation of the manuscript.

This work was supported by Public Health Service grants CA 50898 from the National Cancer Institute and AI 20069 from the National Institute of Allergy and Infectious Disease as well as by a grant from the Presbyterian Health Foundation.

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