Regulation of Cytokine and Growth Factor Gene Expression in Human Bone Marrow Stromal Cells Transformed With Simian Virus 40


Marrow stromal cells are thought to regulate hematopoiesis by producing colony-stimulating factors (CSFs) and other cytokines, either constitutively or in response to mediators such as interleukin-1α (IL-1α) or tumor necrosis factor-α (TNFα). The mechanisms by which these inflammatory cytokines induce CSF expression in stromal cells are not fully defined. In this study, we used human marrow stromal cells transformed by simian virus 40 (SV-MSCs) to study growth factor and cytokine gene regulation in response to IL-1α and TNFα. IL-1α induced significant and prolonged increases in steady-state mRNA levels for interleukin-6 (IL-6), interleukin-1β (IL-1β), granulocyte-macrophage CSF (GM-CSF), and, to a lesser extent, granulocyte-CSF (G-CSF); this induction was not dependent on new protein synthesis. Nuclear run-on analyses showed that IL-1α transcriptionally activated the genes for IL-6, GM-CSF, and IL-1β, while TNFα transcriptionally induced expression of IL-6 and IL-1β. Furthermore, mRNA for IL-6 and IL-1β was dramatically superinduced by the combination of cycloheximide and TNFα. When SV-MSCs were cultured in semisolid medium, they formed colonies of blast-like cells that, when replated on plastic, resumed adherent growth. These “colony-derived” cell lines, unlike the parental SV-MSCs from which they were derived, constitutively expressed colony-stimulating activity and mRNA for GM-CSF, G-CSF, IL-6, and IL-1β. In this report, we show that the expression of IL-6 and IL-1β mRNA in the colony-derived cell lines was due, at least in part, to constitutive transcriptional activation of these genes (similar to the findings in IL-1α- and/or TNFα-stimulated parental SV-MSCs). However, in contrast to the transcriptional activation of the GM-CSF gene seen in cytokine-induced parental SV-MSCs, GM-CSF transcripts accumulated in the colony-derived cell lines by a posttranscriptional mechanism.

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kine gene expression in SV-MSCs; in addition, we further define the mechanisms by which the colony-derived cell lines constitutively produce CSA. The data suggest that expression of CSFs and other cytokines in SV-MSCs is heterogeneously regulated by both transcriptional and posttranscriptional mechanisms.

MATERIALS AND METHODS

Materials. Actinomycin D and cycloheximide (CHX) were purchased from Sigma Chemical Co (St Louis, MO). Deoxyribonuclease I (DPRF grade) was obtained from Worthington Biomedical Corp (Freehold, NJ); radioisotopes were from New England Nuclear (Wilmington, DE); and multiprime DNA labeling system was from Amersham (Arlington Heights, IL). Recombinant human IL-1α and TNFα were gifts of the Immunex Corp (Seattle, WA).

Cell culture. Marrow aspirates were obtained from normal donors after written informed consent under protocols approved by the Institutional Review Board of the Fred Hutchinson Cancer Center. SV40 transformation of human stromal cells in long-term marrow culture was done as previously described. Cultures were maintained in McCoy's 5A medium supplemented with 10% fetal calf serum and were passaged weekly. All experiments were done with cells that had reached confluence, and mediators were added to the medium at the indicated times before cell harvest.

Northern analysis. Cells were harvested by trypsinization and total cellular RNA was isolated by acid guanidium thiocyanate-phenol-chloroform extraction, as described. Briefly, cell pellets were resuspended vigorously in 4 mol/L guanidium isothiocyanate, 25 mmol/L sodium citrate pH 7.0, 0.5% sarkosyl, and 1 mol/L 2-mercaptoethanol, and allowed to sit on ice for 30 minutes. Next, 0.1 volume of 2 mol/L sodium acetate, pH 4.1 was added, followed by 1.0 volume of phenol saturated with diethylpyrocarbonate-treated water. After vigorous mixing, 0.2 volumes of 49:1 chloroform:isoamyl alcohol was added and the resultant emulsion was allowed to cool on ice for 15 minutes. Phases were separated by centrifugation at 4,000 rpm in a tabletop centrifuge at room temperature. Most of the aqueous phase was removed and added to an equal volume of isopropanol. RNA was precipitated overnight at -20°C, pellets were resuspended in diethylpyrocarbonate-treated water, and RNA concentration was estimated by OD at 260 nm. RNA was size-fractionated in 1.2% agarose, 6.5% formaldehyde gel and subsequently transferred to nitrocellulose as described. Integrity of RNA was assessed by staining gels with ethidium bromide and visualization of 28S and 18S ribosomal bands under ultraviolet light. After transfer, nitrocellulose filters were baked at 80°C for 2 hours and then prehybridized for at least 2 hours at 42°C in a solution containing 50% deionized formamide, 200 µg/mL yeast tRNA (Boehringer Mannheim, from brewer's yeast), 5X SSPE (1 x SSPE = 0.15 mol/L NaCl, 10 mmol/L NaH2PO4, 1 mmol/L EDTA), 0.1% sodium dodecyl sulfate (SDS), and 2X Denhardt's solution (1X Denhardt's = 0.02% each ficol, polyvinylpyrrolidone, and bovine serum albumin). Hybridizations were done at 42°C using human cDNAs for IL-6, GM-CSF, G-CSF, and IL-1β (provided by the Immunex Corp, Seattle, WA). The cDNA inserts were isolated from their respective vectors by restriction digestion and gel purification (Geneclean kit from Bio101, La Jolla, CA) before labeling with [32P]-dCTP using the random primer method. To control for loading, transfer, and integrity of RNA, most blots were stripped and reprobed with a chicken β-actin cDNA; no changes in β-actin mRNA levels were seen with the various cytokine treatments used in this study. After at least 12 hours of hybridization, blots were washed three times (30 minutes each) at room temperature in 2X SSC, 0.1% SDS, then twice (30 minutes each) in 0.1X SSC, 0.1% SDS at 45°C to 60°C, depending on the probe. Autoradiography was done at -70°C using Kodak X-omat AR film and dual intensifying screens.

Nuclear run-on analysis. Nuclei were isolated, and run-on analyses were done essentially as described. Briefly, cells from two large flasks (20 to 40 x 10⁶ cells) were trypsinized and washed once with ice-cold phosphate-buffered saline (PBS), then resuspended in RSB buffer (10 mmol/L Tris, pH 7.4, 10 mmol/L NaCl, 3 mmol/L MgCl2) at 4°C, and dounced 10 times in a type B pestle on ice. After centrifugation at 4°C, the pellet was resuspended in ice-cold RSB plus 0.5% NP-40, and the suspension was dounced five times on ice, again in a type B pestle. Nuclei were spun down at 4°C for 10 minutes at 1,000 rpm and carefully resuspended in 150 µL of nuclear freezing buffer (50 mmol/L Tris pH 8.0, 5 mmol/L MgCl2, 40% glycerol, 0.5 mmol/L dithiothreitol). They were immediately frozen and stored at -70°C. Run-on reactions and isolation of newly-labeled RNA were done as described. Plasmids containing the same cDNA inserts as used for Northern blot analysis were denatured by incubating at 65°C for 1 hour in 0.3 mol/L NaOH, followed by neutralization with an equal volume of 2 mol/L ammonium acetate. Five micrograms of each plasmid was applied to nitrocellulose filters using a slot-blot apparatus (Schleicher & Schuell, Keene, NH), and blots were baked for 2 hours at 80°C. Hybridization to immobilized, denatured cDNAs was done in 10 mmol/L TES (Sigma), 0.2% SDS, 10 mmol/L EDTA, 250 µg/mL Escherichia coli tRNA (Boehringer Mannheim MRE 600 strain, RNase-free) 0.3 mol/L NaCl, and IX Denhardt's solution. Counts and volumes of labeled RNA were equalized for each series when a comparison of different conditions was done. Hybridizations were at 65°C for 36 to 48 hours. After hybridization, nitrocellulose strips were washed four times in 2X SSC, 0.1% SDS, 30 minutes each at room temperature. Final wash was in 0.1X SSC, 0.1% SDS at 65°C, followed by autoradiography at -70°C as outlined above.

RESULTS

Basal expression of growth factors in SV-MSCs. Under basal culture conditions in 10% fetal bovine serum, SV-MSCs, similar to some other stromal cell lines, constitutively expressed mRNA for M-CSF and TGF-β (data not shown). Some but not all cell lines also expressed very small amounts of steady-state message for IL-6 and IL-1β. We have not observed constitutive expression of GM-CSF or G-CSF mRNA in SV-MSCs by Northern blot analysis of total cellular RNA; however, by nuclear run-on analysis, the genes for IL-6 and G-CSF were constitutively transcribed, albeit at low rates (see below).

Response of SV-MSCs to IL-1α and TNFα. When SV-MSCs were treated with recombinant human IL-1α, a rapid and significant increase in steady-state mRNA was noted for IL-6, IL-1β, and GM-CSF (Fig 1). The induction of message for all three cytokines occurred within 1 hour and was maximal at 8 hours after IL-1α addition. In contrast, G-CSF mRNA was induced more slowly and to a lesser degree. When SV-MSCs were treated with recombinant human TNFα, a more modest but equally rapid induction of IL-6 and IL-1β was noted (Fig 2). Although detectable message for IL-6 was present 2 hours after TNFα stimulation, the maximum induction was not seen until 48 hours. This contrasted with the kinetics of IL-6 induction by IL-1α, where the peak induction occurred at 8 hours, with a gradual decline in message levels thereafter. Treatment of SV-MSCs with TNFα alone did not increase G-CSF steady-state mRNA levels, and increased GM-CSF steady-state mRNA
steady-state mRNA in SV-MSCs; in contrast, under the experimental conditions used, all experiments testing the effects of CHX and TNFa were stopped at 24 hours. When SV-MSCs were pretreated with CHX and then stimulated with TNFa, a synergistic superinduction of message for IL-6 and IL-1β was evident as early as 2 hours after addition of TNFa (Fig 2). This effect is explored in more detail below.

Mechanism of cytokine induction in SV-MSCs. The rapid induction of IL-6, IL-1β, and GM-CSF mRNA by IL-1α suggested that IL-1α might be acting at a transcriptional level in inducing these genes. To initially examine this question, cells were pretreated with actinomycin-D, an inhibitor of RNA synthesis, before stimulation with IL-1α. Pretreatment with actinomycin-D completely abolished the response to IL-1α (data not shown), suggesting that new transcription was required for the induction of IL-6, IL-1β, and GM-CSF mRNA by IL-1α. To investigate specifically whether IL-1α induced new transcription of the IL-6, IL-1β, GM-CSF, or G-CSF genes, nuclei were isolated from control SV-MSCs and SV-MSCs treated for 8 hours with IL-1α, and newly transcribed mRNA was assayed by nuclear run-off study.

Effects of protein synthesis inhibition with CHX. To further explore the mechanism(s) responsible for this effect, cells were pretreated with CHX, an inhibitor of protein synthesis, and then stimulated with IL-1α or TNFa. Treatment of SV-MSCs with CHX either alone or in combination with IL-1α for up to 24 hours did not change the morphology of the cells or result in a decrease in cell viability (as assessed by trypan blue dye exclusion). In contrast, treatment of cells with the combination of TNFa and CHX for 24 hours (but not 8 hours) caused significant loss of cell viability. For this reason, all experiments testing the effects of CHX and TNFa in combination were stopped at 8 hours, a time at which cells were 100% viable and unchanged morphologically. The addition of CHX alone increased steady-state message for IL-6 and IL-1β (Fig 1), but did not result in evident induction of mRNA for GM-CSF or G-CSF. It is clear from Fig 1 that pretreatment of SV-MSCs with CHX did not prevent the induction of IL-6, IL-1β, GM-CSF, or G-CSF mRNA by IL-1α. There was a shift in kinetics in all cases (with maximum increase in mRNAs at 24 hours instead of 8 hours), but the overall level of induction was as high (IL-1β, GM-CSF) or higher (IL-6, G-CSF) than in cells stimulated with IL-1α alone. In addition, G-CSF steady-state mRNA was superinduced by the combination of IL-1α and CHX, but this superinduction was delayed and most evident only at 24 hours.

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analysis. This assay measures the relative density of RNA polymerase II molecules bound to specific genes, and is an indirect measure of transcriptional activity across the segment of the gene represented by the immobilized cDNA probe. Figure 3 indicates that, in control cells, there was a small amount of constitutive transcription of the IL-6 and G-CSF genes, but no significant basal transcription of GM-CSF or IL-1β. When SV-MSCs were exposed to IL-1α for 8 hours, increases in transcription were noted for the IL-6 (10-fold), GM-CSF, IL-1β, and G-CSF (twofold) genes (Fig 3; compare lane A with lane B). Quantitating the fold induction for GM-CSF and IL-1β was difficult due to the essentially unmeasurable levels of basal transcription of these genes. However, IL-1α clearly induced major increases in transcriptional activity across the GM-CSF and IL-1β genes in SV-MSCs, increases that correlated well with changes seen on analysis of steady-state mRNA (Fig 1). Note that transcription of the β-actin gene, used here as a control, remained essentially constant. Similar experiments with TNFα showed that this cytokine also increased transcription of the IL-6 and IL-1β genes (Fig 3, lanes C and D). Finally, TNFα induced a small (twofold) increase in transcription of the GM-CSF gene, but caused little or no change in the basal transcription rate of G-CSF.

The induction of IL-6 and GM-CSF steady-state mRNA by IL-1α and/or TNFα in SV-MSCs was quite prolonged, with continued message evident as long as 48 to 72 hours after stimulation. Figure 4 presents the results of an experiment in which SV-MSCs were treated with IL-1α and TNFα simultaneously, and nuclei and cytoplasmic RNA were isolated 72 hours after addition of the cytokines. Northern analysis revealed that steady-state levels of cytoplasmic mRNA for IL-6 and GM-CSF remained significantly elevated even at 72 hours after stimulation. Furthermore, the accompanying nuclear run-on analysis showed that this prolonged induction of the IL-6 and GM-CSF genes by IL-1α and TNFα was mediated, at least in part, by a prolonged increase in transcription of those genes.

Mechanism of CHX-induced changes in CSF gene expression. Accumulation of IL-6 and IL-1β mRNA in SV-MSCs after treatment with CHX alone (Fig 1) indicated that continuous protein synthesis was necessary to prevent constitutive accumulation of IL-6 and IL-1β transcripts in these cells. This suggested that either a labile transcriptional repressor(s) or a labile degradative protein(s) was involved in regulating basal IL-6 and IL-1β gene expression in SV-MSCs. It seemed unlikely that global translational inhibition alone was responsible for the accumulation of IL-6 and IL-1β mRNA, since similar increases in GM- and G-CSF transcripts were not seen after CHX treatment alone. When cells were pretreated with CHX and then stimulated with TNFα, there was a superinduction of steady-state mRNA for IL-6 and IL-1β (Fig 2). To pursue the mechanisms of this effect, nuclear run-ons were done with nuclei isolated from control SV-MSCs and cells treated for 4 hours with TNFα alone,
CHX alone, or the combination of CHX and TNFα. The nuclear run-on analysis (Fig 5) showed modest transcriptional induction of IL-6 and IL-1β by TNFα alone, and modest transcriptional activation of IL-6 (but not IL-1β) by CHX alone. When cells were pretreated with CHX and then stimulated with TNFα for 4 hours, there was no significant further transcriptional activation of either IL-6 or IL-1β, despite a marked increase in steady-state mRNA for both genes (Figs 2 and 5). In fact, the small transcriptional induction of IL-1β by TNFα was blocked by pretreatment of cells with CHX (Fig 5, lane D). The strong and relatively even hybridization of newly-transcribed β-actin mRNA indicated that 4 hours of treatment with TNFα and CHX did not result in a nonspecific global decrease in transcription. These results suggested that the superinduction of IL-6 and IL-1β steady-state mRNAs by the combination of TNFα and CHX was due primarily to increases in stability of the respective transcripts.

To pursue further the effect of protein synthesis inhibition in this system, transcript stabilities were determined after treatment of SV-MSCs with TNFα, either alone or after pretreatment with CHX. After 4 hours of treatment with either or both agents, actinomycin-D was added to block transcription, and message levels were measured at 1, 2, and 4 hours after actinomycin-D addition; cell viability as assessed by trypan blue dye exclusion remained unchanged throughout the course of these experiments. As noted in Fig 6, there was a significant increase in IL-6 and IL-1β mRNA half-life in cells treated with both CHX and TNFα, compared with cells treated with TNFα alone. In cells treated with both TNFα and CHX, there was essentially no diminution in signal for IL-6 or IL-1β between 0 and 4 hours. In contrast, the half-life of IL-6 mRNA in TNFα-treated cells was approximately 4 to 5 hours, and that of IL-1β mRNA was 3 to 4 hours. Similar experiments were done to assess whether CHX had an equivalent effect on expression of the GM-CSF and/or G-CSF genes. As noted in Fig 6, GM-CSF mRNA was quite labile in cells treated with TNFα alone (half-life less than 1 hour), and no G-CSF mRNA was detected (the absence of detectable G-CSF mRNA despite constitutive transcription of this gene suggests that the G-CSF transcript is exceedingly labile in these cells, even when they are treated with TNFα). Pretreatment of SV-MSCs with CHX resulted in stabilization of GM-CSF mRNA (half-life greater than 4 hours) and appearance of measurable levels of G-CSF mRNA (half-life, 1 to 2 hours). Since nuclear run-on experiments did not show any increase in transcription of the G-CSF gene after treatment of SV-MSCs with TNFα and/or CHX (Fig 3 and data not shown), the effect of CHX on G-CSF gene expression also appeared to be due to an increase in G-CSF mRNA stability.

Mechanisms of constitutive CSF production in CSA-producing or colony-derived cell lines. We recently described and isolated lines of transformed human marrow stromal cells that, unlike the cells described above, constitutively produced CSA. To derive these lines, adherent SV-MSCs were cultured in semisolid medium in the presence of various exogenous mediators, such as IL-1α or TNFα. The colonies that formed were pooled and replated in liquid culture, where they again formed adherent layers of cells with typical stromal morphology (but now constitutively producing CSA). We were interested in determining the mechanism(s) by which these colony-derived or CSA-producing stromal cell lines had upregulated production of CSA, in effect using them as a model to gain further insight into the molecular mechanisms of growth factor expression in human stromal cells. Figure 7 presents the results of Northern blot analysis of total cellular RNA from a CSA-producing line compared with RNA from a typical SV-MSC line, cultured under identical conditions. In the CSA-producing or colony-derived line, there were substantial increases in steady-state mRNA levels for GM- and G-CSF, IL-1β, and IL-6. To determine the mechanism(s) of this constitutive mRNA expression, nuclear run-on assays were
done with nuclei from several CSA-producing lines and from respective control SV-MSC lines. Figure 7 (bottom panel) shows that both the IL-6 and IL-1β genes were transcriptionally activated in the colony-derived or CSA-producing lines (compare lane B with lane A in both experiments). In contrast, and despite at least a 10- to 20-fold increase in steady-state mRNA for GM-CSF and G-CSF in the colony-derived lines, there was little or no evidence for transcriptional activation of these genes. Thus, accumulation of IL-6 and IL-1β transcripts in the CSA-producing lines was due at least in part to constitutive, endogenous activation of transcription of those genes, while G- and GM-CSF mRNA accumulated by a posttranscriptional mechanism.

DISCUSSION

Bone marrow stromal cells are believed to play a key role in regulating hematopoiesis, possibly by the constitutive or induced production of CSFs, interleukins, and other hematopoietic regulatory molecules. Therefore, it is important to characterize the molecules produced by stromal cells and to understand the mechanisms that regulate their expression. In the current study, SV-40-transformed human marrow stromal cells were shown to respond to IL-1α and TNFα by increasing expression of several growth factor and cytokine genes. In particular, IL-1α caused rapid and significant increases in steady-state mRNA levels for IL-1β, IL-6, GM-CSF, and G-CSF. New protein synthesis was not necessary to mediate this effect, suggesting a direct effect of IL-1α on CSF mRNA expression. Finally, nuclear run-on analyses indicated that in SV-MSCs, IL-1α caused major increases in transcription of the IL-6, GM-CSF, and IL-1β genes.

One cannot conclude from this study that the effects of IL-1α or TNFα on steady-state CSF/cytokine mRNA levels were mediated solely through increased transcription of these genes; in fact, as stressed by others, inflammatory cytokines may also regulate CSF gene expression (and thus, hematopoiesis) by increasing stability of CSF mRNA. An accurate comparison of transcript stability before and after treatment of SV-MSCs with cytokines was not possible because of the virtual absence of CSF transcripts in uninduced cells. However, the half-lives after stimulation with IL-1α (data not shown) or TNFα (Fig 6) were generally measured in hours rather than minutes, suggesting relatively stable messages. One of the major findings in this study, however, was the marked transcriptional activation of the GM-CSF gene in SV-MSCs by IL-1α; this result is consistent with investigations in other systems, where increased transcription of the GM-CSF gene has been noted after treatment of cells with several different mediators, including IL-1. It is interesting to note that transcriptional activation of GM-CSF has been observed most commonly in endothelial cells, whereas posttranscriptional regulation seems to play a major role in macrophages, fibroblasts, and T-cells; in this regard, SV-MSCs share some phenotypic features with endothelial cells (eg, expression of type IV collagen and thrombomodulin). In summary, data from this study and others suggest that there are likely to be multiple, perhaps cell- or tissue-specific, mechanisms of control of GM-CSF expression.
TNFα also induced expression of the IL-6, IL-1β, and GM-CSF genes in SV-MSCs. This effect was mediated, at least in part, by an increase in transcription of these genes, and was not prevented by protein synthesis inhibition with CHX. The superinduction of IL-6 and IL-1β steady-state mRNA after treatment of SV-MSCs with the combination of CHX and TNFα is reminiscent of findings in other systems with so-called "early response" genes, such as c-fos and c-myc, which are transcriptionally activated by serum and other growth factors, and which are superinduced by pretreatment of cells with protein synthesis inhibitors. Labile transcriptional regulators appear to be involved in regulating the response of these and other early response genes to such agents as serum, PDGF, and TPA. On the other hand, CHX (either alone or in combination with other agents) has been shown in other systems to inhibit mRNA degradation and thus prolong mRNA half-life. In the current study, treatment of SV-MSCs for 4 hours with CHX alone increased steady-state mRNA for both IL-6 and IL-1β, and increased transcription of the IL-6 (but not the IL-1β) gene (see Fig 5). This result suggests that a labile transcriptional repressor may be involved in limiting basal expression of the IL-6 gene, at least in SV-MSCs. However, the dramatic superinduction of both IL-6 and IL-1β transcripts by the combination of CHX and TNFα was not accompanied by further increases in transcription of either gene (in fact, CHX blocked the minor TNFα-induced increase in transcription of IL-1β; Fig 5, lane D), suggesting that the major effect of CHX in this system was on mRNA stability. Indeed, as noted in Fig 6, the half-lives of the IL-6, IL-1β, and GM-CSF mRNAs were significantly longer in cells treated with TNFα and CHX than in cells treated with TNFα alone. The lack of similar superinduction with the combination of IL-1α and CHX is somewhat puzzling, but presumably reflects differences in the patterns of cellular proteins that are posttranslationally modified after treatment of SV-MSCs with either IL-1α or TNFα. These cellular proteins themselves may have markedly different stabilities in the presence of CHX.

SV-MSCs selected for colony formation in semisolid medium became constitutive producers of colony-stimulating activity and constitutive expressors of steady-state mRNA for IL-6, IL-1β, GM-CSF, and G-CSF (Fig 7). We analyzed the mechanisms for this effect and discovered that while significant transcriptional activation of the IL-6 and IL-1β genes had occurred, there was little or no change in the transcription rate of the GM- or G-CSF genes. Thus, it appeared that GM- and G-CSF transcript half-lives were increased in the CSA-producing or colony-derived lines. Prolongation of GM-CSF mRNA half-life has been reported as a mechanism of constitutive GM-CSF production in, among other systems, a mouse monocytic tumor line and in human leukemia cells. The constitutive transcriptional activation of the IL-6 and IL-1β genes in the colony-derived lines could be secondary to endogenous expression of IL-1β in these cells. In this regard, Seiff et al recently reported constitutive IL-1 production in retrovirus-immortalized endothelial cells; interestingly, these cells also produced GM- and G-CSF. Further analysis of these and other constitutively expressing lines may lead to insights into the molecular events that control growth factor and cytokine gene regulation in human stromal cells.

The validity of using cultured SV-MSCs as a model to study events occurring in the hematopoietic microenvironment (in particular, to make inferences regarding growth factor and cytokine gene regulation in vivo) is supported by several observations. First, the profile of constitutive (uninduced) CSF gene expression in these cells is similar to that reported for other human or murine stromal cell lines, suggesting that immortalization with SV40 has not altered the mechanisms that regulate basal CSF gene expression in these cells. Furthermore, the changes in CSF steady-state mRNA levels reported here are in general agreement with investigations in other systems using nontransformed murine or human mesenchymal cells. Second, in a recent study by Williams et al, immortalization with SV40 large T antigen did not prevent murine stromal cells from supporting stem cell proliferation, suggesting that the SV40
large T antigen itself has minimal effects on the differentiated phenotype of marrow stromal cells. Third, there is no evidence that SV40 virus affects CSF gene expression either directly or indirectly; in experiments not shown, the transcription rate of the SV40 large T antigen did not change after stimulation of SV-MSCs with IL-1α or TNFα, suggesting that the effects of these cytokines were not mediated by intermediate changes in expression of this viral protein. In summary, we feel that SV-MSCs offer a reasonable model system to study the molecular mechanisms of cytokine and growth factor gene regulation in cells of the human hematopoietic microenvironment. Attempts to reproducibly establish clonable, nontransformed human stromal cell lines that support hematopoiesis are currently in progress.

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Regulation of cytokine and growth factor gene expression in human bone marrow stromal cells transformed with simian virus 40

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