Characterization of Murine Bone Marrow and Spleen-Derived Stromal Cells: Analysis of Leukocyte Marker and Growth Factor mRNA Transcript Levels


Stromal cells are believed to regulate lympho-hematopoietic activity through direct cell–cell interactions and the release of growth factors. Many questions remain, however, about their lineage derivation and functional heterogeneity. We previously prepared a panel of stromal cell lines from murine spleen and bone marrow and characterized them based on their ability to support lymphocyte growth in long-term cultures. These cells are now compared with respect to their expression of various immunoglobulin superfamily and cytokine genes by Northern blot analysis. These results indicate that although stromal cells appear to be mesodermal in origin, they are not closely related developmentally to the hematopoietic progenitor cells they support. The potential production of at least six cytokines is suggested. Most potent lymphocyte supporting clones also made interleukin 7 constitutively. Previous findings had suggested that these clones responded to exogenous stimuli and this has now been demonstrated in terms of induced expression of IL-6 and G/M-CSF mRNA. Interleukin 6 mRNA levels were markedly upregulated by exposure of cells to LPS, TNF, IL-1, IL-6, IL-7, and EGF. G/M-CSF mRNA levels were “superinduced” by the combination of LPS and cycloheximide, a protein synthesis inhibitor. These responses are similar to ones documented by investigators working with endothelial cells and fibroblasts. Together, these data suggest that stromal cells are a multifunctional component of the lymphopoietic microenvironment and may be active participants in a complex, cytokine-mediated regulatory network.

The hematopoietic microenvironment (HIM) includes a heterogeneous population of macrophages, fibroblasts, and adipocytes referred to as “stromal” cells. A number of investigators have successfully cloned stromal cell lines from long-term bone marrow cultures (LTBMC), which can support myeloid and lymphoid differentiation and/or proliferation in vitro. Stromal cells support lympho-hematopoietic progenitors through direct cell–cell interactions and the release of cytokines.

The importance of cell surface molecules in stromal cell function has been based on observations made in LTBMC where hematopoietic cells are routinely found adhering to the stromal layers. Diffusion chamber experiments demonstrate decreased myelopoiesis and absent lymphopoiesis if the stroma and hematopoietic precursor cells are physically separated. Specific surface proteins or proteoglycans in the extracellular matrix are presumed to mediate this interaction. Glycosyl-phosphatidylinositol (GPI) linked plasma membrane proteins are candidates for this role.

In a manner similar to fibroblasts, monocytes, and endothelial cells, stromal cells produce a number of cytokines based on mRNA analyses and functional assays. Likewise, stromal cells display cell surface receptors for various cytokines. Following stimulation with these agents, stromal cells have been reported to increase their proliferative rate and alter their steady state expression of cytokine mRNA. Together, these data imply that, in vivo, stromal cells may operate within a communication network mediated by multiple growth factors and their receptors.

A number of murine bone marrow and spleen derived stromal cell lines were recently developed in our laboratory. These clones differ significantly in ability to support B lineage lymphocyte proliferation in vitro, based on studies using stromal cell dependent B-lymphocyte lines and stromal cell depleted bone marrow (reference 27 and manuscript in preparation). In this report, we describe studies examining the repertoire of cell surface marker and growth factor gene expression in representative clones by Northern blot analysis. These results have implications regarding our understanding of the lineage derivation of stromal cells and offer an explanation for their heterogeneity with respect to lymphocyte support function. Responsiveness of the most potent B lymphoid supporting stromal cell clone to exogenous factors is also investigated.

Materials and Methods

Cell culture. The cell lines employed in this study included the lymphoma lines WEH13, EL4, and W279, the neuronal cell line N2A, the macrophage line P388D, the myocyte line G7, and the murine fibroblast lines CCL1 and NIH 3T3. All cell lines were obtained from the ATCC and maintained in culture as specified. The stromal cell lines (isolated as described in) were either spleen derived (SS1, SNS1) or bone-marrow derived (BMS1, BMS2). Stromal cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 1% glutamine, and 100 U/mL penicillin and streptomycin. All cells were cultured at 37°C in 5% CO2. Cells were maintained in 100 mm culture dishes (polystyrene, Corning #25020).

When growth factors were added, the cells were plated at subconfluent levels and incubated for 24 to 48 hours prior to growth factor addition. Cells were treated with factors for 8 to 48 hours as indicated in the figure legends and then harvested for RNA as specified.

From the Oklahoma Research Foundation, Oklahoma City. Submitteed September 9, 1988; accepted February 23, 1988. Supported by grant AI-20669 from the National Institutes of Health and a grant from the Presbyterian Medical Research Foundation.

Address reprint requests to J.M. Gimble, Oklahoma Medical Research Foundation, 825 N.E. 13th St, Oklahoma City, OK 73104.

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described below. The factors employed included transforming growth factor β, 10 ng/mL; hydrocortisone, 10⁻⁷ mol/L; epidermal growth factor (EGF), 50 ng/mL; 1.25 vitamin D, 10⁻⁴ mol/L; tumor necrosis factor (TNF) 10⁻⁴ mol/L; interleukin-1 (IL-1), 2.5 U/mL; interleukin-6 (IL-6); interleukin-7 (IL-7), 500 U/mL; cyto-ehemidine, 25 μg/mL; Actinomycin, 10 μg/mL.

**RNA preparation.** RNA was harvested by the method of Chom- zynski and Sacchi.33 For cell cultures, the media was removed, the cells immediately suspended in 4 mol/L guanidium isothiocyanate buffer, scraped off the plate with a rubber policeman, placed on ice and immediately homogenized through a no. 22 gauge needle.Murine tissues were homogenized in guanidium isothiocyanate using a polytron apparatus. The material was acid/phenol extracted, precipitated twice with 2-propanol and resuspended in DEPC treated water. The total RNA concentration was determined by OD 260/280 absorbance and confirmed by direct analysis on a formalde-gy/agarose gel. Poly A mRNA was harvested from embryonic tissues using a polytrion apparatus. The material was acid/phenol extracted, precipitated twice with 2-propanol and resuspended in DEPC treated water. The total RNA concentration was determined by OD 260/280 absorbance and confirmed by direct analysis on a formalde-hy/agarose gel. Poly A mRNA was selected on oligo-dT columns (Collaborative Research) according to the method of Aviv and Leder.29

**Northern blots.** Northern blots were prepared by a modification of the method of Thomas.35 Either poly A mRNA (approximately 2 μg) or total RNA (10 μg) were ethanol precipitated, suspended in 5.5% formaldehyde/50% formamide loading buffer, heated at 65°C for 5 minutes and then loaded onto a 1.5% agarose/0.5% formalde-hyde gel. Samples were electrophoresed at 50 V for 5 to 6 hours and then transferred to a nylon membrane (Gene Screen Plus, NEN, or Nytran, Schleicher and Schuell). RNA was cross linked to the membrane by UV exposure and prewashed at 65°C for 1 hour in 1X SSC, 0.1% SDS.

**Random labeling.** Radiolabeled DNA probes were prepared using the method of Feinberg and Vogelstein.39 Probes were routinely labeled to >10⁸ cpm/μg DNA using a commercially available kit from Boehringer Mannheim (Indianapolis) or BRL (Gaithersberg, MD).

**Hybridization.** Blots were prehybridized overnight at 42°C in 50% formamide, 5X SSC, 5X Denhardt’s solution, 0.1% SDS, 5 mmol/L EDTA, 100 μg RNA per lane. The radiolabeled probe was heated 10 minutes at 90°C and added to the blot at a concentration of 1 to 2 million cpm per lane. In 40% formamide, 10% dextran sulfate, 5X SSC, 1X Denhardt’s solution, 20 mmol/L Na Phosphate pH 6.8, 0.2% SDS, 5 mmol/L EDTA, 100 μg/mL tRNA and sheared DNA, 10 μg polyadenylacid. The blots were hybridized 16 to 18 hours at 42°C and washed at low stringency (2X SSC, 0.1% SDS, 30 minutes, 50°C) and then high stringency (0.1X SSC, 0.1% SDS, 30 minutes, 50°C). The blots were wrapped in cellophane while still wet and exposed at −70°C. Autoradiographs were developed between 1 and 7 days.

**Densitometry.** Densitometric readings were performed using a LKB Model 2202 Ultrascan Densitometer.

**Materials.** DNA probes were generously provided by a number of investigators: P. Burrows, UAB, Birmingham, AL—murine μ heavy chain constant region36; S. Clark, Genetics Institute, Cam- bridge, MA—human cDNA of IL-1α, IL-1β and IL-6 (637 bp insert); R. Derynick, Genentech, S. Francisco—human TGFβ 1, 1.05 Kb Eco RI insert cDNA (λ BcI)32; J. Gurney, University of Chicago—murine neurofilament cDNA, 2.0 kb EcoRI insert (C19)35; Freitenger, UNC, Chapel Hill, NC—murine MHC Class II α and β cDNA (pAACA6 and pAAAC8)36; S. Gillis, Immunex, Seattle—murine G/M-CSF cDNA and murine IL-3 cDNA (400 bp insert); R. Flavell and G.L. Waneck, Biogen, Cambridge, MA—MHC Class I Q7 cDNA (pB3G37)48; T. Honjo, Osaka, Japan—murine IL-5 cDNA (pSp6k-mTRF23)44; Kishimoto and Springer, Dana-Farber Inst, Boston—human LFA 1 β subunit cDNA42; F. Melcher, Basel Inst, Switzerland—murine β 52; P. Ralph, Cetus Corp, Emeryville, CA—murine M-CSF cDNA, 3.9 kb insert, (pGEM2-MCSF10, culture collection #CMCC 2760, Cetus Corp); J. Roberts, Mt. Sinai Medical School, New York—rat POMC (plasmid SAL65—exon 3)44; Smale and Baltimore, Whitehead Inst, Cambridge, MA—murine TdT full length cDNA (pTdT-1); J. Teele, UTSA, San Antonio, TX—murine IL-4 cDNA probe42; M. Thomas, Washington University, St. Louis—murine Ly5 cDNA46; and P. Tucker, Northwestern Medical School, Dallas—murine T cell receptor β J-C region (plasmid pDO β2).35 Additional probes included murine N-CAM cDNA clone T3 corresponding to bp 1636-2249 (C. Goridis). 2.2 kb murine genomic Thy1.1 Xbal probe (J. Silver); murine IL-7 cDNA probe 1046 (Namen)48; murine E-cadherin and chicken N-cadherin cDNA probes (Takei-chi).30

Cytokines and growth factors were obtained courtesy of the following investigators and companies at the indicated concentrations: Human recombinant IL-7, 220,000 U/mL, Dr A. Namen, Immunex (Seattle); Human recombinant IL-6, Dr S. Clark, Genet- ics Institute (Cambridge, MA); Tumor Necrosis Factor, 2.3 mg/ mL, Dr D. Stern, Columbia University (New York); purified TGF β 1, 0.1 mg/mL, Dr L. Ellingsworth, Collagen Corp (Palo Alto, CA); 1.25 VitD3, 0.1 mmol/L, Dr M. Uskokovic, Hoffman LaRoche (Nutley, NJ). The following factors were purchased from the indicated companies: purified human IL-1, 100 U/mL, Genzyme (Boston); epidermal growth factor, 100 μg/mL, Collaborative Research Inc (Bedford, MA); Hydrocortisone, Elkins-Sinn (Cherry Hill, NJ); and Lipopolysaccharide W S. typhosa 0901, Difco Labo- ratories (Detroit). All other factors and chemicals were purchased from Sigma Chemical Co (St Louis).

**RESULTS**

**Leukocyte marker genes.** Northern blots were prepared with Poly A + selected mRNA from representative murine cell lines (stromal, lymphoid, myeloid, myocyte, neuronal, and fibroblast) and tissues (kidney, liver, brain). The non-stromal cell lines were included as controls and for comparative purposes. The stromal cell lines employed were bone marrow (BMS1, BMS2) or spleen derived (551, SNS1). These cells were cloned by selecting for adherent cells in short-term cultures followed by treatment with 5-fluouroura-cil.27 The cell lines differ in their ability to support myeloid and lymphoid proliferation and differentiation (Pietrangeli et al, manuscript in preparation). Based on studies with stromal cell depleted bone marrow and stromal-cell dependent B lineage lymphocyte lines, the lympho-hemopoietic support ability of the cell lines is as follows: BMS2 > BMS1 > SS1 > SNS1. The cell line NIH 3T3, selected as a contact inhibited fibroblast from embryonic tissues, can function as a myeloid support stroma (reference 52 and unpublished observations) and it was also included with the stromal cell lines for comparison.

Northern blots were prepared with approximately 2 μg of Poly A + mRNA per lane. To normalize between cell types, a commonly expressed gene (actin) was examined. Individual northern blots were successively hybridized with radiola- beled cDNA probes for genes encoding cell surface proteins, with particular emphasis on members of the immunoglobulin superfamily (Fig 1). The results of these studies are summa-rized in Table 1. The stromal cells expressed N-cadherin, a Ca ++ dependent intercellular adhesion molecule, but did not display its counterpart, E-cadherin.30 The bone marrow and
CHARACTERIZATION OF MURINE STROMAL CELLS

Fig 1. Poly A+ mRNA northern blot analysis (A,B,C). Northern blots were prepared on nylon membranes as described in Materials and Methods. Each lane contains approximately 2 μg of Poly A+ mRNA. The cell lines include lymphoma (W279, WEHI 3, EL4), fibroblast (CCL1, NIH 3T3), myocyte (G7), stromal (SNS1, BMS1, BMS2), and macrophage (P388D). The blots were hybridized with the indicated probes. The size in kilobases of the N-CAM mRNA transcripts is indicated to the right of panel A.

spleen derived stromal cells, in addition to NIH 3T3 cells, expressed mRNA for a limited number of Ig superfamily genes. While there was no evidence of gene expression for "classical" leukocyte molecules (μ heavy chain, T cell receptor β, MHC Class II, Tdt, λ 5, Ly 5), the stromal cells all contained transcripts for MHC Class I, Neural Cell Adhesion Molecule (N-CAM) and Thy 1. Each of these families of surface proteins includes an isoform, which is membrane bound via a glycosyl-phosphatidylinositol linkage. The N-CAM probe detected multiple transcripts due to alternative splicing of the mRNA. Comparison of the N-CAM mRNA transcripts between stromal cells, myocytes (G7) and neuronal tissues (N2, brain) demonstrated similarities in the myocyte and stromal cell expression patterns. Each expressed transcripts of 6.7 kb, 5.2 kb, and 2.9 kb. Unlike the neuronal tissues, neither exhibited the 7.4 kb mRNA.

Growth factors. The same Poly A+ mRNA Northern blots were hybridized with radiolabeled probes for represen-
Table 1. Leukocyte Marker mRNAs

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Abbreviation: ND, not done.
*Member of Ig gene superfamily.

Table 2. Growth Factor mRNAs

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Abbreviation: ND, not done.

Data are based on Northern Blot analysis of Poly A+ mRNA. The cell lines are fibroblasts (3T3), bone marrow (BMS1, BMS2) and spleen (SS1, SNS1)-derived stromal cells.

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Abbreviation: ND, not done.

Regulation of transcript levels by exogenous factors. Studies were performed to determine the effects of exogenous factors on the abundance of mRNA transcripts in the bone-marrow derived stromal cell, BMS2. Cells were grown to 50% to 70% confluency in 100 mm culture dishes prior to the addition of various stimuli. The factors employed were IL-6, IL-7, LPS, TNF, TFGβ2, hydrocortisone, and cycloheximide. After culturing the cells for between 6 and 48 hours, the total RNA was harvested and examined by Northern blot analysis. Hybridization with the gene probe for IL-6 revealed induction of transcript levels after 48 hours in the presence of specific growth factors (Fig 2). Based on densitometric analysis, factor induction was as follows: LPS (47-fold), TNF (44-fold), IL-6 (18-fold), IL-7 (13-fold), IL-1 (tenfold), EGF (fivefold). In contrast, hybridization of the same Northern blot with a radiolabeled probe for Thy 1 revealed little variation in the mRNA levels following incubation with exogenous factors.

Cycloheximide, which acts to block new protein synthesis, has been reported to “superinduce” the levels of gene tran-

Fig 2. BMS2 total RNA: 48-hour incubation with exogenous cytokines. As described in Materials and Methods, BMS2 cells were incubated for 48 hours in the presence of various cytokines: hydrocortisone, 10-7 mol/L; 1.25 vitamin D, 10-8 mol/L; interleukin-6; interleukin-7, 500 U/mL; transforming growth factor β, 10 ng/mL; lipopolysaccharide, 10 μg/mL; tumor necrosis factor, 10-8 mol/L; interleukin-1, 2.5 U/mL. The total RNA was analyzed on Northern blots hybridized successively to radiolabeled probes for IL-6 and Thy 1.
scripts when added in combination with a stimulating cytokine. Actinomycin, which blocks the initiation of RNA synthesis, would be expected to decrease the levels of mRNA transcripts. BMS2 cells were incubated for 24 hours in the presence of LPS, cycloheximide and actinomycin, either alone or in combination (Fig 3A). The Northern blots of these experiments were hybridized successively with probes for IL-6 and G/M-CSF. In the case of IL-6, treatment with cycloheximide or LPS alone increased the mRNA levels by 19- or 13.6-fold, respectively. At the concentrations employed, the two agents together had a less than additive effect, resulting in a 22-fold increase. The presence of actinomycin, either alone or with LPS, prevented any increase in mRNA levels. Unlike IL-6, G/M-CSF mRNA was not detected in resting BMS2 cells. While the addition of cycloheximide or LPS alone had little effect, the two agents in combination induced G/M-CSF mRNA levels 23.8-fold over control levels, consistent with a "superinduction." The action of cycloheximide on IL-6 mRNA levels was time-dependent (Fig 3B), progressing from a 2.4-fold effect at 6 hours to 17.3-fold effect at 24 hours of incubation.

Analysis of a similar Northern blot (Fig 4) with a probe for TGFβ revealed that the level of TGFβ mRNA was increased 1.5-fold following incubation of BMS2 cells with TGFβ itself or cycloheximide. Similarly, M-CSF mRNA levels were induced fourfold following incubation with cycloheximide.

DISCUSSION

A panel of murine bone marrow and spleen-derived stromal cell clones, together with NIH 3T3 cells, have been analyzed with respect to leukocyte marker and cytokine mRNA expression. These cell lines differ significantly in ability to support B lymphoid cell proliferation in vitro. Nevertheless, the pattern of gene expression among these cells was remarkably similar. They differed only for IL-7, which was associated with the most potent B lymphoid-supporting stromal cells. Stromal cells appear to be mesodermal in origin, but are distinct from the lymphoid and myeloid lineages. Stimulation of stromal cells with exogenous cytokines markedly influenced the mRNA levels of the cytokines IL-6 and G/M-CSF.

Stromal cells express several cell surface proteins within the immunoglobulin gene superfamily based on Northern blot analysis. These include N-CAM, Thy 1, and the MHC Class I molecule Qa2. This is in agreement with immunoperoxidase and Western blot analyses of the stromal cells. Each of these protein families includes an isoform linked to the plasma membrane through a phosphatidyl inositol (PI) moiety. Earlier studies with phosphatidylinositol specific phospholipase C (PI-PLC) had suggested that lymphoid/stromal cell adhesion is at least partly dependent on PI-linked molecules; treatment of long-term cultures with PI-PLC released up to 60% of the lymphocytes from the stromal layer. In neural tissues, N-CAM is known to mediate cell adhesion through a homophilic interaction. The N-CAM structural gene consists of 19 or more exons and its regulation involves a mechanism of alternative splicing, creating multiple mRNA transcripts encoding a group of related proteins. The N-CAM protein has been localized primarily to neural and embryonic or regenerating muscle tissues. Stromal cell expression of N-CAM mRNA most closely resembles that of muscle, lacking the distinctly neural 7.4 kb transcript. This observation is consistent with a mesodermal origin for the stromal cells.

The presence of Thy 1 mRNA in stromal cells, together with the evidence of positive antibody staining, is of interest. The primary bone marrow cultures used to clone the cells were originally Thy 1 negative (and unpublished observations). Other reported stromal cell clones either lacked Thy 1 ...
selected for a small subset of bone marrow cells that were low levels on gene family expressed on epithelial derived tissues.65 In contrast, N-cadherin, which is found on cells of mesoderm molecule 1 positive or that it induced Thy I expression. Procedure used to clone these stromal cell lines either or absence of cycloheximide alone. The northern blot was successively hybridized with radiolabeled probes for TGFβ or M-CSF.

or expressed only low levels on the surface.63 Only 5% of total bone marrow cells are Thy 1 positive.64 This suggests that the procedure used to clone these stromal cell lines either selected for a small subset of bone marrow cells that were Thy 1 positive or that it induced Thy 1 expression de novo.

Stromal cells also expressed the calcium-dependent adhesion molecule N-cadherin, which is found on cells of mesodermal origin such as muscle and neural tissue.65 In contrast, the stromal cells did not express E-cadherin, a member of the same gene family expressed on epithelial derived tissues.65 Absent in the stromal cell mRNA were certain transcripts common in lymphoid and myeloid cells, including μ heavy chain, the common leukocyte antigen Ly 5, lambda 5, LFA 1, terminal deoxynucleotidyl transferase (TdT) and the MHC Class II α and β chains. These findings at the mRNA level are in complete agreement with immunohistochemical studies performed on the stromal cell lines.27 Together, these results imply that stromal cells are of mesodermal origin but lie outside the lymphoid and myeloid lineages.

Numerous investigators have described cytokine produc-

tion by murine and human derived bone marrow stromal cell clones.3,5,7,10,21,66 Additional studies examined cytokine gene regulation in human fibroblast, monocyte, and umbilical vein endothelial cells.17,21,23,24 The presence of cytokine production has been assessed by biologic assays of conditioned medium or Northern blot analyses. However, failure to detect a growth factor by these methods does not necessarily confirm its absence in a particular cell type. Recently, Roberts et al demonstrated that the stromal cell surface glycosaminoglycan, heparan sulfate, binds to the active forms of IL-3 and G/M-CSF.67 Similar observations have been made with other growth factors.68 Thus, stromal cells could present locally high concentrations of cytokotins within their extracellular matrix without releasing significant levels into the conditioned medium. In addition, destabilizing sequences are known to occur within cytokine transcripts, leading to relatively brief mRNA half lives.66 Cycloheximide treatment prior to RNA purification has been reported to stabilize these transcripts, presumably by blocking the synthesis of labile proteins involved in their recognition and degradation in vivo. Both of these phenomenon could contribute to a “false negative” assessment of a given cell’s cytokine production.

As the list of factors regulating gene expression has increased, the opportunity has developed to compare the potential cytokine repertoire among functionally different stromal cell clones. Constitutive expression of M-CSF, IL-6, TGFβ and neuroleukin was detected in the four bone marrow and spleen-derived stromal cells. Only the bone marrow derived clones produced readily detectable levels of IL-7. Each of these growth factors has been implicated in lymphoid or myeloid regulation. Neuroleukin, a product of lectin-stimulated T lymphocytes, stimulates immunoglobulin secretion from B lymphocytes in addition to having a trophic effect on neuronal cells.38,69 Interleukin-6 has been demonstrated to act as a potent stimulant of early B lineage cell proliferation (references 9 and 49, and Lee G et al, submitted). Interleukin-6 has multiple actions, including those of a B cell stimulating factor.70 Moreover, it will synergize with IL-3 to stimulate myeloid and erythroid proliferation.71 M-CSF, a product of endothelial cells, monocytes, and stromal cells, is known to stimulate myeloid development.6,10,19,20 While TGFβ is known to enhance mesenchymal and epithelial cell proliferation and synthesis of extracellular matrix proteins, it has also been reported to inhibit hemato poiesis (reference 72 and Hayashi et al, submitted). In the pre-B cell line 70Z/3, TGFβ acts as an antagonist to agents known to induce certain B lymphocyte differentiation events.73 Thus, stromal cells may use this growth factor as a negative regulator of B lymphocyte production.

The mRNA data correlates directly with functional analyses of the stromal cell lines. Based on colony forming assays, the stromal cell clones were found to release G/M-CSF like factors into the medium (Pietrangeli et al, manuscript in preparation). Experiments using stromal cell dependent lymphocyte lines, as well as stromal cell-depleted bone marrow cells, consistently demonstrated that the bone marrow-derived stromal cells were superior to spleen in support of
lymphocyte proliferation (unpublished observations). Interleukin-7 mRNA production generally paralleled those findings.

Levels of cytokine mRNA in BMS2 cells were subject to positive regulation by exogenous factors. Addition of IL-1, IL-6, IL-7, TNF, EGF, or LPS all increased IL-6 mRNA levels. Cycloheximide also had a positive effect, although it did not lead to a "superinduction" when combined with LPS. In contrast, G/M-CSF mRNA was only increased to detectable levels by LPS and cycloheximide in combination. These observations resemble those of other laboratories examining the regulation of growth factor genes. In human and murine fibroblast, endothelial, monocyte, and stromal cell lines, treatment with IL-1 alpha, TNF, LPS, and cycloheximide have each been reported to increase IL-6 mRNA levels. The combination of TNF and cycloheximide caused up to a 300-fold "superinduction". Similarly, TNF and IL-1 have been observed to increase G/M-CSF mRNA levels in human endothelial cells, lung fibroblasts, and bone marrow stromal cells.

Less dramatic effects of exogenous factors were observed on TGFβ and M-CSF mRNA levels. Treatment of BMS2 cells with cycloheximide or TGFβ alone led to a minor (1.5-fold) increase in TGFβ mRNA levels. These results are similar to those for other cell types. TGFβ treatment induced its own mRNA levels 2.6-fold in NIH 3T3 cells, presumably through a transcriptional mechanism. Levels of M-CSF mRNA were only elevated by cycloheximide treatment. These results differ from a number of studies on human endothelial cells where IL-1 and TNF treatment induced M-CSF mRNA levels and functional activity. However, studies involving IL-1 induction of a human bone marrow stromal cell clone did not reveal a significant change in the constitutive production of M-CSF mRNA. Thus, a single cytokine gene may be subject to different regulatory pathways depending on the specific cell type under investigation.

The murine stromal cells described here have the potential for expressing at least six different cytokine genes. The levels of these gene transcripts can be regulated by exogenous cytokines. BMS2 cells are of particular interest due to their potency as a lymphoid support stroma. Moreover, these cells are noteworthy for their differentiation potential. Like many other stromal cell lines described in the literature, BMS2 cells are preadipocytes. The differentiation of BMS2 cells may be regulated by a novel stromal cell-derived bone marrow stromal cells in response to exogenous factors and how this, in turn, may influence lympho-hematopoiesis in vivo.

ACKNOWLEDGMENT

The authors acknowledge the following people for their assistance: for DNA probes, Drs Baltimore, Clark, Derynch, Freilerger, Gurney, Honjo, Kishimoto, Melcher, Roberts, Smale, Springer, Taile and Tucker; for cytokines, Drs Nawroth, Ellingsworth, Clark, Stern and Uskokovic; for technical assistance, Margaret Robinson; for preparation of the manuscript, Nancy Brown and Kathy Giles.

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JM Gimble, C Pietrangeli, A Henley, MA Dorheim, J Silver, A Namen, M Takeichi, C Goridis and PW Kincade

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