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-hematopoiesis 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 was demonstrated. All clones constitutively expressed mRNA for macrophage colony stimulating factor, interleukin-6, transforming growth factor β and neureulokin. The 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.

MATERIALS AND METHODS

Cell culture. The cell lines employed in this study included the lymphoma lines WEHI13, EL4, and W279, the neuronal cell line N2A, the macrophage line P388D, the myocyte line G7, and the murine fibroblast lines CCL 1 and NIH 3T3. All cell lines were obtained from the ATCC and maintained in culture as specified. The stromal cell lines (isolated as described in9) 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 analysis.

From the Oklahoma Research Foundation, Oklahoma City. Submitted 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.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1989 by Grune & Stratton, Inc.
0006-4971/89/7401-0008$3.00/0

described below. The factors employed included transforming growth factor \( \beta \), 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\(^{-6}\) mol/L; interleukin-1 (IL-1), 2.5 U/mL; interleukin-6 (IL-6); interleukin-7 (IL-7), 500 U/mL; cytoheximide, 25 \( \mu \)g/mL; Actinomycin, 10 \( \mu \)g/mL.

**RNA preparation.** RNA was harvested by the method of Chomczynski and Sacchi. For cell cultures, the media was removed, the cells immediately suspended in 4 mol/L guanidine 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 guanidine 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 formaldehyde/agarose gel. Poly A \(^+\) mRNA was selected on oligo-DT columns (Collaborative Research) according to the method of Aviv and Leder.

**Northern blots.** Northern blots were prepared by a modification of the method of Thomas.

**Hybridization.** Blots were prehybridized overnight at 42\(^\circ\)C in 50% formamide, 5X SSC, 5X Denhardt's solution, 0.1% SDS, 5 mmol/L EDTA, 100 \( \mu \)g RNA, 100 \( \mu \)g sheared DNA, 10 \( \mu \)g polyadenylic acid. The radiolabeled probe was heated 10 minutes at 90\(^\circ\)C and added to the blot at a concentration of 1 to 2 million cpm per mL 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 \( \mu \)g/mL RNA and sheared DNA, 10 \( \mu \)g polyadenylic acid. The blots were hybridized 16 to 18 hours at 42\(^\circ\)C and washed at low stringency (2X SSC, 0.1% SDS, 30 minutes, 50\(^\circ\)C) and then high stringency (0.1X SSC, 0.1% SDS, 30 minutes, 50\(^\circ\)C). The blots were washed in cellophane while still wet and exposed at 70\(^\circ\)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 \( \mu \) heavy chain constant region; S. Clark, Genetics Institute, Cambridge, MA—human cDNAs of IL-1a, IL-1p and IL-6 (637 bp insert); R. Derynck, Genentech, San Francisco—human TGF\( \beta \), 1.05 Kb Eco RI insert cdNA (\( \lambda \) BC1); M. Gurney, University of Chicago—murine neuroleukin cdDNA, 2.0 kb EcoRI insert (C19;)
Flringer, UNC, Chapel Hill, NC—murine MHC Class II \( \alpha \) and \( \beta \) cdna (pAAC6 and pAAC8); S. Gillis, Immunex, Seattle—murine G/M-CSF cdDNA and murine IL-3 cdDNA (400 bp insert); R. Flavell and G.L. Waneck, Biogen, Cambridge, MA—MHC Class I Q7 cdDNA (pBG36); T. Honjo, Osaka, Japan—murine IL-5 cdDNA (pSP6k-mTRF23); Kishimoto and Springer, Dana-Farber Inst, Boston—human LFA 1 \( \beta \) subunit cdDNA; F. Melcher, Basel Inst, Switzerland—murine \( \lambda \) 5; P. Ralph, Cetus Corp, Emeryville, CA—murine M-CSF cdDNA, 3.9 kb insert, (pGEM2MCSF10, culture collection #CMCC 2760, Cetus Corp); J. Roberts, Mt. Sinai Medical School, New York—rat POMC (plasmid SAL65—exon 3); Smale and Baltimore, Whitehead Inst, Cambridge, MA—murine TdT full length cdDNA (pTdT-1); J. Teele, UTSA, San Antonio, TX—murine IL-4 cdDNA probe; M. Thomas, Washington University, St. Louis—murine Ly5 cdDNA; and P. Tucker, Northwestern Medical School, Dallas—murine T cell receptor \( \beta J \)-region (plasmid pDO \( \beta \)). Additional probes included murine N-CAM cdDNA clone T.3 corresponding to bp 1636-2249 (C. Goridis); 2.2 kb murine genomic Thy1.1 Xbal Xhol probe (J. Silver); murine IL-7 cdDNA probe 1046 (Namen); murine E-cadherin and chicken N-cadherin cdNA probes (Takeichi).

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, Genetics Institute (Cambridge, MA); Tumor Necrosis Factor, 2.3 mg/mL, Dr D. Stern, Columbia University (New York); purified TGF \( \beta \) 1.1 \( \mu \)g/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 \( \mu \)g/mL, Collaborative Research Inc (Bedford, MA); Hydrocortisone, Elkins-Sinn (Cherry Hill, NJ); and Lipopolysaccharide W. S. typhosa 0901, Difco Laboratories (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 (SNS1, SNS1). These cells were cloned by selecting for adherent cells in short-term cultures followed by treatment with 5-fluorouracil. The cell lines differ in their ability to support myeloid and lymphoid proliferation and differentiation (Pietrangelo 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 \( \mu \)g 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 radiolabeled 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 summarized in Table 1. The stromal cells expressed N-cadherin, a Ca\(^{++}\) dependent intercellular adhesion molecule, but did not display its counterpart, E-cadherin. The bone marrow and
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

<table>
<thead>
<tr>
<th>Probe</th>
<th>3T3</th>
<th>BMS 1</th>
<th>BMS 2</th>
<th>SS1</th>
<th>SNS1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thy 1*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N-CAM*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MHC class I*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MHC class II*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>IgA*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lambda 5*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>TCR β*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>TdT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Ly5</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
</tbody>
</table>

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.

Abbreviation: ND, not done.

*Member of Ig gene superfamily.

Table 2. Growth Factor mRNAs

<table>
<thead>
<tr>
<th>Probe</th>
<th>3T3</th>
<th>BMS 1</th>
<th>BMS 2</th>
<th>SS1</th>
<th>SNS1</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β 2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M-CSF</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>IL-6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-7</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Neuroleukin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>POMC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IL-1α</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>IL-1β</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>IL-3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>IL-4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IL-5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Inducible</td>
<td>ND</td>
</tr>
</tbody>
</table>

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.

Abbreviation: ND, not done.
CHARACTERIZATION OF MURINE STROMAL CELLS

scripts when added in combination with a stimulating cytokine.\(^6\) 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 TGF\(\beta\) revealed that the level of TGF\(\beta\) mRNA was increased 1.5-fold following incubation of BMS2 cells with TGF\(\beta\) 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.\(^25\)\(^6\) Each of these protein families includes an isof orm linked to the plasma membrane through a phosphatidylinositol (PI) moiety.\(^4\) 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.\(^5\) 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.\(^56\) 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.\(^62\) 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,\(^27\) is of interest. The primary bone marrow cultures used to clone the cells were originally Thy 1 negative (\(^27\) and unpublished observations). Other reported stromal cell clones either lacked Thy 1
or expressed only low levels on the surface. Only 5% of total bone marrow cells are Thy 1 positive. 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. In contrast, the stromal cells did not express E-cadherin, a member of the same gene family expressed on epithelial derived tissues. 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. Together, these results imply that stromal cells are of mesodermal origin but lie outside the lymphoid and myeloid lineages.

Numerous investigators have described cytokine production by murine and human derived bone marrow stromal cell clones. Additional studies examined cytokine gene regulation in human fibroblast, monocyte, and umbilical vein endothelial cells. 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. Similar observations have been made with other growth factors. Thus, stromal cells could present locally high concentrations of cytokines 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. 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 tropic effect on neuronal cells. Interleukin-7 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. Moreover, it will synergize with IL-3 to stimulate myeloid and erythroid proliferation. M-CSF, a product of endothelial cells, monocytes, and stromal cells, is known to stimulate myeloid development. 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 tropic effect on neuronal cells. Interleukin-7 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. Moreover, it will synergize with IL-3 to stimulate myeloid and erythroid proliferation. M-CSF, a product of endothelial cells, monocytes, and stromal cells, is known to stimulate myeloid development.

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
CHARACTERIZATION OF MURINE STROMAL CELLS

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.10,17,24,74 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.10,29,30,75,76 The combination of TNF and cycloheximide caused up to a 300-fold "superinduction".76 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.10,17,19,21-22,74

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.77 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.19,22 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.10 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.3,5,6,78,82 The differentiation of BMS2 into adipocytes is subject to regulation by a number of agonists and antagonists, including hydrocortisone, TGFβ, IL-1 alpha, and TNF (manuscript in preparation). Future studies will continue to explore the differentiation potential of these mesodermal-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, Derynck, Freiinger, Gurney, Honjo, Kishimoto, Melcher, Roberts, Smale, Springer, Teale and Tucker; for cytokines, Drs Nawroth, Ellingsworth, Clark, Stern and Uuskokovic; for technical assistance, Margaret Robinson; for preparation of the manuscript, Nancy Brown and Kathy Giles.

REFERENCES

18. Sieff CA, Tsai S, Faller DV: Interleukin-1 induces cultured


33. Thomas PS: Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 77:5201, 1980


40. Faehrer K, Hogan BLM, Flavell RA: Transcription of H-2 and Qa genes in embryonic and adult mice. EMBO J 6:1265, 1987


44. Chen CLC, Dionne FT, Roberts JL: Regulation of the pro-opiomelanocortin mRNA levels in rat pituitary by dopaminergic compounds. Proc Natl Acad Sci USA 80:2211, 1983


52. Roberts RA, Spooner E, Parkinson EK, Lord BI, Allen TD, Dexter TM: Metabolically inactive 3T3 cells can substitute for marrow stromal cells to promote the proliferation and development of multipotent haemopoietic stem cells. J Cell Physiol 132:203, 1987


55. He H-T, Barbet J, Chaux J-C, Goridis P: Phosphatidylinositol is involved in the membrane attachment of NCAM-120, the smallest component of the neural cell adhesion molecule. EMBO J 5:2489, 1986
63. Whitlock CA, Tidmarsh GF, Muller-Sieburg C, Weissmann IL: Bone marrow stromal cell lines with lymphopoietic activity express high levels of a pre-B neoplasia-associated molecule. Cell 48:1009, 1986
78. Li CL, Johnson GR: Stimulation of multipotential, erythroid and other murine haematopoietic progenitor cells by adherent cell lines in the absence of detectable multi-CSF (IL-3). Nature 316:633, 1985
80. Song ZX, Shadduck RK, Innes DJ, Wahed A, Quesenberry PJ: Hematopoietic factor production by a cell line (TC-1) derived from adherent murine marrow cells. Blood 66:273, 1985
Characterization of murine bone marrow and spleen-derived stromal cells: analysis of leukocyte marker and growth factor mRNA transcript levels

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