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Genetics

Molecular cloning of a cDNA encoding interleukin 11, a stromal cell-derived lymphopoietic and hematopoietic cytokine

(colony-stimulating factor/hematopoiesis/microenvironment)

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ABSTRACT **Hematopoiesis occurs in close association with a complex network of cells loosely termed the hematopoietic microenvironment. Analysis of the mechanisms of microenvironmental regulation of hematopoiesis has been hindered by the complexity of the microenvironment as well as the heterogeneity of hematopoietic stem cells and early progenitor cells. We have established immortalized primate bone marrow-derived stromal cell lines to facilitate analysis of the interactions of hematopoietic cells with the microenvironment in a large animal species. One such line, PU-34, was found to produce a variety of growth factors, including an activity that stimulates the proliferation of an interleukin 6-dependent murine plasmacytoma cell line. A cDNA encoding the plasmacytoma stimulatory activity was isolated through functional expression cloning in mammalian cells. The nucleotide sequence contained a single long reading frame of 597 nucleotides encoding a predicted 199-amino acid polypeptide. The amino acid sequence of this cytokine, designated interleukin 11 (IL-11), did not display significant similarity with any other sequence in the GenBank data base. Preliminary biological characterization indicates that in addition to stimulating plasmacytoma proliferation, IL-11 stimulates the T-cell-dependent development of immunoglobulin-producing B cells and synergizes with IL-3 in supporting murine megakaryocyte colony formation. These properties implicate IL-11 as an additional multifunctional regulator in the hematopoietic microenvironment.**

The hematopoietic microenvironment (HM) consists of an organized array of endothelial cells lining the marrow vasculature with a network of fibroblasts, macrophages, and adipocytes in the perisinusoidal spaces (1, 2). Hematopoietic stem and progenitor cells reside in close association with the various cell types of the HM. The interactions between the hematopoietic cells and the microenvironment are still poorly understood in large measure due to the heterogeneity of the cellular compartments of the HM. However, both direct contact between the stromal and hematopoietic cells and hematopoietic growth factor production by the stromal cells are believed to contribute to the regulation of blood cell production. Long-term marrow cultures (LTMCs) provide an *in vitro* system for studying the interactions of the stromal cells with hematopoietic stem and progenitor cells (3, 4). These systems can be further dissected by the establishment of continuous stromal cell lines through the use of retroviral vectors that transfer immortalizing oncogenes into recipient infected cells (M. Rios and D.A.W., unpublished data; refs. 5 and 6). These lines can be used to study both stromal cell hematopoietic growth factor production and direct intercellular interactions between stromal and hematopoietic cells.

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We have recently established several nonhuman primate stromal cell lines with the ultimate goal of the analysis of the role of the HM in the maintenance of reconstituting hematopoietic stem cells for long periods of time in culture. One fibroblastic line, designated PU-34, supports long-term hematopoiesis by normal human or primate progenitor cells in culture (S. R. Paul and D.A.W., unpublished data). As a first step in analyzing the mechanism by which PU-34 supports hematopoiesis, we analyzed the ability of PU-34 cells to produce hematopoietic growth factors. In addition to a variety of known hematopoietic colony-stimulating factors (CSFs) and interleukins (ILs), PU-34 cells were found to produce a mitogenic activity for the IL-6-dependent plasmacytoma cell line T1165 (7). Here we report the molecular cloning of a cDNA encoding this cytokine through functional expression cloning using the simian COS-1 cell system (8–10).¶ Analysis of the nucleotide sequence of the cDNA has revealed that this cytokine is unrelated to any of the other known cytokines. In addition to its ability to support the proliferation of T1165 cells, the newly discovered cytokine stimulates the production of IgG-secreting B cells in spleen cell cultures and augments the IL-3-dependent development of megakaryocyte colonies in bone marrow cell clonal culture. These diverse biologic effects are reminiscent of some of the other pleiotropic cytokines, especially IL-6 and IL-7. Therefore, we propose that this molecule be designated interleukin 11 (IL-11).

METHODS

Establishment of Permanent Stromal Cell Lines. The PU-34 stromal cell line was derived from a long-term primate marrow culture by immortalization with a defective amphotropic transforming retroviral vector, U19BL, as described elsewhere (S. R. Paul and D.A.W., unpublished data). The U19 retrovirus plasmid was constructed as reported (5) and contains simian virus 40 large tumor antigen sequence and the neomycin phosphotransferase sequence encoding G418 resistance expressed off the Moloney murine leukemia virus long terminal repeat. An amphotropic producer clone was generated by infection of the packaging cell line ψAM (11) with ecotropic viral harvest from ψ2U19-5 (5) followed by selection in G418 (0.75 mg/ml). One clone (ψAMU19-BL) produces recombinant simian virus 40 vector virus at a titer

Abbreviations: HM, hematopoietic microenvironment; LTMC, long-term marrow culture; IL, interleukin; CFU, colony-forming unit(s); NP, 4-hydroxy-3-nitrophenylacetyl; GM-CSF, granulocyte–macrophage colony-stimulating factor.

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¶The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M37006 (human) and M37007 (primate)].

of 5×10^3 G418-resistant colony-forming units (CFU) per ml when assayed on NIH 3T3 cells. LTMCs were established by standard methods and maintained in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal calf serum, 10% horse serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) (GIBCO) (complete long-term culture medium). The LTMC adherent layer was infected 7 and 10 days after establishment with 2 ml of ψAMU19-BL viral stock in the presence of Polybrene (8 μg/ml) (Aldrich) for 2.5 hr at 33°C. Beginning 3 days after infection, the cultures were selected in G418 (0.5 mg/ml). Fourteen days after infection, G418-resistant colonies were picked and expanded in multiwell plates (Corning). One cell line, designated PU-34, was extensively analyzed based on its ability to support progenitor cells in long-term cultures.

Bioassays. PU-34 conditioned medium was evaluated for hematopoietic growth stimulatory activity by the use of multiple factor-dependent cell lines. Conditioned medium was harvested 48 hr after addition of recombinant IL-1α (2 units/ml) (Cistron, Pinebrook, NJ) to a subconfluent 25-cm^2 tissue culture flask of PU-34 cells. One murine plasmacytoma cell line, T1165 (7), normally responsive to IL-6, was used for bioassay during expression cloning of a cDNA library generated from PU-34. The T1165 proliferation assay was performed by a modification of the method described by Nordan and Potter (7). T1165 cells, routinely maintained in RPMI medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, penicillin (100 units/ml), streptomycin (100 μg/ml) (GIBCO), 50 μM 2-mercaptoethanol (Sigma), and recombinant human IL-6 (20 units/ml) (Genetics Institute) were washed and incubated at a concentration of 10^4 cells per well in 200 μl of IL-6-free medium in 96-well tissue culture plates for 48 hr at 37°C in the presence of multiple dilutions of PU-34 or cos cell conditioned medium. During the final 6 hr of incubation, the cells were pulsed with 0.5 mCi of [^{3}H]thymidine per well (1 Ci = 37 GBq; DuPont). After incubation, the cells were harvested onto a glass fiber filter, washed, and assayed on an LKB flatbed scintillation counter. Neutralizing goat anti-human IL-6 antibody was included to abrogate the effect of IL-6 in the PU-34 or cos cell conditioned medium.

The murine plaque-forming assay was performed by incubating 7.5×10^6 spleen cells from naive C57BL/6 mice with 3×10^6 4-hydroxy-3-nitrophenylacetyl (NP)-modified horse erythrocytes in 0.75 ml of Mishell–Dutton medium (12) supplemented with 5% fetal calf serum with test samples for 5 days. NP-coupled horse erythrocytes or sheep erythrocytes were prepared as described (13). These cultures were fed daily by addition of 0.1 ml of supplemental medium containing 5% fetal calf serum without test samples. NP-responsive B cells were identified at the end of the culture period using the NP-coupled sheep erythrocyte plaque assay as described by Dresser and Greaves (14) with the percentage response calculated by comparing the numbers of plaques obtained from cultures supported with the test samples with those cultures supplemented with medium alone. In a typical experiment, background responses in the absence of exogenous factors yielded 6000 NP-specific plaque-forming cells per 7.5×10^6 cells plated.

The murine CFU megakaryocyte assay was performed by plating 2.5×10^5 murine bone marrow cells in 0.4 ml of IMDM supplemented with 20% fetal calf serum in six-well dishes. Clot formation was initiated by addition of 0.25 mg of fibrinogen and 0.25 unit of thrombin (Sigma) at 37°C. Test samples at various dilutions were added to the fibrin clot and cultures were subsequently incubated for 6 days at 37°C. The clots were fixed with 2.5% glutaraldehyde and stained with acetylcholinesterase (0.5 mg/ml) as described (15, 16). Positive colonies (containing megakaryocytes) were enumerated under direct microscopy.

Molecular Cloning and RNA Analysis. PU-34 cells were stimulated for 24 hr with IL-1α at a concentration of 2 units/ml and poly(A)$^+$ RNA was prepared from these cells by standard methods. A cDNA expression library was prepared from 5 μg of poly(A)$^+$ RNA prepared from IL-1α-induced PU-34 cells and ligated via *Xho* I linkers into the COS-1 expression vector pXM as described (10). The ligation reaction was used to transform competent *Escherichia coli* (strain HB101) to generate a library of ≈500,000 ampicillin-resistant colonies. Bacterial colonies were replicated onto nitrocellulose filters and subdivided, and plasmid DNA was isolated by standard methods. Five micrograms of each plasmid DNA was used to transfect COS-1 cells by the DEAE-dextran protocol with the addition of 0.1 mM chloroquine (10). Culture supernatant from transfected COS-1 cells was harvested 72 hr after transfection and assayed for biologic activity (see above). One positive pool was obtained and subdivided to obtain a single positive plasmid. The insert of this cDNA was sequenced by the dideoxynucleotide chain-termination method on supercoiled templates with synthetic oligonucleotide primers (17).

RNA blot analysis was performed on 5 μg of poly(A)$^+$ RNA after fractionation on a 7% (vol/vol) formaldehyde/1% agarose gel. Size-fractionated RNA was transferred to nylon filters (NEN) and probed with ^{32}P-labeled cDNA probes. Prehybridization, hybridization, and posthybridization washes of filters were performed as recommended by the manufacturer. Filters were exposed to x-ray film in the presence of a calcium tungstate intensifying screen at −70°C. Probes used included human IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, granulocyte–macrophage (GM)-CSF, G-CSF, M-CSF, leukemia inhibitory factor, and primate IL-11.

Protein Analysis. COS-1 cells were pulse labeled 24 hr after transfection with 0.3 mCi of [^{35}S]methionine in 1.0 ml of methionine-free Dulbecco's modified Eagle's medium for 4 hr. Ten-microliter samples of labeled COS-1 conditioned medium were analyzed by SDS/PAGE with the Laemmli buffer system on a 12% gel as described (10). The reference protein sample was purchased from Bethesda Research Laboratories.

RESULTS

Generation of the Cell Line and Bioactivity of PU-34 Conditioned Medium. Infection of primate LTMCs with the defective retroviral vector U19-BL followed by selection in G418 led to the formation of multiple G418-resistant stromal cell clones. One cell line, PU-34, demonstrated the capacity to maintain multipotent human and primate progenitor cells for up to 3 weeks in culture and was used for further analysis of cytokine activity. In addition to known growth factor activities including IL-6, IL-7, GM-CSF, M-CSF, G-CSF, and LIF/HILDA (data not shown), the IL-1α-stimulated PU-34 conditioned medium proved capable of stimulating the proliferation of the T1165 murine plasmacytoma cell line (7), even in the presence of a neutralizing antiserum against human IL-6 (Fig. 1*A*). This assay was used to screen an expression library for cDNAs capable of directing the expression of putative novel T1165-stimulatory activity when transfected into monkey COS-1 cells.

Generation of the PU-34 cDNA Library and pC1R6 cDNA Clone. The cDNA expression library was prepared from 5 μg of poly(A)$^+$ RNA and ligated into the COS-1 expression vector pXM as described above (9). Three hundred pools of this library, each comprising 300–500 individual clones, was transfected into COS-1 cells by DEAE-dextran DNA transfection with the addition of 0.1 mM chloroquine. Culture supernatant from COS-1 cells was harvested 72 hr after transfection and assayed for T1165 stimulatory activity. Of 300 pools screened, we identified 17 pools that contained

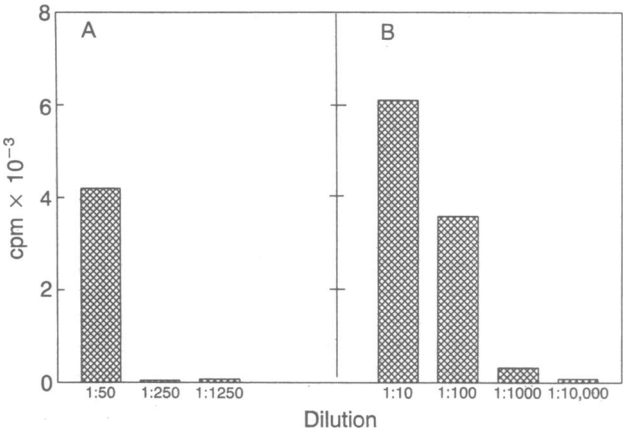

FIG. 1. Identification of T1165 growth-promoting activity produced by IL-1α-induced PU-34 cells. Conditioned medium from IL-1α-induced PU-34 (*A*) and from pC1R6-transfected COS-1 cells (*B*) was tested for the ability to stimulate [^{3}H]thymidine uptake (cpm) by the plasmacytoma T1165 cells at the indicated final dilutions. The PU-34 conditioned medium was tested in the presence of an excess of a neutralizing goat anti-human IL-6 antiserum. Data obtained from purification of COS-1 cell-derived IL-11 indicate the concentration of cytokine is 100 ng/ml with a specific activity of 3×10^6 units per mg of protein (data not shown).

detectable levels of IL-6 (as determined by neutralization with anti-IL-6 antibody) and 1 that contained T1165-stimulatory activity resistant to inhibition by antibody. This pool was subdivided and the transfection process was repeated until a single plasmid, pC1R6, was obtained that encoded the T1165 proliferation activity. The conditioned medium from pC1R6-transfected COS-1 cells stimulated measurable incorporation of [^{3}H]thymidine by T1165 cells, even at final dilutions up to 1:1000 (Fig. 1*B*). The nucleotide sequence of the pC1R6 cDNA shown in Fig. 2 contains a single long open reading frame of 597 nucleotides encoding a predicted 199-amino acid polypeptide. Located immediately adjacent to the putative initiation codon is a stretch of 17–20 hydrophobic amino acids that resembles a conventional protein secretory leader sequence. Although the initial cDNA clone, pC1R6, proved to be incomplete, analysis of additional cDNAs revealed that this transcript contains ≈420 base pairs of 3′ noncoding sequence with multiple copies of the RNA instability sequence, ATTTA, believed to be an important regulatory element for cytokine gene expression (18). Comparison of the nucleotide and derivative amino acid sequences revealed no significant similarity to any sequences in GenBank.

SDS/PAGE analysis of conditioned medium from [^{35}S]-methionine-labeled pC1R6-transfected COS-1 cells revealed the presence of a prominent 20-kDa species that was not present in mock-transfected controls (Fig. 3*A*), consistent with the molecular mass expected for an ≈180-amino acid secreted protein. This size estimate as well as the lack of heterogeneity of the expressed protein are in accordance with the absence of the consensus sequence (Asn-Xaa-Thr/Ser) (19) for the addition of asparagine-linked carbohydrate. Interestingly, the predicted amino acid sequence of the mature protein includes no cysteine residues, a feature not found with any other cytokine gene.

Evaluation of Expression of IL-11 and Isolation of the Human IL-11 cDNA. Northern blot analysis of mRNA from several different cell lines revealed the existence of two distinct transcripts, ≈2.5 and ≈1.5 kb long, which hybridized with the pC1R6 probe (Fig. 3*B*). This difference results from additional 3′ noncoding sequences in the larger transcript as demonstrated by isolation and analysis of additional cDNA clones (data not shown). The expression of both transcripts

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GGGAAGGGTT AAAGGCCCCC GGCTCCCTGC CCCCTGCCCT GGGGAACCCC TGGCCCTGCG GGGAC  (65)

ATG AAC TGT GTT TGC CGC CTG GTC CTG GTC GTG CTG AGC CTG TGG CCA GAT ACA (119)
MET Asn Cys Val Cys Arg Leu Val Leu Val Val Leu Ser Leu Trp Pro Asp Thr

    C                                C                T
GCT GTT GCC CCT GGG CCA CCA CCT GGC TCC CCT CGA GCT TCC CCA GAC CCT CGG (173)
Ala Val Ala Pro Gly Pro Pro Pro Gly Ser Pro Arg Ala Ser Pro Asp Pro Arg
                                  (Pro)      (Val)
                                                    C
GCC GAG CTG GAC AGC ACC GTG CTC CTG ACC CGC TCT CTC CTG GAG GAC ACG CGG (227)
Ala Glu Leu Asp Ser Thr Val Leu Leu Thr Arg Ser Leu Leu Glu Asp Thr Arg
                                                      (Ala)
       G   GC          G
CAG CTG ACT ATA CAG CTG AAG GAC AAA TTC CCA GCT GAC GGG GAC CAC AAC CTG (281)
Gln Leu Thr Ile Gln Leu Lys Asp Lys Phe Pro Ala Asp Gly Asp His Asn Leu
      (Ala Ala)       (Arg)
                              T
GAT TCC CTG CCC ACC CTG GCC ATG AGC GCG GGG GCA CTG GGA GCT CTA CAG CTC (335)
Asp Ser Leu Pro Thr Leu Ala MET Ser Ala Gly Ala Leu Gly Ala Leu Gln Leu

  A G                                                              C
CCG AGT GTG CTG ACA AGG CTG CGA GCG GAC CTA CTG TCC TAC CTG CGG CAT GTG (389)
Pro Ser Val Leu Thr Arg Leu Arg Ala Asp Leu Leu Ser Tyr Leu Arg His Val
   (Gly)
                  C           GGT                                  C
CAG TGG CTG CGT CGG GCA ATG GGC TCT TCC CTG AAG ACC CTG GAG CCT GAG CTG (443)
Gln Trp Leu Arg Arg Ala MET Gly Ser Ser Leu Lys Thr Leu Glu Pro Glu Leu
                     (Gly)
                  G   A
GGC ACC CTG CAG ACC CGG CTG GAC CGG CTG CTG CGC CGG CTG CAG CTC CTG ATG (497)
Gly Thr Leu Gln Thr Arg Leu Asp Arg Leu Leu Arg Arg Leu Gln Leu Leu MET
            (Ala)
                              CA      G
TCC CGC CTG GCC CTG CCC CAG CTG CCC CCA GAC CCG CCG GCG CCC CCG CTG GCG (551)
Ser Arg Leu Ala Leu Pro Gln Leu Pro Pro Asp Pro Pro Ala Pro Pro Leu Ala
                        (Pro)
                  G
CCC CCC TCC TCA ACC TGG GGG GGC ATC AGG GCC GCC CAC GCC ATC CTG GGG GGG (605)
Pro Pro Ser Ser Thr Trp Gly Gly Ile Arg Ala Ala His Ala Ile Leu Gly Gly
            (Ala)
                                          A   G
CTG CAC CTG ACA CTT GAC TGG GCC GTG AGG GGG CTA CTG CTG CTG AAG ACT CGG (659)
Leu His Leu Thr Leu Asp Trp Ala Val Arg Gly Leu Leu Leu Leu Lys Thr Arg

CTG TGACCCGAGG CCCAGAGCCA CCACCGTCCT TCCAAAGCCA CATCTTATTT ATTTATTTAT   (722)
Leu

TTCGGTACTG GGGGCGAAAC AGCCAGGTGA TCCCCCTGCC TTTAGCTCCC CCTAGTTAGA     (782)

GACAGTCCTT CCGTGAGGCT GGGGGGCATC TGTGCCTTAT TTATACTTAT TTATTTCAGG     (842)

AGCGGGGGTG GGCTCCTGGG TCCCCGAGGA GGAGGGAGCT GGGGTCCCGG ATTCTTGTGT     (902)

CCACAGACTT CTGCCCTGGC TCCTCCCCCT CGAGGCCTGG GCAGGAATAC ATACTATTTA     (962)

TTTAAGCAAT TACTTTTCAT GTTGGGGTGG GGAGGGAGGG GAAAGGGAAG CCTGGGTTTT    (1022)

TGTACAAAAA TGTGAGAAAC CTTTGTGAGA CGGAGAACAA GGAATTAAAT GTGTCATACA    (1082)

TAAAAAAAAA AAAAAAAAAA                                                (1102)
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FIG. 2. Analysis of the nucleotide sequences of the primate and human IL-11 cDNA. The complete nucleotide and predicted amino acid sequences of the single long open reading frame are as indicated. The differences found in the human IL-11 nucleotide sequence are indicated above the primate sequence and the resulting changes in amino acid sequences are below the primate sequence. The nucleotide sequence from 1–721 from the primate sequence was obtained from pC1R6; the remainder from 721–1092 from a second primate cDNA was isolated by hybridization with pC1R6.

by PU-34 cells proved to be IL-1α-regulated, since neither transcript was evident in the absence of IL-1α induction. In addition to PU-34, a human fetal lung fibroblast cell line (MRC 5) (20) was found to express both transcripts after stimulation with phorbol 12-myristate 13-acetate (50 ng/ml) and IL-1α (1 unit/ml) while the human simian virus 40-transformed trophoblast cell line TPA30-1 (21) constitutively expressed only the 2.5-kb transcript (Fig. 3*B*). Neither transcript was identified by RNA blot analysis of the human T-cell lines C10-MJ2 (22), C5-MJ2 (23), and Mo (24) from lectin-stimulated human peripheral blood lymphocytes or from human placenta (data not shown). Thus far, the only identified sources of IL-11 are mesenchymal-derived adherent cells. Analysis of the human cDNA sequence isolated from the MRC 5 cell line revealed that the primate and human coding regions share ≈97% identity at the nucleotide level (Fig. 2).

Biological Activities of IL-11. Because of the diversity and multiplicity of activities observed with many of the cytokines such as IL-6 and IL-7, we have begun testing the PU-34 cell-derived cytokine for activity in a variety of hematopoietic and immunological assay systems. In initial studies, the T1165-stimulatory factor was found to significantly enhance the formation of immunoglobulin-secreting B cells in a standard murine spleen cell plaque-formation assay, even at final dilutions as high as 1:500 (Fig. 4*A*). This system measures the development of B cells in culture that respond to a specific immunogen, NP-modified horse erythrocytes in the context

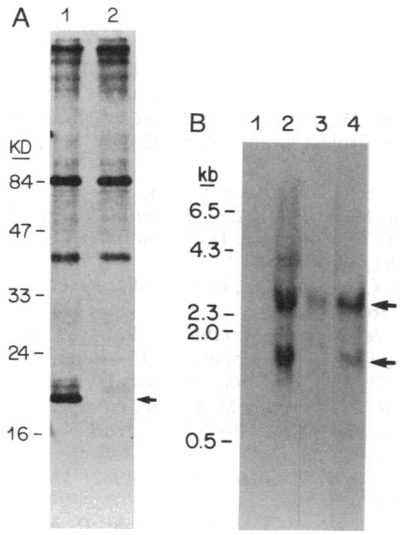

FIG. 3. Analysis of the expression of pC1R6 protein by transfected COS-1 cells (*A*) and pC1R6-hybridizing transcripts by different cell lines (*B*). (*A*) Protein expression was assessed by SDS/PAGE analysis of conditioned medium from pC1R6-transfected (lane 1) or mock-transfected (lane 2) COS-1 cells pulse-labeled with [^{35}S]-methionine. (*B*) pC1R6 transcript expression was assessed by Northern blot analysis of mRNA prepared from uninduced PU-34 cells (lane 1) or IL-1α-induced PU-34 cells (lane 2), TPA 30-1 cells (lane 3), and IL-1α and phorbol 12-myristate 13-acetate-induced MRC 5 cells (lane 4).

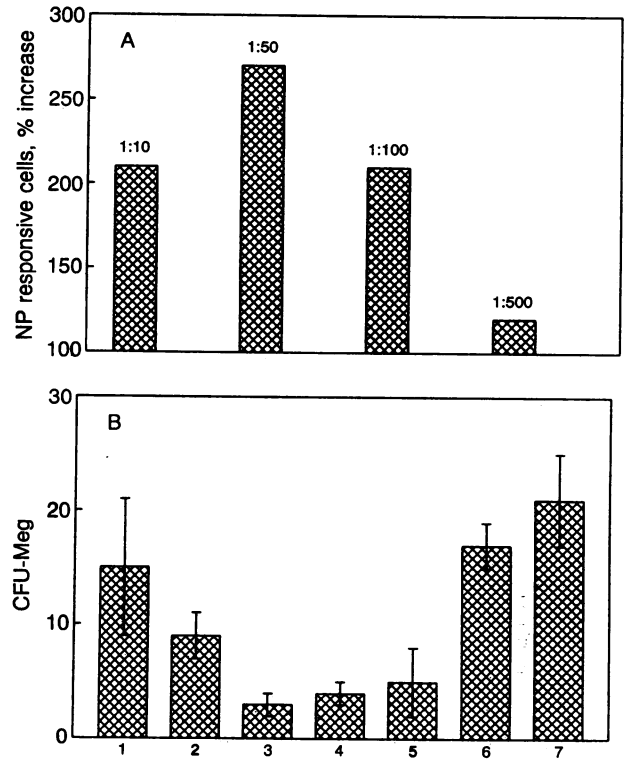

FIG. 4. Enhancement of the development of murine NP-reactive B cells (*A*) and IL-3-dependent murine megakaryocyte colonies (*B*) by pC1R6-transfected COS-1 cell conditioned medium. (*A*) The percent of control response is the increase in the development of NP-responsive B cells in 5-day cultures of naive spleen cells stimulated with NP horse erythrocytes supported by the indicated dilution of pC1R6-transfected COS-1 cell conditioned medium compared to control cultures supplemented with medium alone. (*B*) The colony number represents the total number of megakaryocyte (Meg) colonies (acetylcholinesterase-positive cells) in 6-day cultures of mouse bone marrow cells supported by: 1, a 1:10 dilution of canine aplastic anemia serum (positive control); 2, murine IL-3 (150 units/ml); 3, no stimulus; 4 and 5, 1:10 and 1:50 dilutions, respectively, of pC1R6-transfected COS-1 cell conditioned medium alone; 6 and 7, 1:10 and 1:50 dilutions, respectively, of pC1R6-transfected COS-1 cell conditioned medium supplemented with murine IL-3 (150 units/ml). Apparent decreasing bioactivity with an increasing concentration of cytokine is a phenomenon occasionally seen in biologic assays of COS cell supernatants. This may be due to inhibitory effects of a high concentration of the cytokine or other cos-1 cell-derived proteins.

of the normal cellular constituents of the spleen. Thy 1 complement-mediated depletion of T cells from the spleen cell cultures resulted in complete abrogation of the response (data not shown), demonstrating that the increase in NP-responding B cells, even in the presence of the PU-34-derived cytokine, depends at least in part on the presence of T cells. The activity of the cytokine is therefore not attributable to a direct B-cell mitogenic effect because B-cell mitogens such as lipopolysaccharide stimulate the formation of NP-specific plaque-forming cells in the absence of T cells (R.M.O., unpublished data).

Analysis of the effects of the PU-34-derived cytokine in a variety of hematopoietic culture systems revealed striking effects on megakaryocyte development (Fig. 4*B*). With murine bone marrow cells as targets, the cytokine had little effect alone, but it stimulated megakaryocyte colony formation supported by IL-3 3-fold. IL-6 has also been found to augment IL-3-dependent megakaryocyte colony formation (25, 26) and the effect of IL-6 and the PU-34-derived cytokine on megakaryocyte development appear qualitatively and quantitatively similar (data not shown). These results indicate that the cytokine may play an important role in regulating megakaryocytopoiesis.

DISCUSSION

Hematopoietic stem cells proliferate and differentiate in close contact with multiple adherent cells comprising the HM. These cellular interactions as well as local production of hematopoietic growth factors and other cytokines are believed to play critical roles in stem cell proliferation and self-renewal as well as inductive microenvironment restriction of differentiation. Although long-term marrow cultures provide a useful model for studying these interactions, this system remains a complex combination of multiple cell types. We have used retroviral vectors to establish permanent cell lines representing various cell types of the murine HM (6). These lines provide simplified systems for analysis of the interactions between stromal and hematopoietic cells. The analysis of growth factor production by some stromal cell lines has previously led to the discovery of additional cytokine activities including the recent identification and the molecular cloning of the gene encoding IL-7 (27) and another thymic stromal cell line-derived T-cell growth factor activity that is not yet molecularly defined (28). Our identification of a mitogen for an IL-6-dependent plasmacytoma cell line (T1165) from a primate stromal cell line, PU-34, provides further confirmation of the general power of this approach for the identification of additional cytokines. Our early analysis of the biologic properties of the T1165-stimulatory cytokine has already revealed a multiplicity of activities reminiscent of some of the other interleukins, especially IL-6 (29) and IL-7 (27).

The initial experiments described here have already begun to provide some insight into the regulatory significance of IL-11. The isolation of IL-11 from a marrow stromal fibroblast cell line implicated this cytokine in the regulation of marrow hematopoiesis or lymphopoiesis. The role in lymphopoiesis was originally suggested by the mitogenic activity of IL-11 for the IL-6-dependent plasmacytoma cell line

T1165. This expectation has been at least partially confirmed in a spleen plaque assay system that measures the development of antibody-producing cells in primary cultures of murine splenocytes. However, this effect required the presence of functional T cells, suggesting that this effect of IL-11 may be at least in part indirectly mediated by T cells. This observation presents the intriguing possibility that IL-11, like IL-6, may be an important regulator of T-cell function.

The identification of IL-11 through the use of an assay previously believed to be specific for IL-6, a cytokine with many effects on hematopoietic cells, has prompted us to test this cytokine in a variety of other hematopoietic assays. The initial screen led to the observation that IL-11 significantly augments the formation of megakaryocyte colony formation supported by IL-3. Interestingly, the response of IL-11 in this culture system is similar to that reported for IL-6. Both IL-6 and IL-11 enhance IL-3-dependent megakaryocyte colony formation *in vitro* while IL-7, another cytokine that acts on progenitor cells of this lineage, has been reported to selectively support megakaryocyte maturation (30). The results from *in vitro* culture systems suggest that the early stages of megakaryocytopoiesis are regulated by the interactions of early hematopoietic growth factors such as IL-3 or possibly IL-4 with synergistic factors such as IL-6 and IL-11; the later stages appear to be regulated by maturation factors such as IL-7. The availability of all of these recombinant cytokines, now including IL-11, will soon make it possible to test this model in various animal systems.

The mechanisms utilized by PU-34 cells to maintain multipotent progenitors in culture, like those operative in long-term marrow cultures, remain to be defined. Whether or not IL-11, or any of the other cytokines elaborated by PU-34 cells, contribute to the maintenance of multipotent progenitors in culture remains to be determined. The development of neutralizing antibodies against recombinant IL-11 should facilitate the analysis of the function of IL-11 in long-term hematopoietic cultures supported by PU-34 cells as well as long-term cultures supported by primary stromal cells. The recombinant IL-11 protein, the neutralizing antibodies against IL-11, and the IL-11 cDNA clone together will facilitate the analysis of how this pleiotropic cytokine fits into the ever-expanding lympho–hematopoietic cytokine network and how it interacts with the other members of this family.

Note Added in Proof. Although in the original polyacrylamide gel (Fig. 3*A*), we estimated the size of COS-1-produced IL-11 to be 20 kDa, more recent gels show the apparent molecular mass to be closer to 23 kDa.

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