Progenitor Cell Hyperplasia With Rare Development of Myeloid Leukemia in Interleukin 11 Bone Marrow Chimeras

By Robert G. Hawley,*§ Andrew Z. C. Fong,*
Bo Y. Ngan,‡ Véronique M. de Lanux,*§
Steven C. Clark,‖ and Teresa S. Hawley*

*From the *Division of Cancer Research and the ‡Department of Pathology, Sunnybrook Health Science Centre, and the §Department of Medical Biophysics, University of Toronto, Toronto, Ontario M4N 3M5 Canada; and ‖Genetics Institute, Cambridge, Massachusetts 02140*

Summary

Post 5-fluorouracil-treated murine bone marrow cells infected with a recombinant retrovirus (murine stem cell virus–interleukin 11 [MSCV-IL-11]) bearing a human IL-11 gene were transplanted into lethally irradiated syngeneic mice. Analysis of proviral integration sites in DNA prepared from hematopoietic tissues and purified cell populations of long-term reconstituted primary and secondary recipients demonstrated polyclonal engraftment by multipotential stem cells. High levels (100–1,500 U/ml) of IL-11 were detected in the plasma of the MSCV-IL-11 mice. Systemic effects of chronic IL-11 exposure included loss of body fat, thymus atrophy, some alterations in plasma protein levels, frequent inflammation of the eyelids, and often a hyperactive state. A sustained rise in peripheral platelet levels (~1.5-fold) was seen throughout the observation period (4–17 wk). No changes were observed in the total number of circulating leukocytes in the majority of the transplanted animals (including 10 primary and 18 secondary recipients) despite a >20-fold elevation in myeloid progenitor cell content in the spleen. The exceptions were members of one transplant pedigree which presented with myeloid leukemia during the secondary transplant phase. A clonal origin of the disease was determined, with significant expansion of the MSCV-IL-11-marked clone having occurred in the spleen of the primary host. Culturing of leukemic spleen cells from a quaternary recipient led to the establishment of a permanent cell line (denoted PGMD1). IL-11–producing PGMD1 myeloid leukemic cells are dependent on IL-3 for continuous growth in vitro and they differentiate into granulocytes and macrophages in response to granulocyte/macrophage colony-stimulating factor. The inability of autogenously produced IL-11 to support autonomous growth of PGMD1 cells argues against a mechanism of transformation involving a classical autocrine loop.

Interaction between hematopoietic and stromal cells in the bone marrow is important for the maintenance of steady-state hematopoiesis (1). IL-11 is a recently identified multifunctional stromal cell–derived factor whose actions on cells of the hematopoietic and immune systems implicate it as a potential contributor to this process (2).

A cDNA encoding IL-11 was originally isolated from the PU-34 primate bone marrow stromal cell line based on an activity in the culture supernatant that stimulated the proliferation of IL-6–dependent T1165 murine plasmacytoma cells (3, 4). The identification of IL-11 through the use of an IL-6 bioassay prompted the testing of this cytokine in other IL-6–responsive systems. The results of these studies have confirmed that IL-11 and IL-6 possess overlapping biologic properties. Like IL-6, IL-11 has megakaryocytopoietic and thrombopoietic functions (4–8). In addition, IL-11 has been shown to synergize with IL-3, IL-4, or the ligand for the c-kit receptor to promote the growth of blast colony-forming cells (CFC)[1], an effect previously described for IL-6 and G-CSF (9–11). IL-11 also shares with IL-6 the ability to augment antigen-specific antibody responses (12). Outside the hematopoietic system, activities common to both IL-11 and IL-6 have been described on hepatocytes, preadipocytes, and neuronal cells (13–16).

There have been a limited number of reports of in vivo effects of IL-11 on hematopoiesis. Administration of IL-11 to mice accelerated platelet and neutrophil recovery after bone

[1] *Abbreviations used in this paper:* CFC, colony-forming cells; LIF, leukemia inhibitory factor; MSCV, murine stem cell virus; neo, neomycin phosphotransferase; pgk, phosphoglycerate kinase.

marrow transplantation (7). By comparison, short-term treatment of normal mice with IL-11 resulted in modest increases in peripheral platelet levels above those of vehicle-treated controls (~1.3-fold) with minimal effects noted on circulating neutrophil numbers (8, 17, 18). These findings are comparable to those obtained by in vivo administration of IL-6 (19–21), but contrast sharply with the striking changes we observed in mice chronically exposed to high plasma concentrations of IL-6 after engraftment with bone marrow cells constitutively expressing a retrovirally introduced IL-6 gene (22). The IL-6 transplant mice uniformly developed a fatal myeloproliferative syndrome characterized by neutrophil excess (up to 400 $\times$ 10^3 cells/mm^3), microcytic anemia, thrombocytosis followed by thrombocytopenia, and marked alterations in plasma protein levels. In general, these hematologic changes were greater than had previously been seen in other IL-6 animal models (23, 24). Thus, in the present study, we used our retroviral delivery system to examine the long-term hematologic consequences of dysregulated IL-11 expression.

Materials and Methods

Murine Stem Cell Virus (MSCV)–IL-11 Retrovirus Vector and Cell Lines. The MSCV-IL-11 vector (see Fig. 1 *A*) was constructed by inserting a 0.8-kb EcoRI-BglII fragment of a human IL-11 cDNA (4) into the corresponding sites of the polylinker in the MSCV vector (version 2.1 has unique EcoRI, HpaI, XhoI, and BglII sites) (22). The salient features of the MSCV vector design include variant LTRs and modified 5′ untranslated sequences conferring efficient LTR-directed expression in primitive cells, an extended packaging region (ψ^+) for high viral titer containing mutations that abolish the synthesis of any gag-related polypeptides and eliminate the need for splicing to express inserted genes, and the absence of all envelope sequences, thereby abrogating the possibility of homologous recombination with corresponding sequences of endogenous retroviruses. The neomycin phosphotransferase (*neo*) gene in MSCV v2.1 contains a synthetic initiation sequence favorable for translation in mammalian cells and has been placed under the transcriptional control of an internal phosphoglycerate kinase (pgk) promoter for constitutive expression in a wide range of cell types (25). Plasmids were constructed using standard techniques (26).

Recombinant virus was produced from clones of GP+E-86 ecotropic helper-free packaging cells (27), generated as described previously by infection of tunicamycin-treated cells with supernatant from transient transfectants (22). The cell line used for all gene transfer experiments, GP+E-86/MSCV-IL-11 c3, produces structurally intact MSCV-IL-11 virus with a titer of 4 $\times$ 10^6 G418-resistant CFU/ml when assayed on NIH3T3 fibroblasts. Cells were maintained in DME with 4.5 g/l glucose (GIBCO BRL, Gaithersburg, MD) supplemented with 10% calf serum (Hyclone Laboratories, Logan, UT) in a humidified atmosphere containing 5% CO_2 at 37°C.

Retrovirus Infection and Transplantation of Bone Marrow. Female BALB/c mice (Charles River Laboratories, Montreal, Canada) were used at 6–8 wk of age as bone marrow donors and recipients. Bone marrow processing, infection, and transplantation were carried out according to established procedures (22, 28). In brief, bone marrow was flushed from hind limbs of donors injected 4 d previously with 150 mg/kg 5-fluorouracil (Hoffmann-La Roche Ltd., Mississauga, Canada) with ice-cold IMDM containing 50 μM 2-ME and 10% heat inactivated FCS (GIBCO BRL). After erythrocyte lysis in 0.17 M ammonium chloride, nucleated cells were added to 100 mm petri dishes (Fisher Scientific Co., Pittsburgh, PA) at a density of 5 $\times$ 10^5 cells/ml in IMDM supplemented with 50 μM 2-ME, 10% heat-inactivated FCS, 10% conditioned medium from X630-rIL3 cells (a source of IL-3; a gift of F. Melchers, Basel, Switzerland) (29), and 10% conditioned medium from Sp2/mIL-6 cells (a source of IL-6) (30). After 48 h, the bone marrow cells were collected and added to subconfluent monolayers of GP+E-86/MSCV-IL-11 c3 cells in 100-mm tissue culture dishes (Nunc, Roskilde, Denmark) at a density of 5 $\times$ 10^5 cells/ml in fresh medium supplemented with 8 μg/ml polybrene. After a further 48 h, nonadherent bone marrow cells were harvested and incubated in cocultivation medium containing 0.75 mg/ml (actual concentration) G418 (GIBCO BRL). These cells were collected 24 h later and injected via the tail vein into recipients that had received 7 Gy of irradiation (gammacell 1000; Atomic Energy of Canada Ltd., Kanata, Ontario, Canada). Each mouse received between 5 $\times$ 10^5 and 2 $\times$ 10^6 cells (seven donor equivalents per recipient). For serial transplantations, 10^6 bone marrow cells from affected animals were injected intravenously into lethally irradiated (7 Gy) recipients. Mice were housed one to four per sterilized microisolator cage on laminar flow racks. The colony was periodically tested for a panel of viruses by analysis of serum samples (Charles River Laboratories Diagnostic Services, Wilmington, MA). Animals tested negative for sendai virus, pneumonia virus of mice, mouse hepatitis virus, minute virus of mice, mouse polio virus and reovirus type 3. The animals were also found to be free of mycoplasma.

Hematologic and Pathologic Analysis. Blood was collected from the retroorbital sinus at weekly/biweekly intervals after transplant and immediately before death. Total leukocytes, total erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, total platelets, and mean platelet volume were determined on a System 9000 Hematology Series Cell Counter (Serono-Baker Instruments, Allentown, PA) equipped with a veterinarian software package (22). Controls were mice transplanted with hematopoietic cells infected with other MSCV viruses as well as age-matched littermates.

Mice were killed by cervical dislocation and necropsy examinations were performed immediately after death. Tissues were preserved in 10% neutral-buffered formalin overnight, then embedded in paraffin, sectioned, and stained with hematoxylin and eosin before examination by light microscopy (22). Tissues examined included spleen, thymus, lungs, heart, liver, pancreas, stomach, intestines, kidneys, lymph nodes, sternum (bone marrow), and eyelids.

In Vitro Progenitor Cell Assay. The number of CFC was determined by plating nucleated bone marrow (at 10^4 and 2 $\times$ 10^4 cells/ml) and spleen cells (at 2 $\times$ 10^4 and 4 $\times$ 10^4 cells/ml) in duplicate 1-ml cultures (35-mm Lux suspension plates; Nunc, Inc., Naperville, IL) containing α-MEM, 0.8% methylcellulose, 30% FCS, 1% BSA, 100 μM 2-ME, 3 U/ml erythropoietin, and 1% PWM-stimulated spleen conditioned medium (a source of IL-3 and GM-CSF; Terry Fox Laboratory, Vancouver, Canada) as described (31). Where indicated, G418 was added to a final concentration of 1 mg/ml. Colonies (consisting of more than 50 cells) of granulocytes, macrophages, erythroid cells, and mixtures of these with megakaryocytes were enumerated using an inverted microscope on day 7 and recorded together as CFC.

Isolation of Primary Cell Populations. To generate populations enriched for mast cells (adherent and nonadherent), bone marrow or spleen cells were cultured in 100-mm tissue culture dishes in IMDM supplemented with 50 μM 2-ME, 10% heat-inactivated FCS, 10% conditioned medium from X630-rIL3 cells, and 0.75

mg/ml G418 for 3–4 wk. Populations of macrophages were prepared by culturing bone marrow or spleen cells for 48 h in IMDM supplemented with 50 μM 2-ME, 10% heat-inactivated FCS, 10% conditioned medium from X630-rIL3 cells, and 20% conditioned medium from L929 cells (a source of CSF-1), transferring the nonadherent cells to dishes containing IMDM supplemented with 50 μM 2-ME, 10% heat-inactivated FCS, 30% conditioned medium from L929 cells, and 0.75 mg/ml G418, and culturing for periods of up to 4 wk. T cell populations were obtained by culturing spleen cells in RPMI 1640 medium (GIBCO BRL) supplemented with 50 μM 2-ME, 10% heat-inactivated FCS, 10% conditioned medium from X630-rIL2 cells (a source of IL-2; a gift of F. Melchers) (29), 10% conditioned medium from X630-rIL4 cells (a source of IL-4; a gift of F. Melchers) (29), 5 μg/ml Con A, and 0.75 mg/ml G418 for more than 3 wk. The various cell populations were judged to be >90% pure by cytochemical and immunological criteria (32, 33).

Bioassays. IL-11 activity in culture supernatants and plasma samples was detected by T10 microproliferation assay (a gift of F. Bennett, Genetics Institute) (34). The cells were propagated in RPMI 1640 medium supplemented with 50 μM 2-ME, 10% heat-inactivated FCS, and 20 U/ml recombinant human IL-11. Purified recombinant human IL-11 (a gift of P. Schendel, Genetics Institute) was produced in *Escherichia coli* and had a sp act of 2.5×10^6 U/mg protein. For the assay, T10 cells were harvested by centrifugation, washed twice in medium lacking IL-11, and then used at 10^4 cells/200 μl well in the presence of twofold dilutions of samples to be tested. Cells were labeled with 0.2 μCi [^{3}H]TdR (New England Nuclear, Boston, MA) from 48–56 h. The incorporated radioactivity was determined for triplicate cultures by liquid scintillation counting (betaplate; Wallach, Gaithersburg, MD) after transfer of cellular debris to glass fiber filters with a cell harvester (Skatron Inc., Sterling, VA). The standard deviation of means of triplicate cultures was always <15%. Each assay was standardized with recombinant human IL-11 and values (U/ml) were calculated by probit analysis. 1 U/ml of IL-11 is the amount that induces half-maximal [^{3}H]TdR incorporation. When included, 1/100 dilution of a rat mAb against murine IL-6 (a gift of L. Sachs, Weizmann Institute, Rehovot, Israel) (35) completely neutralized IL-6–induced proliferation of T10 cells.

IL-6 activity in plasma samples was tested for by B9 microproliferation assay (36) essentially as described (22).

Microwell cultures of 32D and OTT1 cells were similarly used to assay IL-3 and GM-CSF as described (37). OTT1 murine myeloid cells which are dependent for their survival and proliferation on one of IL-3, GM-CSF, or IL-5 were obtained from primary cultures of murine bone marrow infected with a retrovirus vector carrying a murine IL-1α cDNA and the bacterial *neo* gene (33). The subclone (c12) used is propagated in IMDM supplemented with 50 μM 2-ME, 10% heat-inactivated FCS, and 10% conditioned medium from X630-rIL3 cells, murine Pam 212 keratinocyte cells (a source of GM-CSF; a gift of R. McKenzie and D. Sauder, Sunnybrook Health Science Centre) (38) or X630-rIL5 cells (a source of IL-5; a gift of F. Melchers) (29).

Cellulose Acetate Electrophoresis of Plasma Samples. Electrophoretic separations of plasma proteins were performed in barbital-sodium barbital buffer according to the manufacturer's instructions (Sepratek System; Gelman Sciences, Inc., Ann Arbor, MI). Briefly, electrophoresis was for 20 min at 225 V on cellulose acetate strips soaked in the buffer for 10 min before sample application. After electrophoresis, the strips were stained and fixed for 10 min in 0.5% Ponceau S/7.5% trichloroacetic acid, destained for 15 min in 5% acetic acid, and cleared in 40.0% aqueous *N*-methyl pyrrolidone before photography.

Nucleic Acid Analysis. Southern and Northern blot analyses were carried out according to standard procedures (26). High molecular weight DNA (10 μg) was digested with EcoRI (which cleaves uniquely within the vector) and proviral junction fragments were identified by Southern blotting using a 1.0-kb BglII-SmaI fragment of the *neo* gene as probe (39). Total cellular (10 μg) or poly(A)$^+$ (1 μg) RNA was analyzed by Northern blotting for viral RNA transcripts using a 0.8-kb EcoRI-BglII fragment of a human IL-11 cDNA (4) and a 1.0-kb BglII-SmaI fragment of the *neo* gene as probes. Endogenous IL-6 transcripts were detected with a probe consisting of a 0.6-kb EcoRI-BglII fragment of a murine IL-6 cDNA (40). Other probes used were restriction fragments obtained from the following plasmids: p17.4 (carrying a cDNA encoding the murine Mac-1 β_2 integrin subunit; 63056; American Type Culture Collection, Rockville, MD [ATCC]); K4-1.1 (carrying a murine intercellular adhesion molecule 1 (ICAM-1) cDNA; 63043, ATCC); pMC1 (carrying murine c-*myb* genomic sequences; 41056, ATCC); pSVc-myc1 (carrying the second and third exons of the murine c-*myc* gene; 41029, ATCC); and p465.20 (carrying a murine *jun*B cDNA; 63025, ATCC). The amount and integrity of RNA loaded was monitored by hybridization with a probe recognizing glyceraldehyde-3-phosphate dehydrogenase sequences (41).

Results

Infection of IL-6–dependent B Cell Lines. The T10 cell line is a derivative of the IL-6–dependent T1165 plasmacytoma that will also grow in medium supplemented with IL-11 (34). To demonstrate MSCV-IL-11–encoded production of functional IL-11, T10 cells were infected by exposure to helper-free recombinant virus produced by a clonal isolate of GP+E-86 packaging cells and examined for conversion to factor-independent growth. IL-6–dependent murine B9 hybridoma cells (36) were also infected with MSCV-IL-11 since testing of several factor-dependent cell lines revealed IL-11 to have mitogenic activity for these cells (our unpublished results). G418-resistant populations of MSCV-IL-11-infected T10 cells were readily established and could be maintained in the absence of an exogenous source of IL-11, indicating successful transmission of functional vector sequences. Notably, G418-resistant populations of MSCV-IL-11–infected B9 cells were also converted to growth factor autonomy. Conditioned medium prepared from culture supernatants of T10 and B9 infectants supported the long-term growth of T10 cells. Northern blot analysis of poly(A)$^+$ RNA from infectants with an IL-11 probe revealed, as expected, a 3.6-kb transcript originating from the MSCV-IL-11 LTR (Fig. 1 *B*). Rehybridization of the blot with a murine IL-6 probe failed to detect any endogenous IL-6 mRNA species (Fig. 1 *B*). To investigate whether autogenous IL-11 synthesis could facilitate in vivo growth of IL-6–dependent cells, syngeneic BALB/c mice were inoculated subcutaneously (10^6 cells/site) with pools of infectants and corresponding parental cell lines. As shown in Table 1, compared to parental T10 cells, T10 infectants formed tumors at a much higher frequency and with a much shorter latency period. The in vivo growth potential of IL-6–dependent B9 cells (42) was also enhanced by constitutive IL-11 synthesis. MSCV-IL-11–infected B9 cells exhibited a decreased latency period of tumor development that was comparable

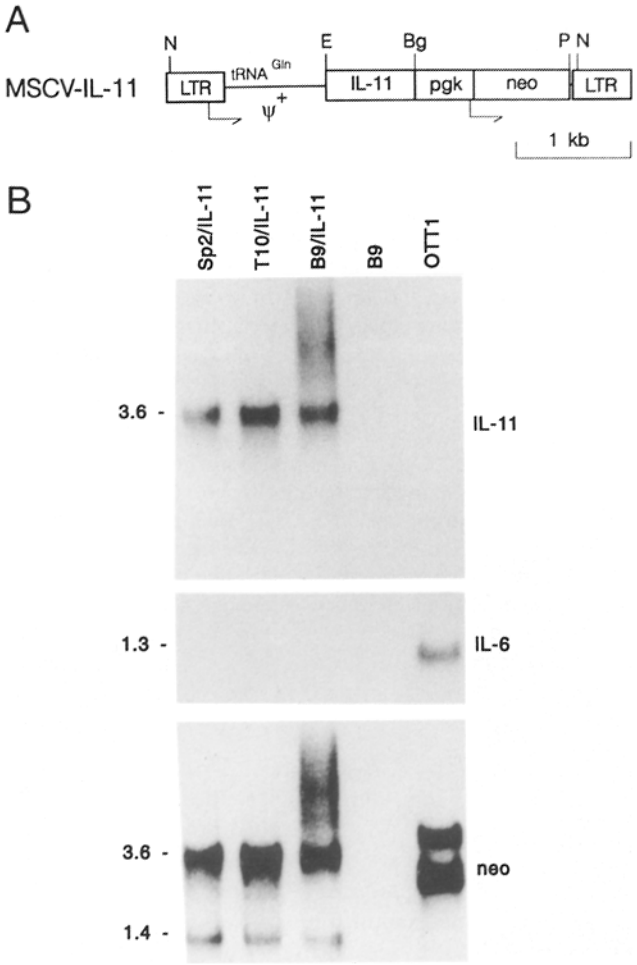


Figure 1. Schematic representation of the MSCV-IL-11 retrovirus vector and its expression in IL-6–dependent B cell lines. (*A*) The IL-11 gene in MSCV-IL-11 is transcribed from the retroviral LTR as a 3.6-kb transcript which also contains neo sequences; the *neo* gene is transcribed from the pgk promoter as a 1.4-kb mRNA. Also indicated is the extended packaging region (ψ^+) for high viral titer and the modified 5′ untranslated region ($tRNA^{Gln}$) which facilitates LTR-directed transcription of exogenous genes in primitive cells. Restriction sites: (*Bg*) BglII; (*E*) EcoRI; (*N*) NheI; (*P*) polylinker (BamHI, SalI, HindIII, and ClaI). See Materials and Methods for details. (*B*) Northern blot analysis of poly(A)$^+$ RNA (1 μg) from pools of MSCV-IL-11–infected T10 and B9 cells showing absence of the endogenous 1.3-kb IL-6 mRNA. Other RNAs were from Sp2/0 hybridoma cells infected with MSCV-IL-11, parental B9 cells, and OTT1 murine myeloid cells. Sizes were determined by comparison to 28S and 18S rRNAs (4.5 and 1.8 kb, respectively).

with that observed for B9 derivatives producing autocrine retrovirally encoded IL-6. Collectively, the results show that IL-11 can act as an autocrine growth factor for certain IL-6–dependent murine B cell lines via a mechanism that does not involve endogenously synthesized IL-6.

IL-11 Expression in Long-Term Reconstituted Mice. Lethally irradiated BALB/c mice received intravenous inoculations of MSCV-IL-11–infected bone marrow cells (10^6 cells) according to a protocol previously used (22) to generate transplant mice having excessive circulating levels of IL-6. Plasma obtained from all primary and secondary MSCV-IL-11 mice examined

Table 1. *Tumorigenicity of MSCV-IL-11–infected B cells*

Cells	Latency period	Tumor take	Tumor weight
	d		*g*
T10/IL-11	11*	10/10	1.1 ± 0.2
T10	62	4/10	0.8 ± 0.5
B9/IL-11	11	10/10	1.1 ± 0.3
B9/IL-6‡	11	10/10	1.0 ± 0.4
B9	16	10/10	0.7 ± 0.3

BALB/c mice (6–8 wk of age) were inoculated subcutaneously with 10^6 cells of the indicated cell lines and pools of infectants; two sites per mouse.
* Time after injection when animals were killed.
‡ Pool of IL-6–independent derivatives of B9 cells created by infection with the L6PNL retrovirus bearing a murine IL-6 gene (32).

5–16 wk after transplantation, but not from control mice, exhibited significant activity in the T10 bioassay (mean values of 500 U/ml IL-11 equivalents; range, 100–1,500 U/ml; n = 10). The T10 stimulatory activity could not be inhibited by a neutralizing antibody to murine IL-6 (Fig. 2 top). This finding plus the fact that plasma from both MSCV-IL-11 and control mice had minimal stimulatory activity when assayed on IL-6–dependent B9 cells (<0.1 ng/ml IL-6) ruled out the possibility that the T10 response was mediated by endogenous IL-6. Recent experiments demonstrating >90% inhibition of plasma-induced T10 proliferation by a neutralizing anti-IL-11 antibody (a gift of E. Alderman, Genetics Institute) have established the identity of the active species as IL-11 (data not shown).

Northern blot analysis of total spleen RNA demonstrated sustained expression of the 3.6-kb viral IL-11 mRNA for periods of up to 16 wk in secondary recipients (Fig. 3 *A*). No endogenous IL-6 mRNA transcripts were detected upon rehybridization of the blot with a probe specific for murine IL-6 coding sequences (Fig. 3 *A*).

The clonal composition of the reconstituted hematopoietic systems of MSCV-IL-11 transplant mice was examined by Southern blot analysis of EcoRI-digested DNA with a neo probe. Since EcoRI cleaves the MSCV-IL-11 provirus once (Fig. 1 *A*), each band represents a unique integration site. As illustrated in Fig. 3 *B*, genomic DNAs from hematopoietic tissues (bone marrow and spleen) and purified G418-resistant cell populations (macrophages, mast cells and T cells) of a representative primary recipient (mouse 96, reconstituted for 12 wk) and two secondary recipients (mice 95.1 and 95.2, reconstituted for 12 wk with bone marrow from a primary mouse reconstituted for 16 wk) contained many fragments that hybridized to the neo probe. The presence of common bands in lanes containing DNA samples from lymphoid and myeloid lineage cells, as well as bands identifiable in only some of the lanes, indicated polyclonal repopulation by primitive MSCV-IL-11–infected hematopoietic cells, a subset of which were multipotential stem cells.

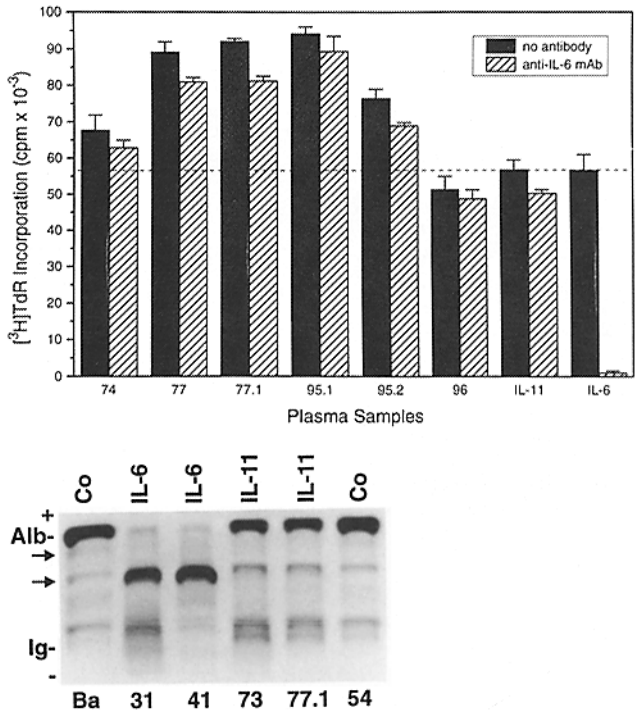


Figure 2. Plasma analyses of MSCV-IL11 mice. (*Top*) Plasma was tested for IL11 activity in the T10 proliferation assay in the absence or presence of a neutralizing antibody to murine IL6 (anti-IL6 mAb; dilution 1/100). Results are presented for 1% final concentrations of plasma collected before death. Values plotted are the mean and SE of three determinations. The numbers designate individual animals: mice 74, 77, and 96 are primary recipients; mice 77.1, 95.1, and 95.2 are secondary recipients. (*Dashed line*) Maximal proliferation of T10 cells to purified recombinant human IL11 (56,680 ± 2,670 cpm). Representative T10 responses to IL11 (5 U/ml) and IL6 (10% conditioned medium from Sp2/mIL6 cells) are shown. (*Bottom*) Cellulose acetate electrophoresis of plasma obtained from mice 73 and 77.1 revealing alterations in levels of albumin (*Alb*) and protein(s) in the α_1-(*top arrow*) and α_2- (*bottom arrow*) regions by comparison with the protein patterns of normal BALB/c (*Ba*) and transplant (mouse 54) controls (*Co*). Note that whereas the qualitative changes are similar to those observed in mice 31 and 41 overexpressing IL6 (22), there are quantitative differences. (*Ig*) Immunoglobulin fraction.

Hematologic Changes in MSCV-IL-11 Transplant Mice. The main consequence of persistent IL-11 expression was a sustained rise in platelet levels (1.5–1.6-fold higher than corresponding values obtained for normal and control transplant mice, respectively) throughout the observation period (Table 2; Fig. 4). Since this increase could not be attributed to IL-6, the possibility was considered that IL-11 may have induced elevations of IL-3 or GM-CSF, two factors with known in vivo actions on megakaryocytes (43, 44). However, no IL-3 or GM-CSF activity was detectable by 32D or OTT1 microproliferation assays (33, 37) in the plasma of any mice tested. On the basis of results from in vitro studies (4–6), the observed changes in platelet levels might be due in part to IL-11 acting in collaboration with low amounts of these factors. To address this issue, current experiments are examining cytokine gene mRNA profiles in MSCV-IL-11 mice by RT-PCR.

Long-term exposure to high circulating levels of IL-11 resulted in minimal changes in other peripheral blood cell values (Table 2). Differential leukocyte counts showed a neutrophilic predominance (mean, 70%) but there was no increase in total leukocyte numbers. Although in vitro erythropoietic properties of IL-11 have been recognized (45), the in vivo effects of IL-11 on circulating erythrocyte values were minor. In fact, hematocrits were slightly reduced compared with those of normal mice because of a small decrease in mean corpuscular volume. The significance of this is unknown, however, since hematocrit values did not differ meaningfully from values obtained for control transplant mice.

In view of the relatively normal peripheral blood cell counts in MSCV-IL-11 mice, it was notable that enhanced hematopoiesis was observed in bone marrow and spleen (Fig. 5). This was most striking in the case of the spleen where there was a concomitant 12–56-fold elevation in total progenitor cell content (Table 3). As spleen weight was increased about twofold (Table 3), the actual total increase per spleen in progenitor cell numbers was 20–100-fold. Detailed cytological analyses of colonies formed in methylcellulose-containing medium were not carried out, but no conspicuous differences between MSCV-IL-11 and control mice in the relative distribution of the various progenitor subpopulations were apparent upon gross inspection of cultures. Although high percentages (31–68%) of CFC found in the spleens of both primary and secondary recipients were G418 resistant (Table 3), substantial expansion of G418-sensitive progenitors also occurred, indicating that paracrine mechanisms contributed to the phenomenon. Associated with the general rise in splenic hematopoiesis was a minor elevation in spleen megakaryocyte numbers in correspondence with the higher blood platelet levels.

Other Effects of Dysregulated IL-11 Expression. A uniform finding in MSCV-IL-11 mice was a 15–20% reduction in body weight (Table 3), often accompanied by a hyperactive state. Upon autopsy, it was observed that the decrease in body weight was due to the loss of subcutaneous and intraabdominal fat, an effect presumably of the lipoprotein lipase inhibitory activity of IL-11 (14, 15). MSCV-IL-11 mice routinely developed inflammatory eyelid lesions characterized grossly by marked swelling and erythema. Histologic analysis revealed acute inflammation on the surface of the conjunctival mucosa. Other consistent changes included thymus atrophy and frequent distention of the stomach or bowel with noticeable alterations in muscle tone. No significant changes were noted in liver or kidney and histologic examination of these organs failed to reveal any abnormalities.

Cellulose acetate electrophoresis of plasma samples from MSCV-IL-11 mice revealed changes in acute phase protein levels, including slight reductions in albumin levels with increases in protein levels in the α_2-macroglobulin/haptoglobin region, that were much less pronounced than those observed in mice overexpressing IL-6 (Fig. 2, *bottom*). Measurement of amounts of plasma proteins by rocket immunoelectrophoresis (46) indicated that albumin levels were in the normal range whereas haptoglobin levels were significantly higher

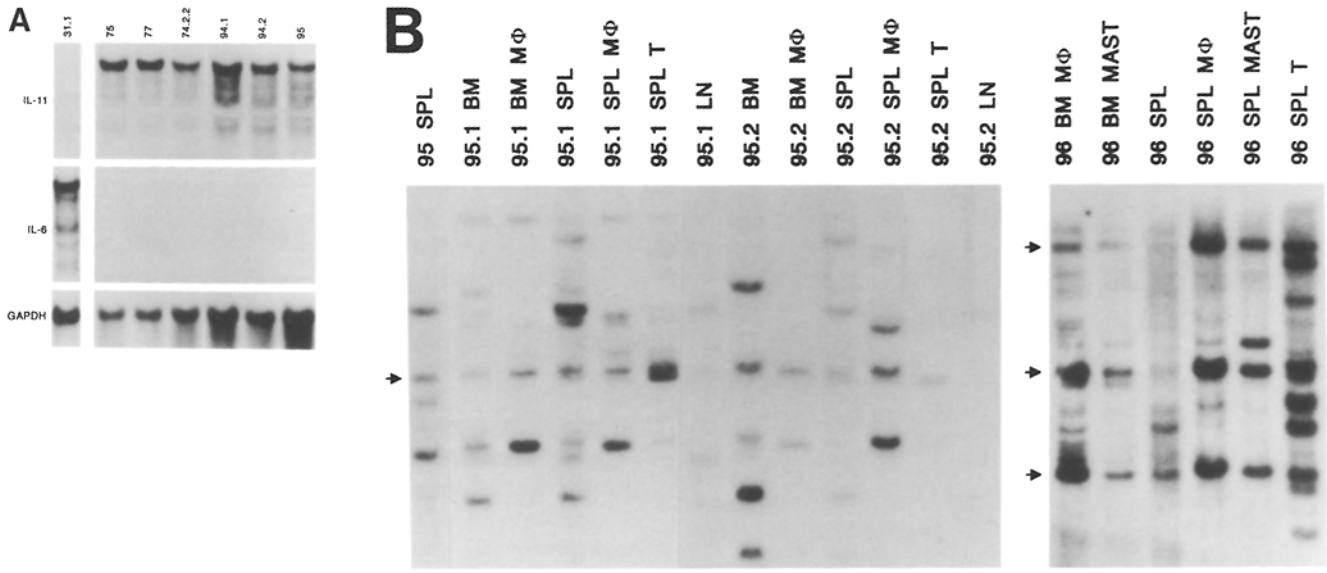


Figure 3. Expression and distribution of MSCV-IL-11 proviruses in hematopoietic tissues of long-term reconstituted mice. (*A*) Total spleen RNA (10 μg) was examined by Northern blot analysis for the presence of viral IL-11 (*top*) and endogenous IL-6 (*middle*) transcripts. Hybridization of the blot with a probe specific for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) indicated that similar amounts of RNA were loaded (*bottom*). Samples were from primary (mice 75, 77, and 95), secondary (mice 94.1 and 94.2), and tertiary (mouse 74.2.2) recipients. Spleen RNA from a secondary recipient of MSCV-IL-6–infected bone marrow (mouse 31.1; reference 22) was included as a positive control for IL-6 sequences. (*B*) Proviral integrants were enumerated in EcoRI-digested DNA from various hematopoietic tissues and cell lineages by Southern blotting with a neo probe. Analyses of mouse 95 and its secondary recipients (mice 95.1 and 95.2) and mouse 96 are presented. DNA (10 μg) was from the following tissues and cell populations: (*BM*) bone marrow; (*BM Mø*) bone marrow–derived macrophages; (*BM MAST*) bone marrow–derived mast cells; (*LN*) lymph node; (*SPL*) spleen; (*SPL MF*) splenic-derived macrophages; (*SPL MAST*) splenic-derived mast cells; and (*SPL T*) splenic-derived T cells. (*Arrows*) Proviruses common to myeloid and lymphoid lineages.

Table 2. *Hematologic Values in MSCV-IL-11 Transplant Mice*

Hematologic parameter	BALB/c (n = 42)	MSCV-X (n = 28)	MSCV-IL-11 (n = 43)
WBC ($\times 10^3/mm^3$)*	9.0 ± 3.0	7.6 ± 2.1	7.7 ± 2.4
RBC ($\times 10^6/mm^3$)	10.6 ± 0.5	10.1 ± 0.8	11.0 ± 0.6
HGB (g/dl)	16.3 ± 0.8	16.0 ± 1.2	15.6 ± 1.0
HCT (%)	49.2 ± 2.5	45.5 ± 3.4	44.0 ± 3.2
MCV (fl)	46.2 ± 0.8	45.1 ± 1.4	39.8 ± 1.4
MCH (pg)	15.4 ± 0.6	15.8 ± 0.7	14.1 ± 0.7
MCHC (g/dl)	33.3 ± 1.5	35.1 ± 1.2	35.5 ± 1.4
RDW (%)	22.7 ± 0.8	21.8 ± 0.8	22.8 ± 0.6
PLT ($\times 10^3/mm^3$)	1,200 ± 124	1,097 ± 131	1,811 ± 192
MPV (fl)	4.7 ± 0.6	5.4 ± 0.5	4.7 ± 0.4

Data recorded are the mean and SE of values. MSCV-IL-11 data were obtained from analysis of three primary recipients (mice 94, 95, and 96) and four secondary recipients (mice 94.1, 94.2, 95.1, and 95.2) at intervals 4–16 wk after transplant. MSCV-X data were obtained from similar analysis of six primary recipients of bone marrow infected with other MSCV constructs. BALB/c data were obtained from analysis of 42 normal mice (8–12 wk of age).

* WBC, total leukocytes; RBC, total erythrocytes; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; PLT, total platelets; MPV, mean platelet volume.

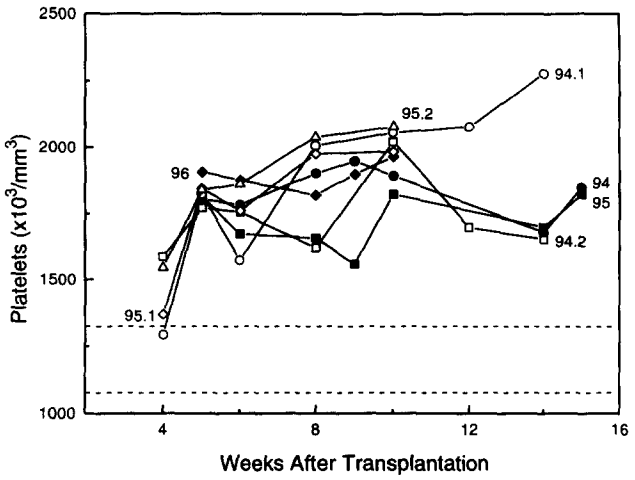


Figure 4. Elevated platelet levels in MSCV-IL-11 mice. Platelet analyses were performed as described in Material and Methods on peripheral blood collected at the time points indicated after bone marrow transplantation. Platelet counts for representative primary (mice 94, 95, and 96) and secondary (mice 94.1, 94.2, 95.1, and 95.2) recipients are presented. Platelet counts of control transplant mice stabilized by 4 wk after transplantation and were slightly lower than normal BALB/c values (range, *dashed lines*). See Table 2 for values of all hematologic parameters examined.

(mean values of 9.4 μg/ml; range, 7.2–11.0 μg/ml; $n = 8$) than normal (<1 μg/ml). These results indicate that IL-11 can function as a regulator of the hepatic acute phase response in mice (13).

Myeloid Leukemia Development after Serial Transplantation of MSCV-IL-11–infected Hematopoietic Cells. 6 wk after transplantation with bone marrow cells from mouse 74, decreased platelet levels were detected in two secondary recipients. By 8 wk, both animals were severely thrombocytopenic (platelet counts ~130 × 10^3/mm³). One mouse (mouse 74.1) had lower than normal peripheral leukocyte counts (3.6 × 10^3/mm³) whereas the other animal (mouse 74.2) presented with leukocytosis (43.6 × 10^3/mm³). At necropsy, both mice were found to have considerably enlarged spleens (spleen weights of 0.364 and 0.498 g). Bone marrow (2 × 10^6) or spleen (5 × 10^6) cells from these mice were leukemogenic within 4 wk of transplant into sublethally irradiated (4.5 Gy) tertiary recipients. The characteristic features of the malignant disease included leukocytosis with neutrophil bias (>200 × 10^3 cells/mm³) and evidence of blasts in the peripheral blood, anemia (mean hematocrits, 27%), thrombocytopenia (mean platelet counts, 56 × 10^3/mm³), and splenomegaly (mean spleen weights, 0.65 g). Histopathologic analysis of the spleen from diseased mice showed effacement of the splenic architecture by cytologically abnormal cells (Fig. 6 *B*).

Attempts to establish factor-dependent or autonomously growing cell lines in liquid culture from spleen cells of tertiary 74-series recipients were unsuccessful. However, in vitro culture of spleen cells from one quaternary recipient (mouse 74.1.1.1) yielded an IL-11–producing yet IL-3–dependent cell line (denoted PGMD1, Fig. 6 *C*). An immature myeloid phenotype was determined by flow cytometric analysis of cell

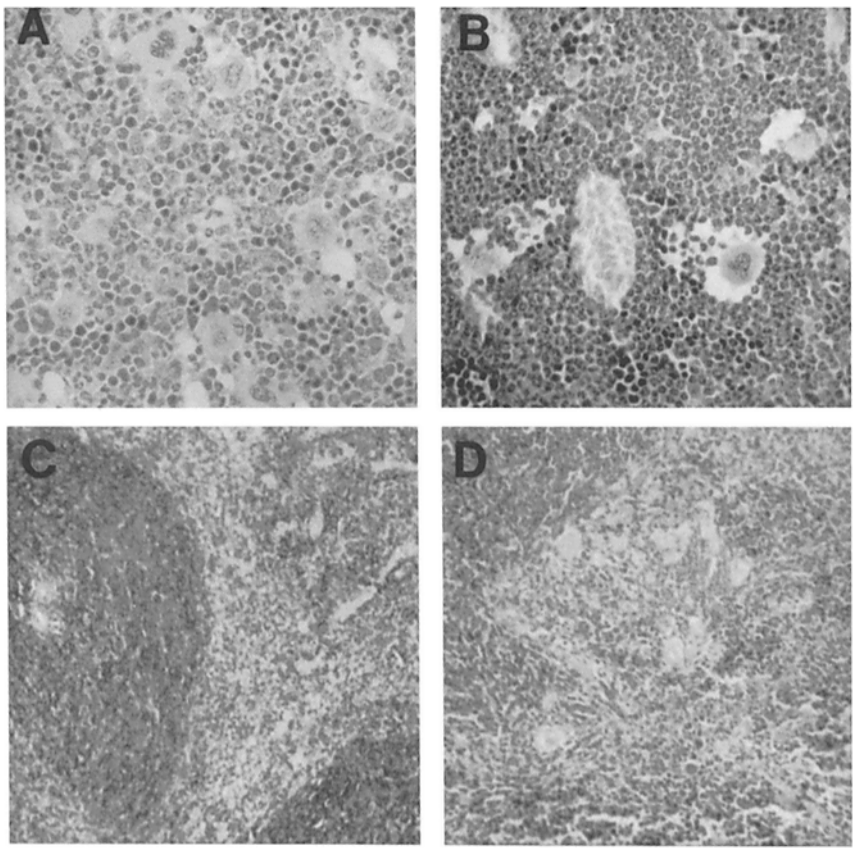


Figure 5. Histology of bone marrow and spleen of MSCV-IL-11 mice. (*A*) Normal bone marrow (×900). (*B*) Hyperplastic bone marrow of mouse 95 (×900). (*C*) Normal spleen (×450). (*D*) Spleen of mouse 96 showing expansion of red pulp with myeloid elements (×450).

Table 3. *Progenitor Cell Changes in MSCV-IL-11 Transplant Mice*

Mouse	Time of reconstitution	Body weight	Spleen weight	CFC in bone marrow (%G418R)	CFC in spleen (%G418R)
	wk		*g*		
73.1*	17	16.7	0.098	5,600 (52)‡	900 (56)‡
73.2	10	ND	0.170	4,250 (45)	2,500 (47)
74	12	17.0	0.260	5,750 (51)	3,900 (58)
75	12	16.6	0.170	5,000 (55)	3,300 (33)
76	7	ND	ND	5,350 (50)	4,800 (52)
77	11	ND	ND	5,500 (48)	2,000 (38)
77.1	5	17.5	0.200	8,500 (50)	2,900 (68)
78	9	ND	0.180	6,000 (54)	1,750 (64)
79	9	ND	ND	5,000 (36)	2,250 (37)
94	16	ND	0.170	5,600 (47)	1,860 (61)
94.1	16	ND	0.175	15,000 (50)	4,000 (50)
94.2	16	16.8	0.155	15,000 (40)	2,000 (44)
95	16	ND	0.183	12,000 (46)	1,450 (43)
95.1	12	17.7	0.125	10,000 (30)	1,625 (31)
95.2	12	18.3	0.176	8,500 (31)	1,700 (35)
96	12	17.8	0.162	3,000 (42)	4,000 (39)
<MSCV-IL-11>	12 ± 4	17.3 ± 0.6	0.17 ± 0.04	7,500 ± 3,700 (45 ± 8)	2,600 ± 1,100 (47 ± 12)
<MSCV-X>	53 ± 3	20.5 ± 1.4	0.08 ± 0.01	3,400 ± 600 (48 ± 8)	46 ± 31 (50 ± 21)
<BALB/c>	–	21.1 ± 1.5	0.10 ± 0.02	2,800 ± 700	215 ± 150

* Secondary recipients of MSCV-IL-11–infected bone marrow are denoted by the number of the primary recipient followed by a unique numeric extension. MSCV-X and BALB/c values represent the mean and SE of values obtained for six other MSCV transplant mice (as per Table 2 footnotes) and for six normal mice, respectively.
‡ CFC frequency per 10^6 cells.

surface markers. PGMD1 cells express high levels of the leukocyte common antigen Ly-5 (CD45), high levels of the Fc receptor for aggregated IgG (CD32), low levels of the myeloid integrin Mac-1 (CD11b), and low levels of the cell adhesion molecule ICAM-1 (CD54), but no detectable amounts of Ia antigen. Intravenous inoculation of PGMD1 cells into sublethally irradiated syngeneic BALB/c mice gave rise to myeloid leukemia (four of five mice that received 5×10^6 cells developed tumors by 6 mo). The fact that in vivo–passaged PGMD1 cells maintained their factor dependency upon reintroduction into liquid culture (data not shown) suggests that the leukemic disease in 74-series mice occurs via a paracrine mode of growth stimulation.

Southern blot analysis of proviral insertion pattern showed that DNA from the PGMD1 cell line and spleen DNAs from all 74-series mice contained four predominant bands (Fig. 6 *A*). We interpret this result to mean that the myeloproliferative disease that developed in this transplant pedigree originated in the clonal descendant of a hematopoietic precursor harboring four MSCV-IL-11 proviruses. Although the clone represented the major MSCV-IL-11–positive cell population in the spleen of primary mouse 74, it did not appear to be overtly malignant at this time as indicated by the absence of abnormal cells in histologic sections or of atypical colony formation in methylcellulose culture (Table 3). Even after the development of frank malignancy, further clonal evolution was apparently necessary before successful adaptation for growth in culture. Culture supernatant from PGMD1 cells tested negative for the presence of infectious virus in a sensitive marker virus mobilization assay (32, 47), arguing against insertional mutagenesis by packaging line–generated or spontaneously activated endogenous retroviruses.

Unlike other IL-3–dependent murine myeloid leukemic cell lines (48), PGMD1 cells can be induced to differentiate into macrophages and/or granulocytes by GM-CSF or G-CSF (Fig. 6 *D*; other data not shown). Analysis of gene expression patterns during GM-CSF–induced myeloid differentiation revealed upregulated expression of IL-6, Mac-1, ICAM-1, and *junB*

mRNAs and decreased expression of *c-myb* and *c-myc* mRNAs (Fig. 6 *E*). This genetic response is reminiscent of that observed when autonomously proliferating M1 murine myeloid leukemic cells are stimulated to terminal differentiation by IL-6 (49).

Discussion

IL-11 as a Thrombopoietic Factor in Mice. To investigate the in vivo hematopoietic activities of IL-11, we generated chimeric mice whose hematopoietic systems had been reconstituted for extended periods of time with genetically modified bone marrow cells constitutively expressing IL-11. The major effect of high circulating IL-11 levels was a chronic 1.5-fold increase in peripheral platelet counts. This finding confirms and extends previous reports (4–8) of megakaryocytopoietic and thrombopoietic activity of IL-11. In a recent publication (8), C57BL/6 mice injected subcutaneously with 1.5 μg of IL-11 twice a day for 7 d showed a 1.3-fold elevation in peripheral platelet counts compared with vehicle-treated controls. In that study, rapid clearance of IL-11 from the circulation upon cessation of the dosing regimen resulted in platelet counts returning to normal values by day 15. Because there were no other consistent hematologic changes, the modest thrombopoietic response elicited could have been due to suboptimal circulating IL-11 concentrations. In the present series of experiments, it is unlikely that IL-11 amounts are limiting in this respect. Although spleen megakaryocyte numbers were slightly elevated in MSCV-IL-11 mice, observations in splenectomized mice would suggest that splenic megakaryocytopoiesis does not contribute substantially to the increase in peripheral platelets effected by IL-11. Rather, as documented previously (8), the higher peripheral platelet counts are probably the result of IL-11–induced enhancement of megakaryocyte progenitor numbers and IL-11–stimulated endoreplication of megakaryocytes in the bone marrow.

It is noteworthy that sustained high level IL-11 expression had little impact on peripheral leukocyte or erythrocyte values, the exceptions being a neutrophilic shift in the differential and a reduction in mean corpuscular volume, respectively. In other work (7, 17), daily administration of IL-11 to mice during bone marrow reconstitution accelerated the recovery of peripheral leukocytes, mainly neutrophils, in addition to platelets. Moreover, early recovery of erythrocytes was observed in another study in mice (50) transplanted with bone marrow cells infected with a different IL-11–expressing retrovirus. One interpretation of these results is that IL-11 acts synergistically with certain cytokines induced after hematopoietic injury or expressed by the reconstituting cells that are not normally present in high concentrations during steady-state hematopoiesis. This interpretation would be consistent with the demonstrated synergistic interactions between IL-11 and multiple cytokines in various in vitro assays and a general lack of observed activity when it is used as a single agent (2). Future work will investigate the rate of recovery of peripheral leukocytes and erythrocytes in transplant recipients receiving MSCV-IL-11–infected bone marrow.

It should be noted that a transient 1.5-fold increase in leukocyte counts, primarily due to a sevenfold increase in neutrophils, was seen in $B6D2F_1$ mice that received subcutaneous injections of 4 μg of IL-11 twice a day for 10 d (18). As well, bone marrow cells from $B6D2F_1$ mice formed significant numbers of macrophage colonies in methylcellulose when plated into medium containing IL-11 and erythropoietin (9), indicating that IL-11 can directly support the proliferation of some committed macrophage progenitors in that mouse strain. A possible reason therefore for the numbers of circulating leukocytes remaining relatively unchanged in this animal model could be strain-specific differences in IL-11 responsiveness. The use of the BALB/c mouse strain might explain why blood erythrocyte counts were not markedly elevated despite the ability of IL-11 to promote maturation of late erythroid progenitors in bone marrow cultures derived from mice of some other strains (45). This explanation is not completely satisfactory, however, in light of the enhanced splenic hematopoiesis observed. If anything, the in vivo situation is probably complex and other possibilities, for example, that constant high level IL-11 expression induces activities which are inhibitory to steady-state hematopoiesis (51), need also to be considered. Erythrokinetics would also be expected to be negatively affected by IL-11–induced alterations in splenic function and plasma composition (52).

Pleiotropy and Redundancy. Interestingly, the in vivo effects of high sustained concentrations of IL-11 were less like those of IL-6 (22) and were more like those of another pleiotropic cytokine, leukemia inhibitory factor (LIF) (53). Injection of mice with LIF (2 μg three times daily for 14 d) resulted in elevated numbers of circulating platelets, a moderate increase in spleen weight with an associated rise in the numbers of various subsets of splenic progenitors, a small decrease in hematocrit, and thymus atrophy. Nonhematopoietic effects included behavioral changes (hypermobility and irritability), weight loss due to reduction in fatty tissue, distended stomachs, and increased erythrocyte sedimentation rate and elevated calcium/albumin ratios characteristic of the acute phase protein reaction. There are differences, however, between IL-11 and LIF. For example, LIF appears to be more toxic than IL-11 since several of the mice injected with LIF died. Moreover, chronic exposure of mice to high levels of LIF (plasma concentrations of ~1,000 U/ml) by engraftment of a hematopoietic cell line engineered to express this cytokine resulted in the development within 12–70 d of a fatal cachexia (54).

Nonetheless, the two cytokines clearly share some biologic activities. The molecular basis for this overlap is likely to be related to the recent finding that both IL-11 and LIF transduce signals via a common transmembrane glycoprotein, gp130, originally identified as a component of the IL-6 receptor and now known be utilized by two other cytokines, oncostatin M and ciliary neurotrophic factor (55, 56). Because the two cytokines share a common signal transduction pathway, any cell that expresses the binding subunits for IL-11 and LIF might be expected to behave similarly in response to both cytokines. It is also possible that the similar in vivo effects of high concentrations of IL-11 and LIF are due to one factor binding to the high affinity receptor of the other, as has been

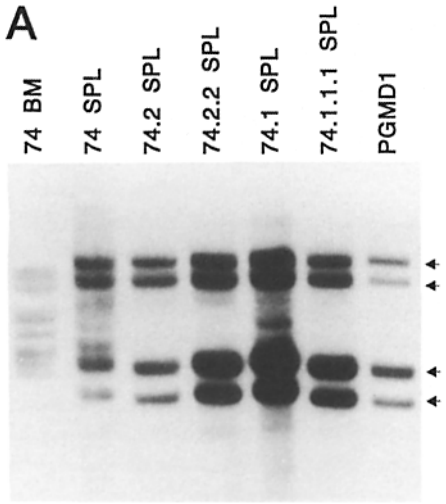
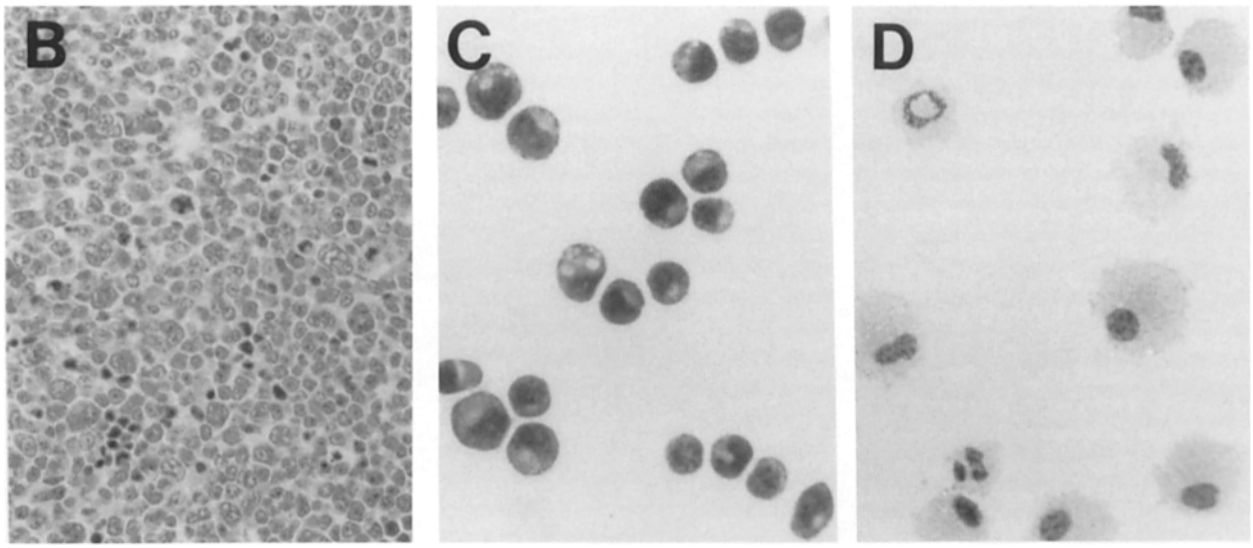


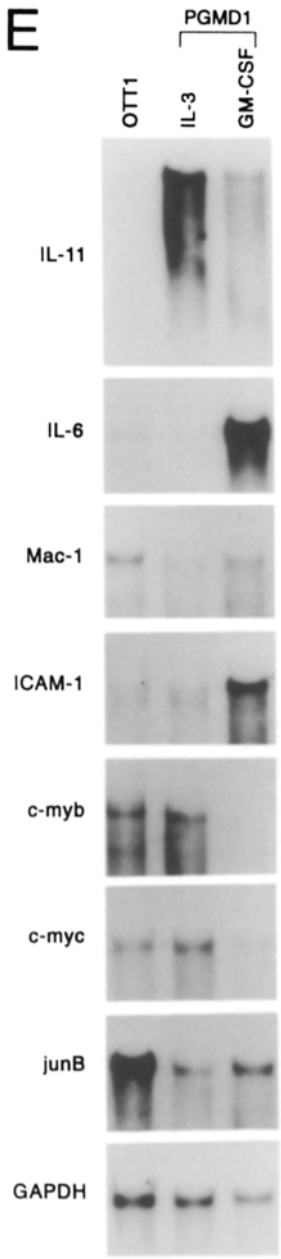
Figure 6. Analysis of myeloid disease in 74-series mice. (*A*) Southern blotting of EcoRI-digested DNA with a neo probe shows clonality of the disease. (*Arrows, right*) The four diagnostic proviral integrants in the PGMD1 myeloid leukemic cell line derived from spleen cells of quaternary recipient 74.1.1.1. See Fig. 3 for abbreviations used. (*B*) Histologic section of spleen of tertiary recipient 74.2.2 showing obliteration of normal follicular architecture (×1,200). (*C*) Cytospin preparation of PGMD1 cells showing blast morphology. (*D*) Cytospin preparation of PGMD1 cells grown for 5 d in GM-CSF–containing medium showing macrophages and cells of the granulocytic lineage. (*E*) Northern blot analysis of poly(A)$^+$ RNA (1 μg) from PGMD1 cells maintained in IL-3–supplemented medium or after GM-CSF treatment for 2 d. The blot was sequentially hybridized with probes specific for the IL-11, IL-6, Mac-1 β_2 integrin subunit, ICAM-1, c-*myb*, c-*myc*, *jun*B, and GAPDH genes as described in Materials and Methods. RNA from OTT1 murine myeloid cells was included for comparison purposes.



recently reported for oncostatin M which can bind to the high affinity LIF receptor (57).

Alternatively, as it has also been demonstrated recently that both IL-11 and LIF induce the synthesis of IL-6 by human monocyte-macrophages (58, 59), the question is raised whether some of the in vivo activities of these cytokines might be mediated in part by IL-6. A strong argument for relative lack of paracrine IL-6 involvement is the absence of the pathophysiology seen by us (22) in mice whose hematopoietic systems were reconstituted with bone marrow cells infected with a version of the MSCV vector carrying an IL-6 gene. Direct evidence that IL-6 does not play a major role in the in vivo IL-11 response are the findings that circulating levels of endogenous IL-6 were not elevated in MSCV-IL-11 mice compared with normal levels and that the abnormally high IL-11 levels did not induce detectable amounts of IL-6 mRNA in the spleen, a major organ source of IL-6 during endotoxemia (60). The fact that plasma samples from MSCV-IL-11 mice treated with neutralizing antibody to IL-6 stimulated T10 cell proliferation more effectively than IL-11 alone does, however, indicates the presence of a novel activity in the plasma and leaves open the possibility that some of the changes observed might be ascribable to other factors acting secondarily (61).

IL-11 and Malignant Growth. IL-11 was originally identified as a factor with mitogenic activity for IL-6–dependent murine T1165 plasmacytoma cells (4). We and others (62, and our unpublished results) observed that IL-11 also stimulates the proliferation of IL-6-dependent murine B9 hybridoma cells. In the absence of IL-6, IL-11 poorly supports the growth of either cell line and the vast majority of the cells die by apoptosis (63). The T10 derivative of T1165 cells was established by selection for a variant that would grow in medium supplemented with only IL-11 (34). We showed that IL-11 can act as an autocrine growth factor for both IL-11–dependent T10 cells and IL-11–responsive B9 cells. The in vitro mechanism of action of IL-11 on these IL-6–dependent murine B-lineage cells appears to be direct and not due to the



induction of endogenous IL-6. Previous results demonstrated that acquisition of autostimulatory IL-6 production enhanced the tumorigenicity of other IL-6–dependent murine plasmacytoma/hybridoma cells (64, 65). The acquisition of the ability to produce autogenous IL-11 similarly potentiated the tumorigenicity of T10 and B9 cells, although, in the case of B9, the effect was less pronounced owing to the ready ability of these cells to grow in vivo in response to host IL-6 (42). These findings raise the possibility that IL-11 might be involved, conceivably in an autocrine manner, in the in vivo growth of some murine B cell tumors. At this time, however, the relevance of these observations to human tumor biology is unclear. In the human system, IL-6 has been demonstrated to promote the growth of multiple myeloma cells, either in a paracrine or autocrine fashion, but IL-11 has not yet been found to be a myeloma growth factor (34).

In contrast to the situation with established cell lines, accumulated data has indicated that autocrine stimulation alone is insufficient to transform primary cells (66). Prolonged growth factor expression in vivo causes hyperplasia that infrequently leads to neoplastic transformation (67, 68). In this series of transplants involving 10 primary and 20 secondary recipients of MSCV-IL-11–infected bone marrow, myeloid leukemia developed in two secondary recipients of bone marrow from the same donor. By comparison, none of over 300 BALB/c mice transplanted in our laboratory with bone marrow cells infected with other recombinant retroviruses have developed a myeloid malignancy (22, 32, and our unpublished results). As the PGMD1 leukemic cell line requires a source of IL-3 for propagation, however, it has been difficult to demonstrate a direct stimulatory role for IL-11 (69). Some degree of growth enhancement by IL-11 is assumed based on studies with normal progenitor cells (9) and more recent results showing that IL-11 acts synergistically with IL-3 or GM-CSF in stimulating the in vitro growth of the blast cells from patients with acute myeloblastic leukemia (70). On the other hand, since a paracrine mechanism of growth promotion appears to be operating in vivo, it is possible that constitutive IL-11 synthesis by the leukemic cells might stimulate production of the necessary growth factors by host cells (71). In any event, the MSCV-IL-11–marked clone represented the major population of donor cells in the spleen of primary mouse 74 but did not appear to be overtly malignant at that time. This suggests that whereas IL-11–supported expansion of progenitors might have initiated the process (72), secondary lesions were presumably necessary for progression to frank malignancy (73).

The Bone Marrow Chimera Approach. We have demonstrated efficient infection of hematopoietic stem cells, precursors capable of long-term multilineage reconstitution of lethally irradiated primary and secondary recipients, by functional MSCV-IL-11 viruses. Although we have not shown that the same MSCV-IL-11 provirus is transcriptionally active in both myeloid and lymphoid progeny of a single stem cell, the maintenance of high level expression of a nonselectable gene in vivo for (the equivalent of) at least 7 mo makes this in vivo gene transfer strategy a practical alternative to transgenic technology for analysis of gene function in the hematopoietic system.

We thank Jack Gauldie for performing the rocket immunoelectrophoresis analyses of acute phase protein levels, and Norman Iscove and Sandy Goldman for critically reviewing the manuscript.

This work was supported by grant 2818 from the National Cancer Institute of Canada and grant MT-11749 from the Medical Research Council of Canada. R. G. Hawley is a Career Scientist of the Ontario Cancer Treatment and Research Foundation.

Address correspondence to Dr. R. G. Hawley, Division of Cancer Research, Reichmann Research Building, S218, Sunnybrook Health Science Centre, 2075 Bayview Avenue, Toronto, Ontario M4N 3M5 Canada.

Received for publication 12 May 1993 and in revised form 7 July 1993.

References

1. Allen, T.D., T.M. Dexter, and P.J. Simmons. 1990. Marrow biology and stem cells. *In* Colony-Stimulating Factors: Molecular and Cellular Biology. T.M. Dexter, J.M. Garland, and N.G. Testa, editors. Marcel Dekker, Inc., New York. 1–38.
2. Turner, K.J., and S.C. Clark. 1993. Interleukin-11: biological and clinical perspectives. *In* Hematopoietic Growth Factors in Clinical Applications. R. Mertelsmann and F. Herrmann, editors. Marcel Dekker, Inc., New York. In press.
3. Paul, S.R., Y.-C. Yang, R.E. Donahue, S. Goldring, and D.A. Williams. 1991. Stromal cell-associated hematopoiesis: immortalization and characterization of a primate bone marrow–derived stromal cell line. *Blood.* 77:1723.
4. Paul, S.R., F. Bennett, J.A. Calvetti, K. Kelleher, C.R. Wood, Jr., R.M. O'Hara, A.C. Leary, B. Sibley, S.C. Clark, D.A. Williams, and Y.-C. Yang. 1990. Molecular cloning of a cDNA encoding interleukin 11, a stromal cell–derived lymphopoietic and hematopoietic cytokine. *Proc. Natl. Acad. Sci. USA.* 87:7512.
5. Bruno, E., R.A. Briddell, R.J. Cooper, and R. Hoffman. 1991. Effects of recombinant interleukin 11 on human megakaryocyte progenitor cells. *Exp. Hematol.* 19:378.
6. Teramura, M., S. Kobayashi, S. Hoshino, K. Oshimi, and H. Mizoguchi. 1992. Interleukin-11 enhances human megakaryocytopoiesis in vitro. *Blood.* 79:327.
7. Du, X.X., T. Neben, S. Goldman, and D.A. Williams. 1993. Effects of recombinant human interleukin-11 on hematopoietic reconstitution in transplant mice: acceleration of recovery of peripheral blood neutrophils and platelets. *Blood.* 81:27.
8. Neben, T., J. Loebelenz, L. Hayes, K. McCarthy, J. Stoudemire, R. Schaub, and S.J. Goldman. 1993. Recombinant human interleukin 11 stimulates megakaryocytopoiesis and increases peripheral platelets in normal and splenectomized mice. *Blood.* 81:901.
9. Musashi, M., Y.-C. Yang, S.R. Paul, S.C. Clark, T. Sudo, and M. Ogawa. 1991. Direct and synergistic effects of interleukin 11 on murine hemopoiesis in culture. *Proc. Natl. Acad. Sci. USA.* 88:765.
10. Musashi, M., S.C. Clark, T. Sudo, D.L. Urdal, and M. Ogawa. 1991. Synergistic interactions between interleukin-11 and interleukin-4 in support of proliferation of primitive hematopoietic progenitors of mice. *Blood.* 78:1448.
11. Tsuji, K., S.D. Lyman, T. Sudo, S.C. Clark, and M. Ogawa. 1992. Enhancement of murine hematopoiesis by synergistic interactions between steel factor (ligand for c-*kit*), interleukin-11, and other early acting factors in culture. *Blood.* 79:2855.
12. Yin, T., P. Schendel, and Y.-C. Yang. 1992. Enhancement of in vitro and in vivo antigen-specific antibody responses by interleukin 11. *J. Exp. Med.* 175:211.
13. Baumann, H., and P. Schendel. 1991. Interleukin-11 regulates the hepatic expression of the same plasma protein genes as interleukin-6. *J. Biol. Chem.* 266:20424.
14. Kawashima, I., J. Ohsumi, K. Mita-Honjo, K. Shimoda-Takano, H. Ishikawa, S. Sakakibara, K. Miyadai, and Y. Takiguchi. 1991. Molecular cloning of cDNA encoding adipogenesis inhibitory factor and identity with interleukin-11. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 283:199.
15. Yin, T., K. Miyazawa, and Y.-C. Yang. 1992. Characterization of interleukin-11 receptor and protein tyrosine phosphorylation induced by interleukin-11 in mouse 3T3-L1 cells. *J. Biol. Chem.* 267:8347.
16. Mehler, M.F., Rozental, M. Dougherty, D.C. Spray, and J.A. Kessler. 1993. Cytokine regulation of neuronal differentiation of hippocampal progenitor cells. *Nature (Lond.).* 362:62.
17. Hangoc, G., T. Yin, S. Cooper, P. Schendel, Y.-C. Yang, and H.E. Broxmeyer. 1993. In vivo effects of recombinant interleukin-11 on myelopoiesis in mice. *Blood.* 81:965.
18. Quesniaux, V.F.J., P. Mayer, E. Liehl, K. Turner, S.J. Goldman, and B. Fagg. 1993. Review of a novel hematopoietic cytokine, interleukin-11. Cytokine-induced Pathology, Interleukins and Hemopoietic Growth Factors. *Int. Rev. Exp. Pathol.* 34A:205.
19. Ulich, T.R., J. del Castillo, and K. Guo. 1989. In vivo hematologic effects of recombinant interleukin-6 on hematopoiesis and circulating numbers of RBCs and WBCs. *Blood.* 73:108.
20. Ishibashi, T., H. Kimura, Y. Shikama, T. Uchida, S. Kariyone, T. Hirano, T. Kishimoto, F. Takatsuki, and Y. Akiyama. 1989. Interleukin 6 is a potent thrombopoietic factor in vivo in mice. *Blood.* 74:1241.
21. Patchen, M.L., T.J. MacVittie, J.L. Williams, G.N. Schwartz, and L.M. Souza. 1991. Administration of interleukin-6 stimulates multilineage hematopoiesis and accelerates recovery from radiation-induced hematopoietic depression. *Blood.* 77:472.
22. Hawley, R.G., A.Z.C. Fong, B.F. Burns, and T.S. Hawley. 1992. Transplantable myeloproliferative disease induced in mice by an interleukin 6 retrovirus. *J. Exp. Med.* 176:1149.
23. Suematsu, S., T. Matsuda, K. Aozasa, S. Akira, N. Nakano, S. Ohno, J. Miyazaki, K. Yamamura, T. Hirano, and T. Kishimoto. 1989. IgG_1 plasmacytosis in interleukin 6 transgenic mice. *Proc. Natl. Acad. Sci. USA.* 86:7547.
24. Brandt, S.J., D.M. Bodine, C.E. Dunbar, and A.W. Nienhuis. 1990. Dysregulated interleukin 6 expression produces a syndrome resembling Castleman's disease in mice. *J. Clin. Invest.* 86:592.
25. Hawley, T.S., L.A. Sabourin, and R.G. Hawley. 1989. Comparative analysis of retroviral vector expression in mouse embryonal carcinoma cells. *Plasmid.* 22:120.
26. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
27. Markowitz, D., S. Goff, and A. Bank. 1988. A safe packaging line for gene transfer: separating viral genes on two different plasmids. *J. Virol.* 62:1120.

28. Hawley, R.G., L. Covarrubias, T. Hawley, and B. Mintz. 1987. Handicapped retroviral vectors efficiently transduce foreign genes into hematopoietic stem cells. *Proc. Natl. Acad. Sci. USA.* 84:2406.
29. Karasuyama, H., and F. Melchers. 1988. Establishment of mouse cell lines which constitutively secrete large quantities of interleukin 2, 3, 4 or 5, using modified cDNA expression vectors. *Eur. J. Immunol.* 18:97.
30. Harris, J.F., R.G. Hawley, T.S. Hawley, and G. Crawford-Sharpe. 1992. Increased frequency of both total and specific monoclonal antibody producing hybridomas using a fusion partner that constitutively expresses recombinant IL-6. *J. Immunol. Methods.* 148:199.
31. Nakahata, T., and M. Ogawa. 1982. Clonal origin of murine hemopoietic colonies with apparent restriction to granulocyte-macrophage-megakaryocyte (GMM) differentiation. *J. Cell. Physiol.* 111:239.
32. Hawley, T.S., B.F. Burns, and R.G. Hawley. 1991. Leukocytosis in mice following long-term reconstitution with genetically-modified bone marrow cells constitutively expressing interleukin-1α or interleukin-6. *Leuk. Res.* 15:659.
33. Hawley, T.S., W.A. McLeish, and R.G. Hawley. 1991. Establishment of a novel factor-dependent myeloid cell line from primary cultures of mouse bone marrow. *Cytokine.* 3:60.
34. Paul, S.R., B.A. Barut, F. Bennett, M.A. Cochran, and K.C. Anderson. 1992. Lack of a role of interleukin 11 in the growth of multiple myeloma. *Leuk. Res.* 16:247.
35. Shabo, Y., and L. Sachs. 1988. Inhibition of differentiation and affinity purification with a monoclonal antibody to a myeloid cell differentiation-inducing protein. *Blood.* 5:1543.
36. Aarden, L.A., E.R. De Groot, O.L. Schaap, and P.M. Lansdorp. 1987. Production of hybridoma growth factor by human monocytes. *Eur. J. Immunol.* 17:1411.
37. Hapel, A.J., H.S. Warren, and D.A. Hume. 1984. Different colony-stimulating factors are detected by the "interleukin-3"-dependent cell lines FDC-P1 and 32D cl-23. *Blood.* 64:786.
38. Chodakewitz, J.A., T.S. Kupper, and D.L. Coleman. 1988. Keratinocyte-derived granulocyte/macrophage colony-stimulating factor induces DNA synthesis by peritoneal macrophages. *J. Immunol.* 140:832.
39. Beck, E., G. Ludwig, E.A. Auerswald, B. Reiss, and H. Schaller. 1982. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5. *Gene.* 19:327.
40. Van Snick, J., S. Cayphas, J.-P. Szikora, J.-C. Renauld, E. Van Roost, T. Boon, and R.J. Simpson. 1988. cDNA cloning of murine interleukin-HP1:homology with human interleukin 6. *Eur. J. Immunol.* 18:193.
41. Piechaczyk, M., J.M. Blanchard, L. Marty, C. Dani, F. Panabieres, S. El Sabouty, P. Fort, and P. Jeanteur. 1984. Post-transcriptional regulation of glyceraldehyde-3-phosphate-dehydrogenase gene expression in rat tissues. *Nucleic Acids Res.* 12:6951.
42. Hawley, T.S., B. Lach, B.F. Burns, L.T. May, P.B. Sehgal, and R.G. Hawley. 1991. Expression of retrovirally transduced IL-1α in IL-6-dependent B cells: a murine model of aggressive multiple myeloma. *Growth Factors.* 5:327.
43. Metcalf, D., C.G. Begley, G.R. Johnson, N.A. Nicola, A.F. Lopez, and D.J. Williamson. 1986. Effects of purified bacterially synthesized murine multi-CSF (IL-3) on hematopoiesis in normal adult mice. *Blood.* 68:46.
44. Metcalf, D., C.G. Begley, D.J. Williamson, E.C. Nice, J. DeLamarter, J.-J. Mermod, D. Thatcher, and A. Schmidt. 1987. Hemopoietic responses in mice injected with purified recombinant murine GM-CSF. *Exp. Hematol.* 15:1.
45. Quesniaux, V.F.J., S.C. Clark, K. Turner, and B. Fagg. 1992. Interleukin-11 stimulates multiple phases of erythropoiesis in vitro. *Blood.* 80:1218.
46. Gauldie, J., C. Richards, D. Harnish, P. Landsdorp, and H. Baumann. 1987. Interferon β2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc. Natl. Acad. Sci. USA.* 84:7251.
47. Kaleko, M., J.V. Garcia, R.A. Osborne, and A.D. Miller. 1990. Expression of human adenosine deaminase in mice after transplantation of genetically-modified bone marrow. *Blood.* 75:1733.
48. Morishita, K., D.S. Parker, M.L. Mucenski, N.A. Jenkins, N.G. Copeland, and J.N. Ihle. 1988. Retroviral activation of a novel gene encoding a zinc finger protein in IL-3-dependent myeloid leukemia cell lines. *Cell.* 54:831.
49. Lord, K.A., A. Abdollahi, S.M. Thomas, M. DeMarco, J.S. Brugge, B. Hoffman-Liebermann, and D.A. Liebermann. 1991. Leukemia inhibitory factor and interleukin-6 trigger the same immediate early response, including tyrosine phosphorylation, upon induction of myeloid leukemia differentiation. *Mol. Cell. Biol.* 11:4371.
50. Wood, C.R., S.R. Paul, S. Goldman, M. Muench, R. Palmer, G. Pedneault, G.E. Morris, M. Bree, L. Hayes, J. Hoysradt, et al. 1992. IL-11 expression in donor bone marrow cells improves hematological reconstitution in lethally irradiated recipient mice. *J. Cell. Biochem.* 16C(Suppl.):93.
51. Gasparetto, C., J. Laver, M. Abboud, A. Gillio, C. Smith, R.J. O'Reilly, and M.A.S. Moore. 1989. Effects of interleukin-1 on hematopoietic progenitors: evidence of stimulatory and inhibitory activities in a primate model. *Blood.* 74:547.
52. Williams, W.J., E. Beutler, A. Erslev, and M.A. Lichtman. 1990. Hematology. McGraw-Hill, Inc., New York. 1882 pp.
53. Metcalf, D., N.A. Nicola, and D.P. Gearing. 1990. Effects of injected leukemia inhibitory factor on hematopoietic and other tissues in mice. *Blood.* 76:50.
54. Metcalf, D., and D.P. Gearing. 1989. Fatal syndrome in mice engrafted with cells producing high levels of the leukemia inhibitory factor. *Proc. Natl. Acad. Sci. USA.* 86:5948.
55. Taga, T., M. Narazaki, K. Yasukawa, T. Saito, D. Miki, M. Hamaguchi, S. Davis, M. Shoyab, G. Yancopoulos, and T. Kishimoto. 1992. Functional inhibition of hematopoietic and neurotrophic cytokines by blocking the interleukin 6 signal transducer gp130. *Proc. Natl. Acad. Sci. USA.* 89:10998.
56. Yang, Y.-C., and T. Yin. 1992. Interleukin-11 and its receptor. *Biofactors.* 4:15.
57. Gearing, D.P., M.R. Comeau, D.J. Friend, S.D. Gimpel, C.J. Thut, J. McGourty, K.K. Brasher, J.A. King, S. Gillis, B. Mosley, et al. 1992. The IL-6 signal transducer, gp130: an oncostatin M receptor and affinity converter for the LIF receptor. *Science (Wash. DC).* 255:1434.
58. Gruss, H., M. Brach, and F. Herrmann. 1992. Involvement of nuclear factor-κB in induction of the interleukin-6 gene by leukemia inhibitory factor. *Blood.* 80:2563.
59. Anderson, K.C., C. Morimoto, S.R. Paul, D. Chauhan, D. Williams, M. Cochran, and B.A. Barut. 1992. Interleukin-11 promotes accessory cell-dependent B-cell differentiation in humans. *Blood.* 80:2797.
60. Ulich, T.R., K. Guo, D. Remick, J. del Castillo, and S. Yin. 1991. Endotoxin-induced cytokine gene expression in vivo. *J. Immunol.* 146:2316.
61. Le, J., L.F.L. Reis, and J. Vilcek. 1988. Tumor necrosis factor

and interleukin 1 can act as essential growth factors in a murine plasmacytoma line. *Lymphokine Res.* 7:99.
62. Burger, R., and M. Gramatzki. 1993. Responsiveness of the interleukin (IL)-6–dependent cell line B9 to IL-11. *J. Immunol. Methods.* 158:147.
63. Sabourin, L.A., and R.G. Hawley. 1990. Suppression of programmed death and G_1 arrest in B-cell hybridomas by interleukin-6 is not accompanied by altered expression of immediate early response genes. *J. Cell. Physiol.* 145:564.
64. Tohyama, N., H. Karasuyama, and T. Tada. 1990. Growth autonomy and tumorigenicity of interleukin 6–dependent B cells transfected with interleukin 6 cDNA. *J. Exp. Med.* 171:389.
65. Vink, A., P. Coulie, G. Warnier, J.-C. Renauld, M. Stevens, D. Donckers, and J. Van Snick. 1990. Mouse plasmacytoma growth in vivo: enhancement by interleukin 6 (IL-6) and inhibition by antibodies directed against IL-6 or its receptor. *J. Exp. Med.* 172:997.
66. Lang, R.A., and A.W. Burgess. 1990. Autocrine growth factors and tumourigenic transformation. *Immunol. Today.* 11:244.
67. Suematsu, S., T. Matsusaka, T. Matsuda, S. Ohno, J. Miyazaki, K. Yamamura, T. Hirano, and T. Kishimoto. 1992. Generation of plasmacytomas with the chromosomal translocation t(12;15) in interleukin 6 transgenic mice. *Proc. Natl. Acad. Sci. USA.* 89:232.
68. Rich, B.E., J. Campos-Torres, R.I. Tepper, R.W. Moreadith, and P. Leder. 1993. Cutaneous lymphoproliferation and lymphomas in interleukin 7 transgenic mice. *J. Exp. Med.* 177:305.
69. Kobayashi, S., M. Teramura, I. Sugawara, K. Oshimi, and H. Mizoguchi. 1993. Interleukin-11 acts as an autocrine growth factor for human megakaryoblastic cell lines. *Blood.* 81:889.
70. Hu, J.P., A. Cesano, D. Santoli, S.C. Clark, and T. Hoang. 1993. Effects of interleukin-11 on the proliferation and cell cycle status of myeloid leukemia cells. *Blood.* 81:1586.
71. Leslie, K.B., H.J. Ziltener, and J.W. Schrader. 1991. The role of interleukin-1 and granulocyte-macrophage colony-stimulating factor in the paracrine stimulation of an in vivo–derived murine myeloid leukemia. *Blood.* 78:1301.
72. Preston-Martin, S., M.C. Pike, R.K. Ross, P.A. Jones, and B.E. Henderson. 1990. Increased cell division as a cause of human cancer. *Cancer Res.* 50:7415.
73. Fearon, E.R., and B. Vogelstein. 1990. A genetic model for colorectal tumorigenesis. *Cell.* 61:759.