Transplantable Myeloproliferative Disease Induced in Mice by an Interleukin 6 Retrovirus

By Robert G. Hawley,*‡ Andrew Z. C. Fong,* Bruce F. Burns,§ and Teresa S. Hawley*

*From the *Division of Cancer Research, Sunnybrook Health Science Centre, and the ‡Department of Medical Biophysics, University of Toronto, Toronto, Ontario M4N 3M5; and the §Department of Anatomical Pathology, Ottawa Civic Hospital, Ottawa, Ontario K1Y 4E9, Canada*

Summary

Lethally irradiated mice transplanted with bone marrow cells infected with a novel recombinant retrovirus (murine stem cell virus–interleukin 6 [MSCV-IL-6]) bearing a mouse IL-6 gene developed a fatal myeloproliferative disease within 4 wk of engraftment. The hematologic manifestations of the syndrome included elevated peripheral leukocyte counts (up to 430 × 10^3 cells/mm^3) with a predominance of neutrophilic granulocytes, microcytic anemia, and thrombocytosis or thrombocytopenia. The mice showed extensive neutrophil infiltration of the lungs, liver, and occasionally lymph nodes, plus splenomegaly resulting from enhanced splenic myelopoiesis (30–60-fold increase in progenitor numbers). Despite the chronic stimulation of neutrophil excess by IL-6, bone marrow from affected mice was capable of repopulating the hematopoietic tissues (bone marrow and spleen) of lethally irradiated hosts during repeated serial transplantation. In the longest documented case, the progeny of a single MSCV-IL-6-marked cell transferred the myeloproliferative disease to two secondary, four tertiary, and two quaternary recipients (the clone endured for a total of 72 wk). These results, demonstrating considerable proliferative longevity of the IL-6-producing cells, support an in vivo role of IL-6 in the maintenance of hematopoietic precursors. Dysregulated IL-6 production also had significant systemic effects. The mice displayed increased mesangial cell proliferation in the kidney, frequent liver abnormalities, and marked alterations in plasma protein levels. Unlike previous studies where constitutive expression of exogenous IL-6 genes resulted in lymphoproliferative disorders characterized by massive plasmacytosis, minimal plasma cell expansion occurred in the MSCV-IL-6 mice during the observation period. Potential explanations for the differences in disease phenotypes observed in the present and previous studies are different cell types expressing the exogenous IL-6 genes, higher sustained circulating levels of IL-6 achieved using the MSCV-IL-6 retroviral delivery system, and/or the premature death (3–15 wk after transplantation) of the MSCV-IL-6 mice before the onset of plasmacytosis. This animal model should prove useful for further investigation of the function of IL-6 in normal and abnormal hematopoiesis and in inflammatory responses.

Mature blood cells have short life spans. Consequently, they are replenished continuously throughout life from a small population of undifferentiated precursors (stem cells) (1). Besides their differentiative potential, stem cells have extensive self-maintaining (self-renewal) capacity. It is this unique property of self-renewal that distinguishes stem cells from committed hematopoietic progenitor cells. Although a large body of data has been accumulated identifying the growth factors (cytokines) that act on progenitor cells (2), little is known about the factors that govern stem cell numbers or the regulatory mechanisms controlling the production of progenitor cells by stem cells in vivo.

In adults, hematopoiesis occurs mainly in the bone marrow where specialized stromal cells are believed to maintain steady-state levels of hematopoietic stem cells through the concerted actions of multiple membrane-bound and short-range factors (3). The recently described c-*kit* ligand (KL; also referred to as stem cell factor, or mast cell growth factor), whose defective expression in mice of the Steel genotype results in severe anemia and stem cell deficiency, is clearly an important contributor to basal hematopoiesis (4). Indirect evidence suggests that IL-6, a pleiotropic cytokine produced by a variety of cell types, including bone marrow stromal cells (5), may also be important for normal hematopoietic development (6, 7). Originally identified because of its immune and inflammatory properties (8), subsequent studies have revealed an impressive range of activities of IL-6 on early hematopoietic cells. Most relevant in this regard has been the demonstra-

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/92/10/1149/15 $2.00
Volume 176 October 1992 1149–1163

tion that IL-6 can act in concert with IL-3 to increase the in vitro number of stem cells providing long-term, multilineage engraftment of lethally irradiated mice (9, 10). The combination of IL-6 with IL-3 or KL also increases exogenous numbers of stem cells with short-term repopulating potential, as well as progenitors capable of forming splenic myeloerythroid colonies (CFU-spleen [S][1]) (9–12). Numerous other studies have documented the ability of IL-6 to stimulate the in vitro production of multipotential and restricted progenitors of the myeloid, erythroid, and megakaryocytic lineages and their progeny (5, 13–19). In the mouse, IL-6 by itself directly supports the proliferation and differentiation of some granulocyte/macrophage progenitor cells (14, 15, 20).

Compared with in vitro studies, there have been limited reports of in vivo effects of IL-6 on the hematopoietic stem/progenitor cell compartment. Administration of IL-6 to mice increased recovery of bone marrow and spleen CFU-S numbers and myeloerythroid progenitor numbers after chemotherapy- or radiation-induced hematopoietic injury (21, 22). Similar treatment of normal mice with IL-6 enhanced production of CFU-S and granulocyte/macrophage progenitors transiently in the spleen (22, 23), with the number of these cell types in bone marrow being either slightly elevated or remaining relatively unchanged in different treatment regimens (22, 24, 25). Modest increases in the circulating numbers of neutrophils and platelets and mild erythropoietic effects have also been observed after in vivo administration of IL-6 (24–26).

An alternative approach to examine the effects of exogenous gene products on hematopoietic processes is to introduce the corresponding genes directly into stem cells with retrovirus vectors (27). The recombinant retroviruses can then be used as tags to follow the fates of stem cell clones during serial bone marrow transplantation (28–34). When cytokine genes have been transferred into hematopoietic cells in this manner, their dysregulated expression routinely elicits hyperplastic hematologic disorders that are frequently fatal (35–39). There has been limited documentation, however, of the ability to transfer the disorders to secondary recipients (37–39), thus precluding systematic evaluation of the consequences of long-term cytokine exposure on the stem cell pool. A basic question that remains unanswered for any factor is whether chronic overproduction of mature hematopoietic cells depletes stem cells or diminishes their self-renewal capacity.

Because sustained high-level expression of nonselectable genes after serial transplantation of retrovirus-infected stem cells has been infrequent (9, 40–42), we used an improved infection protocol (9) and a new high-titer retrovirus vector for the current studies in an attempt to reduce the possibility of deficiencies in the gene transfer methodology (inefficient introduction of transcriptionally active genes into stem cells or extinction of gene expression with time [29, 38]) complicating the interpretation of the data. A previously described retrovirus vector from this laboratory (L6PNL) bearing a mouse IL-6 gene and the bacterial neomycin phosphotransferase (*neo*) gene (43) was modified by incorporating those sequences from the murine embryonic stem cell virus (MESV) that have recently been shown to facilitate the expression of exogenous genes in embryonic stem cells (44). The resulting IL-6 murine stem cell virus (MSCV-IL-6) was utilized herein to evaluate the repopulating potential of IL-6-producing, bone marrow precursors.

Materials and Methods

MSCV-IL-6 Retrovirus Vector and Cell Lines. To simplify plasmid constructions with MSCV vectors, the MESV retrovirus backbone of P5Gneo (a gift of M. Grez, Heinrich-Pette-Institut, Hamburg, Germany) (44) was subcloned into a pUC8-based plasmid (45). The majority of the LTR sequences in L6PNL (43) and all internal sequences from an NheI site in the 5′ LTR to a KpnI site in the 3′ LTR were replaced with the corresponding P5Gneo sequences contained on a 1.9-kb NheI-XhoI fragment and a 3.2-kb XhoI-KpnI fragment. To construct MSCV-IL-6 (Fig. 1), the gag-neo sequences of the resulting plasmid PUC5Gneo were deleted from an SpeI site (position 292) to a HindIII site present in the envelope region, and a 3.0-kb SpeI-HindIII fragment of L6PNL-containing gag-IL-6-pgk-neo sequences was inserted. The net result of these manipulations was the substitution of the viral transcriptional controlling sequences and 5′ untranslated region in L6PNL shown to be involved in the restriction of viral gene expression in embryonic stem cells with the corresponding elements from MESV that are permissive for gene expression in these cells.

A general MSCV vector was derived from MSCV-IL-6 by excising the IL-6 sequences with EcoRI. The salient features of the MSCV vector design include variant LTRs (from PCMV, a mutant of the myeloproliferative sarcoma virus expressed in PCC4 embryonal carcinoma cells) (46) and a modified 5′ untranslated region (from dl-587rev, an endogenous mouse retrovirus containing a $tRNA^{Gln}$ primer binding site instead of $tRNA^{Pro}$) (47) for efficient LTR-directed expression in embryonic stem cells, a functional splice donor, and 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

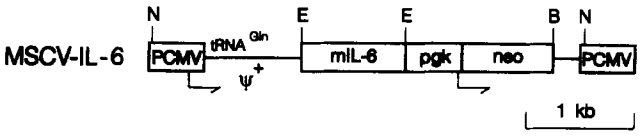

Figure 1. Schematic representation of MSCV-IL-6 retrovirus vector. The LTRs of MSCV-IL-6 (denoted PCMV) and the 5′ untranslated region (indicated by $tRNA^{Gln}$) permit efficient LTR-directed expression of exogenous genes in embryonic stem cells. Arrows indicate the LTR and pgk-1 (pgk) promoters utilized for expression of the mouse IL-6 (mIL-6) gene and the bacterial *neo* gene, respectively. Also indicated are the NheI (N) and BamHI (B) restriction sites, which permit assessment of vector sequence transmission and quantitation of proviral integration sites, respectively. Not shown are the splice donor and acceptor sites within the extended packaging region (Ψ^+) that result in a spliced IL-6 mRNA normally present as a minor species in MSCV-IL-6-infected cells. As described in Materials and Methods, a prototype MSCV retrovirus vector was generated by excision of the IL-6 gene with EcoRI (E).

[1] *Abbreviations used in this paper:* CFC, colony-forming cells; CFU-S, colony forming unit-spleen; MCV, mean corpuscular volume; MESV, murine embryonic stem cell virus; MoMLV, Moloney murine leukemia virus; MPV, mean platelet volume; MSCV, murine stem cell virus; neo, neomycin phosphotransferase; pgk-1, phosphoglycerate kinase-1.

genes (48). The *neo* gene in MSCV 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-1) promoter for constitutive expression in a wide range of cell types (49). In the final version of MSCV, all envelope sequences have been deleted by incorporation of a synthetic oligonucleotide fragment from LNCX (48), and unique restriction enzyme sites (EcoRI, HpaI, XhoI, BglII) have been provided for insertion of genes 5′ of the pgk-1 promoter (oligonucleotides synthesized by the University of Ottawa Biotechnology Research Institute [3808; Applied Biosystems, Inc., Foster City, CA]). Subcloning was carried out using standard recombinant DNA procedures (50). All MSCV vectors and the exact details of their construction are available upon request.

Recombinant virus was produced from clonal isolates of GP+E-86 ecotropic helper-free packaging cells (51), generated as described previously by infection of tunicamycin-treated cells with supernatant from transient transfectants (43). The cell line used for all gene transfer experiments, GP+E-86/MSCV-IL-6 c2.12, produces structurally intact MSCV-IL-6 virus with a titer of 2×10^6 G418-resistant CFU/ml when assayed on NIH3T3 fibroblasts. Cells were maintained in DME with 4.5 g/liter glucose supplemented with 10% FCS (HyClone Lab) in a humidified atmosphere containing 5% CO_2 at 37°C.

Retrovirus Infection and Transplantation of Bone Marrow. Female BALB/c mice (Charles River Breeding Laboratory) were used at 6–8 wk of age as bone marrow donors and recipients. Bone marrow processing, infection, and transplantation were carried out using a slightly modified version of published procedures (9, 43, 52). Bone marrow was flushed from hind limbs of donors injected 4 d previously with 150 mg/kg 5-fluorouracil with ice-cold IMDM containing 50 μM 2-ME and 10% heat-inactivated FCS (Gibco Laboratories, Grand Island, NY). After erythrocyte lysis in 0.17 M ammonium chloride, nucleated cells were added to 100-mm petri dishes (Fisher Scientific) at a density of 5×10^5 cells/ml in IMDM supplemented with 50 μM 2-ME, 10% heat-inactivated FCS, 10% conditioned medium from X630-rIL-3 cells (a source of IL-3; a gift of F. Melchers, Basel, Switzerland) (53), and 10% conditioned medium from Sp2/mIL-6 cells (a source of IL-6) (54). After 48 h, the bone marrow cells were collected and added to subconfluent monolayers of GP+E-86/MSCV-IL-6 c2.12 cells in 100-mm tissue culture dishes (Nunc Roskilde, Denmark) at a density of 5×10^5 cells/ml in IMDM supplemented with 50 μM 2-ME, 10% heat-inactivated FCS, 10% X630-rIL-3-conditioned medium, and 8 μg/ml polybrene. After a further 48 h, nonadherent bone marrow cells were harvested, centrifuged, and resuspended in fresh cocultivation medium containing 0.75 mg/ml G418 (Gibco Laboratories, Grand Island, NY). These cells were collected 24 h later and injected via the tail vein into recipients that had received 7 Gy of irradiation (Gammacell 40; Atomic Energy of Canada). In some experiments, the G418 selection was omitted. Each mouse received between 5×10^5 and 2×10^6 cells. 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 three per sterilized microisolater cage on laminar flow racks. The colony was periodically tested for a panel of viruses by analysis of serum samples (Charles River Professional Services, Wilmington, MA). Animals repeatedly 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 intervals after transplant and immediately before death. Total leukocytes (WBC), total erythrocytes (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), total platelets, and mean platelet volume (MPV) were determined on a cell counter (System 9000 Hematology Series; Serono-Baker Instruments) equipped with a veterinarian software package (43). Granulocytic predominance of leukocytes suggested by the automated differential leukocyte count was confirmed by performing a manual differential count of Wright-stained blood smears. Controls were mice transplanted with hematopoietic cells infected with a derivative of MSCV carrying a PKC gene (55; our unpublished results) as well as age-matched littermates.

Mice were killed by cervical dislocation when moribund and necropsy examinations were performed immediately after death. Tissues were preserved in B5 fixative for 2 h, stored in 10% neutral-buffered formalin, then embedded in paraffin, sectioned, and stained with hematoxylin and eosin before examination by light microscopy (43, 56). Tissues examined included spleen, thymus, lungs, heart, liver, pancreas, kidneys, lymph nodes, and sternum (bone marrow).

In Vitro Progenitor Cell Assay. The number of colony-forming cells (CFC)was determined by plating nucleated bone marrow (at 10^3 and 10^4 cells/ml) and spleen cells (at 10^3, 10^4, 10^5 cells/ml) in duplicate 1-ml cultures (35-mm Lux suspension plates; Miles) 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 (Terry Fox Laboratory, Vancouver, Canada) as described (57). Where indicated, G418 was added to a final concentration of 1 mg/ml. Colonies (consisting of >50 cells) of erythroid cells, granulocytes, macrophages, and mixtures of these were enumerated using an inverted microscope on day 7 and recorded together as CFC.

Bioassays. IL-6 activity in culture supernatants and plasma samples was detected by B9 microproliferation assay (58) essentially as described (43). The cells were propagated in IMDM supplemented with 50 μM 2-ME, 5% heat-inactivated FCS, and 2% conditioned medium from Sp2/mIL-6 cells (54). For the assay, B9 cells were harvested by centrifugation, washed twice in medium lacking IL-6, 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) at 64–72 h. The incorporated radioactivity was determined for triplicate cultures by liquid scintillation counting (Betaplate, Wallac) after transfer of cellular debris to glass fiber filters with a cell harvester (Skatron). The standard deviation of the mean of triplicate cultures was always <15%. Each assay was standardized with human rIL-6 (R&D Systems), and values (ng/ml human IL-6 equivalents) were calculated by probit analysis. When included, a rat mAb against mouse IL-6 (59) (a gift of L. Sachs, Weizmann Institute, Rehovot, Israel) completely neutralized the B9 proliferative activity in the plasma samples. Microwell cultures of OTT1 (responsive to GM-CSF, IL-3, and IL-5), FDC-P1 (responsive to GM-CSF and IL-3), and 32D (responsive to IL-3) cells were similarly used to assay GM-CSF and IL-3 as described (60, 61).

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). 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% TCA, destained for 15 min in 5% acetic acid, and cleared in 40.0% aqueous *N*-methyl pyrrolidone before scanning by laser densitometry (Ultrascan XL; Pharmacia LKB, Piscataway, NJ).

Nucleic Acid Analysis. High molecular weight DNA (10 μg) was digested with BamHI (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 (62). Structural integrity of MSCV-IL-6 proviruses was assessed by Southern blot analysis of NheI-digested DNA. Total cellular RNA (10 μg) was analyzed by Northern blotting for viral RNA transcripts using a 0.6-kb EcoRI-BglII fragment of the mouse IL-6 cDNA as probe (63). After exposure to film, the blots were stripped and rehybridized with the neo probe to assess the relative levels of LTR- and pgk-1-initiated transcripts. The blots were subjected to a final hybridization with a 1.2-kb PstI fragment of the rat glyceraldehyde-3-phosphate dehydrogenase gene (64). Southern and Northern blot analyses were carried out according to standard procedures (50).

Results

Rationale for Construction of the MSCV-IL-6 Retrovirus Vector. We previously reported the engraftment of lethally irradiated BALB/c mice with bone marrow cells infected with the L6PNL retrovirus carrying a mouse IL-6 gene transcribed from the Moloney murine leukemia virus (MoMLV) LTR (43). The presence of the bacterial *neo* gene in the L6PNL virus permitted the isolation of G418-resistant populations of macrophages, mast cells, and T cells from hematopoietic tissues of recipients for lineage analyses and RNA expression studies up to 10 mo after transplantation. The results demonstrated transfer of functional L6PNL retroviruses into repopulating stem cells capable of long-term multilineage reconstitution. In all cases, however, the levels of viral IL-6 mRNA were found to be considerably lower than the corresponding levels of neo mRNA, which originated from an internal pgk-1 promoter in the L6PNL virus. Low-level expression of the introduced IL-6 gene evoked minor hematologic changes in the animals: a mild myeloproliferative effect with moderate increases in circulating neutrophil numbers. These findings contrasted with the considerable effects reported by Brandt et al. (39) in unirradiated, stem cell–defective W/W^v mice receiving bone marrow infected with a different IL-6 retrovirus. Most of the mice in the latter study developed a syndrome characterized by massive plasma cell hyperplasia. A similar lymphoproliferative disease had previously been observed in transgenic mice expressing a human IL-6 gene under the control of an Ig heavy chain enhancer (65).

Besides differences in infection regimens, differences in retrovirus constructs could account for the discordant results obtained in these studies. Since the LTRs and 5′ untranslated region of the MESV retrovirus vector were recently found to alleviate the restriction block experienced by MoMLV-based vectors in embryonic stem cells (44), we felt it desirable to incorporate these MESV components into the L6PNL vector before undertaking further experiments aimed at examining the effects of chronic IL-6 overproduction on early hematopoietic cells. The modified IL-6 retrovirus vector, designated MSCV-IL-6 to reflect its derivation from MESV, is illustrated in Fig. 1.

The effective titer of helper-free recombinant MSCV-IL-6 produced by a clonal isolate of GP+E-86 packaging cells was 2×10^5 G418-resistant CFU/ml when assayed on the extremely nonpermissive P19 embryonal carcinoma cell line (66). This frequency of functional *neo* gene transfer into P19 cells represents an ~40-fold improvement over that previously obtained with other retrovirus constructs (49) and is only 10-fold less than the titer obtained on fibroblasts. More important, unlike the situation experienced with L6PNL proviruses in P19 cells, where virtually no LTR-initiated IL-6 transcripts could be detected, viral IL-6 RNA species were present in MSCV-IL-6-infected P19 cells at levels comparable to the neo transcripts originating from the pgk-1 promoter (data not shown).

Fatal Hematologic Disease Develops in Mice Receiving MSCV-IL-6-infected Hematopoietic Cells. Lethally irradiated BALB/c mice received intravenous inoculations of MSCV-IL-6-infected bone marrow cells (~10^6 cells). Bone marrow infections were carried out according to protocols described previously except that exposure to virus-producing monolayers was preceded by a 48-h incubation in medium supplemented with IL-3 and IL-6. Inclusion of the prestimulation step has been shown to improve the efficiency of gene transfer into long-term repopulating cells (9). Under these experimental conditions, each inoculum contained $1–4 \times 10^5$ progenitor cells as determined by 7-d methylcellulose CFC assay (~50% of which were G418 resistant), a 10-fold increase over previous numbers (43). Beginning 3 wk after transplantation, the MSCV-IL-6 mice developed leukocytosis (peripheral leukocyte count routinely in the range of $100–300 \times 10^3$ cells/mm^3). By 4 wk, only 9 of 20 primary animals undergoing hematopoietic reconstitution were still alive, and these animals were visibly ill (showing substantial weight loss, ruffled fur, and a hunched posture). The animals with the most severe symptoms (7/9) were those that had received infected bone marrow that had been transiently cultured in the presence of G418 before injection.

Serial Transplantation of IL-6-induced Hematologic Disease. Seven moribund mice were immediately killed for autopsy, and their bone marrow was transplanted to lethally irradiated secondary recipients (two to three secondaries per primary) in an attempt to transfer the syndrome via infected stem cells. Mice 31 and 32, which had been transfused with infected bone marrow that had not been exposed to G418 ex vivo, were killed 9 and 10 wk posttransplant, respectively, when their peripheral leukocyte counts were $>300 \times 10^3$ cells/mm^3. All of the secondary recipients receiving bone marrow from the affected primary animals presented with leukocytosis 3–4 wk after transplant. Despite the elevated leukocyte levels, some of the secondary recipients survived for periods of up to 13 wk. These data suggested the possibility that functional integration of MSCV-IL-6 viruses had occurred at the level of repopulating stem cells. Consistent with this notion was the finding that the hematologic disease could be serially transferred to lethally irradiated tertiary (total of 10) and quaternary (total of two) recipients. In all cases, Southern blot analysis with a neo probe revealed the presence of nonrearranged MSCV-IL-6 proviruses in NheI-digested DNA from bone marrow or spleen (NheI cleaves once within each LTR yielding a fragment 3.9 kb in size; see Fig. 1). Al-

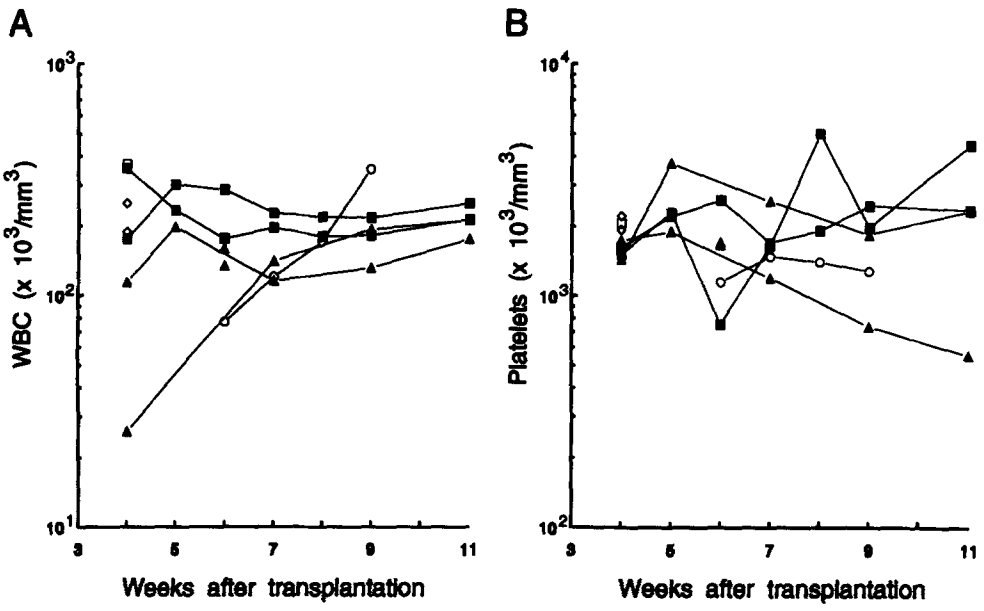

Figure 2. Leukocytosis and variable platelet levels in MSCV-IL-6 mice. Peripheral blood of mouse 31 (○), and its secondary (■), tertiary (▲), and quaternary (◆) recipients was analyzed at the time points indicated after bone marrow transplantation. (*A*) Leukocyte (WBC) counts were routinely >100 × 10^3 cells/mm³ compared with control values, which were 2.9–16.0 × 10^3 cells/mm³ during an equivalent observation period. (*B*) Platelet counts varied widely (range, 62–5,008 × 10^3 cells/mm³), with mice frequently displaying thrombocytosis or thrombocytopenia. By comparison, platelet counts of controls stabilized by 4 wk after transplantation (range, 884–1232 × 10^3 cells/mm³). See Tables 1 and 2 for values of hematologic parameters of 31 series mice and controls before death.

together, nine separate transplant pedigrees involving a total of 43 animals were established. The hematologic and pathologic features of the syndrome were similar for all mice examined. Most of the data presented were provided by two transplant pedigrees, one arising from infected bone marrow that had been selected in G418 (41 series) and one originating from infected bone marrow in which the selection had been omitted (31 series).

Hematopathologic Changes Associated with Myeloproliferative Disease in MSCV-IL-6 Mice. Sustained leukocytosis was observed in the MSCV-IL-6 mice for a maximum of 15 wk after transplant (Fig. 2 *A*, Table 1), with the mice either dying or killed for health concerns by this time (usually when one of the affected cohorts died). Differential cell counts performed on Wright-stained blood smears indicated that the increase in leukocytes was due primarily to excess numbers of mature neutrophilic granulocytes (Table 1, Fig. 3 *D*). With one exception, all MSCV-IL-6 animals developing the myeloproliferative syndrome also developed a microcytic anemia (mean hematocrit of MSCV-IL-6 animals was 30% compared with 44% for concurrent controls receiving bone marrow infected with another MSCV construct or unirradiated littermates; Table 2). The exception, mouse 31.3, presented with a macrocytic anemia (MCV of 74.8 fl compared with 37.5 fl for other MSCV-IL-6 mice and 42.4 fl for controls; Table 2). Consistent with previously reported megakaryocytopoietic and thrombopoietic effects of IL-6 (16, 18, 24), many MSCV-IL-6 mice had higher than normal platelet levels throughout the observation period (Fig. 2 *B*). However, other MSCV-IL-6 mice showed minor changes in platelet levels and a significant fraction of the mice eventually became thrombocytopenic (Table 2). In most cases, MPV was increased (Table 2), a phenomenon typically seen in myeloproliferative disease (67). Suboptimal levels of synergistic factors required for efficient stimulation of platelet production (68) would account for the variable effects on platelet levels by IL-6. The variability in platelet numbers probably also reflects additional hematologic complications arising as a consequence of systemic IL-6 action on organs such as the liver or kidney (see below).

On autopsy (see Fig. 3 *A*), the most noticeable tissue change in the MSCV-IL-6 mice was an enlarged spleen (5–10-fold larger than normal) frequently containing white nodules. In view of the striking lymph node expansion (up to 1 cm in diameter) due to plasma cell infiltration seen previously in mice expressing exogenous IL-6 genes (39, 65), it was notable that in the vast majority of MSCV-IL-6 mice minimal lymph node swelling was observed. When moderate enlargement of the mesenteric lymph node occurred, it was mainly in serially transplanted animals surviving 8 wk or longer. Autopsy also frequently revealed a small or undetectable thymus and pale diffuse areas of the liver.

Upon histologic examination, the increased cellularity in the spleen was found to be due predominantly to expansion of the red pulp with an increased number of primitive myeloid cells and neutrophils in various stages of differentiation (Fig. 3 *B*). Megakaryocytes were the other cell type frequently recognized (Fig. 3 *C*). Inspection of multiple arbitrary microscopic areas gave values as high as 40–50 megakaryocytes per 10 high-power fields, compared with control spleens, which contained one to two megakaryocytes per 10 high-power fields. Comparably minor changes were observed in sternal marrow, similar to previous findings in mice having lower circulating IL-6 levels (39, 43), with an increased percentage of neutrophils being the most prominent finding. Neutrophilic infiltrates were prevalent in the alveolar septae of the lung without evidence of bacterial infection (Fig. 3 *E*), and in the periportal areas of the liver (Fig. 3 *F*). Some neutrophils were also present in hepatic sinusoids, and focal neutrophilic infiltrates were found in an occasional expanded lymph node (Fig. 3 *G*) and, less commonly, in the interstitium

Table 1. *Peripheral Leukocyte Counts and CFC Frequency in MSCV-IL-6 Mice*

Mouse	Time of reconstitution	IL-6	WBC	Differential*				CFC in bone marrow‡ (%G418R)	CFC in spleen‡ (%G418R)
				Ly	Gr	Mo	Eo		
	wk	*ng/ml*	*× 10³ cells/mm³*						
31	9	75	350	2	96	1	1	ND	ND
31.1	13	22	370	4	94	2	0	6,800 (<1)	13,000 (<1)
31.2	13	ND	250	2	97	1	0	ND	ND
31.3	4	43	350	1	98	1	0	3,000 (<1)	7,600 (3)
31.1.1	6	62	135	5	92	2	1	12,000 (<1)	12,800 (<1)
31.1.2	6	42	160	4	92	4	0	12,300 (<1)	5,500 (<1)
31.3.1	11	65	176	7	90	3	0	7,700 (1)	9,000 (<1)
31.3.2	15	130	310	3	94	3	0	10,000 (2)	7,600 (1)
31.3.1.1	4	58	250	2	95	3	0	7,700 (1)	10,300 (<1)
31.3.1.2	4	54	190	4	95	1	0	9,000 (<1)	7,000 (1)
41	4	65	49.2	2	98	0	0	35,000 (17)	6,500 (18)
41.1	9	14	270	1	98	1	0	13,500 (30)	10,300 (26)
41.2	9	45	120	4	96	0	0	10,000 (55)	8,000 (69)
41.3	9	18	430	3	95	2	0	10,800 (74)	17,000 (73)
41.3.1	7	48	125	3	96	1	0	10,700 (67)	6,000 (50)
41.3.2	7	7	120	6	94	0	0	12,500 (56)	9,200 (49)
51	8	ND	11.0	77	20	3	0	8,500 (<1)	90 (7)
52	8	ND	5.6	73	27	ND	ND	ND	ND
53	8	ND	7.6	83	17	ND	ND	ND	ND
54	12	ND	12.1	84	16	ND	ND	7,700 (<1)	200 (<1)
55	12	ND	13.1	84	16	ND	ND	ND	ND
56	12	ND	8.5	82	18	ND	ND	ND	ND
BALB/c	–	<0.1	11.9 ± 2.1	83 ± 5	17 ± 6	1 ± 1	1 ± 1	5,300 ± 1,300	175 ± 120

MSCV-IL-6-infected bone marrow from mouse 31 was serially transplanted to three secondary recipients (mice 31.1, 31.2, and 31.3), four tertiary recipients (mice 31.1.1, 31.1.2, 31.3.1, and 31.3.2), and two quaternary recipients (mice 31.3.1.1 and 31.3.1.2). Mouse 31.2 died 14 wk after transplantation. MSCV-IL-6-infected bone marrow from mouse 41 was serially transplanted to three secondary recipients (mice 41.1, 41.2, and 41.3) and two tertiary recipients (mice 41.3.1 and 41.3.2). Mice 51–56 are primary recipients of bone marrow infected with another MSCV construct. BALB/c values represent the mean and standard error of values obtained for six normal mice. WBC, leukocytes; Ly, lymphocytes; Gr, mature neutrophils (~2% band forms); Mo, monocytes; Eo, eosinophils.

* Manual differential leukocyte counts were performed on 200 cells. Automated differential leukocyte counts are presented for mice 52–56.

‡ CFC frequency is expressed per 10^6 bone marrow or spleen cells. Numbers in parentheses indicate the percentage of progenitor cells that were G418 resistant.

Figure 3. Hematopathologic analysis of MSCV-IL6 mice with myeloproliferative disease. (*A*) Dissection of affected secondary recipient demonstrating enlarged spleen with white nodules (*lower arrow*), swollen mesenteric lymph node (*middle arrow*), and small thymus (*upper arrow*). Also apparent are discolored splotchy areas of the liver. (*B*) Section of spleen showing considerable expansion of red pulp with neutrophils and immature myeloid elements (×600). (*C*) Section of spleen illustrating increased numbers of megakaryocytes (×600). (*D*) Peripheral blood smear showing predominance of mature neutrophils (×1,000). (*E*) Section of lung showing extensive interstitial neutrophilic infiltration (×600). (*F*) Section of liver showing periportal neutrophilic infiltration as well as some neutrophils in hepatic sinusoids (×250); abnormalities in hepatocytes are more clearly evident in the high-power image of Fig. 5. (*G*) Section of lymph node showing focal accumulations of neutrophils intermixed with diffuse infiltrate of plasma cells (×600). (*H*) Section of thymus showing normal-appearing lymphocytes in medulla (*top*) and cortex (*bottom*) (×600). (*I*) Smear of factor-independent, bone marrow–derived colony showing macrophages and cells of granulocytic lineage at various stages of differentiation (×1,500).

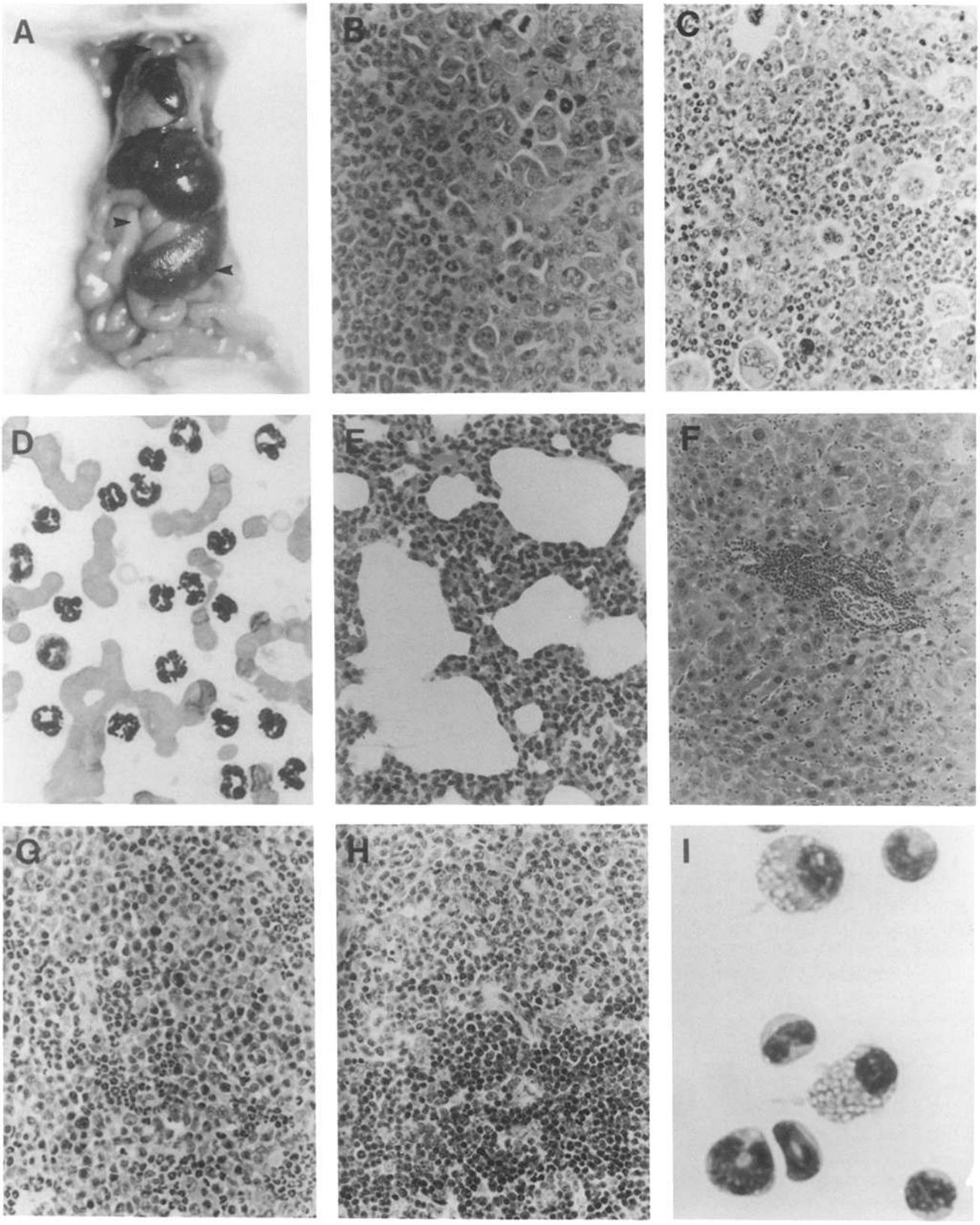
A
B
C
D
E
F
G
H
I

Table 2. *Other Hematologic Changes Associated with IL-6-induced Myeloproliferative Disease*

Mouse	RBC	Hemoglobin	Hematocrit	MCV	Platelets	MPV
	× 10⁶ cells/mm³	*g/d*	*%*	*fl*	*× 10³ cells/mm³*	*fl*
31	8.76	13.2	33.2	37.8	1,248	9.0
31.1	7.00	11.2	27.6	39.6	2,044	6.5
31.2	6.24	10.0	25.6	41.0	1,724	6.9
31.3	3.06	7.6	22.8	74.8	2,060	4.0
31.1.1	8.68	11.2	30.0	34.5	1,708	9.2
31.1.2	8.62	11.4	31.2	36.2	1,666	8.9
31.3.1	8.44	12.0	32.0	37.7	552	10.4
31.3.2	7.84	12.0	32.0	40.7	2,880	6.2
31.3.1.1	8.70	13.6	35.0	40.2	2,214	6.4
31.3.1.2	8.56	12.4	34.0	39.5	1,932	6.8
41	7.84	11.2	31.2	39.7	1,464	8.0
41.1	8.24	12.4	31.2	38.0	140	14.4
41.2	8.40	10.8	29.6	35.2	1,180	10.4
41.3	8.36	12.8	30.0	36.0	820	12.3
41.3.1	9.10	11.8	31.8	34.9	938	10.7
41.3.2	9.02	11.6	30.6	34.0	106	15.0
51	9.79	15.1	41.5	42.4	1,168	5.4
52	10.45	15.9	44.1	42.2	1,117	5.6
53	10.42	16.3	44.8	43.0	1,048	5.4
54	10.37	16.3	43.5	41.9	1,109	6.4
55	10.15	15.9	42.4	41.8	1,080	6.5
56	10.01	16.4	43.1	43.1	1,061	6.4
BALB/c	9.77 ± 0.79	15.4 ± 1.3	43.9 ± 3.6	44.9 ± 0.7	1,096 ± 217	5.8 ± 0.9

MCV, mean corpuscular volume; MPV, mean platelet volume; RBC, erythrocytes. See Table 1 footnotes for details of nomenclature.

of the kidney. Despite the presence of large numbers of circulating neutrophils, there was no histologic evidence of increased neutrophil accumulation in the skin (D. Sauder, personal communication). Compared with the massive neutrophilia seen in these tissues, focal infiltrates of plasma cells were only rarely observed. In these instances, plasma cells were found intermixed with neutrophil infiltrates in expanded lymph nodes (Fig. 3 *G*), with a few small foci of plasma cells infrequently observed in the spleen. No evidence of plasma cell infiltration of lung, liver, or thymus (Fig. 3 *H*) could be found.

In parallel with the histologic findings in spleen and bone marrow, progenitor cell numbers were elevated 30–60-fold and ~2-fold, respectively, in these tissues (Table 1). The frequencies of the various colony types were not determined, but granulocyte and/or macrophage colonies were prominent and proportionally fewer colonies contained erythroid elements than those formed in respective control cultures of spleen or bone marrow cells (data not shown). Interestingly, there was a difference in the percentage of G418-resistant progenitors found in the various mice, depending on whether or not the bone marrow had been subjected to G418 selection. Whereas 20–80% of the progenitors in the bone marrow and spleen of mice receiving preselected bone marrow were G418 resistant, at most a few percent of the progenitors in the two mice receiving nonselected bone marrow grew in G418-supplemented medium (Table 1, discussed below). When plated in the absence of added factors, ~10% of spleen and bone marrow CFC were found to be factor independent regardless of frequency of G418 resistance. 10 individual factor-independent colonies arising from spleen or bone marrow cells of two mice with G418-resistant progenitors were picked, smeared onto microscope slides, Wright-stained, and scored. In both cases, the colonies were comprised of macrophages or neutrophils and macrophages (Fig. 3 *I*), irrespective of the tissue source. These results concur with previous reports of the ability of exogenously supplied IL-6 to directly support the proliferation of some murine granulocyte/macrophage pro-

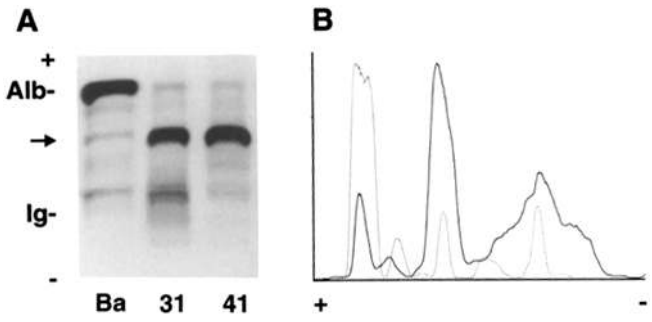

Figure 4. Changes in plasma protein levels in MSCV-IL-6 mice. (*A*) Cellulose acetate electrophoresis of plasma obtained from mice 31 (lane *31*) and 41 (lane *41*) revealing marked alterations in levels of albumin (*Alb*) and protein(s) in the α_2-macroglobulin/haptoglobin region (*arrow*) by comparison with the protein pattern of normal BALB/c plasma (lane *Ba*). (*B*) Densitometric profiles of cellulose acetate electrophoresis protein patterns of mouse 41 (*solid line*) and normal BALB/c (*dashed line*) demonstrating the diffusely increased Ig fraction in mouse 41 plasma.

genitors (14, 15, 20). Since ~30% of the factor-independent CFC were G418 resistant in these instances (data not shown), autocrine as well as paracrine mechanisms of IL-6 stimulation contribute to the myeloproliferative syndrome.

When pools of the G418-resistant factor-independent colonies were transferred to mass culture, the cells proliferated for ~4 wk, at which time they were comprised of adherent macrophages. In a separate experiment, infected spleen cells (2×10^6) were injected subcutaneously into eight sublethally irradiated (4 Gy) mice. No tumors formed during an observation period of 3 mo, whereas six lethally irradiated mice receiving bone marrow transplants from the same affected donor died from the myeloproliferative disorder during this period (data not shown).

Excessive IL-6 Production and Systemic Effects in MSCV-IL-6 Mice. Plasma obtained from MSCV-IL-6 mice before death was analyzed for IL-6 activity by testing the ability of serial dilutions to stimulate the proliferation of IL-6-dependent B9 cells (58). Circulating levels of IL-6 were significantly elevated in all mice (mean, 50 ng/ml; range, 7–130 ng/ml; $n = 15$) compared with control plasma, which had minimal B9 stimulatory activity (<0.1 ng/ml) (Table 1). Failure of the plasma samples to stimulate the proliferation of OTT1, FDC-P1, or 32D cells (60, 61) at any dilution tested demonstrated that the increased number of peripheral neutrophils seen in the mice was not due to high levels of circulating GM-CSF or IL-3 (35, 36).

Cellulose acetate electrophoresis of plasma samples revealed decreased albumin levels and elevated protein levels in the α_2-macroglobulin/haptoglobin region (Fig. 4), indicative of the well-recognized activity of IL-6 as a major regulator of the hepatic acute phase response (69). An increase in haptoglobin levels has been confirmed by rocket immunoelectrophoresis, and the results will be reported elsewhere as part of a larger study of acute phase protein synthesis in MSCV-IL-6 mice (J. Gauldie and R. G. Hawley, unpublished results). Also illustrated by comparison of plasma samples from mice 31 and 41 is a rise in Ig levels between 4 and 9 wk after transplantation (Fig. 4), although less so than the hypergam-

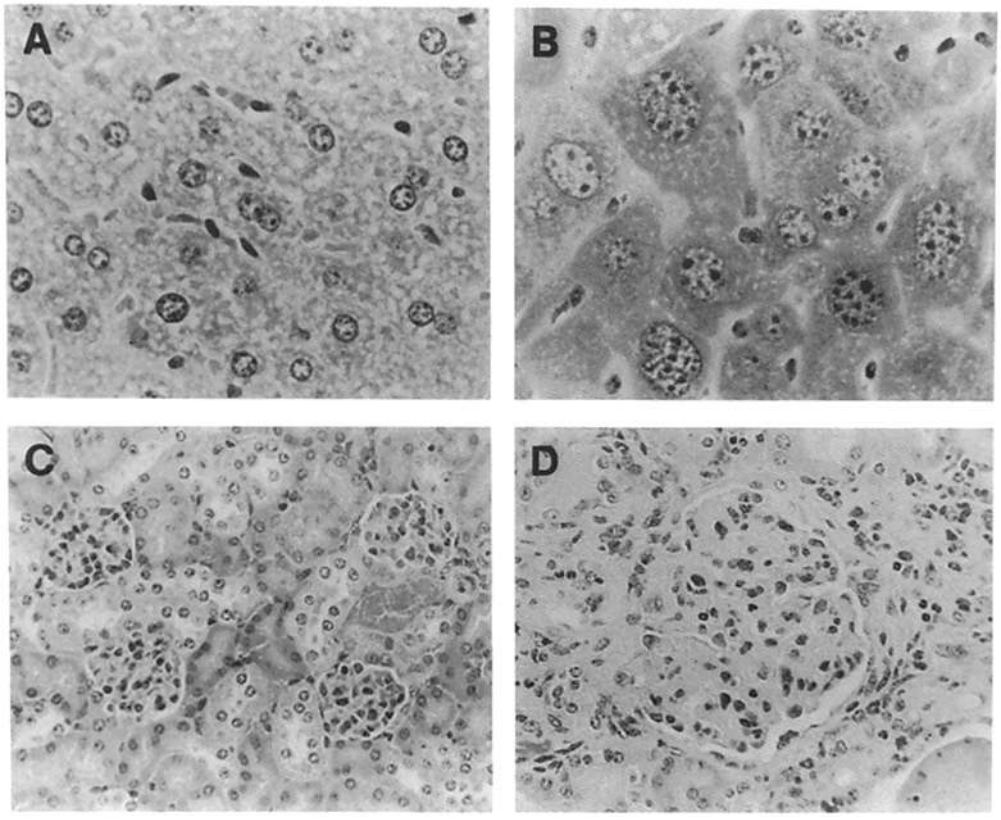

Figure 5. Pathologic changes in liver and kidney of diseased MSCV-IL-6 mice. (*A*) Section of control liver (×1,000). (*B*) Section of liver of MSCV-IL-6 mouse showing abnormal hepatocytes. Note the markedly enlarged nuclei with multiple nucleoli (×1,000). (*C*) Section of control kidney (×600). (*D*) Section of kidney of MSCV-IL-6 mouse showing atrophic tubules and significantly expanded glomerulus due to mesangial cell hyperplasia (×600).

maglobulinemia seen in the lymphoproliferative syndromes elicited by IL-6 overexpression in other studies (39, 65).

In addition to the pathologic consequences of neutrophil infiltration, sustained overproduction of IL-6 had direct effects on liver and kidney. Coincident with the marked changes in plasma levels of hepatic proteins, hepatocytes displayed substantial nuclear and cytoplasmic enlargement (Fig. 5 *B*), the cytoplasm appearing more basophilic than normal, and degenerative changes were also apparent. Mesangial cell proliferation with accompanying glomerular enlargement was the most common abnormality in the kidney (Fig. 5 *D*), as previously reported (39, 65). Other changes in the kidney included the presence of eosinophilic material apparently occluding some of the capillaries and proteinaceous material in dilated and atrophic tubules in some regions of the cortex.

Clonal Origin of IL-6-induced Myeloproliferative Disease. Retrovirus marking studies of stem cells have shown that early after transplantation multiple clones contribute to the hematopoietic system but that with time the progeny of a few clones come to predominate (30, 31, 33). In certain instances when the retrovirally marked bone marrow has been transplanted to secondary recipients, the predominant clone has been transferred (29, 32, 34). Conversely, clonal fluctuation of stem cell pools has been seen at early times during reconstitution, and new clones can become activated by the transplantation process (30, 31, 33, 34, 70). In view of the relative ease with which the myeloproliferative syndrome could be serially passaged in lethally irradiated recipients, we were interested in examining the fate maps of the clones contributing to the hematopoietic systems of the 31 and 41 series mice. DNA from bone marrow and spleen was digested with BamHI, which cleaves the MSCV-IL-6 provirus once (Fig. 1), and were subjected to Southern blot analysis with a neo probe (Fig. 6). In the case of mouse 31, killed 9 wk after transplant, three bands of 5.4, 12, and 14 kb were detected in the DNA from both tissues. Remarkably, these same three proviral junction fragments were detected in the DNA from spleen and bone marrow samples of all 31 series mice examined (Fig. 6). The progeny of a single cell carrying three MSCV-IL-6 proviruses had therefore successfully repopulated the hematopoietic systems of at least nine mice, including two secondary, four tertiary, and two quaternary recipients (tissue samples of mouse 31.2, which died 14 wk after transplantation, were unavailable for analysis). A slightly different pattern was revealed after Southern blot analysis of genomic DNAs from the 41 series recipients. BamHI-digested DNA from bone marrow and spleen from mouse 41 (killed 4 wk after transplant) displayed an extremely large number of proviral flanking sites, indicated by the presence of some predominant bands superimposed on a background smear (Fig. 6). These results indicated that 4 wk after bone marrow transfer, the hematopoietic system of this animal was derived from many MSCV-IL-6-infected cells. Upon serial bone marrow transfer, a single clone harboring one MSCV-IL-6 provirus was found to dominate. BamHI-digested DNA from spleen and bone marrow of three secondary recipients and two tertiary recipients displayed a 5.0-kb proviral junction fragment (plus some additional bands in the case of the secondaries), which was present as a minor band in mouse 41 bone marrow DNA (Fig. 6; other data not shown). Less detailed pedigree analysis carried out with 47 series mice revealed a similar phenomenon whereby multiple clones in the primary animal gave rise to a small number (one or two) of dominant clones after serial transplantation (data not shown).

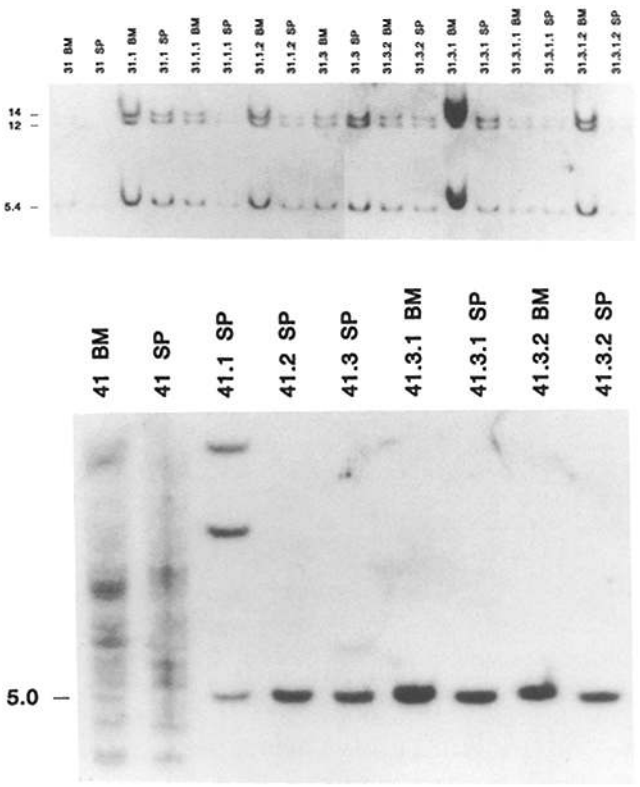

Figure 6. Clonal make-up of hematopoietic tissues of 31 and 41 series mice. Proviral integrants were enumerated in BamHI-digested DNA from bone marrow (BM) and spleen (SP) of MSCV-IL-6 mice by Southern blotting with a neo probe. (*Top*) A major clone identified in bone marrow and spleen of mouse 31 (containing three proviral integrants) persisted in two secondary, four tertiary, and two quaternary affected recipients (the variability in signal intensities is due to unequal sample loading in this experiment). (*Bottom*) A minor clone (identified by a 5.0-kb proviral junction fragment) in bone marrow of mouse 41 was found to have significant repopulating ability during serial bone marrow transplantation, transferring the myeloproliferative disease to three secondary and two tertiary recipients.

High-Level Sustained Transcription of Viral IL-6 Gene in Serially Transplanted MSCV-IL-6 Mice. Northern blot analysis of total spleen RNA demonstrated persistent expression of viral IL-6 mRNA in all affected mice whose hematopoietic systems were reconstituted after serial transplantation of MSCV-IL-6-infected bone marrow (Fig. 7). There were, however, conspicuous differences in expression patterns of MSCV-IL-6 proviruses in 31 and 41 series animals. In all 31 series animals, LTR-directed full-length IL-6 mRNA plus a smaller IL-6 RNA species (4.0 and 3.6 kb, respectively) were readily detected with the IL-6 probe (Fig. 7 *A*). The smaller 3.6-kb IL-6 RNA species (in addition to the 4.0-kb IL-6 mRNA) was detected

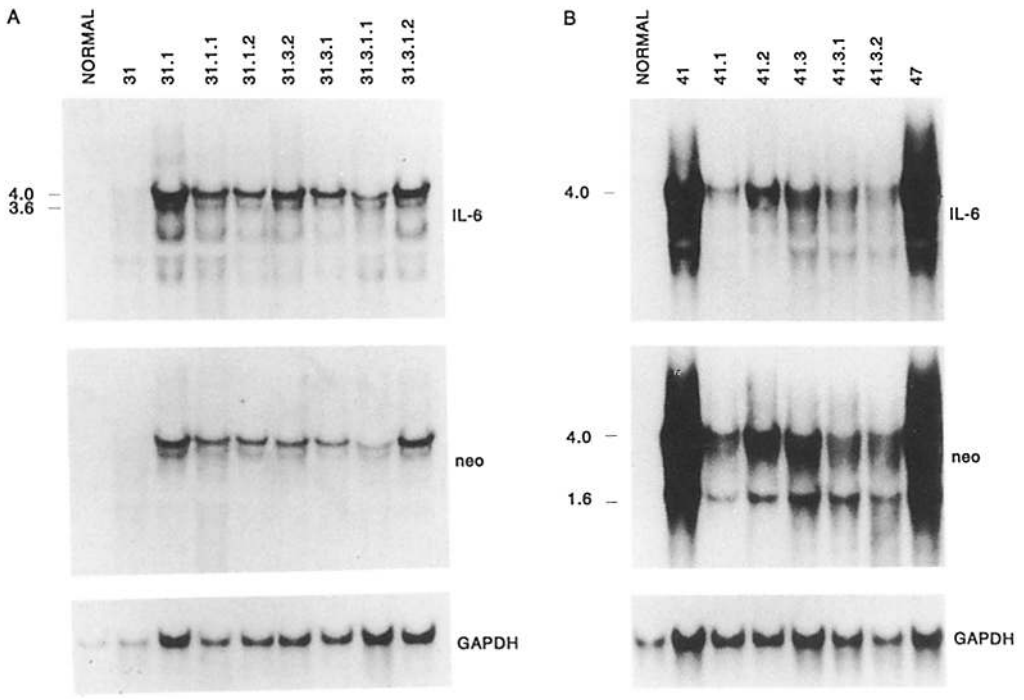

Figure 7. Expression of MSCV-IL-6 proviruses in serially transplanted mice of 31 and 41 series. Total spleen RNA was examined by successive Northern blot analysis for the presence of IL-6 (*top*) or neo (*middle*) transcripts. The blots were rehybridized with a probe specific for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA to monitor the amount and integrity of RNA loaded (*bottom*). (*A*) The major viral RNA species expressed in 31 series mice are IL-6 mRNAs (4.0 and 3.6 kb). (*B*) The 4.0-kb full-length IL-6 mRNA and the 1.6-kb pgk-1-directed neo mRNA are the most abundant viral RNA species in 41 series mice. A similar viral RNA expression pattern is observed in primary mouse 47. Additional RNAs detected with the IL-6 probe originate from the endogenous IL-6 gene.

by the neo probe upon rehybridization of the blot and corresponds to a spliced product frequently observed in retrovirus vectors which include the extended packaging region and retain a functional splice donor site (48). Normally, the full-length IL-6 mRNA is present at significantly higher levels than the 3.6-kb species in MSCV-IL-6-infected cells (Fig. 7 *B*). More curious, however, was the virtual absence of pgk-1-initiated neo transcripts (a faint band at 1.6 kb corresponding in size to neo mRNA was visible upon longer exposure of the blot). The relative paucity of pgk-1-initiated neo transcripts in hematopoietic tissues of 31 series mice contrasts sharply with our previous findings in mice transplanted with bone marrow infected with the MoMLV-based L6PNL IL-6 retrovirus, where the pgk-1-initiated neo transcripts were by far the most abundant RNA species (43). Emerman and Temin (71) have documented the phenomenon of "promoter suppression" in retrovirus vectors having an internal transcriptional unit. Their studies showed that in these "direct orientation" retrovirus vectors (72) suppression of either the LTR promoter or the downstream promoter can occur when there is selection for expression from the other promoter. In our previous studies (and in the majority of the current experiments), *neo* gene expression was selected for by culturing infected bone marrow cells in G418 before transplantation. The possibility that IL-6 gene expression might have been selected for in repopulating cells of 31 series mice is an intriguing notion warranting further study. In any event, the extremely low levels of neo transcripts originating from MSCV-IL-6 proviruses present in the spleen cells of 31 series animals explain the scarcity of G418-resistant progenitor cells in the CFC assays.

In contradistinction to L6PNL mice and 31 series mice, both full-length LTR-directed IL-6 mRNA and pgk-1-directed neo transcripts (4.0 and 1.6 kb, respectively) were present at high levels in splenic RNAs of 41 series mice serially transplanted with G418-selected bone marrow (Fig. 6 *B*). Exclusive of the higher overall levels of MSCV-IL-6 viral transcripts seen in primary recipient mouse 41 (and mouse 47), the level of MSCV-IL-6 gene expression remained stable after multiple transplants.

Discussion

The main finding of this paper is that the sustained in vivo expression of a retrovirally introduced IL-6 gene in hematopoietic precursors results in a novel serially transplantable myeloproliferative disease.

Several of the characteristic features of the IL-6-induced myeloproliferative disease (neutrophilia, anemia, and hypoalbuminemia) were also seen previously in unirradiated W/W^v mice transplanted with bone marrow cells infected with a MoMLV-based IL-6 retrovirus (39). A major difference between our results and those of Brandt et al. (39) is the severity of the disease that develops. In the current experiments, many primary recipients (11/20) died within 4 wk of transplant, whereas only two animals died during the same period in their study. Although a direct comparison of IL-6 levels is precluded by differences in the units of measurement used to report IL-6 activity, the increased morbidity seen in our experiments is probably due to higher concentrations of circulating IL-6 promoted by the MSCV retrovirus configuration (see below). Indirect support for this suggestion is provided by the more striking changes in plasma protein levels seen in the MSCV-IL-6 mice.

In the syndrome that developed in the IL-6 mice of Brandt et al. (39), the neutrophilia was transient and superseded by

excessive production of plasma cells. Plasmacytosis was also the salient feature of a syndrome observed by Suematsu et al. (65) in IL-6 transgenic mice. In the latter case, it can be estimated that the plasma levels of IL-6 are 10-fold lower on average than those found in the MSCV-IL-6 mice. However, higher IL-6 levels alone would not appear to explain the bias towards persistent myeloid expansion seen in our studies. It seems more likely that the different transcriptional regulatory elements directing IL-6 expression are to a large degree responsible for the differences in disease phenotypes. The combination of variant LTR (a derivative of the myeloproliferative sarcoma virus LTR) and the 5′ untranslated region of the MESV vector permits transcription in a range of cell types, including embryonal (44) and myeloid (73) cells. By comparison, the MoMLV LTR used in the bone marrow transplant experiments of Brandt et al. (39) and the Ig heavy chain enhancer used in the transgenic experiments of Suematsu et al. (65) are preferentially active in lymphoid cells (74–77). Earlier studies showing that substitution of the myeloproliferative sarcoma virus LTR with the MoMLV LTR greatly reduces the myeloid-transforming ability of the v-*mos* oncogene (78, 79) lend credence to this idea.

In contrast to the fatal myeloproliferative diseases that develop in mice transplanted with GM-CSF and IL-3 virus–infected bone marrow cells (35, 36), where extensive tissue damage results from the surplus of hematopoietic cells produced (mainly neutrophils and macrophages), minimal extravasation of neutrophils from the blood occurs in MSCV-IL-6 mice. This situation is similar to that observed in mice whose hematopoietic cells have been engineered to overexpress G-CSF, where little sign of tissue damage was observed despite sustained elevation of neutrophil numbers (38). Nevertheless, whereas the mice overexpressing G-CSF remain healthy for periods of up to 30 wk after transplantation, the majority of MSCV-IL-6 mice fail to survive >12 wk. Presumably, additional clinical manifestations (anemia, thrombocytopenia, and coagulation abnormalities) associated with the IL-6-induced "hematological stress syndrome" (69), plus complications due to multisystem damage (especially liver and kidney), all contribute to the early demise of the animals. Interestingly, many of the clinical features of the syndrome that develops in the MSCV-IL-6 mice are reminiscent of those observed in patients with alcoholic hepatitis, a distinct clinical entity that resembles severe sepsis and fulminant hepatic failure (80). Patients with advanced disease have neutrophilia, severe coagulopathy, activation of acute phase proteins, and acute renal failure, and frequently die of hepatic failure or gastrointestinal hemorrhage. High plasma levels of IL-6 have been found to correlate with disease severity and mortality (80). Our data support the hypothesis that chronically elevated IL-6 levels play a contributory role in this human disorder.

Considering the fact that transplantation has deleterious effects on the repopulating ability of normal stem cells (29, 81, 82) and that IL-6 exhibits differentiation-inducing activity on granulocyte/macrophage progenitors (15; this report), the proliferative capacity of MSCV-IL-6-infected cells is impressive. It seems reasonable therefore to suggest that IL-6 played a supportive role in the maintenance of clonal longevity. When the experiments were terminated, the longest observed clone of the 31 series had endured for at least 1.5 yr in nine (documented) recipients. Although uninfected hematopoietic cells (of donor or host origin) undoubtedly contributed to the myeloproliferative disease, their presence does not invalidate this result; self-renewal of the repopulating precursors occurred for the equivalent of (most of) a mouse's lifetime. The MSCV-IL-6-infected repopulating cells can thus be regarded as stem cells (83). Whether the cells in question are the most primitive stem cells (i.e., capable of long-term multilineage reconstitution [84, 85]) remains to be clarified. In limited Southern blotting experiments of other MSCV-IL-6 mice, the same unique proviral integration sites identified in DNA from bone marrow and peripheral tissues were detected in DNA from lymph nodes and thymus. However, lymphoid potential of the clones could not be concluded because infiltration by neutrophils in the lymph nodes or contamination of the thymus by adjacent infiltrated thoracic lymph nodes could not be ruled out (our unpublished results). The issue of differentiative potential of MSCV-IL-6-infected precursors can be addressed by isolating purified populations of lymphoid and myeloid cells (43). To investigate this and other biologic properties of these hematopoietic precursors, it would be advantageous to be able to prolong the lifetimes of MSCV-IL-6 mice by reducing the toxic effects associated with high circulating levels of IL-6. Toward this goal, a modified IL-6 retrovirus vector has been constructed carrying an IL-6 gene with a synthetic sequence added to its COOH terminus (KDEL sequence) that is present on proteins retained within the endoplasmic reticulum (86).

In the avian system, autocrine production of chicken myelomonocytic growth factor, a cytokine distantly related to mammalian IL-6, is a key step in myeloid leukemogenesis (87). As the role of IL-6 in human myeloid leukemia remains unclear (88–90), the IL-6-induced myeloproliferative model developed here may prove a useful mammalian system for investigations along these lines. Indeed, IL-6 transgenic mice have already provided evidence that dysregulated IL-6 expression can contribute to the development of lymphoid neoplasms (91).

We thank Manuel Grez for providing the P5Gneo plasmid of MESV, Fritz Melchers for the gift of X630-rIL3 cells, and Norman Iscove for critically reviewing the manuscript. We are also grateful to Jim Peeke for assistance with the hematologic analyses.

This work was supported by grants from the National Cancer Institute of Canada, the Arthritis Society (Canada), and the Leukemia Research Fund (Canada). R. G. Hawley is a Career Scientist of the Ontario Cancer Treatment and Research Foundation.

Address correspondence to 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 2 April 1992 and in revised form 7 July 1992.

References

1. Till, J.E., and E.A. McCulloch. 1980. Hemopoietic stem cell differentiation. *Biochem. Biophys. Acta.* 605:431.
2. Metcalf, D. 1991. Control of granulocytes and macrophages: molecular, cellular, and clinical aspects. *Science (Wash. DC).* 254:529.
3. Gordon, M.Y. 1991. Hemopoietic growth factors and receptors: bound and free. *Cancer Cells (Cold Spring Harbor).* 3:127.
4. Witte, O.N. 1990. Steel locus defines new multipotent growth factor. *Cell.* 63:5.
5. Chiu, C.-P., C. Moulds, R.L. Coffman, D. Rennick, and F. Lee. 1988. Multiple biological activities are expressed by a mouse interleukin 6 cDNA clone isolated from bone marrow stromal cells. *Proc. Natl. Acad. Sci. USA.* 85:7099.
6. Murray, R., F. Lee, and C.-P. Chiu. 1990. The genes for leukemia inhibitory factor and interluekin-6 are expressed in mouse blastocysts prior to the onset of hemopoiesis. *Mol. Cell. Biol.* 10:4953.
7. Schmitt, R.M., E. Bruyns, and H.R. Snodgrass. 1991. Hematopoietic development of embryonic stem cells in vitro: cytokine and receptor gene expression. *Genes & Dev.* 5:728.
8. Van Snick, J. 1990. Interleukin-6: an overview. *Annu. Rev. Immunol.* 8:253.
9. Bodine, D.M., S. Karlsson, and A.W. Nienhuis. 1989. Combination of interleukin 3 and 6 preserves stem cell function in culture and enhances retrovirus-mediated gene transfer into hematopoietic stem cells. *Proc. Natl. Acad. Sci. USA.* 86:8897.
10. Bodine, D.M., P.S. Crosier, and S.C. Clark. 1991. Effects of hematopoietic growth factors on the survival of primitive stem cells in liquid suspension culture. *Blood.* 78:914.
11. Okano, A., C. Suzuki, F. Takatsuki, Y. Akiyama, K. Koike, K. Ozawa, T. Hirano, T. Kishimoto, T. Nakahata, and S. Asano. 1989. In vitro expansion of the murine pluripotent hemopoietic stem cell population in response to interleukin 3 and interleukin 6. *Transplantation (Baltimore).* 48:495.
12. Bodine, D.M., D. Orlic, N.C. Birkett, N.E. Seidel, and K.M. Zsebo. 1992. Stem cell factor increases colony-forming unit-spleen number in vitro in synergy with interleukin-6, and in vivo in S1/S1^d mice as a single factor. *Blood.* 79:913.
13. Ikebuchi, K., G.G. Wong, S.C. Clark, J.N. Ihle, Y. Hirai, and M. Ogawa. 1987. Interleukin 6 enhancement of interleukin 3-dependent proliferation of multipotential hemopoietic progenitors. *Proc. Natl. Acad. Sci. USA.* 84:9035.
14. Wong, G.G., J.S. Witek-Giannotti, P.A. Temple, R. Kriz, C. Ferenz, R.M. Hewick, S.C. Clark, K. Ikebuchi, and M. Ogawa. 1988. Stimulation of murine hemopoietic colony formation by human IL-6. *J. Immunol.* 140:3040.
15. Shabo, Y., J. Lotem, M. Rubinstein, M. Revel, S.C. Clark, S.F. Wolf, R. Kamen, and L. Sachs. 1988. The myeloid blood cell differentiation-inducing protein MGI-2A is interleukin-6. *Blood.* 72:2070.
16. Ishibashi, T., H. Kimura, T. Uchida, S. Kariyone, P. Friese, and S.A. Burstein. 1989. Human interleukin 6 is a direct promoter of maturation of megakaryocytes in vitro. *Proc. Natl. Acad. Sci. USA.* 86:5953.
17. Rennick, D., J. Jackson, G. Yang, J. Wideman, F. Lee, and S. Hudak. 1989. Interleukin-6 interacts with interleukin-4 and other hematopoietic growth factors to selectively enhance the growth of megakaryocytic, erythroid, myeloid, and multipotential progenitor cells. *Blood.* 73:1828.
18. Williams, N., T. De Giorgio, N. Banu, R. Withy, T. Hirano, and T. Kishimoto. 1990. Recombinant interleukin 6 stimulates immature murine megakaryocytes. *Exp. Hematol. (NY).* 18:69.
19. Tsuji, K., K.M. Zsebo, and M. Ogawa. 1991. Enhancement of murine blast cell colony formation in culture by recombinant rat stem cell factor, ligand for c-kit. *Blood.* 78:1223.
20. Suda, T., Y. Yamaguchi, J. Suda, Y. Miura, A. Okano, and Y. Akiyama. 1988. Effect of interleukin 6 (IL-6) on the differentiation and proliferation of murine and human hemopoietic progenitors. *Exp. Hematol. (NY).* 16:891.
21. Takatsuki, F., A. Okano, C. Suzuki, Y. Miyasaka, T. Hirano, T. Kishimoto, D. Ejima, and Y. Akiyama. 1990. Interleukin 6 perfusion stimulates reconstitution of the immune and hematopoietic systems after 5-fluorouracil treatment. *Cancer Res.* 50:2885.
22. 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.
23. Suzuki, C., A. Okano, F. Takatsuki, Y. Miyasaka, T. Hirano, T. Kishimoto, D. Ejima, and Y. Akiyama. 1989. Continuous perfusion with interleukin 6 (IL-6) enhances production of hematopoietic stem cells (CFU-S). *Biochem. Biophys. Res. Commun.* 159:933.
24. 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.
25. Pojda, Z., and A. Tsuboi. 1990. In vivo effects of human recombinant interleukin 6 on hemopoietic stem and progenitor cells and circulating blood cells in normal mice. *Exp. Hematol. (NY).* 18:1034.
26. 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.
27. Chang, J.M., and G.R. Johnson. 1989. Gene transfer into hemopoietic stem cells using retroviral vectors. *Int. J. Cell Cloning.* 7:264.
28. Dick, J.E., M.C. Magli, D. Huszar, R.A. Phillips, and A. Bernstein. 1985. Introduction of a selectable gene into primitive stem cells capable of long-term reconstitution of the hemopoietic system of W/W^v mice. *Cell.* 42:71.
29. Keller, G., C. Paige, E. Gilboa, and E.F. Wagner. 1985. Expression of a foreign gene in myeloid and lymphoid cells derived from multipotent haematopoietic precursors. *Nature (Lond.).* 318:149.
30. Lemischka, I.R., D.H. Raulet, and R.C. Mulligan. 1986. Developmental potential and dynamic behavior of hematopoietic stem cells. *Cell.* 45:917.
31. Capel, B., R. Hawley, L. Covarrubias, T. Hawley, and B. Mintz.

1989. Clonal contributions of small numbers of retrovirally marked hematopoietic stem cells engrafted in unirradiated neonatal W/W^v mice. *Proc. Natl. Acad. Sci. USA.* 86:4564.
32. Capel, B., R.G. Hawley, and B. Mintz. 1990. Long- and short-lived murine hematopoietic stem cell clones individually identified with retroviral integration markers. *Blood.* 75:2267.
33. Jordan, C.T., and I.R. Lemischka. 1990. Clonal and systemic analysis of long-term hematopoiesis in the mouse. *Genes & Dev.* 4:220.
34. Keller, G., and R. Snodgrass. 1990. Life span of multipotential hematopoietic stem cells in vivo. *J. Exp. Med.* 171:1407.
35. Johnson, G.R., T.J. Gonda, D. Metcalf, I.K. Hariharan, and S. Cory. 1989. A lethal myeloproliferative syndrome in mice transplanted with bone marrow cells infected with a retrovirus expressing granulocyte-macrophage colony stimulating factor. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:441.
36. Chang, J.M., D. Metcalf, R.A. Lang, T.J. Gonda, and G.R. Johnson. 1989. Nonneoplastic hematopoietic myeloproliferative syndrome induced by dysregulated multi-CSF (IL-3) expression. *Blood.* 73:1487.
37. Wong, P.M.C., S. Chung, C.E. Dunbar, D.M. Bodine, S. Ruscetti, and A.W. Nienhuis. 1989. Retrovirus-mediated transfer and expression of the interleukin-3 gene in mouse hematopoietic cells result in a myeloproliferative disorder. *Mol. Cell. Biol.* 9:798.
38. Chang, J.M., D. Metcalf, T.J. Gonda, and G.R. Johnson. 1989. Long-term exposure to retrovirally expressed granulocyte-colony-stimulating factor induces a nonneoplastic granulocytic and progenitor cell hyperplasia without tissue damage in mice. *J. Clin. Invest.* 84:1488.
39. 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.
40. Bender, M.A., R.E. Gelinas, and A.D. Miller. 1989. A majority of mice show long-term expression of human β-globin gene after retrovirus transfer into hematopoietic stem cells. *Mol. Cell. Biol.* 9:1426.
41. Lim, B., J.F. Apperley, S.H. Orkin, and D.A. Williams. 1989. Long-term expression of human adenosine deaminase in mice transplanted with retrovirus-infected hematopoietic stem cells. *Proc. Natl. Acad. Sci. USA.* 86:8892.
42. Wilson, J.M., O. Danos, M. Grossman, D.H. Raulet, and R.C. Mulligan. 1990. Expression of human adenosine deaminase in mice reconstituted with retrovirus-transduced hematopoietic stem cells. *Proc. Natl. Acad. Sci. USA.* 87:439.
43. 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. *Leukemia Res.* 15:659.
44. Grez, M., E. Akgün, F. Hilberg, and W. Ostertag. 1990. Embryonic stem cell virus, a recombinant murine retrovirus with expression in embryonic stem cells. *Proc. Natl. Acad. Sci. USA.* 87:9202.
45. Vieira, J., and J. Messing. 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. *Gene (Amst.).* 19:259.
46. Hilberg, F., C. Stocking, W. Ostertag, and M. Grez. 1987. Functional analysis of a retroviral host-range mutant: altered long terminal repeat sequences allow expression in embryonal carcinoma cells. *Proc. Natl. Acad. Sci. USA.* 84:5232.
47. Colicelli, J., and S.P. Goff. 1987. Isolation of a recombinant murine leukemia virus utilizing a new primer tRNA. *J. Virol.* 57:37.
48. Miller, A.D., and G.J. Rosman. 1989. Improved retroviral vectors for gene transfer and expression. *BioTechniques.* 7:980.
49. 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.
50. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
51. 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.
52. 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.
53. 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.
54. 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.
55. Housey, G.M., M.D. Johnson, W.L.W. Hsiao, C.A. O'Brian, J.P. Murphy, P. Kirschmeier, and I.B. Weinstein. 1988. Overproduction of protein kinase C causes disordered growth control in rat fibroblasts. *Cell.* 52:343.
56. 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.
57. 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.
58. 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.
59. 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.
60. 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.
61. 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.
62. 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 (Amst.).* 19:327.
63. 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.
64. Piechaczyk, M., J.M. Blanchard, L. Marty, Ch. Dani, F. Panabieres, S. El Sabouty, P. Fort, and Ph. Jeanteur. 1984. Post-transcriptional regulation of glyceraldehyde-3-phosphate-dehydrogenase gene expression in rat tissues. *Nucleic Acids Res.* 12:6951.
65. Suematsu, S., T. Matsuda, K. Aozasa, S. Akira, N. Nakano,

S. Ohno, J. Miyazaki, K. Yamamura, T. Hirano, and T. Kishimoto. 1989. IgG1 plasmacytosis in interleukin 6 transgenic mice. *Proc. Natl. Acad. Sci. USA.* 86:7547.
66. Wagner, E.F., M. Vanek, and B. Vennstrom. 1985. Transfer of genes into embryonal carcinoma cells by retrovirus infection: efficient expression from an internal promoter. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:663.
67. Williams, W.J., E. Beutler, A. Erslev, and M.A. Lichtman. 1990. Hematology. McGraw-Hill, Inc., New York. 1882 pp.
68. Quesenberry, P.J., H.E. McGrath, M.E. Williams, B.E. Robinson, D.H. Deacon, S. Clark, D. Urdal, and I.K. McNiece. 1991. Multifactor stimulation of megakaryocytopoiesis: Effects of interleukin 6. *Exp. Hematol. (NY).* 19:35.
69. Gauldie, J., C. Richards, D. Harnish, P. Lansdorp, and H. Baumann. 1987. Interferon $\beta 2$/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.
70. Snodgrass, R., and G. Keller. 19887. Clonal fluctuation within the haematopoietic system of mice reconstituted with retrovirus-infected stem cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3955.
71. Emerman, M., and H.M. Temin. 1986. Comparison of promoter suppression in avian and murine retrovirus vectors. *Nucleic Acids Res.* 14:9381.
72. Korman, A.J., J.D. Frantz, J.L. Strominger, and R.C. Mulligan. 1987. Expression of human class II major histocompatibility complex antigens using retrovirus vectors. *Proc. Natl. Acad. Sci. USA.* 84:2150.
73. Franz, T., F. Hilberg, B. Seliger, C. Stocking, and W. Ostertag. 1986. Retroviral mutants efficiently expressed in embryonal carcinoma cells. *Proc. Natl. Acad. Sci. USA.* 83:3292.
74. DesGroseillers, L., E. Rassart, and P. Jolicoeur. 1983. Thymotropism of murine leukemia virus is conferred by its long terminal repeats. *Proc. Natl. Acad. Sci. USA.* 80:4203.
75. Dunbar, C.E., P.S. Crosier, and A.W. Nienhuis. 1991. Introduction of an activated ras oncogene into murine bone marrow progenitors via retroviral gene transfer results in thymic lymphomas. *Oncogene Res.* 6:39.
76. Storb, U. 1987. Transgenic mice with immunoglobulin genes. *Annu. Rev. Immunol.* 5:151.
77. Adams, J.M., A.W. Harris, C.A. Pinkert, L.M. Corcoran, W.S. Alexander, S. Cory, R.D. Palmiter, and R.L. Brinster. 1985. The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature (Lond.).* 318:533.
78. Stocking, C., R. Kollek, U. Bergholz, and W. Ostertag. 1985. Long terminal repeat sequences impart hematopoietic transformation properties to the myeloproliferative sarcoma virus. *Proc. Natl. Acad. Sci. USA.* 82:5746.
79. Stocking, C., R. Kollek, U. Bergholz, and W. Ostertag. 1986. Point mutations in the U3 region of the long terminal repeat of Moloney murine leukemia virus determine disease specificity of the myeloproliferative sarcoma virus. *Virology.* 153:145.
80. Sheron, N., G. Bird, J. Goka, G. Alexander, and R. Williams. 1991. Elevated plasma interleukin-6 and increased severity and mortality in alcoholic hepatitis. *Clin. Exp. Immunol.* 84:449.
81. Harrison, D.E., and C.M. Astle. 1982. Loss of stem cell repopulating ability upon transplantation. Effects of donor age, cell number, and transplantation procedure. *J. Exp. Med.* 156:1767.
82. Mauch, P., and S. Hellman. 1989. Loss of hematopoietic stem cell self-renewal after bone marrow transplantation. *Blood.* 74:872.
83. Lajtha, L.G. 1979. Haemopoietic stem cells: concepts and definitions. *Blood Cells (NY).* 5:447.
84. Iscove, N. 1990. Searching for stem cells. *Nature (Lond.).* 347:126.
85. Uchida, N., and I.L. Weissman. 1992. Searching for hematopoietic stem cells: evidence that Thy-1.1^{lo}Lin$^-$Sca-1$^+$ cells are the only stem cells in C57BL/Ka-Thy-1.1 bone marrow. *J. Exp. Med.* 175:175.
86. Pelham, H.R.B. 1990. The retention signal for luminal ER proteins. *Trends Biochem. Sci.* 15:483.
87. Metz, T., T. Graf, and A. Leutz. 1991. Activation of cMGF expression is a critical step in avian myeloid leukemogenesis. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:837.
88. Hoang, T., A. Haman, O. Goncalves, G.G. Wong, and S.C. Clark. 1988. Interleukin-6 enhances growth factor-dependent proliferation of the blast cells of acute myeloblastic leukemia. *Blood.* 72:823.
89. Akashi, K., M. Harada, T. Shibuya, T. Eto, Y. Takamatsu, T. Teshima, and Y. Niho. 1991. Effects of interleukin-4 and interleukin-6 on the proliferation of CD34+ and CD34− blasts from acute myelogenous leukemia. *Blood.* 78:197.
90. Givon, T., S. Slavin, N. Haran-Ghera, R. Michalevicz, and M. Revel. 1992. Antitumor effects of human recombinant interleukin-6 on acute myeloid leukemia in mice and in cell cultures. *Blood.* 79:2392.
91. 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.