Interleukin-6 mRNA and Protein Increase In Vivo Following Induction of Acute Thrombocytopenia in Mice

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Induction of experimental thrombocytopenia in rodents results in the enhancement of megakaryocytic growth and differentiation. Interleukin-3 (IL-3) and IL-6, cytokines with a broad spectrum of biologic activities, stimulate megakaryocytogenesis in vitro. To determine if expression of these factors might increase in response to experimental thrombocytopenia, we measured steady-state levels of IL-3 and IL-6 mRNA following rabbit antiplatelet serum (APS) injection. Groups of mice were injected intravenously with 0.2 mL APS while control animals received rabbit antilymphocyte serum (ALS), normal rabbit serum (NRS), or phosphate-buffered saline (PBS). At various times up to 72 hours after injection mice were exsanguinated and splenectomized. Platelet counts in the experimental animals were less than 12% of controls. Splenic RNA was hybridized in solution to 32P-UTP-labeled cRNA probes for IL-3 and IL-6. RNase-resistant hybrids were resolved on denaturing gels and visualized autoradiographically. IL-3 hybrids were undetectable at all time points tested, irrespective of the film exposure time or specific activity of the probe. Conversely, IL-6 hybrids were easily visualized and showed peak expression at 1.5 to 2.0 hours. By 3 hours, IL-6 mRNA had returned almost to the level of the controls. Similar results were observed in the bone marrow, although maximal IL-6 mRNA in that tissue was observed 4 hours following APS administration. To determine if this mRNA increment was associated with a concomitant increase in bioactive protein, serum was tested for its ability to stimulate IL-6-dependent B9 cells. At 1.75 hours following injection, experimental animals showed a small but significant increment in IL-6 activity compared with controls (200 ± 30 U/mL IL-6 compared with 129 ± 17 U/mL in ALS-injected controls, 106 ± 17 U/mL in NRS-injected controls and 84 ± 17 U/mL in PBS-injected controls). The data show that IL-6 mRNA and bioactive protein increase in response to acute immunothrombocytopenia, while no increment in IL-3 is detectable. These results suggest that IL-6 may play a role in the physiologic response to acute immunothrombocytopenia.

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In later experiments, IgG was purified from APS and NRS by ammonium sulfate precipitation followed by affinity chromatography on an immobilized protein G high-performance column (Genex, Gaithersburg, MD) and adjusted to a final concentration of approximately 2 mg/mL protein. Rabbit antimonocytic lymphocyte serum IgG (Accurate Chemical and Scientific Corporation, Westbury, NY) was also subjected to affinity chromatography on protein G. This preparation was found to mark platelets by indirect immunofluorescence and flow cytometry and to reduce the platelet count in mice. Therefore, it was diluted with NRS to the extent that binding to lymphocytes was maintained (as determined by flow cytometry) but binding to platelets was minimized. Because injection of lipopolysaccharide has been shown to increase IL-6 levels in mice, all sera and purified IgG were either passed over a DetoxiGel column (Pierce Biochemical Company, Rockford, IL) or tested for endotoxin by Marine Biologicals, Inc (Marmora, NJ) before use. Endotoxin levels of all measured samples were less than 0.125 EU/mL.

Experimental protocol. Experimental mice in groups of three to five were given 0.2 mL APS or the purified APS IgG, while control animals were given the same volume of either antilymphocyte serum (ALS) IgG, NRS, NRS IgG, or phosphate-buffered saline (PBS) via the tail vein. At 1 to 72 hours following injection, animals were killed by cervical dislocation, followed by exsanguination by cardiac puncture and spleenectomy. Immediately before killing, blood was withdrawn from the tail vein for counts. The spleens were flash frozen in liquid nitrogen and stored at −80°C until RNA was isolated. Serum derived from the cardiac blood was stored at −20°C. The spleen was chosen for RNA analysis because (1) it is easily accessible and can be removed and frozen within seconds; (2) megakaryocytopenia increases in the spleen following APS injection; and (3) cells capable of producing IL-3 and IL-6 are present in this organ. In some experiments, femoral bone marrow was used as a source of RNA. Marrow was flushed from the eight femurs of four mice treated as described above using 4 mL of ice-cold Iscove’s medium ( Irvine Scientific, Santa Clara, CA) supplemented with 1% Nutridoma-SP (Boehringer Mannheim, Indianapolis, IN). The cells were centrifuged at 250g for 10 minutes at 4°C, resuspended in guanidine thiocyanate (GT) solution, and processed immediately as described (vida infra).

Isolation of RNA. RNA was purified from mouse spleen and marrow as described. Briefly, mouse spleens from each group ( 160 mg/spleen) were pooled and homogenized with a Polytron (Brinkman Instruments, Westbury, NY) in a solution (20 mg/mL of tissue) of 5 mol/L GT, 50 mmol/L Tris-HCl (pH 7.5), 25 mmol/L EDTA, 8% β-mercaptoethanol (GT solution). Ethanol, 0.3 vol, were mixed thoroughly into the solution followed immediately by centrifugation at 13,800g for 5 minutes at 0°C. The supernatant, which contained the bulk of the protein and DNA, was discarded and the pellet homogenized in the GT solution (10 mg/L of tissue). Cellular debris was removed by centrifugation at 13,800g for 3 minutes. The RNA was precipitated with 0.5 vol of ethanol and 0.1 mol/L acetic acid (final concentration) at −20°C for at least 3 hours and pelleted at 5,000g for 10 minutes. It was redissolved in a solution of 6 mol/L guanidine hydrochloride, 25 mmol/L EDTA, 0.075% β-mercaptoethanol (Gn-HCl solution) and reprecipitated with ethanol-acetic acid as described above. This step was repeated twice, each time progressively decreasing the volume of the Gn-HCl solution to a final volume of 5 mL/L of starting material. The purified RNA was dissolved in diethylpyrocarbonate-treated H2O and the ODNp measured. RNA was shown to be intact and RNase-free by incubation in RNase buffer (vida infra) at 37°C for 2 hours to permit maximal RNase activity followed by agarose gel electrophoresis. Visualization of the 28S and 18S ribosomal bands confirmed that the RNA was intact and suitable for solution hybridization studies.

Probe transcription. Reagents for probe transcription were purchased from Promega Biotech (Madison, WI), restriction endonucleases and DNA ligases from New England Biolabs (Beverly, MA), and [32P]-UTP (800 Ci/mmol) from New England Nuclear (Wilmington, DE). Because expression of L32, a ribosomal protein comprising part of the large subunit of the ribosome, has been shown not to be autogenously regulated,23 an L32-4A probe, obtained from Drs Antonio Celada and Richard A. Maki of the La Jolla Cancer Research Foundation (La Jolla, CA), was used as a control for the amount of RNA loaded on each lane. A 1.6-KbSac I fragment containing the murine L32 cDNA was inserted into a PGEM-1 vector (Promega) anti-sense to the SP6 promoter. This construct was linearized with Ava II to yield a 700-bp probe that protects a 400-bp region of L32 mRNA. Murine IL-3 cDNA was provided by Dr Frank Jirik, Scripps Clinic and Research Foundation (La Jolla, CA). A 362-bp fragment of the IL-3 cDNA bounded by HindIII and Xba I restriction sites was inserted into a pBS-vector (Stratagene Cloning Systems, La Jolla, CA) anti-sense to the T7 promoter. This construct was linearized with HindIII to yield a 394-bp probe that protects a 362-bp region of the IL-3 mRNA. Murine IL-6 cDNA was provided by Dr Jacques Van Snick, Ludwig Institute, Brussels, Belgium. A 650-bp fragment of the IL-6 cDNA bounded by EcoRI and Bgl II restriction sites was inserted into a pgEM 3Z-vector (Promega). This construct was linearized with Ssa I to yield a 284-bp probe that protects a 241-bp region of the IL-6 mRNA.

Probe transcription was performed by a modification of transcription protocol no. 1 (Promega) to adjust for desired specific activities. Because potentially rare transcripts were being investigated, the specific activities of the IL-3 and IL-6 probes were increased by using [32P]-UTP as the only source of UTP (5.2 μmol/L final concentration). Specific activities ranging from 1 to 2.5 × 106 cpm/μg were obtained. However, the L32 mRNA was so abundant that to permit the long exposure times predicted to visualize IL-6 and IL-3 mRNA, the [32P]-UTP used for production of the L32 probe was limited (0.86 μmol/L [32P]-UTP + 11.76 μmol/L UTP final concentration) to obtain specific activities of 5 × 105 to 1 × 106 cpm/μg.

The probe reaction solutions were incubated at 37°C for 60 minutes. Samples of 1 μL were then removed for measurement of specific activity by trichloroacetic acid precipitation. Full-length probe was purified from DNA and shorter transcripts by electrophoresis on a 4% polyacrylamide, 8 mol/L urea gel and elution into 600 μL of 0.6 mol/L ammonium acetate, pH 5.8, 1% sodium dodecyl sulfate (SDS) by incubation at 37°C for 45 minutes followed by ethanol precipitation.24 The pellet was dissolved in 200 μL 4 mol/L GT hybridization buffer (vida infra) for later use.

Hybridization. All reactions were performed in hybridization buffer consisting of 4 mol/L GT, 25 mmol/L Na2 EDTA, pH 6.0. IL-6 or IL-3 probe, 5 × 105 to 1 × 106 cpm, (200 to 1,000 pg) and 1 × 105 cpm of L32 (700 to 1,500 pg) probe was added to 50 μg lyophilized splenic RNA or the total marrow RNA isolated from eight femurs. Hybridization buffer was added to bring the total volume to 30 μL. Following hybridization at 37°C for 15 hours, the hybridization solution was brought up to 300 μL with 10 mmol/L Tris-HCl pH 7.5, 5 mmol/L Na2 EDTA, 300 mmol/L NaCl (RNase buffer), containing 30 μg/mL RNase A and 1 μg/mL RNase T1 (Worthington Biochemicals, Freehold, NJ), and incubated at 37°C for 30 minutes. Proteinase K (Boehringer) was added to a final concentration of 0.3 mg/mL and the solution incubated at 37°C for 15 minutes followed by phenol/chloroform extraction and ethanol precipitation. The hybrids were subsequently denatured at 95°C for 5 minutes and separated on a 4% polyacrylamide, 8 mol/L urea gel.
The blood counts were measured 1.5 to 2 hours following intravenous administration of APS, ALS, NRS, or PBS. The WBC and platelet (PLT) counts are shown as the means ± SD. No difference in counts was observed when either whole antiserum or purified IgG was used. The WBC counts of the APS-treated mice were higher than those of the ALS and PBS groups (P < .05).

The undried gel was covered in cellophane wrap and exposed to preflashed Kodak X-OMAT AR film (Eastman Kodak, Rochester, NY) with an intensifying screen at -80°C for up to 14 days for visualization of hybrids.

**Bioassay of IL-6.** IL-6 activity in mouse serum was assayed using the IL-6-dependent B9 cell line (provided by Dr Lucien Aarden, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam). These cells are sensitive to subpicogram concentrations of IL-6. B9 cells were cultured in Iscove's medium at a concentration of 5,000 cells/well in 96-well microtiter plates (Corning Glass Works, Corning, NY) in a final volume of 200 μL. The cultures were supplemented with 12.5% fetal calf serum (FCS) and 2.5% mouse serum from study animals. Concurrent standards using recombinant human IL-6 (rhIL-6) (R&D Systems, Inc, Minneapolis, MN) were assayed with each experiment. After incubation for 64 hours at 37°C in a 95% air, 5% CO₂, tissue culture incubator, 0.5 μCi tritiated thymidine (³HtdR; 38 Ci/mmol; ICN Radiochemicals, Irvine, CA) was added. Eight hours later cells were harvested with an MB12R multiwell cell harvester (Brandel Biomedical Research and Development, Gaithersburg, MD) and washed with 5% trichloroacetic acid. ³HtdR incorporation into DNA was determined with an LS 1701 liquid scintillation counter (Beckman Instruments, Palo Alto, CA). One unit (U) of IL-6 was defined as that activity which leads to half-maximal ³HtdR incorporation (3 to 5 pg rhIL-6/mL). The reported specificity of B9 cells for IL-6 was confirmed by preincubation of mouse serum positive for IL-6 on the bioassay with a 1:500 dilution of rabbit antimusite IL-6, provided by Dr David Hilbert (National Institutes of Health, Bethesda, MD), and added to B9 cells. Addition of the antibody abrogated the B9 cell response. Attempts to quantitate serum IL-6 immunologically were unsuccessful using amplified (biotin-avidin-alkaline phosphatase) Western blotting techniques, because the amounts of IL-6 in the serum were below the limits of detection using this method.

**Statistics.** Statistical differences between groups were calculated using the Student's t-test.

### RESULTS

**Blood counts following APS injection.** The mean platelet count in the APS-injected mice at the time of killing was $108 \times 10^9/\mu L \pm 17$ (mean ± 1 SD), about 12% of the ALS-, NRS-, and PBS-injected controls ($924 \pm 123, 965 \pm 97, \text{ and } 944 \pm 123$, respectively). The hematocrits of the APS-injected groups were similar to the controls, while the white blood cell (WBC) counts of the APS-treated mice were significantly higher than those of the ALS and PBS groups ($P < .05$; Table 1).

**Measurement of splenic IL-3 mRNA.** IL-3 mRNA was undetectable in all animals tested even after overexposure of the autoradiogram (Fig 1). Lane 2 shows hybridization of the IL-3 cRNA probe to total RNA extracted from the murine cell line WEHI-3, a constitutive producer of IL-3. Lanes 3 through 8 show that no hybrids were detected using 50 μg of total splenic RNA at 1 to 6 hours following injection of mice with APS. Other studies showed no increase in IL-3 mRNA at 12 to 72 hours following injection, using both total and up to 30 μg poly A+ RNA (data not shown). The L32 hybrids in each lane (at 400 bp) show the presence of RNA.

![Autoradiogram of splenic RNA probed in solution with an IL-3 cRNA probe (1-week exposure). Arrows mark L32 hybrid (400 bp), IL-3 probe (392 bp), and IL-3 hybrid (362 bp). Lane 1, IL-3 probe. Lane 2, 1 μg WEHI-3 RNA with IL-3 hybrid. Lanes 3 through 8, 50 μg splenic RNA from mice injected with APS and killed 1 to 6 hours later. No specific 362-bp band could be identified irrespective of exposure time. IL-3 mRNA was also undetectable at 12, 24, 48, and 72 hours following antiserum administration (data not shown). The small amount of WEHI-3 RNA loaded in lane 2 is reflected by the relatively weaker L32 hybrid.](image-url)
Measurement of splenic and marrow IL-6 mRNA. In contrast to the lack of detection of IL-3 mRNA, IL-6 mRNA was detectable in the spleens of all groups tested, including normal untreated mice (data not shown). The time course of IL-6 mRNA expression in response to APS is shown in Fig 2. The proper location of the IL-6 hybrid was confirmed by hybridization to total RNA extracted from the murine cell line J774 after stimulation with lipopolysaccharide and phorbol 12-myristate 13-acetate (PMA),26 lane 3. Peak expression occurred between 1.5 and 2 hours after injection (lanes 5 and 6) and rapidly decreased thereafter. By 3 hours (lane 8), the intensity of the hybrid was almost at the level of the controls, and did not increase beyond control levels when analyzed up to 72 hours following injection (data not shown). Figure 3 shows the levels of IL-6 mRNA in stimulated J774 cells (lane 1), in the APS-injected group (lane 2), and in the control groups (lanes 3, 4, and 5) during the time of peak expression (1.75 hours). The intensity of the IL-6 mRNA band is markedly increased over the controls. Figure 4 shows that IL-6 mRNA was also detected in the bone marrow following APS injection (lanes 5 and 6). The ALS control (lane 4) shows more IL-6 mRNA than either the PBS- or NRS-injected group (lanes 2 and 3, respectively). In contrast to splenic RNA, the maximum levels of IL-6 mRNA in marrow were observed 4 hours following APS injection (lane 6), with an abrupt decrease seen at 6 hours (lane 7). The IL-6 mRNA level at 24 and 48 hours after APS was similar to that observed at 6 hours after APS (data not shown).

Measurement of bioactive IL-6 in serum. Because it is possible that IL-6 mRNA could increase following APS injection without the production of the bioactive molecule, we measured bioactive IL-6 in the serum using the IL-6-responsive B9 cell line (Fig 5). At 1.75 hours following injection, the serum IL-6 level of the APS-injected group was 200 U/mL ± 30 (mean ± SEM), compared with 129 ± 17 U/mL in the ALS-injected group (P = .08), 106 ± 17 in the NRS-injected group (P < .03), and 84 ± 17 U/mL in the PBS-injected group (P < .01). No significant differences were noted among the control groups. No additional increments in serum IL-6 levels were observed by B9 assay at any time from 3 to 72 hours (assayed hourly to 6 hours, and then at 12, 24, 48, and 72 hours following injection; data not shown).

DISCUSSION

Following induction of immunothrombocytopenia in mice, a predictable series of changes occurs in the megakaryocytic lineage. Megakaryocyte size and ploidy increase within 2 to 3 days of administration of antiserum, followed by increases in the number of megakaryocytes in the marrow and the number of megakaryocytic colony-forming cells in both marrow and spleen.26,27 Partly based on this data, in vitro data showing that megakaryocyte progenitors proliferate in response to IL-3 but not to TSF, and a variety of other studies, a number of investigators have suggested that the regulation of megakaryocytopoiesis is two-tiered.8,11,20,29-31 Megakaryocytic proliferation may be controlled in vitro (and perhaps in vivo) by CSFs such as IL-3 and GM-CSF, although recent studies have shown that the activities of these factors are not restricted to proliferation alone.32,33 Conversely, maturation may be controlled by the activities designated as megakaryocyte potentiators, TSF or thrombopoietin, and it is possible that, at least in part, IL-6 is the factor responsible for these activities.8,18 Of the purified factors studied in our laboratory to date, IL-3 has been the most potent megakaryocyte proliferation factor and IL-6 the most potent maturation factor. Accordingly, we chose to measure the levels of these factors in vivo following administration of APS.

It has proven difficult to show the presence of IL-3 in vivo, perhaps because the techniques used for measurement of the factor are insufficiently sensitive. Thus, we

![Fig 2. Autoradiogram of splenic RNA hybridized in solution with an IL-6 cRNA probe (2-day exposure). Arrows mark L32 hybrid (400 bp), IL-6 probe (284 bp), and IL-6 hybrid (241 bp). Lane 1, IL-6 probe. Lane 2, 220-bp marker. Lane 3, 2 μg J774 RNA showing an IL-6 hybrid. Lanes 4 through 8, 50 μg splenic RNA from mice injected with APS and killed 1, 1.5, 2, 2.5, or 3 hours later. IL-6 mRNA returned to baseline levels by 3 hours and remained unchanged when examined hourly to 6 hours, and at 12, 24, 48, and 72 hours following antiserum administration.](From www.bloodjournal.org by guest on October 24, 2017. For personal use only.)
chose to employ the method of solution hybridization using complementary RNA probes. This method has the advantages of favorable kinetics, specificity, extreme sensitivity, and no requirement for probe denaturation. Despite the sensitivity of the method, IL-3 hybrids could not be detected. It is possible that IL-3 mRNA might be detectable in organs other than the spleen, or that the levels of IL-3 produced are below the limits of detection of this method, notwithstanding its sensitivity.

In contrast to IL-3, IL-6 message in spleen was identifiable after 2 to 4 days of autoradiographic exposure. Maximum IL-6 was detected only 2 hours after injection of antiserum in the experimental animals and had decreased to the level of the control animals by 3 hours, remaining at control levels thereafter. This increment in IL-6 mRNA was paralleled by a concomitant small increase in the serum levels of IL-6. In the bone marrow, IL-6 mRNA was maximal at about 4 hours following APS injection. The amount of IL-6 mRNA in marrow appears to be significantly less than that observed in spleen, because the autoradiogram shown was developed after a 2-week exposure. This difference is due in part to the smaller quantity of RNA obtainable from murine marrow, but may also be due to fewer cells capable of producing IL-6 mRNA in marrow compared with spleen. The temporal differences in detection of maximal IL-6 mRNA levels in marrow may be because of a divergence in the rate of penetration and/or processing of the antibody by the marrow compared with the spleen. Alternatively, factors produced secondarily by the antigen-antibody reaction at extramedullary sites may be responsible for induction of marrow IL-6 mRNA production at later time periods.

The role of the IL-6 elevations seen in this study must be interpreted with circumspection. First, the elevations in IL-6 mRNA and in serum bioactivity are small, and may not be easily detectable by the less sensitive Northern analysis or immunoblotting technique, respectively. Second, elevations in serum levels may not reflect marrow concentrations or processing in that tissue. Third, other hematopoietic growth factors of perhaps greater potency and/or specificity to megakaryocytes may increase concurrently. Finally, spleen and marrow IL-6 mRNA may neither be temporally nor quantitatively related to other tissue sources of IL-6 mRNA. Consequently, it is possible that the virtual superimposition of the bioassay and mRNA data reflects varying kinetics of IL-6 production in different tissues, with the penultimate serum levels representing IL-6 translated from mRNA produced at earlier times in organs other than the marrow or spleen.

However, the short-lived peak in IL-6 expression does not necessarily mitigate against its potential importance in stimulating megakaryocytes. This interval may be sufficient for IL-6 binding to immature megakaryocytes, triggering an intra-cellular differentiation program independent of further exogenous stimulation.

It is now known that IL-6 increases in response to a variety of stimuli including inflammation, endotoxemia, elective surgery, and certain neoplasms and has a variety of biologic effects including B-cell maturation, T-cell activation, fever and modulation of the hepatic acute phase response in addition to acting in concert with other growth factors to enhance early hematopoiesis. It is of note that processes associated with increased IL-6 levels such as rheumatoid arthritis, elective surgery, and malignancy are also associated with thrombocytosis.

The effects of administration of APS are not entirely specific to the megakaryocytic lineage. We and others have shown that following APS injection, erythroid, myeloid, and mixed cell progenitors also increase in the marrow and spleen. We hypothesized that the immune response per se may have led to a nonspecific increase in the production of hematopoietic factors. Other studies have shown that thrombocytopenia may be stimulated nonspecifically. In the present study, IL-6 bioactivity levels increased following ALS administration in comparison with PBS (P = .06),
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Fig 4. Autoradiogram of marrow RNA hybrids from experimental and control animals (14-day exposure). The L32 hybrid is at 400 bp (the L32 probe was transcribed to a low specific activity so as to not overwhelm the autoradiogram), the IL-6 hybrid at 241 bp and residual IL-6 probe at 284 bp. Lane 1, 2 μg J774 RNA (the lane is overexposed because of the long exposure time). Lanes 2 through 5, RNA from femoral marrow obtained 2 hours following PBS, NRS, ALS, and APS administration, respectively. In lane 4, the L32 hybrid is more intense than its counterpart in lane 5, showing greater RNA loading in lane 4. Consequently, the APS-related IL-6 hybrid exceeds the ALS-related one. Lane 6, marrow RNA obtained 4 hours after APS. Lane 7, marrow RNA obtained 6 hours after APS. The hybrid observed at 24 and 48 hours after APS was similar to the 6-hour hybrid (data not shown).

Fig 5. Bioassay of IL-6. Mouse serum obtained 1.75 hours following injection of APS (n = 11), ALS (n = 8), NRS (n = 8), or PBS (n = 8) was added to IL-6-responsive B9 cells and assayed as described in Materials and Methods. The mean serum IL-6 concentrations (U/mL ± 1 SEM) of mice in each group are shown. APS, 200 ± 30; ALS, 129 ± 17 (P = .08); NRS, 106 ± 17 (P < .03); and PBS, 84 ± 17 (P < .01). The statistical comparisons are between the APS-treated mice and each of the control groups. One unit of activity represents that amount of IL-6 which promotes half-maximal incorporation of ³H'TdR into B9 cells. Maximum incorporation ranged from 50,000 to 100,000 cpm.

while IL-6 mRNA in marrow following ALS exceeded that of NRS or PBS (Fig 4). It is then conceivable that at least part of the megakaryocytic response to APS administration is unrelated to thrombocytopenia per se, but rather related to some response (perhaps immune) induced by the injection of foreign protein. Although IL-6 was generally increased to a greater extent in the APS-treated mice compared with our selected control antibodies, it is possible that more intense immunologic stimulation, perhaps achievable with other as yet untested antibodies, might promote higher levels of IL-6 expression than we observed with APS. In that circumstance, the effect of such antibody treatment on megakaryocytes would be of interest.

The present study adds to the accumulating data, suggesting that IL-6 plays some role in megakaryocytosis in vitro and in vivo, despite its lack of specificity to that lineage. Nevertheless, with the delineation of additional growth factors shown to influence megakaryocytosis proceeding rapidly,16-38 it is premature to infer that IL-6 represents a unique or necessary megakaryocytic growth factor.
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