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# Stimulation of hematopoiesis *in vivo* by recombinant bacterial murine interleukin 3

(colony-forming units/hematopoiesis/prokaryotic expression vector)

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ABSTRACT **Mouse interleukin 3 (IL-3) cDNA was cloned into a plasmid construction, allowing the synthesis of very high quantities of IL-3 in *Escherichia coli*. The recombinant (r) IL-3, purified to homogeneity, was active *in vitro* on the proliferation and differentiation of various hematopoietic progenitor cells at 1 pM. To maintain detectable blood levels of IL-3, osmotic pumps containing rIL-3 or control solutions were placed under the skin of normal and irradiated C3H/HeJ and (BALB ×B10) $F_1$ mice. The effect of IL-3 on hematopoietic progenitor cell numbers in spleen and bone marrow was evaluated 3 and 7 days later by using an *in vitro* clonal assay. The results demonstrated the following: (*i*) Doses of IL-3 infused at the rate of 2.5–5 ng per g of body weight per hr were sufficient to increase the numbers of hematopoietic progenitors in normal mice by at least 2-fold within 3 days. (*ii*) In mice with progenitor cell levels depressed by sublethal irradiation, 7-day treatment with IL-3 resulted in a 10-fold increase to near normal levels. (*iii*) The erythroid and myeloid lineages appeared to be enhanced to the same extent. (*iv*) Enhancement of hematopoiesis occurred primarily in spleen, but hematopoietic foci were also evident in the liver; in contrast, total cell and progenitor cell numbers were decreased in the bone marrow.**

Interleukin 3 (IL-3) is a hormone-like glycoprotein produced by lectin- or antigen-activated mouse T lymphocytes and, constitutively, by the mouse myelomonocytic cell line WEHI-3. It is thought to stimulate the growth and differentiation of hematopoietic progenitor cells and multipotential stem cells *in vitro* and has been described under a variety of names, among them multi-colony-stimulating factor, burst-promoting activity, mast cell growth factor, and hematopoietic cell growth factor (for review, see refs. 1–3). The cDNA for murine IL-3 has been cloned from the WEHI-3 cell line (4) and from an activated T-cell clone (5); the structure of the chromosomal gene has also been determined (6). Glycosylated mature IL-3 has been purified to homogeneity from medium conditioned by WEHI-3 cells and its *in vitro* activity on cells of hematopoietic origin has been determined (7, 8); the product of the cloned IL-3 cDNA expressed in Cos-7 monkey cells has also been found to be active *in vitro* (9). However, neither the native nor the recombinant molecules could be obtained in sufficient amounts to test their biological activity *in vivo*. In this paper, we report the purification of large quantities of recombinant murine IL-3 produced in *Escherichia coli* as well as the effects of this material on hematopoiesis in normal and sublethally irradiated mice.

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## MATERIALS AND METHODS

**cDNA Library Construction and Screening.** The cDNA library was constructed as described (10), using poly(A)$^+$ mRNA from cultured WEHI-3 cells. The double-stranded cDNA was inserted into pBR322 by G-C tailing (11) and was used to transform competent *E. coli* MC 1061 (12) cells using calcium chloride. The screening was done in two steps, using as probes first a $^{32}$P-labeled synthetic oligonucleotide (13) corresponding to nucleotides 123–150 of the IL-3 cDNA sequence (kind gift of André Chollet, Biogen S.A., Geneva), and second a nick-translated *Xba* I/*Nco* I fragment from the genomic clone pFASV-MIL3 (N.F., unpublished results) corresponding to nucleotides 466–571 of the IL-3 cDNA sequence, labeled by nick-translation (14).

**Bacterial Strains.** Plasmids were propagated in the *E. coli* strain C600 (*thr-1, leu-B6, thi-1, sup-E44, lac-Y1, ton-A21, hsr*$^-$, *hsm*$^+$) lysogenic for wild-type λ. The expression plasmid pBR per pLT4-MIL3 was transformed into C600 (pcI*857*) (15) in which pcI*857* plasmid provides a heat-inducible λ repressor and a kanamycin-resistance marker.

**Plasmids and Site-Specific Mutagenesis.** Plasmid pBR322 was cleaved by *Bam*HI and the site was filled with DNA polymerase (the Klenow fragment) in the presence of the four deoxyribonucleoside triphosphates. A synthetic *Bgl* II linker (5′ -CAGATCTG) was ligated to the linear plasmid and then digested with *Bgl* II and self-ligated. The resulting pBR derivative is *tet*$^-$ and has a *Bgl* II site flanked by two *Bam*HI sites. Plasmid pBR *trp* Cla MIL-3 [2] (see Fig. 2*C*) was prepared from pBR *trp* Cla MIL-3 [1] (see Fig. 2*B*) by site-specific mutagenesis, using the gapped molecule technique (16). In this experiment, the synthetic oligonucleotide 5′ -CGATACTATGGATACCCAC was designed to replace the sequences 5′ -CGATAAGCTTTCAATCAGTGGCCG-GGATACCAC present in pBR *trp* Cla MIL-3 [1]. The relevant nucleotide sequences of the various plasmids were determined by the method of Maxam and Gilbert (17).

**Purification of Recombinant IL-3 (rIL-3).** After fermentation, *E. coli* cells were harvested by centrifugation at 5000 × *g* for 30 min and resuspended in 0.1 M Tris·HCl/0.05 M EDTA, pH 7.5 (3.33 ml of buffer per g of pellet). The cell suspension was then made hypertonic with sucrose (final concentration, 20%; wt/vol) and incubated at 30°C for 30 min after addition of 1 mg of lysosyme per g of pellet, then passed twice through a French press at 8000 psi (1 psi = 6.89 kPa), followed by centrifugation at 20,000 × *g* for 30 min. The pellet was resuspended in 3.3 vol of Tris/EDTA buffer [0.75 M in guanidine·HCl and 1% (vol/vol) in Tween 80], spun at 10,000

Abbreviations: CFC, colony forming cells; CFU-S, colony forming unit in spleen; IL-3, murine interleukin 3; rIL-3, recombinant bacterial murine interleukin 3; bp, base pair(s).

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× *g* for 30 min, washed twice under the same conditions, and twice in 0.15 M NaCl/0.01 M $PO_4$, pH 7.4, then solubilized in 8 M guanidine·HCl/5 mM dithiothreitol, and spun at 10,000 × *g* for 30 min. The supernatant (3.5 ml) was applied on a 2.5 × 100 cm Sephadex G-100 column (Pharmacia, Sweden) equilibrated in 8 M urea/0.1 M Tris·HCl, pH 7.8/5 mM dithiothreitol. Fractions containing pure IL-3, as judged on silver-stained (18) gels after $NaDodSO_4$/polyacrylamide gel electrophoresis (19), were pooled, adjusted to 100 μg of protein per ml with phosphate-buffered saline containing 10% (vol/vol) glycerol (PBS-G) and 5 mM dithiothreitol, then repeatedly dialyzed against PBS-G. As judged by the limulus assay, the preparations used in the present experiments contained <2 ng of lipopolysaccharide per ml of rIL-3 solution ($5 \times 10^5$ units).

**IL-3 Bioassay and Clonal Assay Cultures.** IL-3 activity was determined either on: (*i*) the IL-3-dependent cell lines AD3, established by T. M. Dexter (20), or Ea-3 (kind gift of R. Palacios) (21), using a cell proliferation assay (7) measuring cell survival and proliferation by colorimetric assay (22); or (*ii*) bone marrow clonal assay cultures. Clonal assay cultures were prepared in serum-free methyl cellulose medium as described (23, 24), at a final concentration of $2.5 \times 10^4$ nucleated bone marrow, or $5 \times 10^4$ nucleated spleen cells per ml.

***In Vivo* Perfusions and Other Methods.** Alzet (Alza, Palo Alto, CA) osmotic minipumps were placed subcutaneously in C3H/HeJ [a strain insensitive to endotoxin (25)] or (BALB × B10)$F_1$ mice of our breeding stock. Mice were used in groups of two to four animals ≈10 weeks old, either normal or irradiated from a $^{60}Co$ source with 500 rads immediately before placing the pump. Each experiment consisted of two groups of mice, one with pumps loaded with 200 μl of PBS-G containing 10–20 μg of rIL-3, 200 units of penicillin, and 1 μg of streptomycin; the other group had pumps loaded with the same solution without rIL-3 but with 5 ng of *E. coli* lipopolysaccharide (i.e., >10 to 20 times the possible maximal contamination of the rIL-3 solution). Lymphohematopoietic cells were obtained from the livers as described (26). *In vivo* colony-forming ability of these cells in the spleens of lethally irradiated syngeneic mice (cfu-S) was determined 10 days after intravenous injection into groups of three or five lethally irradiated recipients (27).

## RESULTS

**Isolation of IL-3 cDNA Clones.** The first screening of the WEHI-3 cDNA library selected a positive clone K1, which was found to be ≈400 base pairs (bp) long, beginning with the first ATG of the leader sequence, extending 15 bp beyond the *Bgl* II site at its 3′ end, missing 115 bp of the coding region (Fig. 1 *A* and *B*). A second screening with a probe corresponding to a sequence in the missing region yielded a second clone, K2, containing the 3′ terminal end sequence (Fig. 1*C*) that was found by DNA sequencing to be identical, downstream from the *Bgl* II site, to the IL-3 DNA published sequence (4, 6).

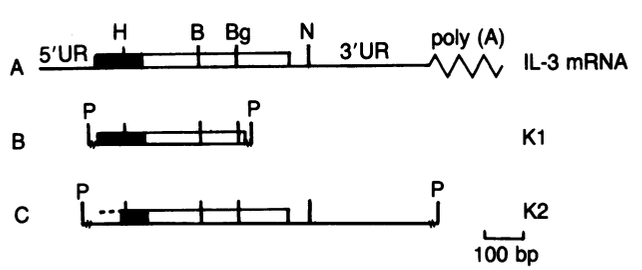


FIG. 1. Maps of murine IL-3 mRNA (1.3 kilobases), (*A*) and of the cDNA clones K1 (*B*) and K2 (*C*). The region of the mRNA encoding the mature protein is shown as an open block and the $NH_2$-terminal hydrophobic leader peptide is shown as a solid box. The untranslated regions are designated by UR. The locations of certain relevant restriction endonuclease are indicated as follows: P, *Pst* I; H, *Hin*dIII; B, *Bam*HI; Bg, *Bgl* II; N, *Nco* I; cleavage sites are indicated above the mRNA. The dashed lines at the ends of cDNA clones K1 and K2 indicate oligo(dC-dG) tails, and the broken lines represent unsequenced regions.

**Expression of the Mature Gene in *E. coli*.** As a preliminary step, the unique *Hin*dIII site of the K1 cDNA immediately upstream from the starting site of the mature protein (Fig. 1*B*) was connected to a *Hin*dIII site located just downstream from the *E. coli trp* attenuator regulatory region (Fig. 2*A*). A *trp–MIL-3* fusion gene was first engineered with the incomplete *K1* gene immediately available, yielding pBR *trp* Cla MIL-3 [1] and [2] (Fig. 2*B*), which has a favorable distance between the *trp* Shine–Dalgarno sequence and the ATG in front of the mature sequence. The missing coding sequences downstream from the *Bgl* II site were then restored by

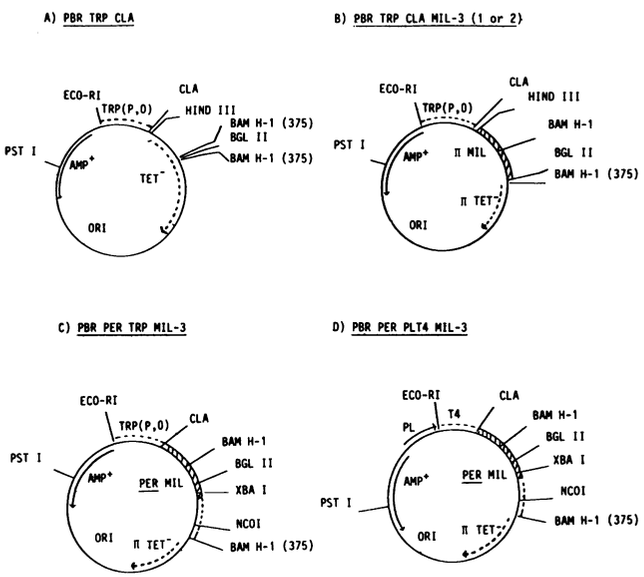


FIG. 2. (*A*) The *trp* sequences in pBR *trp* Cla derive from pBR 1-5A provided to us by K. Bertrand (28). The fragment of 343 bp spanning from *Eco*RI (pBR–*trp* junction) to *Taq* I (between the Shine–Dalgarno sequence and the ATG of the *trp* attenuator region) of pBR 1-5A was cloned between the single *Eco*RI and *Cla* I sites of pBR322. *Taq* I/*Cla* I fusion restores the *Cla* I site. For further manipulations, a *Bgl* II site has been added to the *Bam*HI site of pBR322. The numbers in parentheses refer to the position of the corresponding sites in the standard map of pBR322. (*B*) pBR *trp* Cla MIL-3 [1]. Plasmid in *A* was cleaved by *Hin*dIII/*Bgl* II and the larger resulting fragment was purified. In parallel, the MIL-3-containing *Hin*dIII/*Bgl* II fragment of 289 bp was purified from K1 (see Fig. 1*A*) and the two fragments were ligated. Plasmid pBR *trp* Cla MIL-3 [2] was prepared from pBR *trp* Cla MIL-3 [1] by site-specific mutagenesis (see *Materials and Methods*). (*C*) pBR per *trp* MIL-3. Plasmid pBR *trp* Cla MIL-3 [2] was cleaved by the mixture of *Cla* I and *Bgl* II, and the smaller resulting fragment was purified. In parallel, the *Bgl* II/*Nco* I fragment containing the carboxyl-terminal end of MIL-3 was prepared from the genomic clone and from plasmid K2. Finally, an independent *trp* clone having the same general structure as the plasmids in this figure, but including a unique *Nco* I site in the nontranslated part of a different gene was cleaved by the mixture of *Cla* I and *Nco* I enzymes, and the larger fragment was purified. Three way ligation gave rise to pBR per *trp* MIL-3. (*D*) pBR per pLT4 MIL-3. Plasmid in *C* was digested by the mixture of *Pst* I and *Cla* I enzymes, and the larger fragment was purified. In parallel, an analogous plasmid in which the *trp* sequences were replaced by pL-T4 sequences was also cleaved by the same restriction enzymes, and the smaller resulting fragment was purified and ligated to the fragment described above.

connecting a fragment available in either the K2 cDNA clone or the genomic clone (Fig. 2*C*). Since the expression level obtained from this pBR per *trp* MIL-3 construction was rather low, pL-T4 regulatory sequences were substituted for the *trp* sequences (Fig. 2*D*), leading to much higher levels of expression. The pL (early leftward promoter) sequences provide a strong promoter for *E. coli* RNA polymerase and the T4 (phage T4 protein 32) sequences contain a strong Shine–Dalgarno sequence as well as a weak promoter sequence. The host used for expression includes a λ heat-inducible repressor gene. At the permissive temperature (30°C) the pL promoter is repressed, but a low level of MIL-3 protein is expressed off the weak T4 promoter (2–3% of total cellular protein mass, as judged on $NaDodSO_4$/polyacrylamide gel electrophoresis). At the nonpermissive temperature (42°C), the contribution of the derepressed pL promoter enhances this level to a value of at least 15% of total cellular protein mass.

***In Vitro* Biological Activity of rIL-3.** Pure rIL-3, showing a single silver-stained band after $NaDodSO_4$/PAGE (Fig. 3), was tested for biological activity (Fig. 4) on a mouse IL-3-dependent cell line and in parallel in serum-free mouse bone marrow cultures (colony-stimulating activity). The dose–response curves are slightly different, with half-maximal activity being obtained at ≈0.4 ng/ml (colony numbers) and 0.1 ng/ml (proliferation), respectively, which is very close to the values reported for native WEHI-3 IL-3 purified to homogeneity (1 unit = 0.2 ng/ml) (8). All types of hematopoietic colonies were stimulated to grow in bone marrow cultures including mixed erythroid-containing cells of at least one other lineage in addition to erythroid. Adding erythropoietin to IL-3-stimulated cultures increased the numbers of mixed erythroid colonies by ≈2-fold and induced the formation of up to 90 pure erythroid colonies per $10^5$ cells plated. Very few (1–2 per $10^5$ cells) pure erythroid colonies were seen in the absence of erythropoietin. In liquid bone marrow cultures. rIL-3 induced, as reported for native IL-3 (8), the differentiation and proliferation of homogeneous populations of mast cells.

***In Vivo* Experiments.** Since the half-life of rIL-3 in the blood has been estimated to be 3–4 min (2), osmotic pumps were used, which should ensure for 7 days a subcutaneous perfusion of 0.05–0.1 μg of rIL-3 per hr when loaded with 10–20 μg of rIL-3. Preliminary experiments indicated that rIL-3

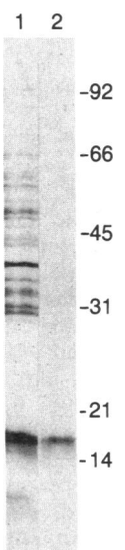


FIG. 3. Silver-stained $NaDodSO_4$/15% polyacrylamide gel. Lane 1, washed *E. coli* pellet; lane 2, purified rIL-3. Molecular sizes of markers are shown in kDa.

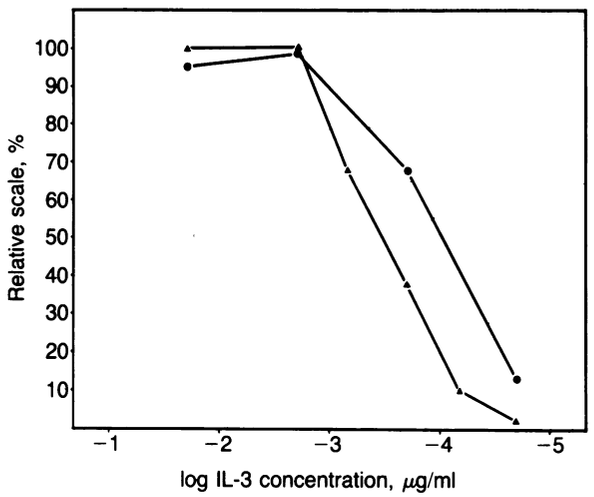


FIG. 4. Biological assays of pure rIL-3. ●, Proliferation of AD3 cells, measured by colorimetric assay; ▲, number of nonerythroid colonies in clonal assay culture of bone marrow cells.

may not remain active for a full week in the pumps. In one experiment, there was no IL-3 activity detected in the blood 3 days after placing the pumps. Thus, in the experiments presented in Table 1, the experimental and control mice also received additional intraperitoneal injections of rIL-3 or control solutions (see legend). In one mouse subjected to daily bleedings it was observed that this additional injection schedule succeeded in maintaining detectable blood levels of IL-3. However, these additional injections were not absolutely required, because similar effects on hematopoiesis were also observed in mice receiving only pumps loaded with 10 μg of rIL-3.

The effect of rIL-3 on the hematopoiesis of normal or irradiated mice was evaluated 3 and 7 days after placing the pumps. In nonirradiated mice bearing pumps, a marked increase (at least 2- to 3-fold, already present after 3 days) was found in the number of spleen cells in IL-3-infused mice compared to controls (Table 1). In contrast, the number of bone marrow cells was strikingly and consistently decreased in IL-3-infused mice (≤50% of the controls). The concentration of colony forming cells (CFC) in spleen and bone marrow was increased after infusion, but the total number of CFC in bone marrow of treated animals was decreased because of the decreased cellular content (Table 1). To determine whether there was an overall increase of progenitors in rIL-3-treated animals or merely a migration from bone marrow to spleen, total CFC numbers in bone marrow and spleen were estimated (Table 1). These calculations indicate that the total number of CFC of normal mice were increased at least 2-fold by maintaining a high IL-3 blood level, and that the increase involves erythroid as well as myeloid progenitors. The evaluation in Table 1 does not take into account the CFC content of blood or the hematopoietic foci that were observed in histologic sections of the livers of IL-3-infused mice. As many as 40 × $10^6$ hematopoietic cells (myeloblasts, erythroblasts, and unidentified blasts) were recovered from some of these livers; injection of these cells into lethally irradiated mice showed that some were able to generate spleen colonies (up to 30 CFU-S per $10^5$ cells).

In mice irradiated with 500 rads immediately before placing the pumps, the spleens of the experimental animals were enormously enlarged on day 7 compared to the atrophic spleens of the control mice (Fig. 5). In contrast, their bone marrow contained slightly fewer cells (Table 1). The increase in total splenic progenitors for nonerythroid colonies was >100-fold. Estimated total progenitor cell content showed that the enhancement resulting from rIL-3 infusion was

Table 1. Content of hematopoietic progenitor cells in IL-3-treated and control mice

| Exp. | Sample | Organ | Cells/ organ, $\times 10^{-6}$ | Progenitors/organ, $\times 10^{-3}$ | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Day 2 (E) | Day 4 (E) | Day 9 (E) | Day 9 (NE) | Day 9 (E-mix) |
| 1. 3-day infusion of normal mice C3H/HeJ | Control | Spleen | 129 | 15.1 ± 2.6 | 3.1 ± 1.3 | 3.9 ± 1.3 | 47.1 ± 8.9 | 6.7 ± 1.3 |
| | IL-3 | Spleen | 426 | 507 ± 26 | 147 ± 8 | 85.2 ± 17.0 | 664 ± 51 | 128 ± 21 |
| | | | (330) | (3360 ± 260) | (4760 ± 280) | (2200 ± 440) | (1410 ± 110) | (1900 ± 320) |
| 2. 7-day infusion of normal mice (BALB × B10)$F_1$ | Control | Femur | 5.93 | 7.4 ± 1.0 | 0.71 ± 0.30 | 2.0 ± 0.5 | 21.6 ± 1.6 | 3.0 ± 0.6 |
| | | Spleen | 220 | 506 ± 22 | 40.0 ± 4.4 | 41.4 ± 4.4 | 90.2 ± 6.6 | 17.6 ± 2.2 |
| | | Total* | 339 | 654 ± 29 | 54.2 ± 7.4 | 81.4 ± 10.4 | 522 ± 33 | 77.6 ± 12.1 |
| | IL-3 | Femur | 1.48 | 1.2 ± 0.2 | 0.44 ± 0.12 | 1.2 ± 0.2 | 10.4 ± 0.6 | 1.8 ± 0.2 |
| | | | (25) | (16 ± 13) | (62 ± 45) | (60 ± 27) | (48 ± 8) | (60 ± 21) |
| | | Spleen | 464 | 1030 ± 30 | 345 ± 18 | 250 ± 32 | 798 ± 60 | 102 ± 23 |
| | | | (211) | (203 ± 8) | (862 ± 46) | (604 ± 78) | (885 ± 67) | (580 ± 131) |
| | | Total* | 494 | 1050 ± 40 | 354 ± 18 | 274 ± 33 | 1010 ± 61 | 138 ± 35 |
| | | | (146) | (160 ± 7) | (653 ± 36) | (337 ± 42) | (193 ± 19) | (178 ± 35) |
| 3. 7-day infusion of irradiated mice C3H/HeJ | Control | Femur | 2.98 | 2.1 ± 0.3 | 0.41 ± 0.12 | 0.06 ± 0.05 | 5.2 ± 0.4 | 0.23 ± 0.08 |
| | | Spleen | 21.0 | 23.5 ± 1.3 | 1.5 ± 0.2 | 0.48 ± 0.13 | 4.3 ± 0.4 | 0.84 ± 0.21 |
| | | Total* | 68.4 | 57.7 ± 5.7 | 8.1 ± 2.4 | 1.48 ± 0.95 | 88.3 ± 9.0 | 4.64 ± 1.7 |
| | IL-3 | Femur | 2.37 | 1.0 ± 0.3 | 0.05 ± 0.05 | 0.05 ± 0.05 | 4.7 ± 0.6 | 0.23 ± 0.12 |
| | | | (80) | (47 ± 27) | (12 ± 41) | (80 ± 156) | (90 ± 7) | (100 ± 76) |
| | | Spleen | 273 | 442 ± 36 | 73.2 ± 5.5 | 35.5 ± 10.9 | 540 ± 38 | 41.0 ± 10.9 |
| | | | (1300) | (1880 ± 150) | (4980 ± 370) | (7400 ± 2280) | (12600 ± 880) | (4880 ± 1300) |
| | | Total* | 320 | 462 ± 36 | 74.2 ± 5.6 | 36.5 ± 11.0 | 634 ± 39 | 45.6 ± 11.2 |
| | | | (467) | (800 ± 63) | (916 ± 76) | (2466 ± 743) | (718 ± 45) | (982 ± 240) |

In each of three experiments shown, four mice (two from each group) were given rIL-3 or control infusions and additional intraperitoneal injections of rIL-3 (10 $\mu$g) or control solutions, respectively, every 6 hr for the last 24 hr (Exp. 1) or 3 days (Exps. 2 and 3). Bone marrow and spleen cells of comparable mice were pooled. Erythropoietin assay cultures for late and intermediate erythroid progenitors (day 2 and day 4) (E) contained erythropoietin at 1 unit/ml (T. C. Fox Cancer Research Center, Vancouver, BC, Canada). Cultures for more primitive day 9 erythroid (E), nonerythroid (NE), and multipotential (E-mix) progenitors contained 10% lymphocyte conditioned medium (23) in addition to erythropoietin at 2 units/ml. Numbers in parentheses represent % of control.
*Based on the assumption that one femur contains 5% of total bone marrow.

≈10-fold, reaching levels comparable to those of normal nonirradiated mice (Table 1).

## DISCUSSION

The pBR pL-T4 MIL-3 plasmid constructed for promoting the expression of IL-3 DNA in *E. coli* directs a very high level of rIL-3 synthesis, thus allowing easy purification to apparent homogeneity of large amounts of active material. The biological activity *in vitro* of the bacterial rIL-3 appears to be identical to that of mature glycosylated IL-3 in terms of target cell specificity, spectrum of effects, and specific activity (8). Thus, the carbohydrate component of the mature molecule is not required for its activity, as has been suggested (29).

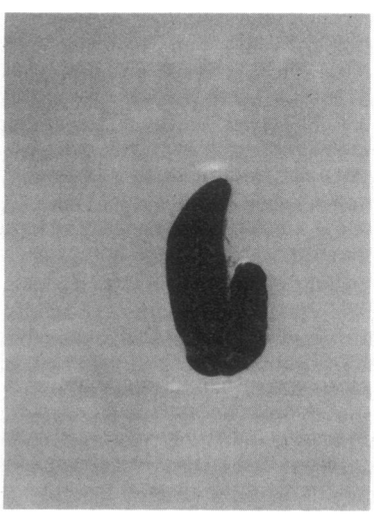

FIG. 5. Spleens of mice 7 days after 500-rad irradiation and rIL-3 infusion (*Left*: 320 × $10^6$ cells) or control (*Right*: 18 × $10^6$ cells).

The main technical obstacle encountered in the present *in vivo* experiments was the difficulty in maintaining constant high blood levels of IL-3, a requirement for obtaining the highest possible effect in a given period of time. Continuous infusions were considered necessary in view of the probable rapid blood clearance of the molecule (2). The subcutaneous osmotic pumps were supplemented with repeated intraperitoneal injections when it became evident that the IL-3 activity detectable in the blood was variable, probably as a result of rIL-3 aggregation within the pumps or in subcutaneous tissue. A more appropriate method to achieve a constant blood level might be intravenous infusion of more dilute solutions, although it is not necessarily the most convenient method to use in mice. Nevertheless, the following conclusions can be drawn: (*i*) it is possible to at least double the total hematopoietic activity of normal animals, as judged by the extrapolated total number of hematopoietic progenitor cells, probably within <3 days; (*ii*) the decreased hematopoietic activity resulting from sublethal irradiation can apparently be compensated for by exogenous IL-3, with hematopoiesis being enhanced >10-fold; (*iii*) total amount of rIL-3 (in subcutaneous perfusion) of <5–10 $\mu$g (the maximal amount delivered in 3 days, or 0.25–0.5 $\mu$g per g of body weight) is probably sufficient to achieve this effect, as deduced from the strongest responses observed without additional intraperitoneal injections.

Two observations deserve emphasis, in relation to this dramatic enhancement of hematopoiesis: (*i*) Erythropoiesis was as strongly enhanced as myelopoiesis. Increased erythropoiesis was also seen in the numbers of relatively mature day 2 erythroid precursors, a step thought to be erythropoietin dependent. This suggests that normal levels of

erythropoietin are sufficient, that IL-3 may induce the production of erythropoietin, or that it may act itself to induce full maturation (30). (*ii*) Not only did the increase in hematopoiesis occur in spleen, it was also accompanied by a marked decrease in progenitor cells in bone marrow. This suggests that progenitor cells, stimulated to enter cell cycle (a possible effect of IL-3), may be mobilized to leave the bone marrow and colonize the spleen and, to a lesser extent, the liver. Support for such a mechanism is provided by the observation that nondividing progenitor cells from bone marrow of 5-fluorouracil-treated mice, injected into lethally irradiated recipients, first colonize bone marrow and only later give rise to spleen colonies (31). Presumably after being stimulated to divide, they become able to leave the bone marrow. It will be of great interest to study the distribution and number of progenitor cells at various times after rIL-3 infusion, as well as the direct effect of IL-3 on differentiation and self-renewal ability of purified multipotential progenitors.

The powerful effect of rIL-3 on hematopoiesis makes obtaining a cDNA for a human equivalent to mouse IL-3, if such exists, an especially important goal to achieve. Based on the present results, it could be expected that the human rIL-3 would be valuable for treating various forms of bone marrow failure—for instance, those resulting from cancer chemotherapy or lethal irradiation required for bone marrow transplantation.

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