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IL-7 Induces Myelopoiesis and Erythropoiesis

Francesca B. Aiello,* †Jonathan R. Keller, ‡Kimberly D. Klarmann, ‡Glenn Dranoff, § Renata Mazzucchelli,* and Scott K. Durum²*

IL-7 administration to mice was previously reported to increase the mobilization of progenitor cells from marrow to peripheral sites. We now report that IL-7 increases the number of mature myeloid and monocytic cells in spleen and peripheral blood. This effect required T cells, and we show that IL-7 treatment in vivo induced GM-CSF and IL-3 production by T cells with memory phenotype. However, additional myelopoietic cytokines were shown to be involved because mice deficient in both GM-CSF and IL-3 also responded to IL-7 with increased myelopoiesis. Candidate cytokines included IFN-γ and Flt3 ligand, which were also produced in response to IL-7. Because IFN-γ-deficient mice also increased myelopoiesis, it was suggested that IL-7 induced production of redundant myelopoietic cytokines. In support of this hypothesis, we found that the supernatant from IL-7-treated, purified T cells contained myelopoietic activity that required a combination of Abs against GM-CSF, IL-3, and anti-Flt3 ligand to achieve maximum neutralization. IL-7 administration increased the number of splenic erythroid cells in either normal, Rag1 or GM-CSF-IL-3-deficient mice, suggesting that IL-7 might directly act on erythroid progenitors. In support of this theory, we detected a percentage of TER-119⁺ erythroid cells that expressed the IL-7Rα-chain and common γ-chain. Bone marrow cells expressing IL-7R and B220 generated erythroid colonies in vitro in response to IL-7, erythropoietin, and stem cell factor. This study demonstrates that IL-7 can promote nonlymphoid hemopoiesis and production of cytokines active in the host defense system in vivo, supporting its possible clinical utility. The Journal of Immunology, 2007, 178: 1553–1563.

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3 Abbreviations used in this paper: Flt3-L, ligand; rhu, human recombinant; HPRT, hypoxanthine phosphoribosyltransferase; F, forward; R, reverse; SCF, stem cell factor; EPO, erythropoietin; int, intermediate; BFU, burst-forming unit; sup, supernatant.
in the Guide for Care and Use of Laboratory Animals (National Institute of Health, Bethesda, MD). Human recombinant (rhu) IL-7 (endotoxin levels <1.3 EU/mg) was provided by S. Giardina (National Cancer Institute, Frederick, MD). Mice were injected i.p. once a day for varying periods with vehicle (HBSS without Ca²⁺, Mg²⁺, and phenol red; BioWhittaker) or with IL-7 diluted in vehicle, at 20 μg/0.2 ml/injection as described previously (24). The day after the last injection mice were euthanized, and tissues and blood were collected and analyzed as indicated.

**Tissue studies**

Myeloperoxidase was visualized in formalin-fixed paraffin-embedded sections by staining with a Vectastain Elite ABC kit (Vector Laboratories) after microwave Ag retrieval using the purified Ig fraction of polyclonal rabbit anti-myeloperoxidase serum (DakoCytomation). Purified Ig fraction of nonimmune rabbit serum was used as an irrelevant matched control. Blood cell analysis was done using the Hemavet System 850 (Drew Scientific). Results were evaluated by a board-certified veterinary pathologist.

**Preparation of cells**

Splenocytes were obtained by mechanical dissociation, erythrocytes were removed by treatment with ACK lysing buffer (BioSource International), and cells were washed with PBS and filtered through a nylon mesh to obtain a single-cell suspension. Bone marrow cells were flushed from femurs and tibias, and after removal of red cells they were washed with PBS and filtered through a nylon mesh. Lymph node cells were obtained by mechanical dissociation, and cells were washed with PBS and filtered through a nylon mesh to obtain a single-cell suspension. CD3⁺ lymph node cells were then obtained by negative selection using mouse T cell enrichment column (R&D Systems) in accordance with the manufacturer’s recommendations, yielding >90% CD3⁺ by cytofluorometry. Lymph node cells were also fractionated into four populations by cell sorting according to the level of CD44 expression: negative, dull, intermediate, and bright. For Ab staining, cells in PBS/0.5% BSA were preincubated with anti-FcγRIII/II receptor-blocking Ab (BD Pharmingen) for 15 min at 4°C, and then stained with PE-conjugated anti-CD44 mAb or with a PE-conjugated isotype-matched mAb (BD Pharmingen) as control for nonspecific staining at 4°C for 25 min. Cells were washed twice with cold PBS/0.5% BSA and separated by fluorescence-activated cell sorting on a FACStar Plus (BD Biosciences). CD45 ‘Mac-1’ bone marrow cells were obtained by cell sorting as described above, by staining with allopbyocyanin-conjugated anti-CD45.2 mAb (eBioscience) using allopbyocyanin-conjugated isotype-matched mAb as a control for nonspecific staining (BD Pharmingen) and with PE or FITC anti-Mac-1 mAb as appropriate (BD Pharmingen) using allopbyocyanin-conjugated isoype-matched mAb as a control for nonspecific staining (BD Pharmingen), TER-119⁺, TER-119⁻CD45⁺, and TER-119⁻Mac-1⁻ cells were obtained by cell sorting by staining with fluorochrome-conjugated anti-TER-119 mAb, alone or in combination with anti-CD45.2 mAb and anti-Mac-1 mAb using fluorochrome-conjugated isotype-matched mAb as a control for nonspecific staining (BD Pharmingen), as indicated in the figures. IL-7Rx-chain-positive cells from bone marrow were obtained by cell sorting by staining with PE-Cys5-conjugated anti-IL-7Rx-chain (eBioscience), or PE-Cys5-conjugated (eBioscience) isotype-matched mAb as a control for nonspecific staining (eBioscience). Cells were separated using a MoFlo high-speed cell sorter (DakoCytomation), yielding >90% TER-119⁺ or IL-7Rx-chain-positive cells by cytofluorometry. Viability was evaluated by trypan blue staining. The culture medium was RPMI 1640 supplemented with 10% FBS (HyClone), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2-ME (50 μM).

**Surface Ag quantitation by flow cytometry**

For one- or two-color immunofluorescence staining, cells in PBS/0.5% BSA were preincubated with anti-FcγRIII/II receptor-blocking Ab for 15 min at 4°C, and sorted on a FACStar Plus. CD3⁺ cells were then stained with anti-CD45.2 mAb and anti-Mac-1 mAb using fluorochrome-conjugated anti-CD44 mAb as control for nonspecific staining (BD Pharmingen) and with PE or FITC anti-Mac-1 mAb as appropriate (BD Pharmingen) using allopbyocyanin-conjugated isotype-matched mAb as a control for nonspecific staining (BD Pharmingen), and then stained with PE-conjugated anti-CD44 mAb or with a PE-conjugated isotype-matched mAb (BD Pharmingen) as control for nonspecific staining at 4°C for 25 min. Cells were washed twice with cold PBS/0.5% BSA and separated by fluorescence-activated cell sorting on a FACStar Plus (BD Biosciences). CD45 ‘Mac-1’ bone marrow cells were obtained by cell sorting as described above, by staining with allopbyocyanin-conjugated anti-CD45.2 mAb (eBioscience) using allopbyocyanin-conjugated isotype-matched mAb as a control for nonspecific staining (BD Pharmingen) and with PE or FITC anti-Mac-1 mAb as appropriate (BD Pharmingen) using allopbyocyanin-conjugated isotype-matched mAb as a control for nonspecific staining (BD Pharmingen), TER-119⁺, TER-119⁻CD45⁺, and TER-119⁻Mac-1⁻ cells were obtained by cell sorting by staining with fluorochrome-conjugated anti-TER-119 mAb, alone or in combination with anti-CD45.2 mAb and anti-Mac-1 mAb using fluorochrome-conjugated isotype-matched mAb as a control for nonspecific staining (BD Pharmingen), as indicated in the figures. IL-7Rx-chain-positive cells from bone marrow were obtained by cell sorting by staining with PE-Cys5-conjugated anti-IL-7Rx-chain (eBioscience), or PE-Cys5-conjugated (eBioscience) isotype-matched mAb as a control for nonspecific staining (eBioscience). Cells were separated using a MoFlo high-speed cell sorter (DakoCytomation), yielding >90% TER-119⁺ or IL-7Rx-chain-positive cells by cytofluorometry. Viability was evaluated by trypan blue staining. The culture medium was RPMI 1640 supplemented with 10% FBS (HyClone), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2-ME (50 μM).
Table II. Spleen cell subpopulations in Rag1-deficient mice after treatment with IL-7 in vivo

<table>
<thead>
<tr>
<th>Cell Populations</th>
<th>Treatment</th>
<th>Cell number ($\times 10^6$)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>B220$^+$</td>
<td>Vehicle</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>IL-7$^a$</td>
<td>14.3 ± 6.1</td>
</tr>
<tr>
<td>B220 IgM$^+$</td>
<td>Vehicle</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>IL-7$^a$</td>
<td>ND</td>
</tr>
<tr>
<td>CD3$^+$</td>
<td>Vehicle</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>IL-7$^a$</td>
<td>ND</td>
</tr>
<tr>
<td>Gr-1$^+$</td>
<td>Vehicle</td>
<td>3.6 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>IL-7$^a$</td>
<td>3.3 ± 0.9</td>
</tr>
<tr>
<td>Mac-1$^+$</td>
<td>Vehicle</td>
<td>3.5 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>IL-7$^a$</td>
<td>5.8 ± 1.8</td>
</tr>
<tr>
<td>TER-119$^+$</td>
<td>Vehicle</td>
<td>0.57 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>IL-7$^a$</td>
<td>2.6 ± 0.4$^b$</td>
</tr>
</tbody>
</table>

$^a$ n = 4; ND, Not detectable.

$^b$ The cell number of each population for individual spleen was calculated by multiplying the percentage of positive cells (determined by flow cytometry) by the total number of cells per spleen. Results are expressed as means ± SEM. (*, Mann-Whitney correlation test, $p < 0.05$).

Production of GM-CSF, IL-3, and IFN-γ by cells from lymph nodes of mice treated with IL-7 in vivo. Cells obtained from lymph nodes of mice injected for 9 days with IL-7 or vehicle as described in Materials and Methods were cultured in medium (2.5 $\times$ 10^6/ml). At the indicated times, sup were collected and assayed by ELISA. Results are expressed as means ± SEM (*, Mann-Whitney correlation test, $p < 0.05$). GM-CSF (A), vehicle (□), IL-7 (▲), representative of four experiments; (B) IL-3, vehicle (□), IL-7 (▲), representative of three experiments; (C) IFN-γ, vehicle (□), IL-7 (▲), representative of four experiments. D, ELISpot assay performed 72 h after the harvesting of lymph nodes, representative of three experiments.

ELISpot assay

The number of cells secreting IFN-γ and GM-CSF was evaluated according to the manufacturer’s instructions. Briefly, MultiScreen HTSIP plates (polycylnylidene difluoride membrane) were incubated with ethanol, washed extensively with PBS, and coated with 100 μl/well anti-murine IFN-γ or GM-CSF mAb (1/100 in PBS, R&D Systems) at 4°C overnight. After washing, membranes were blocked with culture medium for 2 h at 37°C, then cells were added and incubated at 37°C and 5% CO2. After washing with PBS/0.05% Tween 20, biotinylated anti-murine IFN-γ or GM-CSF-detecting Ab (100 μl/well, 1/100 in PBS/1% BSA/0.05% Tween 20) (R&D Systems) were added. Plates were incubated for 2 h at room temperature, washed, and then incubated for 1 h with streptavidin-alkaline phosphatase (100 μl/well, 1/60 in PBS/1% BSA) (R&D Systems). Spots were visualized with 5-bromo-4-chloro-3-indolyl phosphate-NBT phosphate substrate (100 μl/well) (R&D Systems) and subjected to automated evaluation using the ImmunoSpot Imaging Analyzer system (Cellular Technology).

Isolation of RNA and RT-PCR analysis

Total RNA was isolated using the RNeasy Mini Kit (Qiagen) following the manufacturer’s instructions. To avoid DNA contaminations, eluted RNA was incubated with DNase I/RNase free for 1 h at 37°C (DNA-free Kit, Ambion). Total RNA was retrotranscribed using SuperScript II RNase H Reverse Transcriptase (Invitrogen Life Technologies) according to the manufacturer’s protocol. Briefly, RNA was incubated at 65°C for 5 min in a volume of 26 μl containing 0.5 μg of oligo(dT)$_{12-18}$ primer and 1 μl of 10 mM dNTPs mix, and then quickly chilled on ice. The reverse transcription was performed in a total volume of 40 μl containing 50 mM Tris-HCl, 75 mM KCl, 3 mM MgCl$_2$, 5 mM DTT, 40 U of RNase (Invitrogen Life Technologies), and 100 U of SuperScript II (Invitrogen Life Technologies) at 42°C for 55 min followed by heat inactivation at 70°C for 15 min. One microliter of this reaction mixture was used for PCR analysis. Primers to detect IL-7Rα-chain (32), IFN-γ (33), and hypoxanthine phosphoribosyltransferase (HPRT) (34) mRNA were synthesized as published with PCR products of 304, 169, and 172 bp, respectively. Primer sequences forward (F), 5’-TCA TGA GCA GGA GTA TGG-3’ and reverse (R), 5’-AGC TGG AGA GGA GTT TCA TGG-3’ were used for PCR with a PCR product of 358 bp. Primer sequences F, 5’-TCC CTG TTG CTG CTG TTG-3’ and reverse (R), 3’-TCA TGA GCA GGA GTA TGG-5’ were used for PCR with a PCR product of 358 bp. Primer sequences F, 5’-TCC CTG TTG CTG CTG TTG-3’ and reverse (R), 3’-TCA TGA GCA GGA GTA TGG-5’ were used for PCR with a PCR product of 358 bp.

FIGURE 2. Production of GM-CSF, IL-3, and IFN-γ by cells from lymph nodes of mice treated with IL-7 in vivo. Cells obtained from lymph nodes of mice injected for 9 days with IL-7 or vehicle as described in Materials and Methods were cultured in medium (2.5 $\times$ 10^6/ml). At the indicated times, sup were collected and assayed by ELISA. Results are expressed as means ± SEM (*, Mann-Whitney correlation test, $p < 0.05$). GM-CSF (A), vehicle (□), IL-7 (▲), representative of four experiments; (B) IL-3, vehicle (□), IL-7 (▲), representative of three experiments; (C) IFN-γ, vehicle (□), IL-7 (▲), representative of four experiments. D, ELISpot assay performed 72 h after the harvesting of lymph nodes, representative of three experiments.
1.1% methylcellulose (Sigma-Aldrich), 25% FBS (HyClone), 2 mM glu-
ose (Miles Laboratories) in IMDM (Invitrogen Life Technologies), containing
at 37°C and 5% CO2 for 8 days. Murine stem cell factor (SCF) (100 ng/ml),
culture dishes with a 2-mm grid (Nalge Nunc International) and incubated

Immunology, University of Washington, Seattle, WA) (36) and TER-119
thymic stromal cell line Z210R.1 (a gift from Dr. A. Farr, Department of

reverse transcriptase to the first-strand synthesis step was performed. The

culture was performed using a cDNA prepared without the addition of

contamination, a PCR using a cDNA prepared without the addition of

primer pairs. The PCR amplification was performed using the following

conditions: 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C for 30 cycles; 94°C for 1

and 72°C for 1 min, and a cycle of extension of 10 min at 72°C. A

PerkinElmer 9700 thermal cycler was used for the amplification reaction. The

PCR products were resolved on a 1.5% agarose gel and visualized by

ethidium bromide staining under UV light. As a control for DNA genomic

contamination, a PCR using a cDNA prepared without the addition of

reverse transcriptase to the first-strand synthesis step was performed. The

thymic stromal cell line Z210R.1 (a gift from Dr. A. Farr, Department of

Immunology, University of Washington, Seattle, WA) (36) and TER-119

cells from IL-7Rα-chain-deficient mice were used as a negative control for

IL-7Rα-chain gene expression, splenocytes from common γ-chain

deficient mice were used as a negative control for common γ-chain gene

expression, and the IL-7-dependent thymocyte cell lines D1 (37) were used

as negative and positive controls, respectively.

Soft agar colony formation assay
Bone marrow cells from B6 mice, depleted of CD3+ cells by cell sorting,
were plated in IMDM (Invitrogen Life Technologies), 10% heat-inacti-
vated FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin, with 0.35%

weight/volume SePlaque agarose (Cambrex BioScience Rockland). Cells

were plated at a density of 7 × 10⁵ cells/ml/plate in 35 × 10 mm cell

culture dishes with a 2-mm grid (Nalge Nunc International) and incubated

at 37°C and 5% CO2 for 8 days. Murine stem cell factor (SCF) (100 ng/ml),
IL-3 (30 ng/ml) (PeproTech), or SCF plus the sup of T cells purified by

negative selection (as described above) (2.5 × 10⁷/ml) and cultured with

murine IL-7 (10 ng/ml) for 9 days (sup), and neutralizing Abs as follows:
purified goat anti-mouse IL-7 (1 μg/ml), rat anti-mouse GM-CSF mAb, (2
μg/ml), rat anti-mouse IL-3 mAb (2 μg/ml), purified goat anti-mouse

Flt3-L (2.4 μg/ml), and rat anti-mouse IFN-γ mAb (5 μg/ml) were added to

the cell cultures as indicated. The concentration of the Abs exceeded at

least 100 times the 50% neutralizing dose indicated by the manufacturer,
and was calculated with respect to the level of the cytokines in the sup

quantified by ELISA.

Methylcellulose clonogenic assays
Unfractionated or sorted IL-7Rα−, IL-7R−CD3−, and IL-7Rα+ CD3− B220−
bone marrow cells were plated in 35-mm Luc petri dishes (Miles Laboratories) in IMDM (Invitrogen Life Technologies), containing
1.1% methylcellulose (Sigma-Aldrich), 25% FBS (HyClone), 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2-ME (50 μM),
murine IL-3 (30 ng/ml), SCF (100 ng/ml), and human erythropoietin (EPO) (5 U/ml) (PeproTech). Murine IL-7 (50 ng/ml) (PeproTech) was added as
indicated. Cultures performed in triplicate were incubated for 8 days in a fully
humidified atmosphere at 37°C and 5% CO2, and then scored for

colony formation. Hemoglobin containing cells were identified by benzidine
staining (38). HCl (Sigma-Aldrich) in 0.5 M acetic acid and 10 μl of 30%
H2O2 were added to 50 μl of 0.2% benzidine just before use. Colonies
containing cells showing an intense blue staining were scored as positive.

Statistical analysis
Nonparametric Mann-Whitney correlation test for nonpaired data was per-
formed for statistical analysis. Results are means ± SEM.

Results
Effect of IL-7 administration on splenic cell subpopulations
B6 mice were injected daily with 20 μg of rhuIL-7 or with vehicle
for 9 days. As expected, IL-7 treatment greatly increased the
spleen size and cellularity (vehicle = 74.4 ± 10.1 × 10⁶ cells; IL-7 = 264.4 ± 39.7 × 10⁶ cells; p < 0.05). In addition to the
increase of total B cells (B220+), mature B cells (B220+ IgM−), and mature T cells (CD3+), a significant increase in the number of
myeloid cells (Gr-1+), monocyte (Mac-1+) and erythroid (TER-
119+) cells was observed (Table I). Microscopic examination showed that the spleens of IL-7-treated mice contained numerous
immature myeloid cells and mature neutrophils (Fig. 1, A and B),
both positive for the myeloid cell-specific myeloperoxidase
enzyme (Fig. 1, C and D). In the peripheral blood of mice treated for
9 days with IL-7, the number of neutrophils was significantly
elevated compared with mice treated with vehicle (vehicle = 5.0 ± 0.9 × 10³/μl; IL-7 = 19.4 ± 2.4 × 10³/μl; p < 0.05). Following IL-7 treatment, the numbers of blood monocytes
showed an increasing trend (vehicle = 0.1 ± 0.4 × 10³/μl;
FIGURE 6. Spleen cell populations in B6 and GM-CSF-IL-3-deficient mice after treatment with IL-7 in vivo. GM-CSF-IL-3-deficient mice were treated with IL-7 (n = 3) or with vehicle (n = 3) as described in Materials and Methods for 9 days. The cell number of each population for individual spleen was calculated by multiplying the percentage of positive cells (determined by flow cytometry) by the total number of cells per spleen. The increases in spleen-cell population cell numbers in IL-7-treated mice are expressed as fold increase compared with vehicle ± SEM. These values are compared with those observed in B6 mice treated with IL-7 (n = 4) or with vehicle (n = 4) for 9 days.

IL-7 = 0.5 ± 0.1 × 10^6/µl, although the difference did not reach a statistically significant level (p = 0.08). The number of blood erythrocytes did not change (vehicle = 10.3 ± 0.3 × 10^6/µl; IL-7 = 10.0 ± 0.1 × 10^6/µl).

IL-7 effects in Rag1-deficient mice

Because myeloid cells do not express IL-7R (27–29), we sought another cellular target of IL-7 that could secondarily induce myelopoiesis. T cells are a primary target of IL-7, and it was recently observed that T cells mediated IL-7 induction of the maturation of osteoclasts in vivo (39, 40). To evaluate a requirement for T cells in IL-7 effects on myeloid cells, we injected Rag1−/− mice with 20 µg/day of rhuIL-7 or with vehicle. IL-7 treatment moderately increased the spleen size and significantly increased cellularity (vehicle = 7.0 ± 1.3 × 10^6 cells; IL-7 = 22.2 ± 6.8 × 10^6 cells; p < 0.05). This increase primarily represented a rise in immature B cells (B220+ cells that were all IgM−) and erythroid cells (TER-119+) (Table II). As expected, in Rag1−/− mice mature lymphocytes of either B (B220+IgM+) or T (CD3+) lineage were not detectable in either IL-7 or control treatment groups. Notably, the number of Gr-1+ and Mac-1− cells was not significantly increased by IL-7 treatment in Rag1−/− mice. A rise of these cells was not simply delayed because we intentionally injected IL-7 for 16 days, whereas in B6 mice the rise occurred by 9 days. These findings were further confirmed by microscopic examination and immunohistochemical analysis of myeloperoxidase (data not shown). Nine days of IL-7 treatment did not increase the number of neutrophils (vehicle = 1.9 ± 0.4 × 10^5/µl; IL-7 = 0.8 ± 0.4 × 10^5/µl), monocytes (vehicle = 0.4 ± 0.1 × 10^3/µl; IL-7 = 0.4 ± 0.2 × 10^3/µl), or erythrocytes (vehicle = 10.6 ± 0.3 × 10^9/µl; IL-7 = 9.0 ± 0.5 × 10^9/µl) in peripheral blood. These results suggested that the myelopoietic effects of IL-7 in vivo could be mediated by T lymphocytes.

IL-7-induced production of GM-CSF and IL-3 by T cells

We then investigated whether IL-7 administration in B6 mice induced T cells to produce the myelopoietic cytokines GM-CSF and IL-3. Mice were injected daily with 20 µg of rhuIL-7 or with vehicle for 9 days. Lymph node cells were then harvested and cultured, and the sup were assayed for the presence of GM-CSF and IL-3 at different time points (Fig. 2, A and B). Lymph node cells from IL-7, but not from vehicle-treated mice produced GM-CSF and IL-3 in a time-dependent manner. IL-7 treatment in vivo also elicited production of IFN-γ (Fig. 2C). The frequency of cells producing GM-CSF and IFN-γ is shown in Fig. 2D. In vitro treatment of lymph node cells with IL-7 induced production of GM-CSF in a time-dependent manner (Fig. 3), and IL-3 was also induced (medium = <15.6 pg/ml; IL-7 = 90.3 ± 12.9 pg/ml; measured at day 7; p < 0.05). Concentrations of IL-7 were tested in vitro from 0.1–100 ng/ml, for both cytokines the effect of IL-7 was dose-dependent and the maximal dose was 10 ng/ml (data not shown). To determine whether T cells produced myelopoietic cytokines in response to IL-7, T cells were purified from lymph nodes and shown to produce GM-CSF and IL-3 (Fig. 4). Similar results were obtained using either CD4+ or CD8− purified splenic subpopulations of T cells (data not shown).

IL-7 can act on both naive and memory T cells (5–10). To determine whether GM-CSF and IL-3 were produced by one or
the other of these populations, we sorted lymph node cells according to CD44 expression, which is absent on naive T cells and high on memory cells (41, 42). After 7 days of culture with IL-7, CD44+ cells produced GM-CSF, whereas CD44dim, dull, and negative cells did not (Fig. 5). Production of IL-3 was also restricted to CD44bright cells (data not shown). Thus, IL-7 induces the memory subset of T cells to produce myelopoietic cytokines, which may account for our observation that treatment of mice with IL-7 strongly induces generation of myeloid cells.

Effects of IL-7 in GM-CSF−/− IL-3−/− mice

To determine whether GM-CSF and IL-3 accounted for all of the myeloid production elicited by IL-7 in vivo, we treated GM-CSF−/− IL-3−/− mice with IL-7 or vehicle for 9 days. IL-7 treatment increased the spleen size and the total number of splenocytes (vehicle = 62.9 ± 9.0 × 10⁶ cells; IL-7 = 254.4 ± 39.7 × 10⁶ cells; p < 0.05). The distribution of splenic subpopulations showed no difference in the effect of IL-7 on these mice compared with wild-type mice (Fig. 6). This was confirmed by microscopic examination of spleen specimens and immunohistochemical analysis of myeloperoxidase (data not shown). A significant increase of the number of neutrophils (vehicle = 1.1 ± 0.2 × 10⁷/μl; IL-7 = 2.4 ± 0.1 × 10⁷/μl; p < 0.05) and monocytes (vehicle = 0.2 ± 0.07 × 10⁵/μl; IL-7 = 0.6 ± 0.09 × 10⁵/μl; p < 0.05) was observed in peripheral blood, and the number of erythrocytes was not modified (vehicle = 10.3 ± 0.3 × 10⁶/μl; IL-7 = 9.6 ± 0.3 × 10⁶/μl).

IL-7 induced production of other myelopoietic cytokines by T cells

IL-7-induced myelopoiesis required the presence of T cells (Table II) and was still observed in the absence of GM-CSF and IL-3 production (Fig. 6), suggesting that, in addition to GM-CSF and IL-3, IL-7 induced production of other myelopoietic cytokines. For example, lymph node cells ex vivo from B6 mice treated with IL-7 produced IFN-γ (Fig. 2C), which can promote myelopoiesis indirectly (43). Therefore, T cells were purified from lymph nodes of B6 mice (treated with IL-7 in vitro), and IFN-γ, Flt3-L, and M-CSF production and mRNA expression were examined at 24 h at 5 and 7 days (Fig. 7, A–C). IFN-γ and Flt3-L protein levels in sup were greatly increased by IL-7 stimulation (Fig. 7, B and C). RT-PCR analysis showed that IL-7 induced the expression of IFN-γ gene at all time points, whereas Flt3-L gene expression was constitutive, and only slightly up-regulated by IL-7 at day 5 and 7. Thus, the rise of in Flt3-L protein in the sup occurred primarily through posttranscriptional mechanism as described in many other cell types (44-46). M-CSF gene was constitutively expressed by T cells, its expression was maintained only in the presence of IL-7, its product, however, was not detected in the sup of the T cells at any time point.

Effects of IL-7 in IFN-γ−/− mice

IFN-γ can promote myelopoiesis indirectly (43), in contrast it can suppress murine myeloid colony formation in vitro (47). IFN-γ-deficient mice cannot control mycobacterial and Toxoplasma gondii infections, but, after infection, they show an increased myeloid cell proliferation as compared with the control mice (48, 49). To determine whether IFN-γ mediated the IL-7-induction of myelopoiesis, we investigated the response of IFN-γ-deficient mice. We treated IFN-γ-deficient mice with IL-7 or vehicle for 9 days. IL-7 treatment increased the spleen size and the total number of splenocytes (vehicle = 89.0 ± 8.0 × 10⁶ cells; IL-7 = 351.1 ± 83 × 10⁶ cells).

Table III. Induction of myelopoiesis and erythropoiesis by IL-7

<table>
<thead>
<tr>
<th>CD3-Depleted Bone Marrow Cells</th>
<th>Expt. 1a</th>
<th>Expt. 2a</th>
<th>Expt. 3a</th>
<th>Mean ± SEMb</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF</td>
<td>nd</td>
<td>0.5 ± 0.5</td>
<td>91.5 ± 0.5</td>
<td>60.6 ± 12</td>
</tr>
<tr>
<td>SCF + IL-3</td>
<td>26 ± 3</td>
<td>64.5 ± 0.5</td>
<td>54.5 ± 3.6</td>
<td>43.8 ± 5.5</td>
</tr>
<tr>
<td>SCF + supa</td>
<td>25 ± 1.5</td>
<td>64.5 ± 0.5</td>
<td>60 ± 10</td>
<td></td>
</tr>
<tr>
<td>SCF + sup + anti-IL-7/IL-3</td>
<td>26 ± 1.5</td>
<td>64.5 ± 0.5</td>
<td>60 ± 10</td>
<td></td>
</tr>
<tr>
<td>SCF + sup + anti-IL-7/GM-CSF</td>
<td>22 ± 1</td>
<td>64.5 ± 0.5</td>
<td>60 ± 10</td>
<td></td>
</tr>
<tr>
<td>SCF + sup + anti-IL-7/Flt3-L</td>
<td>24 ± 1</td>
<td>64.5 ± 0.5</td>
<td>60 ± 10</td>
<td></td>
</tr>
<tr>
<td>SCF + sup + anti-IL-7/IFN-γ</td>
<td>27 ± 1</td>
<td>64.5 ± 0.5</td>
<td>60 ± 10</td>
<td></td>
</tr>
<tr>
<td>SCF + sup + anti-IL-7/IL-3/IFN-γ</td>
<td>22 ± 4</td>
<td>64.5 ± 0.5</td>
<td>60 ± 10</td>
<td></td>
</tr>
<tr>
<td>SCF + sup + anti-IL-7/Flt3-L</td>
<td>11 ± 2.5</td>
<td>64.5 ± 0.5</td>
<td>60 ± 10</td>
<td></td>
</tr>
<tr>
<td>SCF + sup + anti-IL-7/GM-CSF/Flt3-L</td>
<td>12 ± 0</td>
<td>64.5 ± 0.5</td>
<td>60 ± 10</td>
<td></td>
</tr>
<tr>
<td>SCF + sup + anti-IL-7/IL-3/GM-CSF</td>
<td>8.5 ± 4.5</td>
<td>15.5 ± 0.5</td>
<td>17.5 ± 6.3</td>
<td>13.83 ± 2.3c</td>
</tr>
<tr>
<td>SCF + sup + anti-IL-7/IL-3/GM-CSF/IFN-γ</td>
<td>11.5 ± 0.5</td>
<td>15 ± 5</td>
<td>12.5 ± 2.5</td>
<td>13 ± 1.5</td>
</tr>
<tr>
<td>SCF + sup + anti-IL-7/IL-3/GM-CSF/Flt3-L</td>
<td>5.5 ± 1.5</td>
<td>11 ± 0</td>
<td>9 ± 2.5</td>
<td>8.6 ± 1.26c</td>
</tr>
</tbody>
</table>

* Number of colonies/5 × 10⁶ plated CD3− bone marrow cells ± SEM.
* Mean number of colonies/5 × 10⁶ plated cells ± SEM derived from duplicate-quadruplicate cultures for each experiment.
* Concentrations of cytokines and Abs anti-cytokines as reported in Materials and Methods.
* Supernatant of purified T cells from lymph nodes treated with IL-7 as described in Materials and Methods. The sup of untreated T cells did not induce colony formation.
* Not tested.
* Mann-Whitney correlation test, p < 0.05 (vs CD3− bone marrow cells + SCF + sup: 43.8 ± 5.5).
* Mann-Whitney correlation test, p < 0.05 (vs CD3− bone marrow cells + SCF + sup + anti-IL-7/IL-3/GM-CSF: 13.83 ± 2.3).
cells; \( p < 0.05 \). The distribution of splenic subpopulations showed no difference in the effect of IL-7 on these mice compared with wild-type mice (Fig. 8). Thus, IFN-\( \gamma \), like GM-CSF and IL-3, although induced by IL-7, was not essential to the myelopoietic response.

**Myeloid colony formation in the presence of sup from IL-7-stimulated T cells and inhibitory effect of anti-cytokine Abs**

Our results suggested that a combination of more than two cytokines produced by T cells in response to IL-7 was responsible for its myelopoietic effect. We then investigated whether the sup of purified T cells, stimulated with IL-7, was able to induce the formation of myeloid colonies using bone marrow cells depleted of endogenous T cells (CD3-depleted bone marrow) in a clonogenic assay. We observed that, in the presence of SCF, CD3-depleted bone marrow cells generated a number of myeloid colonies similar to that induced in response to SCF plus IL-3 (Table III). This observation allowed us to test whether combining anti-GM-CSF (2 \( \mu \)g/ml), IL-3 (2 \( \mu \)g/ml), Flt3-L (2.4 \( \mu \)g/ml), and IFN-\( \gamma \) (5 \( \mu \)g/ml) Abs could be inhibitory. To neutralize the effect of residual IL-7 present in the sup, an anti-IL-7 Ab (1 \( \mu \)g/ml) was always added. Preliminary experiments (data not shown) and the results of one of the experiments shown in Table III indicated that none of the Abs was inhibitory when used alone. The combination of anti-GM-CSF and anti-IL-3 Abs significantly inhibited colony formation. The inhibition observed with the combination of anti-GM-CSF and anti-IL-3 Abs significantly increased by adding anti-Flt3-L but not anti-IFN-\( \gamma \) Ab. This result suggests that our observations in vivo could be explained by Flt3-L acting together with GM-CSF and IL-3, all of which were induced by IL-7.

**IL-7Ra-chain expression by TER-119+ erythroid cells**

GM-CSF and IL-3 exhibit erythroid burst-promoting activity (50, 51); however, IL-7 increased the number of TER-119+ cells in GM-CSF-IL-3-deficient mice as well as in T cell-deficient mice (Fig. 5). This effect suggested that IL-7 might also act directly on erythroid progenitors. The Ag recognized by the anti-TER-119 mAb is expressed in the erythroid lineage from erythroblasts to erythrocytes (52). TER-119+ cells are terminally differentiated cells, thus erythroid colonies in normal mice mainly derive from erythroid precursors before the TER-119+ stage (52). We hypothesized that a percentage of TER-119+ cells could have been derived from IL-7R+ progenitors. Because IL-7R is composed of IL-7Ra and common \( \gamma \)-chains, we therefore investigated whether TER-119+ cells (from untreated mice) expressed IL-7Ra and \( \gamma \)-chains. RT-PCR analysis showed that sorted TER-119+ cells, from bone marrow or from spleen, expressed the IL-7Ra-chain gene, whereas the IL-7-unresponsive Z210R.1 cell line, or TER-119+ cells from IL-7Ra-chain-deficient mice, used as negative controls, did not. Depletion of CD45+ or Mac-1+ cells did not affect IL-7Ra-chain gene expression of TER-119+ cells, making it unlikely that these were lymphocytes or monocytes (Fig. 9). The common \( \gamma \)-chain gene was transcribed by TER-119+ cells from bone marrow or from spleen, TER-119+ CD45+ cells, TER-119+ Mac-1+ cells, and by the Z210R.1 cell line, but not by the common \( \gamma \)-chain-deficient mice spleen cells used as a negative control. Analysis by flow cytometry showed that about a third of the bone marrow TER-119+ cells expressed IL-7Ra on their surface, and a similar proportion expressed surface common \( \gamma \)-chain (Fig. 10). Depletion of CD45+ and Mac-1+ cells completely eliminated the nonspecific staining with the isotype-matched control Abs but not their specific TER-119 and IL-7Ra-chain staining (Fig. 11), because an additional specificity control IL-7Ra-chain staining was not observed on TER-119+ bone marrow cells from IL-7Ra-chain-deficient mice, which were used as an additional negative control (Fig. 11). The presence of cells positive for both IL-7R and TER-119 in the bone marrow suggested that IL-7R+ progenitors could respond directly to IL-7 rather than indirectly, as in the case of the myeloid progenitor.

**IL-7 promotes erythroid colony formation in vitro**

The Ag recognized by the anti-TER-119 mAb is expressed in the erythroid lineage from erythroblasts to erythrocytes (52). Erythroid colonies in normal mice mainly derive from erythroid precursors before the TER-119+ stage (52). The expression of IL-7Ra-chain on a percentage of TER+ cells suggested that IL-7 might promote
both total and erythroid colonies from IL-7Rα⁺ progenitors. The increase in the number of total colonies was due to an increase in the number of B lymphoid colonies (data not shown), as also shown by previous studies using IL-7 in combination with SCF in both methylcellulose (53, 54) and agar (55, 56) clonogenic assays. There was no effect of IL-7 on IL-7Rα⁺ progenitors (Table IV). In unfractionated bone marrow cells there was not a significant IL-7 effect, presumably because the frequency of IL-7Rα⁺ progenitors was small compared with IL-7Rα⁻ progenitors. As mentioned above, GM-CSF and IL-3 exhibit erythroid burst-promoting activity (50, 51). Because IL-7Rα⁻ T cells are present within IL-7Rα⁺ bone marrow fraction, they could have been responsible for the IL-7 effect by producing CSF. We therefore performed the assay comparing IL-7Rα⁻ bone marrow cells before and after depletion of CD3⁺ cells, and found that the CD3⁻ depleted IL-7Rα⁻ cells did not exhibit a decreased response to IL-7 (Table V). A number of reports indicate that B cell progenitors can differentiate into osteoclasts, myeloid cells, and macrophages, and that differentiation could be oriented by the factors present in the microenvironment (19, 20, 22, 23). We observed that IL-7 increased the total and erythroid colony formation of IL-7Rα⁺ CD3⁻ B220⁻ bone marrow cells from bone marrow in the presence of EPO, IL-3 and SCF to an extent similar to that observed for unfractionated IL-7Rα⁺ and IL-7Rα⁺ CD3⁻ subpopulations (Table V), suggesting that within this subpopulation some cells could be oriented toward erythroid differentiation.

**Discussion**

IL-7 is required in the lymphoid lineage but when given therapeutically has effects on other hemopoietic cells. Previous studies showed that administration of IL-7 to mice increased the mobilization of pluripotent hemopoietic progenitor cells from the bone marrow to peripheral sites (24–26). In this study, we show that treating mice with IL-7 also substantially increases myelopoiesis and erythropoiesis. The myelopoietic effect of IL-7 was mediated by T cells in vivo, which we showed in vitro can produce at least four myelopoietic cytokines in response to IL-7. The erythropoietic effect of IL-7 was independent of T cells, and our results suggest a direct effect of IL-7 on a subset of IL-7Rα⁺ erythroid progenitors.

IL-7R is composed of IL-7Rα and common γ-chains, both of which are required for signal transduction (57). The common γ-chain is a shared component of several cytokine receptors (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21). The IL-7Rα-chain is shared by the TSLP receptor (58). In the lymphoid, lineage expression of the IL-7Rα-chain is turned on or off at various stages. IL-7Rα-chain is not expressed by primitive myeloid progenitors, immature or mature neutrophils (27–29). In addition, myeloid colony formation supported by IL-3 or by other CSF in methylcellulose clonogenic

### Table IV. Effect of IL-7 on erythroid colony formation of IL-7Rα-chain-positive vs -negative bone marrow cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Cytokines</th>
<th>Coloniesᵃ</th>
<th>Benzidine Positivityᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-7Rα⁺ chain</td>
<td>EPO/IL-3/SCF</td>
<td>3.6 ± 0.6</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>positive</td>
<td>EPO/IL-3/SCF/IL-7</td>
<td>31.0 ± 9.6⁺</td>
<td>6.5 ± 1.7⁺</td>
</tr>
<tr>
<td>IL-7Rα⁻ chain</td>
<td>EPO/IL-3/SCF</td>
<td>25.2 ± 4.3</td>
<td>5.6 ± 0.9</td>
</tr>
<tr>
<td>negative</td>
<td>EPO/IL-3/SCF/IL-7</td>
<td>22.8 ± 3.0</td>
<td>5.1 ± 0.6</td>
</tr>
</tbody>
</table>

ᵃ (Colonies/cell plated) × 10⁴.
ᵇ Colonies containing cells showing intense blue staining were scored as benzidine positive. Results from three experiments are expressed as means ± SE (*, Mann-Whitney correlation test, p < 0.05).
assay is not increased by IL-7 (55, 59), so we sought an indirect effect of IL-7 on myelopoiesis. All mature T cell subpopulations express IL-7Rα, although expression is transiently inhibited by stimulation through the IL-7R (60, 61).

To test the possibility that the IL-7 myelopoietic effect was T cell mediated, we evaluated the effect of IL-7 in Rag1-deficient mice, which lack mature T and B cells (62). Whereas IL-7 administration increased the number of immature B cells it did not induce myelopoiesis, suggesting that T cells might mediate myelopoiesis by producing CSF in response to IL-7 treatment. We found that lymph node cells, obtained from IL-7-treated mice ex vivo, or stimulated with IL-7 in vitro, produced GM-CSF and IL-3, independent of TCR stimulation. These cytokines powerfully promote proliferation of early progenitors and maturation in neutrophils and macrophages (43).

IL-7 maintains survival and homeostatic proliferation of naive and memory T cells in vivo (5–10). In vitro, however, it has been reported that naive T cells do not proliferate in response to IL-7 (63–65), whereas memory T cells have been reported to slowly cycle (Ref. 9 and our unpublished results). We observed that, in vitro, only cells with the memory phenotype, CD44bright, produced GM-CSF and IL-3 in response to IL-7.

Because GM-CSF–IL-3-deficient mice also responded to IL-7 by increasing myelopoiesis, this result suggested that additional myelopoietic factors were also produced by IL-7-stimulated T cells. SCF, IL-3, G-CSF, GM-CSF, M-CSF, Flt3-L, IL-6, and IL-11 promote myelopoiesis directly and indirectly (43, 66–68), whereas IFN-γ, IL-1, and TNF-α work indirectly (43). Homozygous disruption of the G-CSF gene causes a partial reduction of the number of granulocyte-macrophage progenitors and circulating neutrophils, but no defects in myelopoiesis are evident after disruption of the genes encoding the other factors, indicating a high degree of redundancy (43, 66, 69). In keeping with this redundancy, we found that deletion of IFN-γ alone or deletion of the combination of GM-CSF and IL-3 failed to eliminate the myelopoietic response to IL-7. The reported effects of IFN-γ are complex because IFN-γ is also able to suppress murine myeloid colony formation in vitro; however, the magnitude of suppression strictly depends on the levels of CSF present in the culture. It has been shown that after infection, IFN-γ-deficient mice show a myeloid cell increase relative to the control mice (48, 49). However, in our experiment, IFN-γ-deficient mice did not show a myelopoietic response to IL-7 that was more elevated than the control mice, suggesting that, at least in the absence of an infection, the suppressive activity of IFN-γ is not prevailing. GM-CSF–IL-3 and IFN-γ stimulate G-CSF production by several cell types (reviewed in Ref. 43). Normal T lymphocytes do not produce G-CSF (70); however, GM-CSF, IL-3, and IFN-γ stimulate G-CSF production by several cell types (43). T cells produced these cytokines in response to IL-7, thus it cannot be excluded that G-CSF levels could be increased indirectly by IL-7 in vivo. Radiolabeled IL-7 was shown to bind murine bone marrow macrophages (although expression of IL-7Rα was not specifically reported), and to induce phosphorylation of the common γ-chain-associated kinase Jak3 (71), production of cytokines, and tumoricidal activity (72). However, IL-7 did not increase the number of monocytes in Rag1-deficient mice, suggesting that it requires lymphocytes to induce monocyte proliferation, it also could not induce peritoneal macrophages to produce G-CSF in vitro (data not shown). It is very likely that Flt3-L gives an important contribution to the IL-7 myelopoietic effect. Flt3-L is produced constitutively and retained intracellularly within the Golgi and close to the Golgi apparatus (46). In agreement with a previous report (46), we observed that IL-7 induced release of Flt3-L while having little effect on its transcription. Flt3-L alone stimulates the growth of myeloid progenitors (73–75) and also acts in synergy with SCF, IL-3, GM-CSF, IL-6, and, importantly, with IL-7 itself (73–76). Its chronic expression in mice increases peripheral blood cell count affecting all lineages, including neutrophils and monocytes (77). Because our data suggested a combined effect of multiple cytokines on myelopoiesis, we tested the effect of anti-cytokine Abs to neutralize the formation of myeloid colonies induced by the sup of T cells stimulated with IL-7 in vitro. To avoid the interference of T cells possibly responding to residual IL-7 present in the sup, we used CD3-depleted bone marrow as responding cells and always included a neutralizing anti-IL-7 Ab. The combination of anti-GM-CSF and anti-IL-3 was partially inhibitory, the addition of anti-Flt-3 but not anti-IFN-γ Ab increased this inhibition. We did not expect the combination of GM-CSF and IL-3 to significantly inhibit, because GM-CSF–IL-3-deficient mice respond to IL-7 like the control mice. Possible explanations are that the amount of Flt3-L produced in vivo might be greater than in vitro and could override the absence of GM-CSF and IL-3, and/or that in vivo other factors in addition to Flt3-L are produced.

The mAb TER-119 recognizes erythroid cells from the erythroblast stage to the mature erythrocyte stage (52). GM-CSF and IL-3 exhibit erythroid BFU activity in vitro (50, 51), and administration of IL-3 in mice increases the number of splenic erythroid cells (78). Similarly, administration of IL-7 increased the number of splenic TER-119+ erythroid cells. Like GM-CSF and IL-3 (78, 79), IL-7 did not affect the number of erythrocytes in peripheral blood. Perhaps in vivo another factor is limiting for this effect, EPO being one candidate. GM-CSF and IL-3, however, did not mediate the erythropoietic effects of IL-7 because the increase in erythropoiesis was observed in GM-CSF–IL-3-deficient mice. The ability to produce erythroid colonies belongs to erythroid precursors before they express TER-119 (52). The expression of the IL-7Rα-chain and common γ-chain by a percentage of TER-119+ cells suggested that IL-7 may have, as GM-CSF and IL-3, erythroid BFU activity. IL-7 was then added to the combination of cytokines used for the BFU-erythroid assay. The IL-7Rα+ fraction of bone marrow cells showed an impressive response to IL-7, increasing the number of total and hemoglobin-positive colonies. The increase in the number of total colonies was due to an increase in the number of B lymphoid colonies (data not shown), as also shown by previous studies using IL-7 in combination with SCF in methylcellulose (53, 54) and in agar clonogenic assays (55, 56). B/myeloid progenitors have been identified in adult bone marrow (19, 20), and, interestingly, it has been suggested that the macrophage differentiation pathway is favored by CSF such as GM-CSF and M-CSF (22). Interestingly, IL-7-responsive B220+ cells in bone marrow can differentiate into osteoclasts (21). In keeping with a previously mentioned report (40) and with our results, functioning T cells were required in addition to IL-7 to induce osteoclast maturation, because in osteopetrotic mice with impaired T cell activation (80), IL-7 mainly induced an increase in B cells (23). In contrast to IL-7 acting through T cells to induce myelopoiesis, IL-7 erythropoietic effect appeared to occur directly on the progenitors in conjunction with other hemopoietic factors including EPO. The relevant IL-7-responsive progenitor could be related to the pro-B cell, which proliferates in response to IL-7 (81), and a B cell membrane-derived protein was shown to exhibit erythroid burst-promoting activity (82). We found that cells within the IL-7R–CD3+ B220+ population could be oriented to form erythroid colonies. IL-7R+ CD3– B220+ cells in the bone marrow that represent 1.6% of the IL-7R+ population could also exhibit erythroid potential, and this hypothesis is currently under investigation.
IL-7 has been shown to enhance resistance to a variety of microbial infections (reviewed in Ref. 83). The increase we observe in neutrophils and monocytes could contribute to this resistance. Moreover, IFN-γ, which we show is induced by IL-7, has powerful antiviral and antibacterial activities. GM-CSF, also induced by IL-7, could also be protective because GM-CSF-deficient mice show increased susceptibility to diseases caused by bacteria and protozoa (84–86), and treatment with GM-CSF has beneficial effects on the course of these infections (87–89).

Trials are currently underway to evaluate IL-7 in man. If IL-7 acts in humans as predicted from rodent and monkey studies, it would be a promising therapeutic for accelerating recovery of lymphocytes after hematopoietic stem cell transplantation, in restoring CD4+ T cells in AIDS, and for promoting antitumor and antimicrobial immune responses. Our observation that IL-7 induces myelopoiesis and erythropoiesis would be a desirable effect in most clinical situations, and, interestingly, the erythropoietic effect would be predicted to occur also in patients deficient in T cells.

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Disclosures
The authors have no financial conflict of interest.

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2. Noza et al. 1996. IFN-γ-induced CD4+ T cells in AIDS.


