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IL-7 Induces Myelopoiesis and Erythropoiesis[1]

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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.**

Interleukin-7 plays a central role in lymphopoiesis. It is essential for T cell development: mutations in IL-7, IL-7R α, or common γ-chains, or in the receptor-associated kinase Jak3 result in a dramatic block in T cell development in the thymus (1–4). IL-7 is also required for T cell survival after leaving the thymus (5–10). IL-7 induces proliferation of murine B cell progenitors (11), and, in combination with Flt3 ligand (Flt3-L),[3] it is indispensable for B cell development in mice (12).

IL-7 is then considered primarily as a growth and antiapoptotic factor for lymphocytes, and has a potential clinical use for the treatment of immunodeficiencies. Whether IL-7 has important effects on nonlymphoid cells is less explored. The aim of this study was to investigate the effects of IL-7 on nonlymphoid cells in vivo. IL-7 deletion does not affect the development of nonlymphoid hemopoietic lineages (13). However, there are a few reports which suggest that exogenously administered IL-7 can elicit other hemopoietic effects. In vitro, in combination with a number of CSF, IL-7 was shown to increase the number of colonies formed by primitive murine hemopoietic progenitors (Lin$^-$Sca$^+$), but it was ineffective when used alone, and it did not affect myeloid differentiation induced by the CSF (14). IL-7R is expressed on Lin$^-$*Sca*I^{low} lymphoid progenitors, which did not show myeloid differentiation potential (15). However, there is now evidence that myeloid potential can persist in T and B cell lineages even after they have diverged (16–18). B/myeloid progenitors have been identified in adult bone marrow (19–21), and, interestingly, it has been suggested that differentiation is influenced by CSF present in the microenvironment (22, 23). Recently, B220$^+$ cells with myeloid potential responsive to IL-7 have been identified (23). IL-7 has been shown to increase mobilization of long-term reconstituting hemopoietic progenitors to peripheral organs in normal and irradiated mice (24–26). It was not shown whether mature myeloid cells developed in response to IL-7, and it has also not been determined whether the effects on hemopoietic cells are direct effects of IL-7 or secondary to other induced cytokines.

We report in this study that administration of IL-7 to mice increased the numbers of myeloid, monocytic, and erythroid cells in the spleen, and increased numbers of neutrophils and monocytes in peripheral blood. Because primitive myeloid progenitors, as well as mature myeloid cells do not express IL-7R (27–29), we investigated whether some of these effects could be due to cytokines produced in vivo in response to IL-7.

The nonlymphoid effects of IL-7 may have clinical relevance when IL-7 is given to patients, with the aim of promoting lymphocyte survival as proposed, for example, in the treatment of AIDS, transfer of tumor-reactive T cells, and reconstitution of T cells following bone marrow transplantation (30).

Materials and Methods

Mice

C57BL/6 (B6) mice were obtained from the Animal Production Facility of the National Cancer Institute-Frederick Cancer Research and Development Center (Frederick, MD). Rag1-deficient mice, B6 background IFN-γ-deficient mice, and common γ-chain-deficient mice were obtained from The Jackson Laboratory. GM-CSF-IL-3-deficient mice were backcrossed onto the B6 background as previously described (31) and maintained in our facility. Animal care was provided in accordance with procedures outlined

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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.

Table I. *Spleen cell subpopulations in B6 mice after treatment with IL-7 in vivo*

Cell Populations	Treatment	
	Vehicle	IL-7
	Cell number ($\times 10^{-6}$)[a]	
$B220^{+}$[b]	37.3 ± 6.0	179.9 ± 29.8*
$B220^{+}IgM^{+}$[b]	24.5 ± 3.2	129.9 ± 24.5*
$CD3^{+}$[b]	31.0 ± 2.8	64.3 ± 6.1*
Gr-1^{+}[b]	6.0 ± 0.6	27.7 ± 3.1*
Mac-1^{+}[b]	2.3 ± 0.5	8.1 ± 1.8*
TER-119^{+}[c]	3.6 ± 0.8	26.6 ± 9.8*

[a] 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, $p < 0.05$).

[b] Vehicle, $n = 4$; IL-7, $n = 4$.

[c] Vehicle, $n = 3$; IL-7, $n = 3$.

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 Ca^{2+}, Mg^{2+}, 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 antimyeloperoxidase 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-FcγIII/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). $CD45^{-}Mac\text{-}1^{-}$ bone marrow cells were obtained by cell sorting as described above, by staining with allophycocyanin-conjugated anti-CD45.2 mAb (eBioscience) using allophycocyanin-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 allophycocyanin-conjugated isotype-matched mAb as a control for nonspecific staining (BD Pharmingen). TER-119^{+}, TER-$119^{+}CD45^{-}$, and TER-$119^{+}Mac\text{-}1^{-}$ 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-7Rα-chain-positive cells from bone marrow were obtained by cell sorting by staining with PE-Cy5-conjugated anti-IL-7Rα-chain (eBioscience), or PE-Cy5-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-7Rα-chain-positive cells by cytofluorometry. Viability was evaluated by trypan blue staining. The culture medium was RPMI 1640 supplemented with 10% FBS (HyClone), 2 mM glutamine, 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-FcγIII/IIR-blocking Ab for 15 min at 4°C,

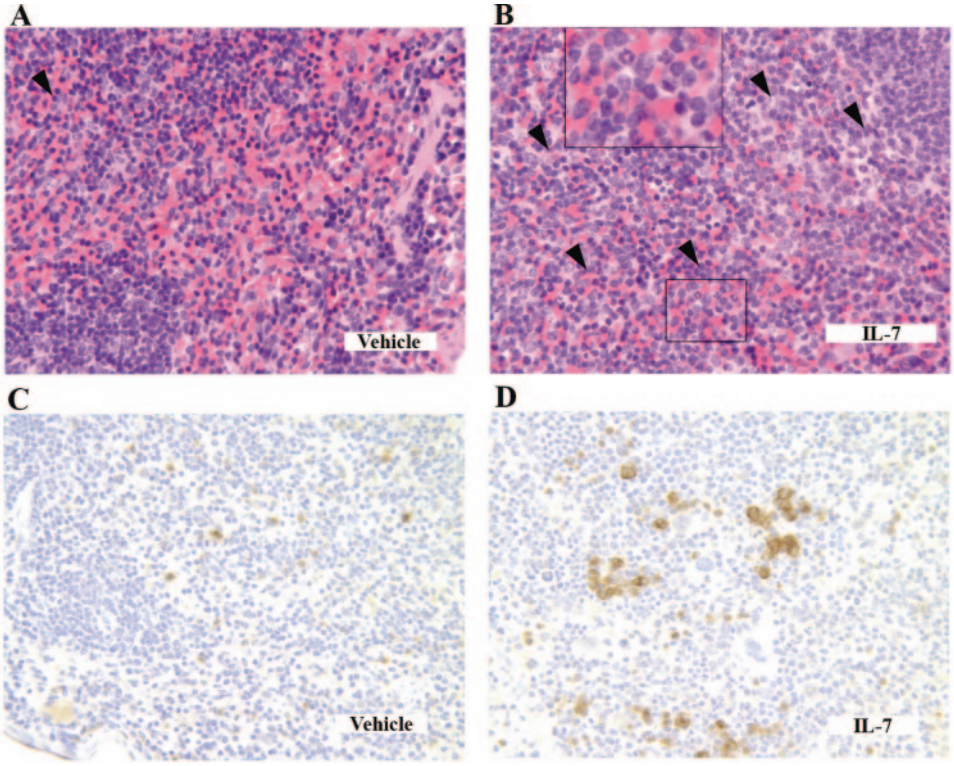

FIGURE 1. Spleen sections from mice injected with IL-7. B6 mice were injected with IL-7 or vehicle for 9 days. H&E stain (*A*), vehicle; H&E stain (*B*), IL-7, arrows indicate areas in the red pulp with myeloid differentiation (original magnification, ×40). *B* (*inset*), Higher magnification; *C*, immunohistochemical staining for myeloperoxidase, vehicle; immunohistochemical staining for myeloperoxidase (*D*), IL-7; (original magnification, ×40). Representative of three experiments.

Table II. *Spleen cell subpopulations in Rag1-deficient mice after treatment with IL-7 in vivo*

Cell Populations	Treatment	
	Vehicle[a]	IL-7[a]
	Cell number ($\times 10^{-6}$)[b]	
$B220^+$	3.0 ± 0.6	14.3 ± 6.1*
$B220^+IgM^+$	ND	ND
$CD3^+$	ND	ND
$Gr\text{-}1^+$	3.6 ± 1.0	3.3 ± 0.9
$Mac\text{-}1^+$	3.5 ± 0.8	5.8 ± 1.8
$TER\text{-}119^+$	0.57 ± 0.3	2.6 ± 0.4*

[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, $p < 0.05$).

and then stained with the following fluorochrome-conjugated mAb: FITC-conjugated anti-IgM, anti-CD3, anti-Mac-1, PE-conjugated anti-B220, anti-Gr-1, anti-TER-119 (BD Pharmingen), and PE-Cy5-conjugated anti-IL-7Rα-chain (eBioscience). Cells stained with FITC, PE (BD Pharmingen), or PE-Cy5-conjugated (eBioscience) isotype-matched mAb served as a control for nonspecific staining. To stain for the common γ-chain, cells were blocked with purified goat IgG (Sigma-Aldrich) for 20 min at 4°C. After indirect staining with unconjugated anti-γ-chain TUGm2 mAb or with the isotype-matched control Ab (BD Pharmingen) followed by PE goat anti-rat $F(ab')_2$ (Serotec), cells were washed and stained with anti-FITC TER-119 mAb or the isotype-matched control mAb. All staining and washing procedures were performed at 4°C in PBS/0.5% BSA. Samples were analyzed on a BD-LSR1 (BD Biosciences).

Assays for cytokine production

Cells were cultured with or without murine IL-7 (50 ng/ml) (PeproTech) as indicated. Cytokine levels in the supernatants (sup) were measured with specific ELISA kits: GM-CSF, IL-3, Flt3-L, M-CSF (R&D Systems), and IFN-γ (Pierce).

ELISPOT assay

The number of cells secreting IFN-γ and GM-CSF was evaluated according the manufacturer's instructions. Briefly, MultiScreen HTSIP plates (polyvinylidene difluoride membrane) were treated with ethanol, washed extensively with PBS, and coated with 100 μl/well anti-murine IFN-γ or GM-CFS mAb (1/60 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% CO_2. After washing with PBS/0.05% Tween 20, biotinylated anti-murine IFN-γ or GM-CSF-detecting Ab (100 μl/well, 1/60 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 phosphatase 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 TTG-3′ and reverse (R), 5′-AGC TGG AGA GGA GTC TCA TGG-3′ were used for M-CSF with a PCR product of 358 bp. Primer sequences F, 5′-TCC CTG TTG CTG CTG TTG C-3′

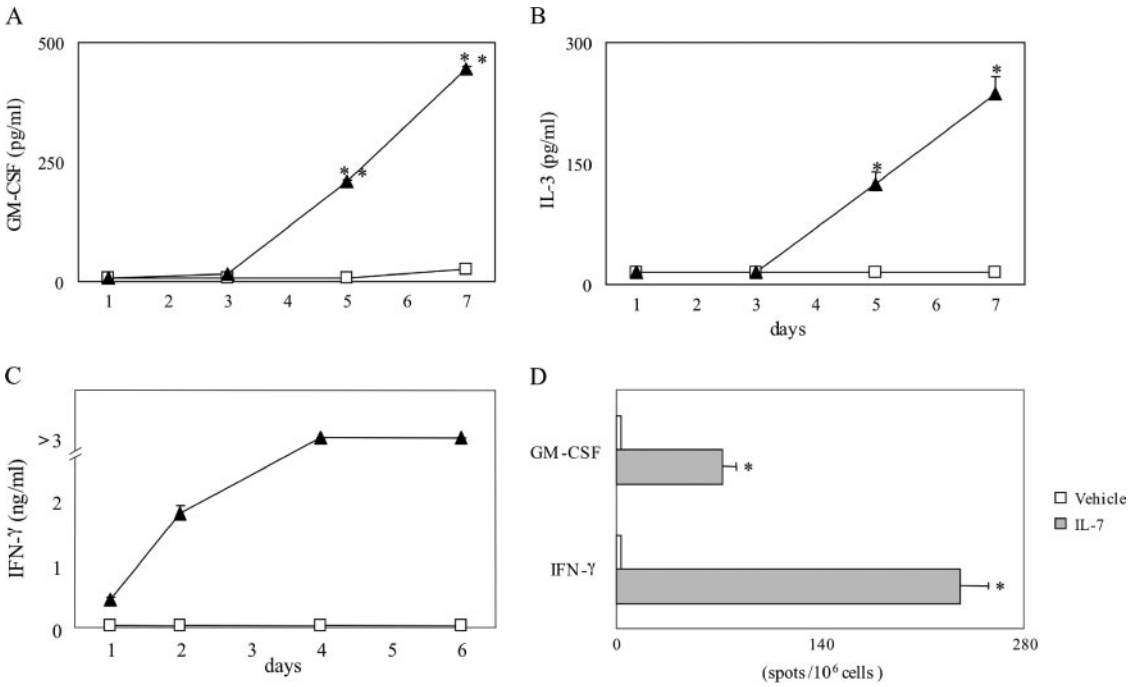

FIGURE 2. Production of GM-CSF, IL-3, and IFN-γ by cells from lymph nodes of mice treated with IL-7 in vivo. Cells obtained from lymph nodes of mice injected for 9 days with IL-7 or vehicle as described in *Materials and Methods* were cultured in medium (2.5×10^6/ml). At the indicated times, sup were 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.

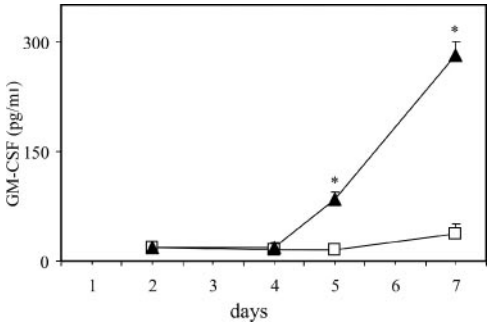

FIGURE 3. Production of GM-CSF by lymph node cells treated with IL-7 in vitro. Cells (2.5×10^6/ml) from lymph nodes of B6 mice were cultured with medium (□) or with IL-7 (50 ng/ml) (▲). At the indicated times, sup were collected and assayed by ELISA. Results from three experiments are expressed as means ± SEM (*, Mann-Whitney correlation test, $p < 0.05$).

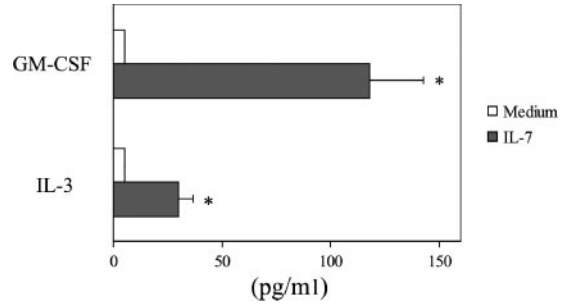

FIGURE 4. Production of GM-CSF and IL-3 by T cells treated with IL-7 in vitro. T cells (1.5×10^6/ml) purified from lymph nodes of B6 mice by negative selection were cultured with medium or with IL-7 (50 ng/ml). After 7 days, sup were collected and assayed by ELISA. Results from three experiments are expressed as means ± SEM (*, Mann-Whitney correlation test, $p < 0.05$).

and R, 5′-TGC AGG TGT CCT TCA GGA GG-3′ were used for Flt3-L, with a PCR product of 373 bp. Common γ-chain primer couples were obtained from the PrimerBank website (35), in particular, primer 7305181a1 and 7305181a3 have been selected as F and R primers, respectively, with a PCR product of 522 bp. All primers were spanning at least one intron-exon border, to exclude amplification of genomic DNA. DNA amplification was conducted in a total volume of 50 μl containing 40 pmol of each primer, 10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 200 mM each dNTPs, and 1 U of AmpliTaq Gold DNA polymerase (Applied Biosystems). PCR thermal profiles were as follows: 5 min at 94°C to activate the polymerase enzyme followed by 37 cycles; 94°C for 30 s, 55°C for 1 min, 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-inactivated FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin, with 0.35% weight/volume SeaPlaque agarose (Cambrex BioScience Rockland). Cells were plated at a density of 7×10^4 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% CO_2 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) (2.5×10^6/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 Lux 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% CO_2, 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% H_2O_2 were added to 500 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 performed 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 × 10^6 cells; IL-7 = 264.4 ± 39.7 × 10^6 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$^+$), monocytic (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 = 0.5 ± 0.2 × 10^3/μl; IL-7 = 1.9 ± 0.4 × 10^3/μl; $p < 0.05$). Following IL-7 treatment, the numbers of blood monocytes showed an increasing trend (vehicle = 0.1 ± 0.04 × 10^3/μl;

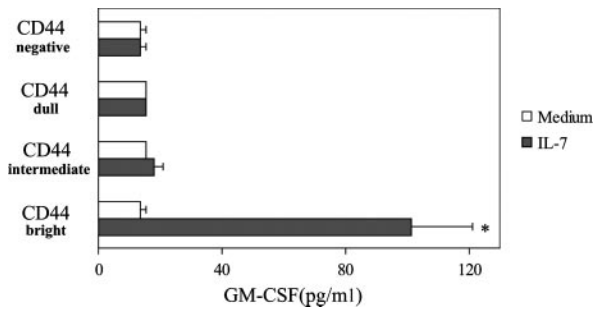

FIGURE 5. Production of GM-CSF by CD44$^+$ cells treated with IL-7 in vitro. Cells expressing different levels of surface CD44$^+$ (0.6×10^6/ml) purified from lymph nodes of B6 mice by cell sorting were cultured with medium or with IL-7 (50 ng/ml). After 7 days, sup were collected and assayed by ELISA. Results from three experiments are expressed as means ± SEM (*, Mann-Whitney correlation test, $p < 0.05$).

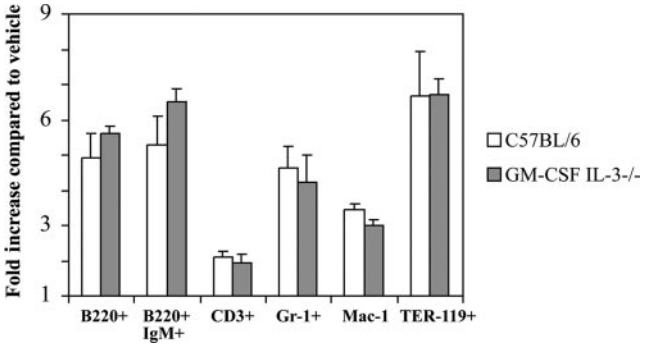

FIGURE 6. Spleen cell populations in B6 and GM-CSF-IL-3-deficient mice after treatment with IL-7 in vivo. GM-CSF-IL-3-deficient mice were treated with IL-7 ($n = 3$) or with vehicle ($n = 3$) as described in *Materials and Methods* for 9 days. The cell number of each population for individual spleen 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 × $10^3/\mu$l), 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 × $10^6/\mu$l; IL-7 = 10.0 ± 0.1 × $10^6/\mu$l).

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 × $10^3/\mu$l; IL-7 = 0.8 ± 0.4 × $10^3/\mu$l), monocytes (vehicle = 0.4 ± 0.1 × $10^3/\mu$l; IL-7 = 0.4 ± 0.2 × $10^3/\mu$l), or erythrocytes (vehicle = 10.6 ± 0.3 × $10^6/\mu$l; IL-7 = 9.0 ± 0.5 × $10^6/\mu$l) 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. 2*C*). The frequency of cells producing GM-CSF and IFN-γ is shown in Fig. 2*D*. 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

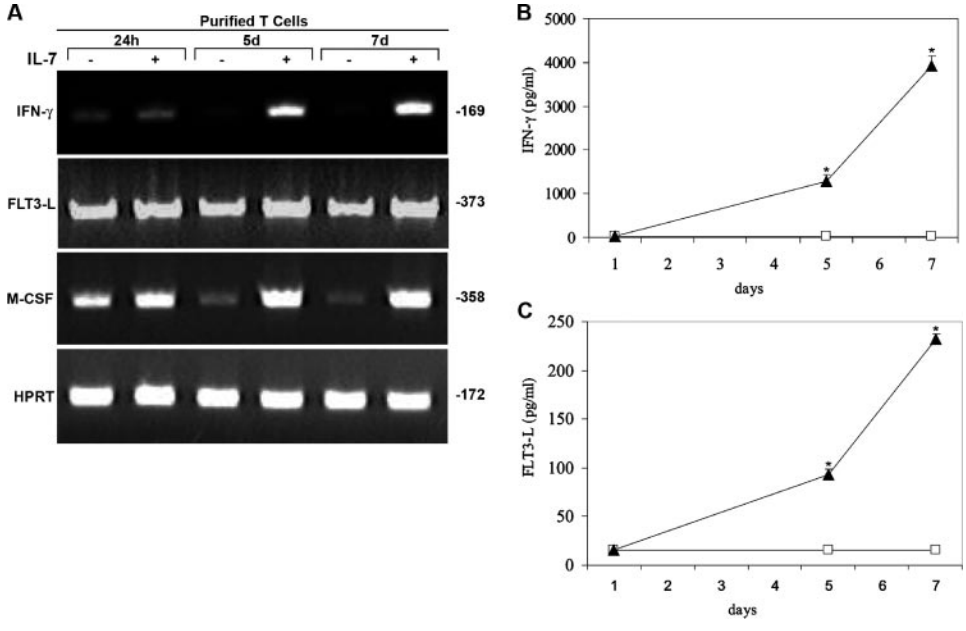

FIGURE 7. Production of IFN-γ and Flt3-L by T cells treated with IL-7 in vitro. Purified T cells (2.5 × 10^6/ml) purified from lymph nodes of B6 mice by negative selection were cultured with medium or with IL-7 (50 ng/ml). Results, representative of three experiments, are expressed as means ± SEM (*, Mann-Whitney correlation test, $p < 0.05$). *A*, RT-PCR analysis for IFN-γ, Flt3-L, M-CSF, and HPRT transcripts. *B* and *C*, Sup were collected and assayed by ELISA at the indicated times: medium (□); IL-7 (▲).

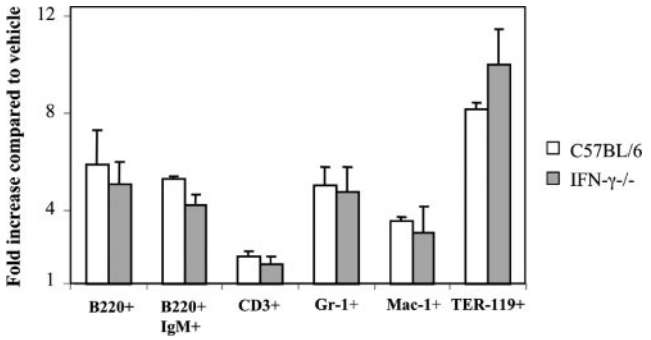

FIGURE 8. Spleen cell populations in B6 and IFN-γ-deficient mice after treatment with IL-7 in vivo. IFN-γ-deficient mice were treated with IL-7 ($n = 3$) or with vehicle ($n = 3$) as described in *Materials and Methods* for 9 days. The increases in spleen-cell population cell numbers in IL-7-treated mice are expressed as fold increase compared with vehicle + SEM ($n = 3$).

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 $CD44^{bright}$ cells produced GM-CSF, whereas $CD44^{int}$, dull, and negative cells did not (Fig. 5). Production of IL-3 was also restricted to $CD44^{bright}$ 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 = $62.9 \pm 9.0 \times 10^6$ cells; IL-7 = $254.4 \pm 39.7 \times 10^6$ 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 = $1.1 \pm 0.2 \times 10^3/\mu l$; IL-7 = $2.4 \pm 0.1 \times 10^3/\mu l$; $p < 0.05$) and monocytes (vehicle = $0.2 \pm 0.07 \times 10^3/\mu l$; IL-7 = $0.6 \pm 0.09 \times 10^3/\mu l$; $p < 0.05$) was observed in peripheral blood, and the number of erythrocytes was not modified (vehicle = $10.3 \pm 0.3 \times 10^6/\mu l$; IL-7 = $9.6 \pm 0.3 \times 10^6/\mu l$).

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. 2*C*), 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 = $89.0 + 8.0 \times 10^6$ cells; IL-7 = $351.1 + 83 \times 10^6$

Table III. *Induction of CFU-C by supernatant from IL-7-stimulated T cells: effect of anti-cytokine Abs*

CD3-Depleted Bone Marrow Cells	Expt. 1[a]	Expt. 2[a]	Expt. 3[a]	Mean ± SEM[b]
SCF[c]	nd	0.5 ± 0.5	0	
SCF + IL-3	26 ± 3	64.5 ± 0.5	91.5 ± 0.5	60.6 ± 12
SCF + sup[d]	25 ± 1.5	54.5 ± 3.6	60 ± 10	43.8 ± 5.5
SCF + sup + anti-IL-7/IL-3	26 ± 1.5	nt[e]	nt	
SCF + sup + anti-IL-7/GM-CSF	22 ± 1	nt	nt	
SCF + sup + anti-IL-7/Flt3-L	24 ± 1	nt	nt	
SCF + sup + anti-IL-7/IFN-γ	27 ± 1	nt	nt	
SCF + sup + anti-IL-7/IL-3/IFN-γ	22 ± 4	nt	nt	
SCF + sup + anti-IL-7/IL-3/Flt3-L	11 ± 2.5	nt	nt	
SCF + sup + anti-IL-7/GM-CSF/Flt3-L	12 ± 0	nt	nt	
SCF + sup + anti-IL-7/IL-3/GM-CSF	8.5 ± 4.5	15.5 ± 0.5	17.5 ± 6.3	13.83 ± 2.3*[f]
SCF + sup + anti-IL-7/IL-3/GM-CSF/IFN-γ	11.5 ± 0.5	15 ± 5	12.5 ± 2.5	13 ± 1.5
SCF + sup + anti-IL-7/IL-3/GM-CSF/Flt3-L	5.5 ± 1	11 ± 0	9 ± 2.5	8.6 ± 1.2**[g]

[a] Number of colonies/75 × 10^3 plated $CD3^-$ bone marrow cells ± SEM.
[b] Mean number of colonies/75 × 10^3 plated cells ± SEM derived from duplicate-quadruplicate cultures for each experiment.
[c] Concentrations of cytokines and Abs anti-cytokines as reported in *Materials and Methods.*
[d] 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.
[e] Not tested.
[f] Mann-Whitney correlation test, $p < 0.05$ (vs $CD3^-$ bone marrow cells + SCF + sup: 43.8 ± 5.5).
[g] 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).

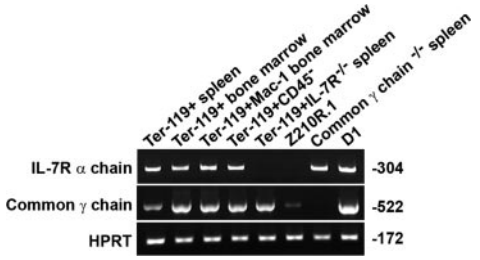

FIGURE 9. Expression of IL-7Rα-chain and common γ-chain genes by TER-119$^+$ cells. RT-PCR analysis for IL-7Rα-chain, common γ-chain, and HPRT transcripts. Purified cells were obtained by cell sorting. *Lane 1*, TER-119$^+$ cells from B6 mice spleens; *lane 2*, TER-119$^+$ cells from B6 mice bone marrow; *lane 3*, TER-119$^+$Mac-1$^+$ cells from B6 mice bone marrow; *lane 4*, TER-119$^+$CD45$^-$ cells from B6 mice bone marrow; *lane 5*, TER-119$^+$ from IL-7-Rα-chain-deficient mice bone marrow; *lane 6*, negative control (thymic stromal cell line Z210R.1); *lane 7*, TER-119$^+$ cells from common γ-chain-deficient mice bone marrow; *lane 8*, positive control (IL-7-dependent T cell line D1). Representative of three experiments.

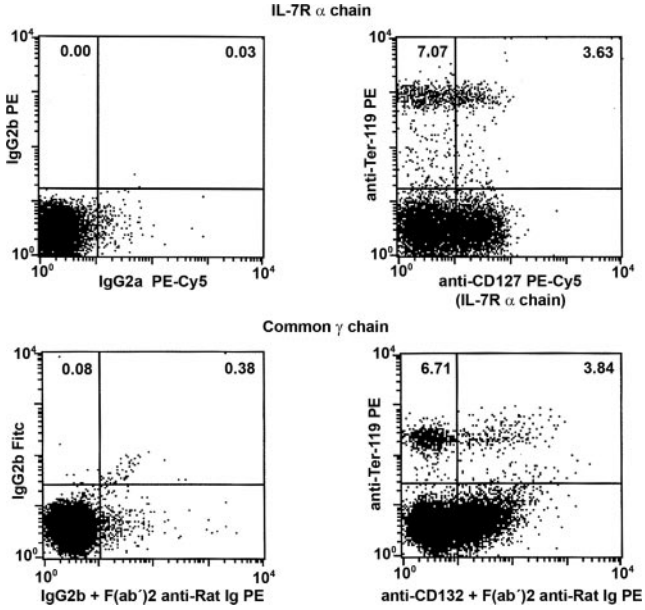

FIGURE 10. Surface expression of IL-7Rα-chain and common γ-chain by B6 bone marrow cells. *Upper panels*, Bone marrow cells of B6 mice stained with fluorochrome-conjugated mAb recognizing TER-119 and IL-7Rα-chain or with corresponding isotype-matched control Abs. *Lower panels*, Bone marrow cells of B6 mice stained with fluorochrome-conjugated mAb recognizing TER-119 and unconjugated mAb anti-common γ-chain plus goat anti-rat fluorochrome-conjugated F(ab′)$_2$ anti-rat Ig or with corresponding isotype-matched control Abs as indicated. Representative of three experiments.

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-γ, 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 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 μg/ml), IL-3 (2 μg/ml), Flt3-L (2.4 μg/ml), and IFN-γ (5 μg/ml) Abs could be inhibitory. To neutralize the effect of residual IL-7 present in the sup, an anti-IL-7 Ab (1 μg/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-γ Ab. This result suggests that our observations in vivo could be explained by Flt3-L acting together with GM-CSF and IL-3, all which were induced by IL-7.

IL-7Rα-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-7Rα and common γ-chains, we therefore investigated whether TER-119$^+$ cells (from untreated mice) expressed IL-7Rα and γ-chains. RT-PCR analysis showed that sorted TER-119$^+$ cells, from bone marrow or from spleen, expressed the IL-7Rα-chain gene, whereas the IL-7-unresponsive Z210R.1 cell line, or TER-119$^+$ cells from IL-7Rα-chain-deficient mice, used as negative controls, did not. Depletion of CD45$^+$ or Mac1$^+$ cells did not affect IL-7Rα-chain gene expression of TER-119$^+$ cells, making it unlikely that these were lymphocytes or monocytes (Fig. 9). The common γ-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 γ-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-7Rα on their surface, and a similar proportion expressed surface common γ-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-7Rα-chain staining (Fig. 11), because an additional specificity control IL-7Rα-chain staining was not observed on TER-119$^+$ bone marrow cells from IL-7Rα-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-7Rα-chain on a percentage of TER$^+$ cells suggested that IL-7 might promote

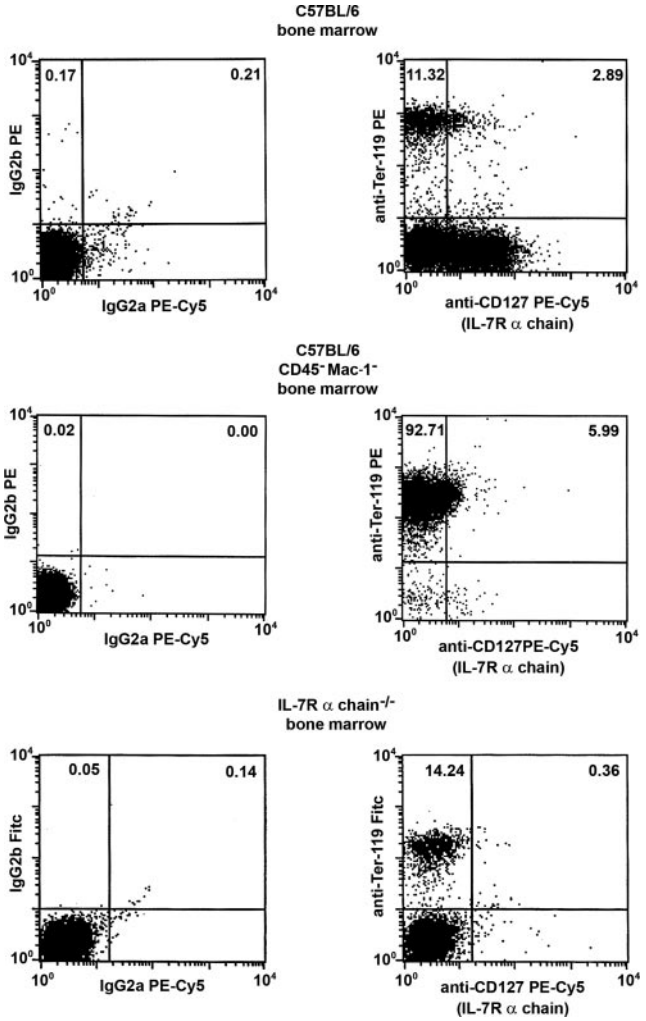

FIGURE 11. Surface expression of IL-7Rα-chain by B6 bone marrow cells after depletion of $CD45^+$ and $Mac\text{-}1^+$ cells. Staining with fluorochrome-conjugated mAb recognizing TER-119 and IL-7Rα-chain or with corresponding isotype-matched control Abs was performed before and after depletion of $CD45^+$ and $Mac\text{-}1^+$ cells by cell sorting. Staining with fluorochrome-conjugated mAb recognizing TER-119 and IL-7Rα-chain or with corresponding isotype-matched control Abs of bone marrow cells from IL-7Rα-chain-deficient mice is shown as negative control. Representative of three experiments.

erythroid colony formation. We therefore sorted IL-7Rα^+ bone marrow cells and performed an in vitro methylcellulose clonogenic assay for burst-forming unit (BFU)-erythroid colonies in the presence and absence of IL-7. As shown in Table IV, IL-7 greatly augmented the effect of EPO, IL-3, and SCF in the generation of

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

Cells	Cytokines	Colonies[a]	Benzidine Positivity[a,b]
IL-7Rα-chain positive	EPO/IL-3/SCF	3.6 ± 0.6	0.9 ± 0.2
	EPO/IL-3/SCF/IL-7	31.0 ± 9.6*	6.5 ± 1.7*
IL-7Rα-chain negative	EPO/IL-3/SCF	25.2 ± 4.3	5.6 ± 0.9
	EPO/IL-3/SCF/IL-7	22.8 ± 3.0	5.1 ± 0.6

[a] (Colonies/cell plated) × 10^4.
[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, $p < 0.05$).

Table V. *Erythroid colony formation by IL-7Rα^+CD3$^-$ and by IL-7Rα^+CD3$^-$B220$^+$ bone marrow cells*

Cells	Cytokines	Colonies[a]	Benzidine Positivity[b]
IL-7Rα^+	EPO/IL-3/SCF	1.8 + 0.2	0.6 + 0.2
	EPO/IL-3/SCF/IL-7	23.2 + 1.3*	4.0 + 0.3*
IL-7Rα^+CD3$^-$	EPO/IL-3/SCF	2.0 + 0.1	0.6 + 0.0
	EPO/IL-3/SCF/IL-7	20.4 + 3.2*	4.2 + 0.3
IL-7Rα^+CD3$^-$ B220$^+$	EPO/IL-3/SCF	2.4 + 0.5	0.9 + 0.2
	EPO/IL-3/SCF/IL-7	33.2 + 0.7*	7.1 + 1.2*

[a] (Colonies/cell plated) × 10^4.
[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. $p < 0.05$).

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-7R$^+$ T cells are present within IL-7R$^+$ bone fraction, they could have been responsible for the IL-7 effect by producing CSF. We therefore performed the assay comparing IL-7R$^+$ bone marrow cells before and after depletion of $CD3^+$ cells, and found that the CD3-depleted IL-7R$^+$ 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-7R$^+$CD3$^-$B220$^+$ cells from bone marrow in the presence of EPO IL-3 and SCF to an extent similar to that observed for unfractionated IL-7R$^+$ and IL-7R$^+$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-7R$^+$ 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

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, $CD44^{bright}$, 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 its 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 white 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 recover of lymphocytes after hemopoietic 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

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