The Stimulatory Effects of Interleukin (IL)-12 On Hematopoiesis Are Antagonized by IL-12-induced Interferon γ In Vivo

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Summary

Interleukin (IL)-12 synergizes with other cytokines to stimulate the proliferation and differentiation of early hematopoietic progenitors in vitro. However, in vivo administration of IL-12 decreases peripheral blood counts and bone marrow hematopoiesis. Here, we used interferon (IFN) γ receptor-deficient (IFNγR$^{-/-}$) mice to investigate whether the in vivo inhibition of hematopoiesis by IL-12 is indirectly mediated by IL-12-induced IFN-γ. IL-12 administered for 4 d (1 μg/mouse per day) resulted in lower peripheral blood counts and a 2-fold decrease in bone marrow cellularity in wild-type mice, but not in IFNγR$^{-/-}$ mice. Bone marrow hematopoietic progenitors were decreased after IL-12 treatment in wild-type mice, but rather increased in IFNγR$^{-/-}$ mice. Splenic cellularity was 2.3-fold higher after IL-12 administration in wild-type mice, largely due to natural killer (NK) cell and macrophage infiltration together with some extramedullary hematopoiesis. In IFNγR$^{-/-}$ mice, spleen cellularity was less increased, there were fewer infiltrating NK cells, but a strong extramedullary hematopoiesis. Thus, alterations mediated by IL-12-induced IFN-γ include reduction in bone marrow cellularity and hematopoietic progenitors, as well as pronounced splenomegaly, largely caused by NK cell infiltration. In the absence of IFN-γ signaling, IL-12 promotes hematopoiesis, consistent with its in vitro activities.

IL-12 is a heterodimeric cytokine that affects the growth and function of T and NK cells (1–4, for review see 5). Studies with human and murine cells have shown IL-12 to promote the development of Th1 cells, induce the secretion of IFN-γ from resting and activated T and NK cells, function as growth factor of T and NK cells, and enhance the cytolytic activity of NK cells and cytotoxic lymphocytes (6–10). In addition, IL-12 synergizes with other cytokines such as kit ligand (KL), IL-3, and IL-11 in stimulating the proliferation and differentiation of early hematopoietic progenitors in vitro (11–14).

In contrast to the stimulation of hematopoietic cells seen in vitro, IL-12 administration reportedly induced neutropenia, lymphopenia, and anemia in mice (unpublished observations reviewed in reference 5). Here, we confirm these results and further document a marked reduction in bone marrow cellularity together with a lower content in hematopoietic precursor after IL-12 administration in mice. The apparent discrepancy between in vitro and in vivo effects of IL-12 on

hematopoiesis may be explained by invoking secondarily produced antagonistic cytokines.

IL-12 was shown to strongly stimulate the transcription and secretion of IFN-γ by T cells and NK cells both in vitro (1, 8, 15) and in vivo (16, 17). IL-12 also induces TNF-α and IL-10 gene transcription in vivo (16). In contrast, IL-3 and IL-4 gene expression are suppressed after IL-12 treatment (16). Enriched human NK cells stimulated with IL-12 were recently reported to inhibit hematopoietic colony formation from bone marrow cells (18). This was attributed to IL-12-induced expression of IFN-γ and TNF-α by NK cells, suggesting that secondary cytokine production by lymphoid cells may play a role in the regulation of hematopoiesis (18). IFN-γ has been reported to be a negative hematopoietic and lymphopoietic regulator, based both on in vitro data (19–22) and on results obtained in vivo in mice and primates (for review see reference 23). The inhibitory effects of IL-12 treatment on in vivo hematopoiesis could therefore be a result of the IL-12-induced production of IFN-γ. In this study, IFN-γ receptor-deficient

mice (24) were used to address this question and to differentiate between the respective effects of IL-12 and IFN-γ on hematopoiesis in vivo.

Materials and Methods

Reagents. The following recombinant human (h) and murine (m) cytokines were used: hIL-1β, mIL-3, and mKL (Sandoz Pharma Ltd., Basel, Swizerland), hIL-11 (Genetics Institute, Cambridge, MA), and mIL-12 (Hoffmann-LaRoche, Nutley, NJ) (16). The antibodies used were rat mAb anti-mouse CD11b (Mac-1; BMA Biomedicals, Augst, Switzerland), mAb NK1.1-FITC conjugate (PharMingen, San Diego, CA), goat Ig anti-rat IgG conjugated to PE (Southern Biotechnology Associates, Birmingham, AL), and rat mAb anti-mouse macrophage IgG (F4/80; American Tissue Culture Collection, Rockville, MD).

In vivo treatments. 7–10-wk-old 129 Sv/Ev wild-type and IFN-γ receptor–deficient mice (24), bred and maintained under specific pathogen–free conditions, were injected intraperitoneally with either saline or mIL-12 (1 μg/mouse per d) for 4 or 7 consecutive days, as indicated. Blood was collected from the retroorbital sinus 24 h after the last injection.

Hematology and Cytology. Standard hemograms were performed with fresh heparinized blood on a hematology analyzer (Sysmex E-2500; Digitana, Switzerland). Reticulocytes were stained using thiazole orange (Becton Dickinson & Co., San Jose, CA) and were quantified by flow cytometry as percentage of erythrocytes. Blood smears stained with DIFF-QUIK® (Dade, Düdingen, Switzerland) were read in parallel. Cytospin preparations of bone marrow and spleen cells were stained with May-Grünwald-Giemsa, and differential analyses were performed on 300–500 cells.

Determination of Plasma IFN-γ Levels. Murine IFN-γ was analyzed by double sandwich ELISA (according to the method of Dr. L. Ozmen, Hoffmann-La Roche, Basel, Switzerland). Briefly, plates were coated with anti-mouse IFN-γ rat mAb AN18 (25; 10 μg/ml in 0.1 M NaHCO₃, pH 9.5) and blocked with 1% BSA in PBS (PBS/BSA). Samples and standards were added in combination with anti-mouse IFN-γ rat mAb XMG 1.2 coupled to peroxidase (26) in PBS/BSA and incubated overnight at room temperature. The reaction was developed with orthophenyldiamine substrate (Sigma Chemical Co., St. Louis, MO) and absorbance at 495 nm was read.

Flow Cytometric Analysis. Bone marrow and spleen cells were washed, stained with anti-mouse CD11b antibodies (45 min), washed three times, and further labeled with anti-rat IgG PE-conjugated Ig (30 min). Alternatively, an NK1.1 antibody–FITC conjugate was incubated for 45 min. Fluorescence analyses were performed on a FACScan® (Becton Dickinson & Co.) using Lysis II software.

Colony Assays from Spleen and Bone Marrow Cells. Cell suspensions from bone marrow and spleen prepared in isotonic balanced salt solution were counted in cytology chambers using Turk's coloration. Methylcellulose cultures were prepared in IMDM containing 4% FCS, BSA fraction V, transferrin, and lipids according to Iscove et al. (27) in the presence of optimized concentrations of erythropoietin (Epo; 0.08 U/ml) with either IL-3 (30 ng/ml) and IL-1 (10 ng/ml), or IL-11 (50 μl COS supernatant/ml) and KL (25 μl COS supernatant/ml), as indicated. Cells from treated mice were pooled for each group, and several concentrations of bone marrow (3–20 × 10⁴ cells/ml) and spleen cells (1–3 × 10⁵ cells/ml) were titrated in triplicate for each pool to ensure sufficient number of colonies per culture. Large (>1 mm) multilineage colonies comprising erythroid, megakaryocytic, and other myeloid cells, erythroid colonies (either pure or containing megakaryocytes) and

GM colonies were counted on day 9. The colonies were identified by their morphology and were periodically picked, spread, and stained (Wright–Giemsa) for confirmation. Results from three to six culture plates are combined for each determination.

Statistics. All results are pooled from two independent experiments and are expressed as mean ± SD with n = 4–8 animals per group. They were compared using the student's t test, and p <0.05 was considered statistically significant.

Results and Discussion

Since in vivo IL-12 administration was reported to induce neutropenia, lymphopenia, and anemia (5), we investigated the effect of IL-12 on hematopoiesis in normal mice. Mice were treated with 1 μg/mouse per d IL-12, a dose shown to give maximal effect on NK cell cytotoxic function (17), daily for 7 d and killed on day 8. Bone marrow cellularity was reduced from 12.5 ± 2.5 million cells per femur in the placebo-treated mice to 4.8 ± 1.1 million cells per femur in mice treated with IL-12 (n = 6). The frequency of bone marrow colony-forming hematopoietic progenitors (CFC) was also reduced by two- to fourfold after IL-12 treatment (not shown). The number of bone marrow cells per femur were already decreased by half, and the CFCs were reduced after 4 d of IL-12 treatment (see below; Figs. 1 and 2). Thus, in line with the depressed blood counts reported after IL-12 administration, we show that a short course of IL-12 was strongly myelodepressive in wild-type mice.

There was an apparent discrepancy between the in vivo myelotoxicity of IL-12 and its positive effects on hematopoietic progenitors in vitro. Indeed, in vitro, IL-12 was shown to synergize with other cytokines such as KL, IL-3, and IL-11 to stimulate the proliferation and differentiation of early hematopoietic progenitors (11, 13, 14, 18). These results were obtained with highly purified progenitors or even single cells, thus under conditions where the IL-12 effects observed were likely to be direct. IL-12 myelotoxicity in vivo could, however, be explained by some indirect mechanisms. Since IL-12 has a profound stimulatory effect on IFN-γ production by T cells and NK cells both in vitro (1, 8) and in vivo (16, 17), we hypothesized that the inhibition of hematopoiesis
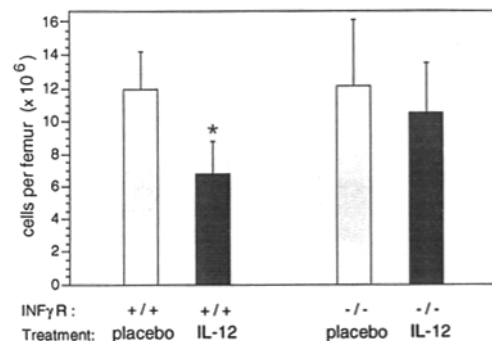

Figure 1. Effect of IL-12 on bone marrow cellularity in wild-type and IFNγR⁻/⁻ mice. Wild-type or IFNγR⁻/⁻ mice were treated with placebo or IL-12 (1 μg/mouse per d) for 4 d. The number of nucleated bone marrow cells per femur was determined on day 5. Student's t test *p = 0.001.
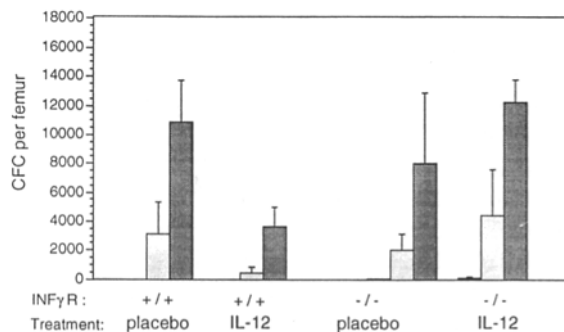
Figure 2. Effect of IL-12 on bone marrow hematopoietic progenitors in wild-type and IFNγR$^{-/-}$ mice. Mice were treated as in Fig. 1. The number of multilineage (*black bars*), erythroid (*light gray bars*), and GM (*dark gray bars*) colony-forming cells per femur on day 5 was determined in methylcellulose cultures containing IL-11, KL, and Epo. Results are from two experiments with pooled bone marrow cells per group. Student's *t* test *p* >0.05. Similar results were obtained in cultures containing IL-3, IL-1, and Epo (not shown).

seen in vivo after IL-12 administration could be mediated by IL-12–induced IFN-γ. IFN-γ plasma levels were indeed strongly increased after 4 d of IL-12 administration to normal mice (from 0.9 ± 1.5 to 20.1 ± 9.1 ng/ml, *n* = 8). Administration of IFN-γ to mice is clearly myelosuppressive (for review see reference 23), and IFN-γ was shown to suppress hematopoietic colony formation in vitro, either directly (19, 21) or when secondarily induced in culture by IL-12 (18).

We then examined the effect of IL-12 treatment in mice rendered unresponsive to IFN-γ after inactivation of the IFN-γ receptor gene (24). First, we established that untreated IFN-γ receptor–deficient (IFNγR$^{-/-}$) mice maintained under SPF conditions have essentially normal hematopoietic parameters in terms of bone marrow cellularity and CFC content, as well as spleen weight, cellularity, and number of CFC (not shown). Wild-type and IFNγR$^{-/-}$ mice were then treated for 4 d with 1 μg/mouse per d of IL-12 and killed on day 5. In wild-type mice, this treatment resulted in mild anemia with reticulocytosis, some lymphopenia, and a 3.3-fold decrease in neutrophils (Table 1). In IFNγR$^{-/-}$ mice, the only

significant change of peripheral blood counts observed after IL-12 treatment was a 1.8-fold increase of neutrophil counts (Table 1), although the levels of circulating IFN-γ were as high as 1,036 ± 322 ng/ml after IL-12 treatment (compared with 0.9 ± 1.5 ng/ml in placebo-treated controls, *n* = 8). There was a significant twofold reduction in bone marrow cellularity in IL-12–treated wild-type mice compared with the placebo-treated controls (Fig. 1). In contrast, bone marrow cellularity was not significantly affected after IL-12 treatment in IFNγR$^{-/-}$ mice (Fig. 1). Thus, the reduction of peripheral blood cells and the overall decrease in bone marrow cellularity seen after IL-12 administration seemed to be mediated by IFN-γ.

Cytological analysis of bone marrow preparations confirmed that the nonmitotic mature bone marrow cell population was markedly depleted in wild-type but not in IFNγR$^{-/-}$ mice treated with IL-12 for 4 d. In wild-type mice, IL-12 treatment resulted in a significant 11.2-fold reduction of erythroid elements and 4.7-fold depletion of lymphoid cells, plus a slight nonsignificant reduction of neutrophilic and eosinophilic precursors, respectively (Table 2). Identifiable monocytic precursors were significantly increased by 2.2-fold, and both myeloblast and erythroblast numbers were normal (Table 2). In IFNγR$^{-/-}$ mice, however, IL-12 treatment caused only a slight, nonstatistically significant decrease in erythroid and lymphoid elements (Table 2). Neutrophilic precursors were 2.9-fold decreased, while monocytic precursors were 1.5-fold increased (Table 2). In contrast to wild-type mice, there was a significant increase in eosinophilic precursors (1.6-fold) and blasts (2.2-fold, predominantly myeloblasts) after IL-12 treatment of IFNγR$^{-/-}$ mice. Thus, although IL-12 induced monocytopoiesis in both wild-type and IFNγR$^{-/-}$ mice, the suppression of most other cell lineages was absent or less severe in IFNγR$^{-/-}$ mice, and blasts were increased only in mice that were unresponsive to IFN-γ.

We next studied the effect of a 4-d IL-12 treatment on hematopoietic progenitors. In wild-type mice, the absolute numbers of erythroid- and GM-CFC per femur were three- to fivefold decreased after IL-12 treatment, although this difference, based on two experiments with pooled bone marrow cells within groups of mice, did not reach statistical

Table 1. *Hematologic Alterations in IL-12–treated Wild-type and IFNγ$^{-/-}$ Mice*

Mouse genotype	In vivo treatment	WBC	RBC	Reticulocytes	Platelets	Lymph-ocytes	Neutrophils	Mono-cytes	Eosinophils
IFNγR$^{+/+}$	Placebo	7.8 ± 2.0	9.2 ± 0.4	0.52 ± 0.08	348 ± 122	6.4 ± 2.4	1.35 ± 0.69	<0.1	0.07 ± 0.06
IFNγR$^{+/+}$	IL-12	5.3 ± 1.8*	8.3 ± 0.8*	0.79 ± 0.23	245 ± 76	4.8 ± 1.6	0.41 ± 0.30‡	<0.1	0.06 ± 0.05
IFNγR$^{-/-}$	Placebo	6.5 ± 1.0	9.2 ± 0.8	0.78 ± 0.23	350 ± 165	5.3 ± 0.4	0.95 ± 0.65§	<0.1	0.14 ± 0.10
IFNγR$^{-/-}$	IL-12	6.8 ± 1.5	9.0 ± 0.7	0.74 ± 0.17	248 ± 90	5.2 ± 0.9	1.76 ± 1.26	<0.1	0.02 ± 0.03

Mice were treated with placebo or IL-12 (1 μg/d) daily for 4 d and blood was collected on day 5.
WBC, total leukocytes (× 10³/μl); *RBC*, total erythrocytes (× 10⁶/μl), reticulocytes (× 10⁶/μl), platelets, neutrophils, monocytes, eosinophils (× 10³/μl).
Student's *t* tests: *p* < *0.05, ‡0.03, §0.04.

Table 2. *Bone Marrow Cytology of Wild-type and IFNγR−/− Mice Treated with IL-12*

Mouse genotype	In vivo treatment	Erythroid	Myeloid	M/E*	Blasts‡	Monocytic	Neutrophilic	Eosino-philic	Lymphocytic
IFNγR+/+	Placebo	348 ± 1.68[a]	4.39 ± 0.78	1.28 ± 0.36[b]	0.35 ± 0.1	0.82 ± 0.22[c]	3.19 ± 0.44[d]	0.37 ± 0.22[e]	3.30 ± 0.62[f]
IFNγR+/+	IL-12	0.31 ± 0.14	3.32 ± 0.4	10.0 ± 4.0	0.39 ± 0.12	1.80 ± 0.26	1.12 ± 0.22	0.14 ± 0.1	0.70 ± 0.32
IFNγR−/−	Placebo	4.26 ± 0.82	6.69 ± 1.14	1.56 ± 0.4	0.32 ± 0.1[g]	1.06 ± 0.30[h]	4.83 ± 1.96[i]	0.58 ± 0.14[j]	2.55 ± 0.92
IFNγR−/−	IL-12	2.61 ± 1.10	4.33 ± 1.0	1.67 ± 0.61	0.69 ± 0.14	1.55 ± 0.32	1.67 ± 0.66	0.95 ± 0.2	2.34 ± 0.58

Mice were treated as described in Table 1. Results represent mean ± SD of total cells per lineage (mitotic and post mitotic pooled) × 10[6] per femur. Myeloid cells include myeloblasts, progranulocytes, monocytic, neutrophilic, and eosinophilic cells.
*Ratio of myeloid to erythroid cells. ‡Blasts includes myeloblasts, progranulocytes, rubriblasts, and prorubricytes.
Student's t tests: a, p <0.02; b, p <0.008; c, p <0.01; d, p <0.2; e, p <0.1; f, p <0.001; g, p <0.03; h, p <0.03; i, p <0.006; j, p <0.04.

significance. In IFNγR−/− mice treated with IL-12, there was no decrease but rather a trend to increase all CFCs (Fig. 2). Even though the total cellularity per femur was twofold decreased in the IL-12–treated wild-type mice, the frequency of CFC was still twofold lower in these mice compared to placebo-treated controls. In contrast, the IL-12 treatment of IFNγR−/− mice resulted in up to a twofold increase of CFC frequency without decreasing the total marrow cellularity. Therefore, a short IL-12 treatment, which depresses bone marrow hematopoietic progenitors in wild-type mice, tends to increase these progenitors in mice rendered unresponsive to IFN-γ.

IL-12 induced splenomegaly in wild-type mice, as spleen weight was 5.5-fold increased in wild-type mice treated for 7 d with 1 μg/mouse per d IL-12 (402.9 ± 57.7 vs. 72.4 ± 12.7 mg in placebo-treated mice, n = 9, p <0.001). A 2.3-fold increased splenic weight was already seen after 4 d of IL-12 treatment in wild-type mice (187.0 ± 30.9 vs. 81.4 ± 15.9 mg, n = 8 and 5, respectively, p <0.001). Splenic cellularity was also 2.3-fold increased in these mice compared with placebo-treated animals (Fig. 3). This was due, at least in part, to monocytic infiltrates and a high NK cell content (histology not shown). FACS® analysis from splenocyte suspensions also showed a doubling of CD11b+ cells and a threefold increase of NK1.1+ cells (Fig. 4). Splenic hematopoiesis was slightly increased after 4 d of IL-12 treatment as seen histologically (not shown) and by some increase in the absolute number of splenic CFC, which did not reach stastistical significance (Fig. 5). Thus, in wild-type mice, IL-12 induces splenomegaly, which is caused by both macrophage and NK cell infiltration in addition to some extramedullary hematopoiesis.

IL-12–induced splenomegaly was less pronounced in IFNγR−/− mice, since 7 d of IL-12 treatment resulted only in a 1.7-fold increase in spleen weight (125.7 ± 25.4 vs. 72.0 ± 13.1 mg, n = 6, p = 0.001). A similar spleen weight increase was already seen after 4 d of IL-12 treatment in IFNγR−/− mice (150.8 ± 25.4 vs. 77.5 ± 17.9 mg, n = 8 and 4, respectively, p <0.001). Splenic cellularity was slightly increased in these animals, but this did not reach statistical

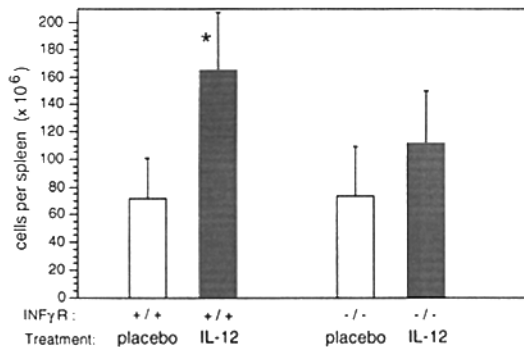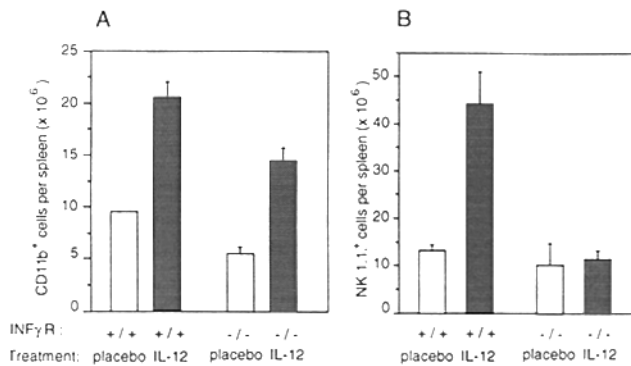

Figure 3. Effect of IL-12 on spleen cellularity in wild-type and IFNγR−/− mice. Mice were treated as in Fig. 1, and the total number of nucleated cells per spleen was determined on day 5. Student's t test *p = 0.002.

Figure 4. Flow cytometric analysis of spleen cells from wild-type and IFNγR−/− mice treated with IL-12. Mice were treated as in Fig. 1. On day 5, splenocytes were harvested, stained with anti CD11b (*A*) or NK1.1 (*B*) antibodies, and analyzed by flow cytometry.

Figure 5. Effect of IL-12 on splenic hematopoiesis in wild-type and IFNγR−/− mice. Spleen cells from wild-type or IFNγR−/− mice treated as in Fig. 1 were harvested on day 5, and the number of multilineage (*black bars*), erythroid (*light gray bars*), and GM (*dark gray bars*) colony-forming cells per spleen was determined in methylcellulose cultures containing IL-11, KL, and Epo. Results are from two experiments with pooled spleen cells per group. Student's *t* test $^*p = 0.04$. Similar results were obtained in cultures containing IL-1, IL-3, and Epo (not shown).

significance (Fig. 3). FACS® analysis from splenocyte suspension showed a doubling of CD11b+ cells but no increase in NK1.1+ cells in IL-12–treated IFNγR−/− mice (Fig. 4). The total number of CFC per spleen was increased in these mice after 4 d of IL-12 treatment, and this was particularly significant for erythroid CFC (Fig. 5). The frequency of CFC on a per cell basis was also 3.7-fold increased in the IL-12 treated IFNγR−/− mice, compared with 1.7-fold only in the wild-type mice. Therefore, the increases in splenic weight, cellularity, and NK cell infiltration after IL-12 treatment were less pronounced in IFNγR−/− mice than in wild-type mice. In contrast, the frequency and the absolute number of splenic CFC were higher after IL-12 treatment in IFNγR−/− than in wild-type mice.

In conclusion, the effects attributable to IFN-γ after IL-12 treatment include the reduction in bone marrow total cellularity, in identifiable myeloid, erythroid, and lymphoid elements and in hematopoietic colony-forming progenitors, and the dramatic increase of splenic cellularity, mainly due to NK cell and macrophage infiltration. In an environment nonresponsive to IFN-γ, IL-12 does not reduce mature bone marrow cells, moderately affects identifiable myeloid, erythroid and lymphoid elements, and tends to increase blasts and colony-forming progenitors in the bone marrow. Splenic hematopoiesis is also increased in IL-12–treated IFNγR−/−

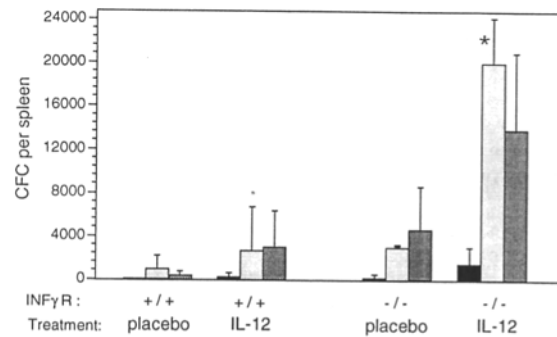mice, although there is a more moderate increase in splenic total cellularity with a markedly lower NK cell content. It seems that, in the absence of IFN-γ signaling, an important costimulatory signal is missing to allow full stimulation of NK cell proliferation by IL-12.

The results obtained in the absence of the IFN-γ negative regulation in mice rendered deficient for IFN-γ receptor are therefore compatible with the direct stimulation of early hematopoietic progenitors by IL-12 seen in vitro (12–14). However, the enhancement of bone marrow progenitors stimulated by IL-12 appears to be balanced in vivo by the production of IFN-γ, which acts in a dominant myelosuppressive fashion. Thus, although IFN-γ does not seem to be essential for steady-state hematopoiesis, as seen in mice deficient for IFN-γ production (28) or signaling (24), we demonstrate here that IFN-γ is clearly a potent negative regulator of hematopoiesis when induced in response to IL-12. Since high levels of IL-12 are produced systemically during endotoxemia, this negative regulatory function of IFN-γ is likely to be of pathophysiological significance for the acute hematological alterations seen after bacterial infections.

References

1. Kobayashi, M., L. Fitz, M. Ryan, R.M. Hewick, S.C. Clark, S. Chan, R. Loudon, F. Sherman, B. Perussia, and G. Trinchieri. 1989. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *J. Exp. Med.* 170:827–845.

2. Stern, A.S., F.J. Podlaski, J.D. Hulmes, Y.-C.E. Pan, P.M. Quinn, A.G. Wolitzky, P.C. Familletti, D.L. Stremlo, T. Truitt, R. Chizzonite, et al. 1990. Purification to homogeneity and partial characterization of cytotoxic lymphocyte maturation factor from human B-lymphoblastoid cells. *Proc. Natl. Acad. Sci. USA.* 87:6808–6812.

3. Wolf, S.F., P.A. Temple, M. Kobayashi, D. Young, M. Dicig, L. Lowe, R. Dzialo, L. Fitz, C. Ferenz, R.M. Hewick, et al. 1991. Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. *J. Immunol.* 146:3074–3081

4. Gubler, U., A.O. Chua, D.S. Schoenhaut, C.M. Dwyer, W. McComas, R. Motyka, N. Nabavi, A.G. Wolitzky, P.M. Quinn, P.C. Familletti, and M.K. Gately. 1991. Coexpression of two distinct genes is required to generate secreted bioactive cytotoxic lymphocyte maturation factor. *Proc. Natl. Acad. Sci. USA.* 88:4143–4147.

5. Brunda, M.J. 1994. Interleukin 12. *J. Leukoc. Biol.* 55:280–288.

6. Robertson, M.J., R.J. Soiffer, S.F. Wolf, T.J. Manley, C. Donahue, D. Young, S.H. Herrmann, and J. Ritz. 1992. Responses of human natural killer (NK) cells to NK cell stimulatory factor (NKFS): cytolytic activity and proliferation of NK cells are differentially regulated by NKSF. *J. Exp. Med.* 175:779–788.

7. Gately, M.K., B. Desai, A.G. Wolitzki, P.M. Quinn, C.M. Dwyer, F.J. Podlaski, P.C. Familetti, F. Sinigaglia, R. Chizzonite, U. Gubler, et al. 1991. Regulation of human lymphocyte proliferation by a heterodimeric cytokine, IL-12 (cytotoxic lymphocyte maturation factor). *J. Immunol.* 147:874–882.

8. Chan, S.H., B. Perussia, J.W. Gupta, M. Kobayashi, M. Posposil, H.A. Young, S.F. Wolf, D. Young, S.C. Clark, and G. Trinchieri. 1991. Induction of interferon-γ production by natural killer cell stimulation factor: characterization of the responder cells and synergy with other inducers. *J. Exp. Med.* 173:869–879.

9. Manetti, R., P. Parronchi, M.G. Giudizi, M.-P. Piccinni, E. Maggi, G. Trinchieri, and S. Romagnani. 1993. Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4–producing Th cells. *J. Exp. Med.* 177:1199–1204.

10. Hsieh, C.S., S.E. Macatonia, C.S. Tripp, S.F. Wolf, A. O'Garra, and K.M. Murphy. 1993. Development of Th1 CD4+ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science (Wash. DC).* 260:547–549.

11. Jacobsen, S.E., O.P. Veiby, and E.B. Smeland. 1993. Cytotoxic lymphocyte maturation factor (interleukin 12) is a synergistic growth factor for hematopoietic stem cells. *J. Exp. Med.* 178:413–418.

12. Ploemacher, R.E., P.L. van Soest, A. Boudewijn, and S. Neben. 1993. Interleukin-12 enhances interleukin-3 dependent multilineage hematopoietic colony formation stimulated by interleukin-11 or steel factor. *Leukemia.* 7:1374–1380.

13. Ploemacher, R.E., P.L. van Soest, H. Voorwinden, and A. Boudewijn. 1993. Interleukin-12 synergizes with interleukin-3 and steel factor to enhance recovery of murine hemopoietic stem cells in liquid culture. *Leukemia.* 7:1381–1388.

14. Hirayama, F., N. Katayama, S. Neben, D. Donaldson, E.R. Nickbarg, S.C. Clark, and M. Ogawa. 1994. Synergistic interaction between interleukin-12 and steel factor in support of proliferation of murine lymphohematopoietic progenitors in culture. *Blood.* 83:92–98.

15. D'Andrea, A., M. Rengaraju, N.M. Valiante, J. Chehimi, M. Kubin, M. Aste, S.H. Chan, M. Kobayashi, D. Young, E. Nickbarg, et al. 1992. Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. *J. Exp. Med.* 176:1387–1388.

16. Morris, S.C., K.B. Madden, J.J. Adamovicz, W.C. Gause, B.R. Hubbard, M.K. Gately, and F.D. Finkelman. 1994. Effects of IL-12 on in vivo cytokine gene expression and Ig isotype selection. *J. Immunol.* 152:1047–1056.

17. Gately, M.K., R.R. Warrier, S. Honasoge, D.M. Carvajal, D.A. Faherty, S.E. Connaughton, T.D. Anderson, U. Sarmiento, B.R. Hubbard, and M. Murphy. 1994. Administration of recombinant IL-12 to normal mice enhances cytolytic lymphocyte activity and induces production of IFN-γ in vivo. *Int. Immunol.* 6:157–167.

18. Bellone, G., and G. Trinchieri. 1994. Dual stimulatory and inhibitory effect of NK cell stimulatory factor/IL-12 on human hematopoiesis. *J. Immunol.* 153:930–937.

19. Broxmeyer, H.E., L. Lu, E. Platzer, C. Feit, L. Juliano, and B.Y. Rubin. 1983. Comparative analysis of the influences of human gamma, alpha and beta interferons on human multipotential (CFU-GEMM), erythroid (BFU-E) and granulocyte-macrophage (CFU-GM) progenitor cells. *J. Immunol.* 131:1300–1305.

20. Zoumbos, N.C., J.Y. Djeu, and N.S. Young. 1984. Interferon is the suppressor of hematopoiesis generated by stimulated lymphocytes in vitro. *J. Immunol.* 133:769–774.

21. Means, R.T., Jr., and S.B. Krantz. 1991. Inhibition of murine erythroid colony forming units by γ interferon can be corrected by recombinant human erythropoietin. *Blood.* 78:2546–567.

22. Gimble, J.M., K. Medina, J. Hudson, M. Robinson, and P.W. Kinkade. 1993. Modulation of lymphohematopoiesis in long-term cultures by gamma interferon: direct and indirect action on lymphoid and stromal cells. *Exp. Hematol.* 21:224–230.

23. Terrell, T.G., and J.D. Green. 1993. Comparative pathology of recombinant murine interferon-γ in mice and recombinant human interferon-γ in cynomolgus monkeys. *Int. Rev. Exp. Pathol.* 34B:73–101.

24. Huang, S., W. Hendriks, A. Althage, S. Hemmi, H. Blüthmann, R. Kamijo, R. Vilcek, R.M. Zinkernagel, and M. Aguet. 1993. Immune response in mice that lack the interferon-γ receptor. *Science (Wash. DC).* 259:1742–1745.

25. Prat, M., G. Gribaudo, P. Comoglio, G. Cavallo, and S. Landolfo. 1984. Monoclonal antibodies against murine gamma interferon. *Proc. Natl. Acad. Sci. USA.* 81:4515–4519.

26. Cherwinsky, H.C., J.H. Schumacher, K.D. Brown, and T.R. Mossman. 1987. Two types of mouse helper T cell clones. 3. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays and monoclonal antibodies. *J. Exp. Med.* 166:1229–1244.

27. Iscove, N.N., A.R. Shaw, and G. Keller. 1989. Net increase of pluripotential hematopoietic precursors in suspension culture in response to IL-1 and IL-3. *J. Immunol.* 142:2332–2337.

28. Dalton, D.K., S. Pitts-Meek, S. Keshav, I.S. Figari, A. Bradley, and T.A. Stewart. 1993. Multiple defects of immune cell function in mice with disrupted interferon-γ genes. *Science (Wash. DC).* 259:1739–1742.