Involvement of Interleukin (IL) 8 Receptor in Negative Regulation of Myeloid Progenitor Cells In Vivo: Evidence from Mice Lacking the Murine IL-8 Receptor Homologue

By Hal E. Broxmeyer,* Scott Cooper,* Grace Cacalano,‡ Nancy L. Hague,* Edward Bailish,§ and Mark W. Moore‡

*From the *Department of Medicine (Hematology/Oncology), the Department of Microbiology/Immunology, and the Walther Oncology Center, Indiana University School of Medicine, Indianapolis, Indiana 46202; ‡Department of Cell Genetics, Genentech, South San Francisco, California 94080; and §Gnotobiotic Laboratory and Department of Surgery, University of Wisconsin Medical School, Madison, Wisconsin 53703*

Summary

Expansion of mature neutrophils has been observed in mice lacking the murine interleukin (IL) 8 receptor homolog [mIL-8Rh(−/−)], and human (hu) IL-8 suppresses proliferation of primitive myeloid cells in vitro and in vivo. To evaluate involvement and relevance of murine IL-8 receptor homolog (mIL-8Rh) in negative regulation of myelopoiesis, we studied mIL-8Rh(−/−) and (+/+) mice raised in a normal or germ-free environment. Immature myeloid progenitors from mIL-8Rh(+/+) mice bred under normal or germ-free conditions were significantly suppressed in vitro by recombinant huIL-8, macrophage inflammatory protein (MIP)-1α, platelet factor (PF) 4, interferon inducible protein (IP) 10, monocyte chemotactic peptide (MCP) 1, and H-ferritin. In contrast, progenitors from mIL-8Rh(−/−) mice were insensitive to inhibition by IL-8, but not to these other chemokines and H-ferritin. Mouse MIP-2, a ligand for mIL-8Rh, suppressed progenitors from normal but not mIL-8Rh(−/−) mice. Under normal environmental conditions, enhanced numbers of myeloid progenitors were found in femur, spleen, and blood of mIL-8Rh(−/−) compared with mIL-8Rh(+/+) mice. Numbers of myeloid progenitors were greatly decreased in mIL-8Rh(−/−) and (+/+) mice in germ-free conditions, and were either not significantly enhanced in mIL-8Rh(−/−) mice compared with (+/+) mice or were only moderately so. Differences in progenitors/organ between a germ-free and normal environment were greater for the mIL-8Rh(−/−) mice. These results document selective insensitivity of myeloid progenitor cells from mIL-8Rh(−/−) mice to inhibition by huIL-8 and mouse MIP-2 and a large expansion of myeloid progenitors in these mice, the latter effect being environmentally inducible. This provides strong support for a negative myeloid regulatory role played by the mIL-8Rh in vivo, whose active ligand may be MIP-2.

IL-8 is a member of the C-X-C subfamily of small molecular weight inducible heparin-binding proteins (1, 2). Members of this family have been implicated in a number of activities associated with attraction and activation of leukocytes with postulated roles as mediators of inflammation (1, 3, 4).

Two high-affinity human (hu)[1] IL-8 receptors have been cloned and characterized (5–8), whereas the murine genome contains a single gene for the putative IL-8 receptor (9, 10). Although neither mouse nor rat IL-8 has been identified (11, 12), antibodies to huIL-8 inhibit lung inflammation in rats (13), suggesting the presence of a molecule in rodents similar to IL-8.

A murine gene with close homology to two known huIL-8 receptors was cloned, and homologous recombination in embryonic stem cells was used to create mice that lacked the murine IL-8 receptor homolog (mIL-8Rh) (9). Interestingly, expansion of neutrophilic myeloid and B lymphoid cells was apparent in mIL-8Rh(−/−) mice. Increased numbers of these hematopoietic cells were noted in lymph nodes, spleen, bone marrow, and in the circulating blood. Changes in these phenotypes were not detected with advanced age up to 20 wk, and heterozygous [mIL-

[1]*Abbreviations used in this paper:* BFU-E, erythroid progenitors; CFU-GEMM, multipotential progenitors; CFU-GM, granulocyte-macrophage progenitors; FBS, fetal bovine serum; hu, human; MCP, monocyte chemotactic peptide; mIL-8Rh, murine IL-8 receptor homolog; MIP, macrophage inflammatory protein; PF, platelet factor; PWMSCM, pokeweed mitogen mouse spleen cell conditioned medium; rmu, recombinant murine; SLF, steel factor.

8Rh(+/−)] mice did not manifest an overt phenotype (9). Neutrophils from mIL-8Rh(−/−) mice were as effective as those from mIL-8Rh(+/+) mice at intracellular and extracellular killing of bacteria and at migration in vitro to a chemotactic and activating agent. However, neutrophils from mIL-8Rh(−/−) mice did not migrate to ligands identified for this receptor: huIL-8 or mouse macrophage inflammatory protein (MIP) 2 (9, 10). It was suggested from these studies that the mIL-8Rh might be involved in the negative regulation of neutrophil production (9). The concept of mIL-8Rh being involved in negative regulation of myeloid blood cells is consistent with the suppressive effects of huIL-8 on immature subsets of human myeloid progenitor cells in vitro (14–17) and on these cells in vivo in mice (16, 17).

This study was undertaken to more definitively evaluate a role for the mIL-8Rh in negative regulation of myeloid blood cell production. The mechanism of neutrophilia in mIL-8Rh(−/−) mice has not been determined (9). This increase could have originated from enhanced numbers of hematopoietic stem or myeloid progenitor cells that escaped negative regulation. Alternatively, this increase may have reflected enhanced proliferative capacity of precursor cells such as myeloblasts, promyelocytes, or myelocytes, and/or increased survival and migration of neutrophils. Based on our previous work (14–17), we chose to concentrate on the level of myeloid progenitor cells. Progenitor cells from mIL-8Rh(−/−) and (+/+) mice, bred under normal conditions (9) or in a germ-free environment (18), were compared for responsiveness in vitro to suppression by huIL-8, as well as to the effects of other chemokines and a nonchemokine suppressor cytokine. Additionally, absolute numbers of myeloid progenitors in the bone marrow, spleen, and circulating blood of these mice were enumerated.

Materials and Methods

Mice. mIL-8Rh(−/−) BALB/c mice were created as described elsewhere by deleting a murine gene with a high degree of homology to the two known huIL-8 receptors by homologous recombination (9). mIL-8Rh(−/−) and control mIL-8Rh(+/+) mice were bred under either normal environmental (9) or germ-free (18) conditions. BDF_1 and C3H/HeJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Cells were obtained from femoral marrow, spleen, or circulating peripheral blood of these mice (16, 17, 19, 20).

Cytokines and Reagents. rhu Epo was purchased from Amgen Biologicals (Thousand Oaks, CA). Hemin was purchased from Eastman Kodak Co. (Rochester, NY). Pokeweed mitogen mouse spleen cell conditioned medium (PWMSCM), a source of growth factors was prepared as described elsewhere (20). Purified preparations of rhuIL-8, platelet factor (PF) 4, monocyte chemotactic peptide 1 (MCP-1), and RANTES were purchased from Pepro Tech (Rocky Hills, NJ). Purified rhuMIP-1α, r murine (rmu) MIP-1α, rmuMIP-2, and rhuMIP-2β were purchased from R&D Systems, Inc. (Minneapolis, MN). Purified rhuGRO-α and rmu steel factor (SLF) were kind gifts from Immunex Corp. (Seattle, WA). Purified rhuIP-10 was purified as described (21) and was a kind gift from Andreas Sarris (MD Anderson Cancer Center, Houston, TX). Purified rhuH-ferritin was a kind gift from Dr. Paolo Arosio (University of Milan, Milan, Italy) (22).

Colony Assay. Cells from bone marrow, spleen and peripheral blood of mIL-8Rh(+/+) and (−/−) mice were plated, respectively, at 5–10 × 10^4, 5 × 10^5, and 10^5 cells/ml in 35-mm tissue culture dishes (Corning Inc., Corning, NY) in 1% methylcellulose culture medium with 30% fetal bovine serum (FBS; Hyclone Laboratories Inc., Logan, UT) in the presence of Epo (1 U/ml), 10% vol/vol PWMSCM and 0.1 mM hemin (17, 19). Colonies deriving from granulocyte-macrophage (CFU-GM), erythroid (BFU-E), and multipotential (CFU-GEMM) progenitors were scored after 7 d of incubation in a humidified environment in an N_2–O_2–CO_2 incubator (model BNP-210; Taboi ESPEC Corp., South Plainfield, NJ) at 5% CO_2 and 5% O_2. Absolute numbers of progenitors per organ were calculated based on the number of viable unseparated nucleated cells per femur or spleen and the number of viable low-density (<1.077 gm/cm^3) peripheral blood cells retrieved after density cut separation using Ficoll–Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ). For assessment of the responsiveness of progenitors to suppression by cytokines, bone marrow cells were plated as above in methylcellulose culture medium with Epo, PWMSCM, and hemin (±50 ng/ml rmuSLF) or in 0.3% agar culture medium with 10% FBS in the presence of 100 U/ml rmuGM-CSF plus 50 ng/ml rmuSLF, in the presence of control diluent (PBS) or 100 ng/ml of IL-8, PF4, IP-10, MIP-1α, MCP-1, or 10^{-9} M H-ferritin or varying concentrations of rmuMIP-2. These concentrations have been previously determined to yield plateau levels of suppression under the growth factor conditions described above. Although complete inhibition of total colony formation is not seen, the decreases reflect complete inhibition of the enhanced growth noted when two or more growth factors are used to stimulate colony formation compared with a single growth factor (14, 15, 21, 23). Statistically significant differences were determined by Student's *t* test.

Results

Responsiveness of Myeloid Progenitor Cells to Negative Regulation. We first assessed whether the neutrophilia observed in mIL-8Rh(−/−) mice might result, at least in part, from insensitivity to negative regulation at the level of myeloid progenitor cells. To this end, bone marrow progenitor cells responsive to stimulation by multiple cytokines (PWMSCM for CFU-GM and Epo plus PWMSCM for BFU-E and CFU-GEMM) were evaluated for their capacity to be suppressed in vitro by IL-8 and other members of the chemokine family previously shown to be myelosuppressive (14–17, 21, 23, 24). Myeloid progenitor cells from femurs of mIL-8Rh(+/+) mice were significantly suppressed by IL-8, PF4, IP-10, MIP-1α, and MCP-1, whether these cells derived from mice raised under normal environmental (Fig. 1) or germ-free (Fig. 2) conditions. Suppression was also evident with H-ferritin, an iron-binding protein with demonstrated myelosuppressive activity (22). The levels of suppression seen were similar to that previously detected with chemokines for marrow progenitors from other strains of mice (16, 17, 23). In contrast, CFU-GM, BFU-E, and CFU-GEMM from mIL-8Rh(−/−) mice were insensitive to suppression by IL-8, whereas these progenitors did respond normally to inhibition by PF4, IP-10,

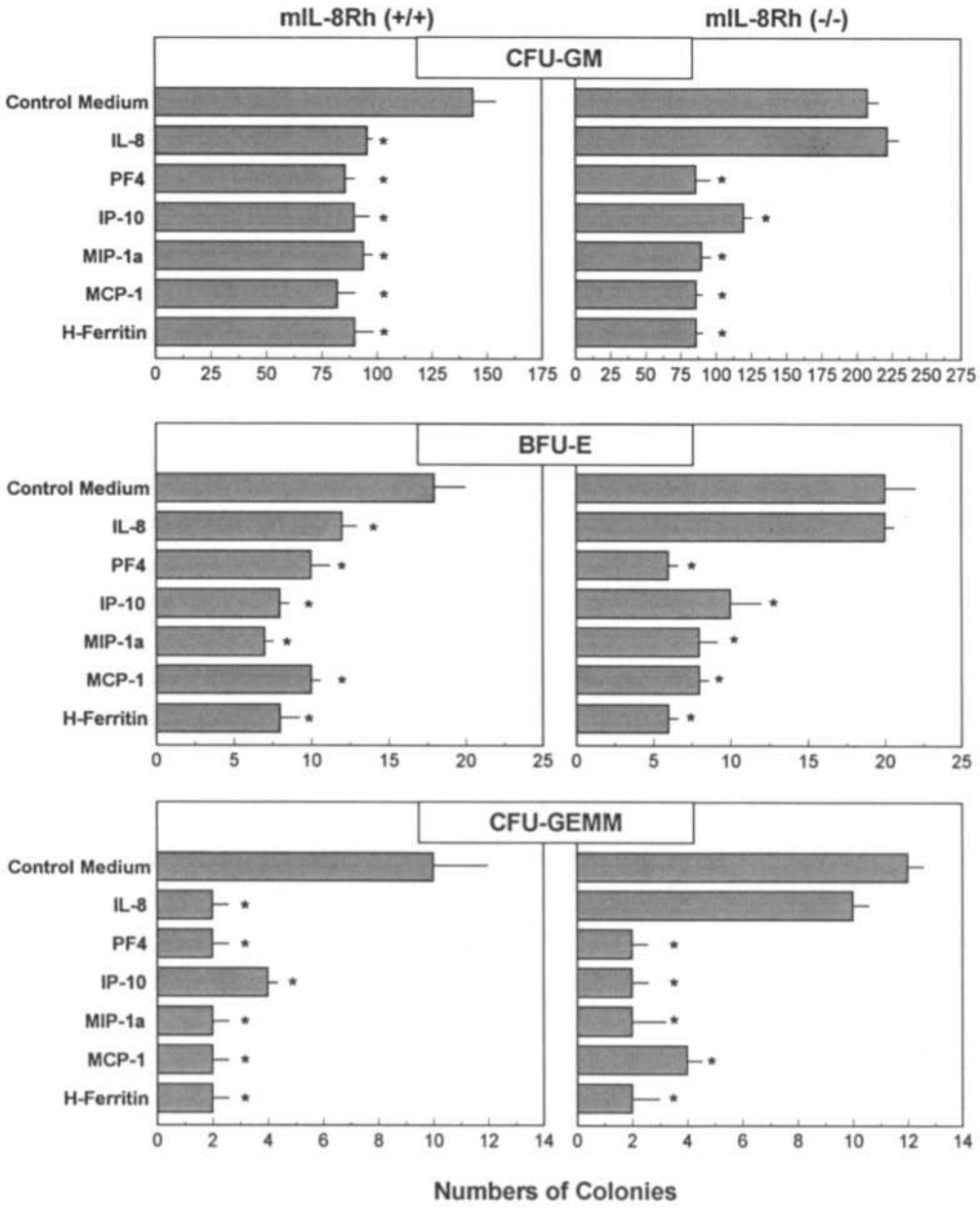

Figure 1. Influence of suppressive cytokines on colony formation by myeloid progenitor cells from the femoral bone marrow of mIL-8Rh(+/+) and mIL-8Rh(−/−) mice raised under normal environmental conditions. Marrow cells were pooled from either eight mIL-8Rh(+/+) or eight mIL-8Rh(−/−) mice before plating in semisolid medium with Epo, PWMSCM, and hemin in the presence of control medium (saline) or 100 ng/ml rhu preparations of IL-8, PF4, IP-10, MIP-1α, or MCP-1, or 10^{-9} M rhuH-ferritin. Results are expressed as mean ± 1 SEM for 10^5 marrow cells/ml. *Designates significant change from control medium, $P < 0.001$; other values are not significant, $P > 0.05$.

MIP-1α, MCP-1, and H-ferritin (Figs. 1 and 2). Because progenitors from mIL-8Rh(−/−) mice were selectively insensitive to inhibition by a known ligand for this receptor, huIL-8, but did respond to ligands for other receptors, the results are consistent with the postulation that the mIL-8Rh is involved in negative regulation of myeloid progenitor cells.

Because IL-8 has not been identified in mice (11, 12), we evaluated whether rmuMIP-2, a known ligand for mIL-8Rh, had suppressive activity. As shown in Fig. 3, rmuMIP-2 had myeloid progenitor cell suppressive activity comparable to that of muMIP-1α, rhuIL-8, and rhuPF4 on marrow cells from BDF_1 and C3H/HeJ mice. The dose–response suppression by rmuMIP-2 was comparable to that of other suppressive chemokines (14, 17, 23, 24). Three chemokines previously reported by us to lack suppressive activity (rhuRANTES, GRO-α, and MIP-2β) (17, 24) were inactive in these assays (Fig. 3). Additionally, rmuMIP-2 (100 ng/ml) significantly suppressed colony formation by bone marrow CFU-GM, BFU-E, and CFU-GEMM from mIL-8Rh(+/+), but not from mIL-8Rh(−/−) mice, with respective decreases in two experiments of 46–47, 34–41, and 49–50% for (+/+) CFU-GM, BFU-E, and CFU-GEMM ($P < 0.001$). Effects of rmuMIP-2 on (−/−) CFU-GM, BFU-E, and CFU-GEMM ranged from −4 to +3% of control numbers ($P > 0.05$).

Quantitation of Myeloid Progenitor Cells in Mice. We next evaluated whether lack of responsiveness of marrow cells from mIL-8Rh(−/−) mice in vitro to a known ligand for this receptor, as shown above, translated into an increased absolute number of myeloid progenitor cells in these mice. The absolute number of myeloid progenitor cells in mar-

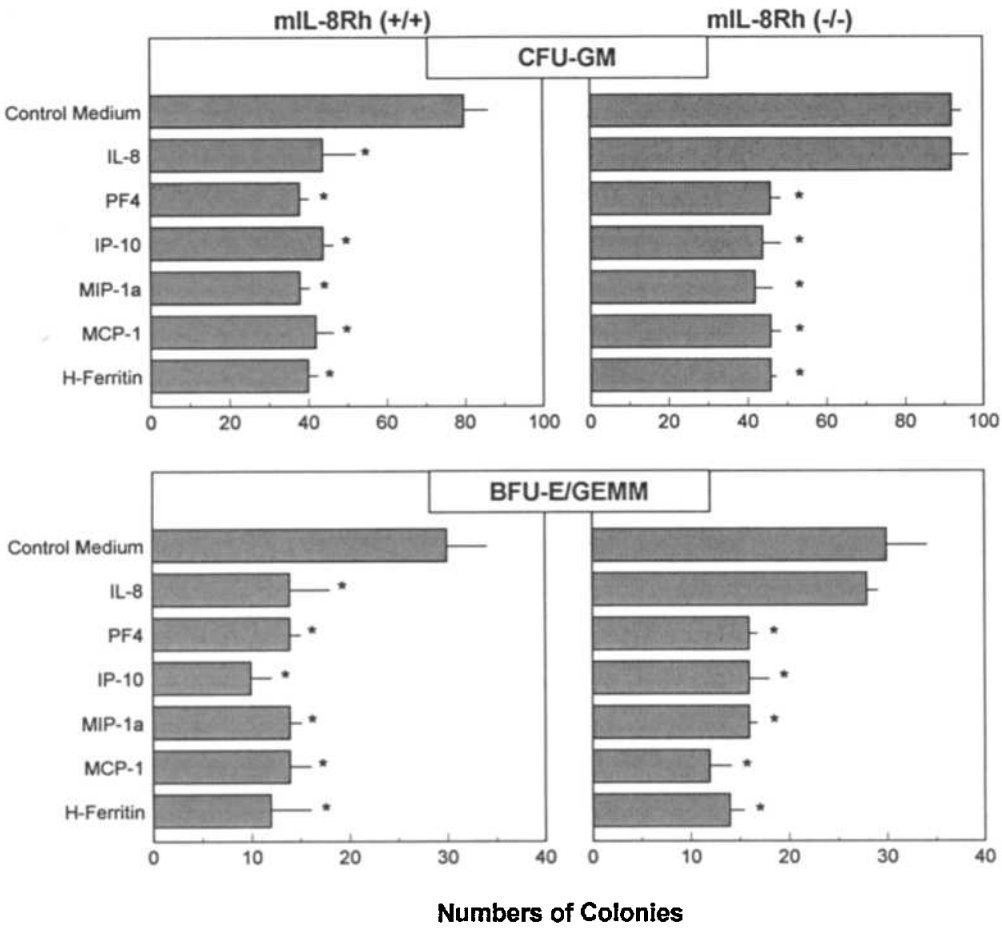

Figure 2. Influence of suppressive cytokines on colony formation by myeloid progenitor cells from the femoral bone marrow of mIL-8Rh(+/+) and mIL-8Rh(−/−) mice bred and raised under germ-free conditions. Marrow cells were pooled from either four mIL-8Rh(+/+) or eight mIL-8Rh(−/−) mice before plating in semi-solid medium as in Fig. 1. Colony formation by BFU-E and CFU-GEMM were combined because of the relatively low numbers of CFU-GEMM colonies in this set of experiments. Results are expressed as mean ± 1 SEM for 10^5 marrow cells/ml. *Designates significant change from control medium, $P < 0.001$, other values are not significant, $P > 0.05$.

row (femur), spleen, and blood of mIL-8Rh(−/−) and (+/+) mice raised under normal environmental conditions were determined (Table 1). Absolute numbers of CFU-GM and CFU-GEMM in the marrow and CFU-GM, BFU-E, and CFU-GEMM in the spleen and blood were significantly higher in the mIL-8Rh(−/−) compared with (+/+) mice. The largest differences between progenitors in the (−/−) and (+/+) mice were noted in spleen and blood and for CFU-GEMM in these organs.

Hematopoiesis is greatly influenced by environmental stresses such as bacteria and bacterial products (25), and because members of the chemokine family are inducible proteins whose production can be enhanced by such types of environmental triggers (1, 3, 4), we also compared absolute numbers of myeloid progenitors from both mIL-8Rh(+/+) and (−/−) mice raised under germ-free conditions (Table 2). The absolute numbers of myeloid progenitors in mIL-8Rh(+/+) mice were significantly ($P < 0.001$) reduced in

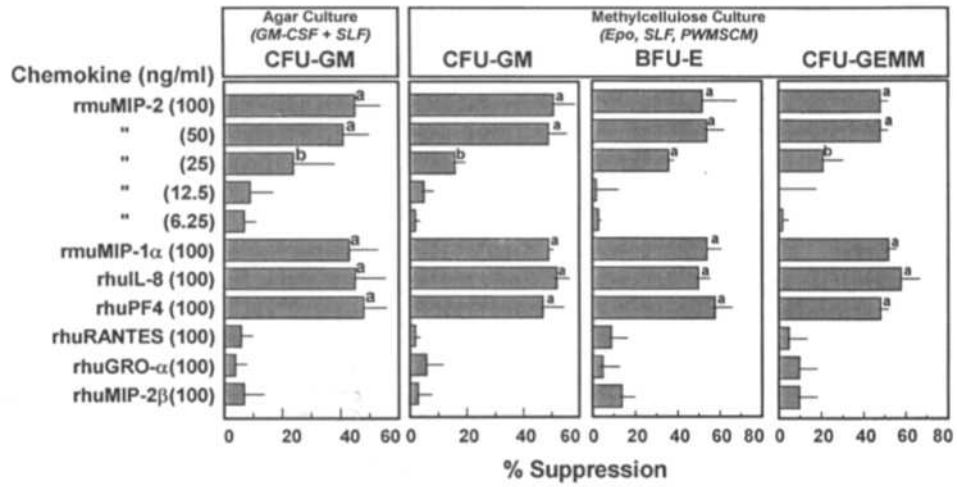

Figure 3. Comparative influence of rmuMIP-2 on colony formation in vitro by myeloid progenitor cells from femoral bone marrow. Results are shown as mean ± SD for three separate experiments (one using BDF_1 cells and two using C3H/HeJ cells). *a*, Significant change from control diluent, $P < 0.01$; *b*, $P < 0.05$.

Table 1. *Comparative Analysis of Marrow, Spleen, and Peripheral Blood Cellularity and Myeloid Progenitors from mIL-8Rh(+/+) and mIL-Rh(−/−) Mice Raised under Normal Environmental Conditions*

mIL-8Rh cells	Nucleated cellularity	CFU-GM	BFU-E	CFU-GEMM
	× 10^{-6}/femur or spleen cells or 1 ml of blood	× 10^{-3}/femur or spleen cells or 1 ml of blood		
Femoral bone marrow				
(+/+)	15.5 ± 1.3	23.3 ± 1.8	4.4 ± 0.3	1.1 ± 0.1
(−/−)	18.9 ± 0.9	45.2 ± 3.8*	4.4 ± 0.4	1.6 ± 0.1*
Fold Δ of (−/−) compared with (+/+)	1.22	1.94	1.0	1.45
Spleen				
(+/+)	68.6 ± 8.0	6.3 ± 1.1	2.6 ± 0.5	0.2 ± 0.1
(−/−)	99.1 ± 18.2*	31.4 ± 7.3*	10.2 ± 3.0*	2.4 ± 0.7*
Fold Δ of (−/−) compared with (+/+)	1.44	4.98	3.92	12.0
Peripheral blood				
(+/+)	4.1 ± 0.5	35.4 ± 5.6	26.0 ± 6.2	0
(−/−)	11.0 ± 4.9*	283.9 ± 97.8*	49.6 ± 8.9*	7.4 ± 2.5*
Fold Δ of (−/−) compared with (+/+)	2.68	8.0	1.91	>74.0

Results shown are an average of eight mice per group expressed as mean ± 1 SEM with each mouse assessed individually. Colony assays were done in methylcellulose with Epo. PWMSCM, and hemin.
*Significant change compared with mIL-8Rh (+/+), $P < 0.05$.

mice raised under germ-free compared with normal environmental conditions (compare Tables 2 and 1). Under germ-free conditions there were similar absolute numbers of BFU-E and CFU-GEMM in marrow and CFU-GM, BFU-E, and CFU-GEMM in spleen and blood for mIL-8Rh(−/−) and (+/+) mice (Table 2). A significant increase was seen in CFU-GM in marrow between mIL-8Rh(−/−) and (+/+) mice raised under germ-free conditions (Table 2), but this difference was less than that seen in mice raised under normal environmental conditions (compare Tables 2

Table 2. *Comparative Analysis of Marrow, Spleen, and Peripheral Blood Cellularity and Myeloid Progenitors from mIL-8Rh(+/+) and mIL-8Rh(−/−) Mice Raised under Germ-Free Conditions*

mIL-8Rh cells	Nucleated cellularity	CFU-GM	BFU-E	CFU-GEMM
	× 10^{-6}/femur or spleen cells or 1 ml of blood	× 10^{-3}/femur or spleen cells or 1 ml of blood		
Femoral bone marrow				
(+/+)	13.4 ± 0.3	8.2 ± 0.5	2.3 ± 0.5	0.3 ± 0
(−/−)	14.6 ± 0.5	11.4 ± 0.7*	3.1 ± 0.4	0.3 ± 0.1
Fold Δ of (−/−) compared with (+/+)	1.09	1.39	1.35	1.0
Spleen				
(+/+)	59.2 ± 2.1	1.5 ± 0.2	2.8 ± 0.3	0.18 ± 0.003
(−/−)	56.2 ± 3.3	2.2 ± 0.4	2.5 ± 0.3	0.13 ± 0.001
Fold Δ of (−/−) compared with (+/+)	0.95	1.47	0.89	0.72
Peripheral Blood				
(+/+)	0.71 ± 0.1	19.8 ± 8.8	38.0 ± 19.1	0
(−/−)	0.36 ± 0.07*	8.5 ± 4.5	3.3 ± 1.6	0
Fold Δ of (−/−) compared with (+/+)	0.5	0.43	0.09	0

Results are an average of four mIL-8Rh(+/+) and eight mIL-8Rh(−/−) mice per group expressed as mean ± 1 SEM with each mouse assessed individually. Colony assays were done exactly as in Table 1.
*Significant change compared with mIL-8Rh(+/+).

and 1). Moreover, and of special interest, calculation of the fold changes in progenitors per organ from a germ-free to normal environment (Tables 2 and 1) demonstrated that the largest differences were noted for (−/−) compared with (+/+) mice. The fold changes from a germ free to normal environment were for (−/−) versus (+/+) mice: bone marrow CFU-GM, 4.0 versus 2.8; spleen CFU-GM, 14.3 versus 4.2; peripheral blood CFU-GM, 33.4 versus 1.8; bone marrow BFU-E, 0.7 versus 1.9; spleen BFU-E, 1.4 versus 0.9; peripheral blood BFU-E, 15.0 versus 0.7; bone marrow CFU-GEMM, 5.3 versus 3.7; spleen CFU-GEMM, 18.5 versus 1.1; peripheral blood CFU-GEMM, >74.0 versus no change (no colonies in either group). Thus, except for bone marrow BFU-E, the fold change was always greater for the (−/−) compared with (+/+) mice.

Taken together, the results demonstrate that mIL-8Rh(−/−) mice have a hyperplasia of myeloid progenitor cells compared with the control mIL-8Rh(+/+) mice, but this increase is environmentally influenced, and greater differences from a germ-free to normal environment are noted for (−/−) compared with (+/+) mice.

Discussion

Hematopoiesis is regulated by both positive and negative factors (26–28). A number of cytokines have been implicated in negative regulation (29, 30). These suppressive cytokines include members of the chemokine family of proteins with c-x-c motifs such as IL-8, PF-4, IP-10, ENA78, and MIP-2α (= GRO-β), and members with c-c motifs such as MIP-1α, MCP-1, and MIP-related protein (MRP) 1 and 2 (14–17, 19, 21, 23, 24, 31). These chemokines have direct suppressive activity in vitro on primitive hematopoietic cells including the immature subsets of CFU-GM, BFU-E, and CFU-GEMM that are stimulated to proliferate by combinations of cytokines such as GM-CSF plus SLF for CFU-GM and Epo plus SLF with or without IL-3 for BFU-E and CFU-GEMM. Chemokines are also suppressive for myeloid progenitors stimulated by multiple cytokines present in conditioned medium such as PWM-SCM. Members of the chemokine family without suppressive activity include those with a c-x-c motif such as MIP-2β (=GRO-γ), GRO-α, and NAP-2 and those with a c-c motif such as MIP-1β and RANTES (14, 17, 23, 24). None of the tested chemokines had suppressive activity in vitro for the more mature myeloid progenitors stimulated to proliferate by a single cytokine. Evidence for an in vivo role for suppressive chemokines and their receptors has thus far come from studies in which administration of chemokines to mice resulted in myelosuppression. Chemokines, including IL-8, found to be suppressive in vitro were also suppressive in vivo. These effects were dose dependent, time related, and reversible (16, 17, 19). Chemokines not suppressive in vitro were inactive in these effects in vivo.

The generation of mIL-8Rh(−/−) mice, in which neutrophilia was present (9), offered us the opportunity to evaluate whether the mIL-8Rh was involved in negative regulation at the level of myeloid progenitor cells. The results clearly demonstrate that myeloid progenitors from mIL-8Rh(−/−) mice have a selective defect in responsiveness in vitro to negative regulation by huIL-8, a ligand for the mIL-8Rh (9, 10). Consistent with this lack of responsiveness, a hyperplasia of myeloid progenitors in marrow, spleen, and circulating blood was noted in these mice. The neutrophilia noted in mIL-8Rh(−/−) mice can thus probably be completely or mostly explained based on enhancement in numbers of CFU-GM and CFU-GEMM in these mice, although it is possible that other effects might be involved. Erythroid hyperplasia has not been noted in mIL-8Rh(−/−) mice, even though increased levels of BFU-E have been detected in the spleen and blood of these mice. This might reflect a level of ineffective erythropoiesis wherein increased erythroid progenitors do not, under the normal environmental conditions in which these mice have been raised, give rise to increased erythrocyte numbers. Our studies were not meant to address the mechanism of B lymphocyte increases in mIL-8Rh(−/−) mice. These increases might reflect increased levels of B lymphocyte progenitors and/or a common myeloid/B lymphocyte progenitor cell.

Because a murine homologue of huIL-8 has not been identified (11, 12) it remained to be determined which ligand or ligands for the mIL-8Rh might function as an in vivo modulator of negative regulation. Besides huIL-8, two murine chemokines, MIP-2 and KC, have been shown to trigger a signal through the mIL-8Rh (9, 10). However, GRO-α, considered to be human counterpart of mKC, is not myelosuppressive in vitro or in vivo (14, 17), although huGRO-α, as well as huMIP-2β (= GRO-γ), can block the in vitro suppressive effects of huIL-8 on human myeloid progenitors (14). Our studies herein demonstrating the myeloid progenitor cell–suppressing effect of rmuMIP-2 in vitro on normal progenitors, but not the progenitors from mIL-8Rh(−/−) mice, suggests that muMIP-2 might be the suppressive chemokine for the mIL-8Rh in vivo. We had previously not found muMIP-2 to be myelosuppressive in vitro (24), but those former studies were done before the potent costimulating cytokine SLF was used to enhance the sensitivity of our suppressor assays.

There is presently little information regarding intracellular signaling in myeloid progenitors by chemokines, and these studies, of necessity, have used growth factor–dependent cell lines so that enough cells of a particular type are available. Whereas human preparations of IL-8, PF4, MIP-1α, and IP-10 suppress proliferation of myeloid growth factor–dependent cell line M07e, the intracellular mechanisms of MIP-1α and IP-10, which act through a cAMP dependent protein kinase A Raf-1 kinase pathway, are different from those of IL-8 and PF4, which do not influence this pathway (32). Thus, how huIL-8 signals through a huIL-8 receptor to elicit a negative proliferative effect is not yet known. However, based on knowledge of the mIL-8Rh compared with human IL-8RA (=CXCR1) and IL-8RB (=CXCR2), it would appear that the rhuIL-8 is mediating its suppressive effects through the IL-8RB.

Chemokines are usually not constitutively present in much, if any, significant quantity, unless their production is induced. Thus, the relevance of a mIL-8Rh and ligand interaction involving negative regulation would probably make most sense in the context of an environmentally induced stress possible under normal environmental conditions, but would be less likely under germ-free conditions. That numbers of myeloid progenitors were significantly decreased in control mice raised under germ-free compared with those control mice raised under normal environmental conditions is consistent with the need for environmentally induced stress for enhanced myeloid cell proliferation. The fact that little or no differences were found in absolute numbers of progenitors between the mIL-8Rh(−/−) and (+/+) mice raised under germ-free conditions enhances the relevance of our findings and strongly implicates involvement of mIL-8Rh and a ligand for this receptor in negative regulation at the progenitor cell level. This is especially so because we detected much greater enhancement in progenitor cell numbers when mIL-8Rh(−/−) compared with mIL-8Rh(+/+) mice were evaluated in a germ-free to a normal environment. huIL-8 has direct suppressive effects on myeloid progenitors in vitro as assessed on purified preparations of $CD34^+$ cells (14) and single isolated $CD34^{+++}$ cells (15); however, whether the effects in vivo of the suppressive murine ligand(s) for the mIL-8Rh acts directly on the progenitors and/or indirectly through an accessory mediated effect remains to be determined.

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Address correspondence to Dr. Hal E. Broxmeyer, Walther Oncology Center, Indiana University School of Medicine, 975 West Walnut Street, Indianapolis, IN 46202.

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