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Hematopoietic growth factors and glucocorticoids synergize to mimic the effects of IL-1 on granulocyte differentiation and IL-1 receptor induction on bone marrow cells in vivo

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Abstract. The mechanisms by which interleukin-1 (IL-1) stimulates hematopoiesis are not clear. We have previously shown that in vivo administration of IL-1 indirectly increases IL-1 receptor (IL-1R) expression on both immature and mature bone marrow (BM) cells, partly due to IL-1-induced hematopoietic growth factor (HGF) production. Because IL-1 also stimulates the hypothalamic pituitary-adrenal axis resulting in the production of glucocorticoids (GC), we assessed whether in vivo treatment with HGF and glucocorticoids upregulates IL-1R. Administration of IL-1 to adrenalectomized mice reduces by 53% IL-specific binding on light density bone marrow (LDBM) cells compared to sham-operated mice. The administration of dexamethasone (dex) alone induced only a slight increase in IL-1R expression but synergized with granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF), IL-3 and IL-6 to upregulate IL-1R expression. Flow cytometry analysis using the RB6-8C5 antibody, which is differentially expressed on myeloid cells, indicated that combined G-CSF and dex treatment acts to promote increased numbers of differentiated myeloid progenitors in the bone marrow. Autoradiographic analysis confirmed that while G-CSF and dex increased IL-1R expression on all myeloid cells, it was particularly pronounced for myelocytes, promyelocytes and metamyelocytes. These results suggest that the ability of IL-1 to enhance granulocyte differentiation in vivo is partly due to its ability to induce a cascade of cytokines and steroids which in turn regulate IL-1 receptor expression.

Key words: IL-1 receptor—CSFs—Glucocorticoids—Myelopoiesis

Introduction. Interleukin-1 (IL-1) is a pleiotropic cytokine affecting the immune, inflammatory, neuroendocrine and hematopoietic systems [1,2]. Much of this diversity is based on the ability of IL-1 to induce production of other biologic mediators such as corticotropin releasing factor, corticosteroids, adrenocorticotropic hormone (ACTH), insulin and hematopoietic growth factors (G-CSF, GM-CSF, IL-3, IL-6, IL-8, transforming growth factor beta [TGF-β] and tumor necrosis factor alpha [TNF-α]) from multiple cell types.

The ability of IL-1 to synergize with HGFs to promote the growth and differentiation of primitive progenitor cells in vitro [3-5] suggests that IL-1 also plays an important role in the regulation of hematopoiesis [6]. In vivo administration of IL-1 induces an initial rapid mobilization of neutrophils from the bone marrow, followed by cycling of hematopoietic progenitor cells resulting in the expansion of the granulocytic compartment [7-11]. These effects presumably contribute to the ability of IL-1 to accelerate the recovery of hematopoietic stem cells and blood neutrophils following myelosuppression by chemotherapeutic drugs or exposure to lethal radiation [12-16].

In order to better understand the mechanism of action of IL-1 in vivo, we demonstrated that the IL-1 mediated upregulation of IL-1 receptors on bone marrow cells after in vivo administration of IL-1 occurs by an indirect mechanism [17]. In contrast, several hematopoietic growth factors, such as GM-CSF, G-CSF, IL-3 and IL-6 but not IL-1, act in vitro to upregulate IL-1R on both progenitor cells [18] and mature myeloid cells [19]. In vivo administration of HGFs, however, only partially mimic the ability of IL-1 to upregulate IL-1R expression [17]. It is, therefore, likely that other factors also contribute to the in vivo upregulation of IL-1R on hematopoietic progenitor cells. Since the in vivo administration of IL-1 also results in an elevation of corticosteroids in plasma [20] and glucocorticoids enhance in culture the expression of IL-1R on monocytes, B lymphocytes and fibroblasts [21,22], GCs might participate in the upregulation of IL-1R on bone marrow cells observed in response to IL-1. In this study, evidence is presented for a role for GCs as well as HGFs in IL-1R regulation.

Materials and methods

Mice. CD2F1 male mice were purchased from the Animal Genetics and Production Branch, National Cancer Institute, NIH (Frederick, MD). Animals were handled as previously described [23]. Animal care was provided in accordance with the procedures outlined in the Guide for Care and Use of Laboratory Animals (NIH Publication #86-23, 1985).

Reagents. Human recombinant IL-1α was supplied by Hoffmann-La Roche (Nutley, NJ). Human recombinant G-CSF was supplied by Amgen Corp. (Thousand Oaks, CA). Human recombinant IL-6 (5x10⁴ U/mL) in pyrogen-free solution was kindly provided by Dr. Menachein Rubinstein (Interpharm
Fig. 1. Comparative effect of in vivo IL-1 on IL-1R expression on BM cells from adrenalectomized (removal of adrenal gland) mice vs. sham-adrenalectomized (similar surgical procedure without removal of the adrenal gland) mice. Mice were adrenalectomized 14 days before injection of 100 ng IL-1. Sixteen to 18 hours after IL-1 administration, BM cells were harvested and tested for the expression of IL-1R as described in Materials and methods. Each bar represents the mean ± SE of 2 experiments from which the level of background binding (280 ± 41) was subtracted. The numbers in parentheses represent the number of mice receiving each treatment.

In vivo procedures. Cytokines and dexamethasone were diluted in pyrogen-free saline on day of injection. Predetermined optimal doses of cytokines were given intraperitoneally (i.p.) at the same time as dexam administered at 50 μg/mouse or at the dose indicated in the text. Bone marrow cells were tested for the expression of IL-1R 16 to 18 hours after treatment. Adrenalectomy was performed under anesthesia 14 days after the experiment. Adrenalectomized mice were given 1.0% NaCl in drinking water.

Measurement of CSF activity in serum. CSF titer in serum was measured as follows. Briefly, mice were bled 2 to 3 hours after IL-1 injection and serum was collected by centrifugation after clot formation. CSF activity was determined using bone marrow colony assay for CSF activity as previously described [20]. Briefly, BM cells were suspended in 1.0 mL IMDM, 10% FCS, in 0.3% Seaplaque agarose (Rockland, ME) in the presence or absence of serum (serial 2-fold dilutions). The cells were plated in 35 mm Lux petri dishes (Miles Laboratories Inc., Naperville, IL) and incubated at 37°C in 5.0% CO2 and scored for colonies (>50 cells) growth after 7 days of incubation. CSF activity was expressed as colony-forming units per milliliter, based on the colony count at 50% of maximum response (Ed50).

Preparation of bone marrow cells. Murine bone marrow cells were aspirated from femurs and low-density mononuclear cells were isolated by separation on Lymphocyte Separation Medium (Organon Teknika Corp., Durham, NC).

Flow cytometry analysis of bone marrow cells. BM cells from saline-, dexamethasone- and/or cytokine-treated mice were labeled with either monoclonal antibody (MAB) RB6-8C5, Thy-1, L3T4, F480 or control IgG in an indirect immunofluorescence assay. Briefly, 105 BM cells were resuspended in RPMI with 10% fetal calf serum and incubated for 30 minutes at 4°C with 1.0 μg of the appropriate MAB. The cells were washed and then incubated with fluorescein-labeled goat antirat antibody for 30 minutes at 4°C. The cells were then washed 2 times with PBS alone, fixed with 1.0% paraformaldehyde in PBS and analyzed using Coulter Profile II.

Preparation of iodinated IL-1. Human rIL-1α was labeled with 125I using chloramine-T reagent as described previously [25]. The radiolabeled IL-1α had a specific activity that ranged from 1 to 3 × 105 cpm/mmol. There was no significant loss of biological activity of radiolabeled IL-1α as determined by the thymocyte mitogenic activity assay.

Receptor binding assay. Fractionated bone marrow cell suspensions were washed once with cold medium and cell pellets were treated for 1 minute on ice with 50 mm glycine-HCl (pH 3.0) to remove potentially bound cytokines. Subsequently, the cells were washed twice with binding medium (RPMI 1.0% BSA supplemented with 0.1% sodium azide and 10 mm Hepes) and incubated at 4°C with 500 pm 125I-labeled IL-1α in a final volume of 0.2 mL. After 1 to 2 hours of incubation, cell-bound radioactivity was separated from unbound 125I-IL-1α by centrifugation of the sample through a mixture of 1:5:1 (vol/vol) dibutyl phthalate/bis(2-ethylhexyl)-phthalate (Eastman Kodak Co., Rochester, NY). Nonspecific binding was determined by incubating bone marrow cells with labeled IL-1α in the presence of 50-fold excess of unlabeled ligand.

Autoradiography. LDBM cells from mice treated 16 to 18 hours with IL-1, G-CSF and/or dexamethasone were prepared as described for receptor binding assay and incubated at 4°C with 1.0 nm 125I-IL-1α. After 1 hour of incubation, cell-bound radioactivity was separated from unbound IL-1α by centrifugation of the sample through a layer of cold FBS. The autoradiography was performed using a modification of a previously described technique [26]. Briefly, 2 × 105 cells were centrifuged onto microscope slides coated with 0.5% gelatin, fixed in methanol for 10 minutes, coated with Kodak NTB2 photographic emulsion and exposed at 4°C for 4 weeks. Slides were developed with Kodak D-19 developer, fixed with Kodak fixer, stained with Jenner-Giemsa. The number of grains was determined for over 50 cells per slide for 2 slides.

Results

Endogenous corticosteroid production is involved in the upregula-
Since we have recently determined that increased IL-IR G-CSF (5.0 μg) and dex synergizes with HGFs in the upregulation of IL-IR. We next examined the ability of exogenous additon of G-CSF plus dex and GM-CSF plus dex were equal to or better than IL-1. TGF-β alone did not increase IL-1R expression and adrenalectomy does not impair IL-stimulated HGF production [2,23], we studied whether adrenalectomy influences corticosteroids participate in the constitutive and IL-1-indcued expression of IL-1R.

Since it has been established that IL-1-induces HGF production [2,23], we studied whether adrenalectomy influences IL-stimulated HGF production. For this, adrenalectomized and control (sham-adrenalectomized) mice were bled to 3 hours after IL-1 injection. IL-1 induced similar levels of CSF production in all mice tested (Table 1), indicating that adrenalectomy does not impair IL-stimulated HGF production. We next examined the ability of exogenous addition of dex to synergize with IL-1 in the regulation of IL-1R expression. The injection of optimal concentrations of dex (50 μg) [27] in the presence of optimal amounts of IL-1 (1.0 μg) [17] did not increase IL-1R expression (Fig. 2). At concentrations of 0.1 and 0.3 μg of IL-1 per mouse, however, dex increased IL-1R expression 2-fold. The magnitude of IL-1 upregulation in these cases, however, did not equal the magnitude seen with optimal IL-1 concentrations.

<table>
<thead>
<tr>
<th>Micea</th>
<th>IL-1 injection (μg/mice)b</th>
<th>CSF titer (U/mL)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-ADX</td>
<td>None</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>Sham-ADX</td>
<td>0.1</td>
<td>590</td>
</tr>
<tr>
<td>Sham-ADX</td>
<td>1.0</td>
<td>705</td>
</tr>
<tr>
<td>ADX</td>
<td>None</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>ADX</td>
<td>0.1</td>
<td>750</td>
</tr>
<tr>
<td>ADX</td>
<td>1.0</td>
<td>853</td>
</tr>
</tbody>
</table>

*aMice were either adrenalectomized or sham-adrenalectomized as described [23].

*bMice were bleed 2 to 3 hours after IL-1 injection as described in Materials and methods.

*cSerum was assayed for CSF activity as described in Materials and methods.

Table 1. IL-1-stimulated CSF production in adrenalectomized and sham-adrenalectomized mice

![Graph showing IL-1 (μg/mouse) versus Dose-dependent interaction between dex and IL-1 administration for increase in IL-1R expression](image)

**Fig. 2.** Dose-dependent interaction between dex and IL-1 administration for increase in IL-1R expression

Mice were injected i.p. with either saline, the indicated doses of IL-1 and maximal dose (50 μg) of dex. Sixteen to 18 hours after treatment radioreceptor assays for the expression of IL-1R on BM cells were done as described in Materials and methods. The data represent CSF titers of pooled serum from 3 mice. Using G-CSF, the dose-dependence of the synergistic interaction between dex and HGFs was studied. The administration of a previously determined [17] optimal dose of G-CSF significaently increased the expression of IL-1R on BM cells (4.3-fold) (Fig. 4). The administration of dex alone induced a 2-fold increase in IL-1R expression but synergized with G-CSF to upregulate IL-1R in vivo in a dose-dependent fashion (Fig. 4). In all experiments performed (n=7), the administration of G-CSF (5.0 μg) and dex (50 μg) upregulated IL-1R expression to a greater extent than seen with an optimal dose (1.0 μg) of IL-1 alone (Fig. 4). This synergy with G-CSF in increasing IL-1R expression was dose-dependent on dex (Fig. 4). To determine if G-CSF and dex can act on isolated hematopoietic cells in vitro, normal BM cells were incubated in vitro with G-CSF and/or different concentrations of dex. G-CSF, GM-CSF, IL-3 and IL-6 as well as TGF-β, a negative regulator of hematopoiesis. Among the cytokines tested, G-CSF, GM-CSF, IL-3 or IL-6 treatment in combination with dex (50 μg) results in a synergistic effect on IL-1R expression on bone marrow cells (Fig. 3). At equivalent doses, rG-CSF was slightly more potent than GM-CSF, followed by IL-6 and IL-3 (Fig. 3). G-CSF plus dex and GM-CSF plus dex were equal to or better than IL-1. TGF-β alone did not increase IL-1R expression and did not synergize with dexamethasone.
**Fig. 3.** Hematopoietic growth factors synergize with dexamethasone to upregulate IL-1R expression

Mice were injected i.p. with either saline, IL-1 (1.0 μg), G-CSF (5.0 μg), GM-CSF (5.0 μg), IL-3 (5.0 μg), IL-6 (5.0 μg), TGF-β (5.0 μg) and/or dex (50 μg). Sixteen to 18 hours after treatment, BM cells were harvested and tested for the expression of IL-1R using radioreceptor assay. The data represent the mean ± SE of determinations using 3 to 15 mice as indicated in parentheses, 2 to 4 experiments for each cytokine. The level of background binding was 315 ± 52, which was subtracted from the data shown here.

**Fig. 4.** Dose-dependent synergistic interaction between dexamethasone and G-CSF administration for the increase in IL-1R expression

Mice were injected i.p. with either saline, maximal dose of IL-1 (1.0 μg), maximal dose of G-CSF (5.0 μg) and/or increasing doses of dex. Sixteen to 18 hours after the treatments, radioreceptor assay for the expression of IL-1R on BM cells was performed as described in Materials and methods. The data represent the mean ± SE of duplicate determinations of at least 2 experiments using pooled cells from 3 to 15 animals. The level of background binding was 259 ± 34, which was subtracted from the data shown here.

CSF and dex also synergize in vitro to increase IL-1-specific binding (Table 2), whereas IL-1 by itself had no effect.

**Distribution of IL-1R on bone marrow cells from G-CSF- and dextreated mice.** We next evaluated whether the in vivo administration of G-CSF and GC increased expression of IL-1R on a specific population or subpopulation of BM cells. Mice received a single injection of saline or a combination of G-CSF and dex. IL-1 binding to BM cells was determined by autoradiography, 16 to 18 hours after injection. Autoradiographic analysis of cells from saline-treated animals showed that most of the labeled cells belonged to the granulocytic series (Table 3, Fig. 5). Seven percent of the undifferentiated blast/early cells were labeled with 8 grains per cell. After treatment with G-CSF and dex, these cells were 19% positive with 28 grains per cell. Promyelocytes and myelocytes (43% positive with 19 grains per cell) were the most heavily labeled cells after G-CSF and dex treatment (87% positive with 57 grains per cell). Thirteen and 16% of eosinophilic and monocytic cells exhibited a similar pattern of labeling with 7 to 8 specific grain per cell. More monocytic cells were labeled after G-CSF and dex treatment (31%) with a small increase in the number of grains per cell (8 to 17). This treatment had no effect on eosinophilic cells. No specific IL-1 labeling was observed on erythroid cells. These results clearly demonstrate that G-CSF and dex treatment results in an increase in IL-1R expression along the myelocytic series and is particularly pronounced for myelocytes followed by metamyelocytes and segmented neutrophils.

G-CSF and dexamethasone promote granulopoiesis. Because the administration of IL-1 induces an initial rapid mobilization of neutrophils from the bone marrow followed by increased cycling of hematopoietic progenitor cells, resulting in the expansion of granulocytes in the marrow [7-11] we examined whether the combination of G-CSF and dex also promoted an expansion of myeloid cells. Bone marrow cells were analyzed by fluorescence-activated cell sorting accordingly to the differential expression of RB6-8C5 antigen on myeloid cells [28].
The RB6-8CS<sup>hi</sup> cells are enriched for the end stage (segmented) neutrophils (>75%) while the RB6-8CS<sup>lo</sup> cells are enriched for myeloblasts, promyelocytes and myelocytes (>80%). The RB6-8CS<sup>lo</sup> population represents 19 to 24% of total bone marrow and contains 50% of CFU-GM progenitors [28]. As previously demonstrated [28], the administration of IL-1 to mice results in a 206% increase in the RB6-8CS<sup>lo</sup> population and a concomitant 41% loss of RB6-8CS<sup>hi</sup> (Fig. 6, Table 4). Treatment of mice with G-CSF and dex induced a 406% increase in the RB6-8CS<sup>lo</sup> immature myeloid population and no reduction in the RB6-8CS<sup>hi</sup> population. In comparison, dex and G-CSF promoted a 206% and 218% increase in RB6-8CS<sup>lo</sup> population, respectively, and a 137% increase and a 45% reduction in the RB6-8CS<sup>hi</sup> population, respectively.

**Discussion**

We have previously demonstrated that injection of mice with IL-1 results in considerable upregulation of type II IL-IR on hematopoietic progenitor cells. Administration of antibody against type I IL-1R on myeloid-enriched progenitors we have clearly demonstrated that this upregulation occurs through an indirect mechanism. Administration of IL-1 in vivo stimulates the hypothalamic-pituitary-adrenal axis, resulting in the production of GC and HGF bone marrow cells. In addition, this synergy between GC and HGF was also seen in vitro. The observations concerning glucocorticoid modulation of IL-1R in vivo using adrenalectomized mice and the synergy between G-CSF and dex are in agreement with a recent observation from Shieh et al. [19]. While the data indicate a role for GC in IL-1 receptor regulation, other mechanisms cannot be excluded.

In addition, we found that GM-CSF and G-CSF were equally potent in synergizing with dex while IL-6 and IL-3 were approximately 50% as potent. Unlike IL-1, HGF and GCs can stimulate IL-1R expression on hematopoietic cells in vitro [33, 34]. Whether this is a direct effect must await the results of technically difficult binding and antibody-blocking assays on single cells.

Because administration of IL-1 rapidly induces an initial mobilization of neutrophils from the bone marrow followed by increased cycling of hematopoietic progenitor cells, resulting in the expansion of granulocytic compartment in the bone marrow [20, 23], we examined whether G-CSF and dex have the same effect. Bone marrow cells were analyzed by fluorescence-activated cell sorting according to the differential expression of RB6-8CS<sup>lo</sup> antigen on myeloid cells [28]. We had previously shown that RB68CS<sup>lo</sup> cells are enriched for the end stage (segmented) neutrophils (>75%) while the RB6-8CS<sup>hi</sup> cells are enriched for myeloblasts, promyelocytes and myelocytes (>80%). While the administration of IL-1 to mice results in an 206% increase in the RB6-8CS<sup>lo</sup> population and a concomitant 41% loss of RB6-8CS<sup>lo</sup>, treatment of mice with GC and dex induced a 406% increase in the RB6-8CS<sup>lo</sup> immature myeloid population and no reduction in the RB6-8CS<sup>hi</sup> population. In addition, autoradiographic analysis of IL-1 binding on bone marrow cells after G-CSF and dex treatment showed that while cells in all stages of granulocytic development had increased IL-1 binding, the most dramatic increase in terms of number of cells positive and grains per cell were the myelocytes, promyelocytes and metamyelocytes.

**Table 2.** In vitro interaction between G-CSF and dexamethasone in the upregulation of IL-1R on bone marrow cells

<table>
<thead>
<tr>
<th>Factor added&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IL-1-specific binding (CPM)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>798 ± 56</td>
</tr>
<tr>
<td>DEX 10&lt;sup&gt;-8&lt;/sup&gt;M</td>
<td>1341 ±30</td>
</tr>
<tr>
<td>G-CSF</td>
<td>4195 ± 273</td>
</tr>
<tr>
<td>G-CSF + DEX 10&lt;sup&gt;-8&lt;/sup&gt;M</td>
<td>5985 ± 409</td>
</tr>
<tr>
<td>G-CSF + DEX 10&lt;sup&gt;-6&lt;/sup&gt;M</td>
<td>8685 ± 22</td>
</tr>
<tr>
<td>IL-1</td>
<td>747 ± 220</td>
</tr>
</tbody>
</table>

<sup>a</sup>DBM cells obtained from normal mice by Ficoll separation were incubated 24 hours in the presence or absence of the 20 ng/mL of IL-1 or G-CSF and the indicated concentrations of dex.

<sup>b</sup>Bone marrow cells were treated for IL-1 binding as described in Materials and methods. The data represent the mean ± SEM of duplicate determination of a representative experiment of 2 experiments. The level of background binding was 402 ± 168 cpm which was subtracted from the total cpm to give the data shown here.

**Table 3.** Distribution of IL-1R on BM cells from G-CSF plus dexamethasone–treated mice

<table>
<thead>
<tr>
<th>Specific IL-1 binding</th>
<th>% labeled</th>
<th>mean grain count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>saline</td>
<td>+ Dex</td>
</tr>
<tr>
<td>Blasts/ early cells</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>Promyelocytes/ myelocytes</td>
<td>43</td>
<td>87</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>29</td>
<td>72</td>
</tr>
<tr>
<td>Later neutrophils</td>
<td>23</td>
<td>39</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Monocyte</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td>Nucleated erythroid</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Data represent background-subtracted grain count over more than 20 cells of each type. The level of background binding of >5 grains was subtracted from the total grain count to give the data shown here.

The RB6-8CS<sup>hi</sup> cells are enriched for the end stage (segmented) neutrophils (>75%) while the RB6-8CS<sup>lo</sup> cells are enriched for myeloblasts, promyelocytes and myelocytes (>80%). The RB6-8CS<sup>lo</sup> population represents 19 to 24% of total bone marrow and contains 50% of CFU-GM progenitors [28]. As previously demonstrated [28], the administration of IL-1 to mice results in a 206% increase in the RB6-8CS<sup>lo</sup> population and a concomitant 41% loss of RB6-8CS<sup>hi</sup> (Fig. 6, Table 4). Treatment of mice with G-CSF and dex induced a 406% increase in the RB6-8CS<sup>lo</sup> immature myeloid population and no reduction in the RB6-8CS<sup>hi</sup> population. In comparison, dex and G-CSF promoted a 206% and 218% increase in RB6-8CS<sup>lo</sup> population, respectively, and a 137% increase and a 45% reduction in the RB6-8CS<sup>hi</sup> population, respectively.
Whether the increased IL-1R binding leads to increased IL-1 responsiveness is being studied. Thus, it is shown that G-CSF and dex in combination in vivo mimic the effects of IL-1 in granulocyte differentiation.

In addition, we have previously shown that an antibody against type I IL-1R not expressed on neutrophils blocked most of the initial mobilization of neutrophils together with HGF production by type I-expressing stromal cells [17,23]. This confirms that chemotactic response of neutrophils to IL-1 is indirect, probably mediated through IL-1-induced potent chemotactic cytokines such as IL-8 [35,36]. In this report, we show that G-CSF alone can mimic the extent of the initial mobilization of bone marrow neutrophils due to IL-1 (Fig 6, Table 4). Unlike IL-1, G-CSF is directly chemotactic for neutrophils in vitro [37]. It is therefore likely that G-CSF participates with other cytokines induced by IL-1 in the mobilization of neutrophils observed after IL-1 administration.

In general, the amplitude of the response to IL-1 correlates with cell surface receptor expression. For example, positive regulators such as PDGF increased the number of IL-1R together with the capacity of the cell to respond to IL-1 [38]. In addition, treatment of hematopoietic progenitor cells with negative regulators such as TGF-β blocked the ability of IL-1 to promote high proliferative potential (HPP) colony formation as well as greatly reduced the expression of IL-1R expression [39]. Clinically, increased IL-1R expression has been noted in sepsis, organ failure and acute disseminated inflammation [40]. In this report, concomitant injection of HGF and GC increased IL-1R expression on myeloid cells in the bone marrow with the most increase seen on the myelocyte and promyelocytes followed by metamyelocytes and segmented neutrophils. Such an increase in IL-1R on a premitotic population would serve to promote cellular differentiation and/or cell division, resulting in an amplification of granulocyte differentiation. Therefore, the ability of IL-1 to enhance granulopoiesis in normal [7-11] as well as in myelosuppressed [12-16] mice may be partly due to the unique ability of IL-1 to induce a complex cascade of cytokines and steroids, which can then act to regulate IL-1 receptor expression.

In conclusion, these results provide new insights into the mechanism of IL-1 restorative effects in the marrow. IL-1 stimulates production of HGF and GC which in turn upregulate the expression of IL-1R and render the cells more responsive to IL-1. This accounts for the initial burst of granulo-
Table 4. Flow cytometric analysis of BM cells from dext- and G-CSF-treated mice using RB6-8C5 antibody

<table>
<thead>
<tr>
<th>Treatments</th>
<th>RB6-8CS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RB6-8CS&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>7.7 ± 2.4 (100)</td>
<td>15.8 ± 1.1 (100)</td>
</tr>
<tr>
<td>IL-1</td>
<td>16.3 ± 4.6 (210)</td>
<td>9.8 ± 1.3 (62)</td>
</tr>
<tr>
<td>Dex</td>
<td>15.4 ± 5.6 (200)</td>
<td>23.3 ± 0.9 (147)</td>
</tr>
<tr>
<td>G-CSF</td>
<td>23.6 ± 2.2 (306)</td>
<td>11.8 ± 1.6 (74)</td>
</tr>
<tr>
<td>G-CSF + Dex</td>
<td>31.3 ± 7.4 (406)</td>
<td>18.0 ± 601 (114)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mice were injected i.p. with either saline, maximal doses of IL-1 (1 ug), G-CSF (5 ug) and/or dex (50 ug). Sixteen to 18 hours after treatment, 10<sup>6</sup> BM cells were labeled in an indirect immunofluorescence assay by using the MAB RB6-8C5 as outlined in Materials and methods. Background staining of the isotype-matched control antibody was >3.5% for each treatment. Data represent mean ± SEM for 2 experiments using pooled cells from 3 mice.

<sup>b</sup>The numbers in parentheses represent the percent of stimulation and inhibition of controls (saline-treated). Using an established protocol (28), 8C5<sup>+</sup> showed fluorescence between channels 0 and 60, 8C5<sup>+</sup> between 60 and 175 and 8C5<sup>+</sup> between 175 and 250.

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