Acceleration of Myeloid Recovery from Cyclophosphamide-induced Leukopenia by Pretreatment with Bacillus Calmette-Guérin

Stephan Ladisch, David G. Poplack, and Joan M. Bull

Introduction

Bone marrow suppression is a common side effect of many antineoplastic chemotherapeutic agents. The resultant granulocytopenia greatly increases the risk of serious infection in patients undergoing cancer treatment (1). Since the efficacy of granulocyte transfusions is still controversial (2), investigators have sought other methods of normalizing the granulocyte count in such patients. Recently, it has been postulated that the immune adjuvant BCG 3 may decrease the severity and duration of chemotherapy-induced leukopenia (7, 8). We have found that pretreatment with BCG accelerates the recovery of the peripheral granulocyte count in mice given the marrow-suppressive drug CTX, an agent widely used in human cancer therapy.

Materials and Methods

Materials. Male C57BL/6 mice, obtained from the Veterinary Resources Branch, NIH, were used for all experiments. The mice were 6 to 7 weeks old and of similar weight at the initiation of the experiments. Lyophilized BCG was obtained from the Institute Pasteur (Paris, France), stored under refrigeration, and reconstituted with 0.9% NaCl solution to a concentration of 10^8 organisms/ml, immediately prior to injection. CTX (Mead, Johnson and Co., Evansville, Ind.) was freshly reconstituted prior to use in each experiment. Endotoxin (E. coli 0111:B4 lipopolysaccharide B) was obtained from Difco Laboratories (Detroit, Mich.) and stored at -20°C.

ABSTRACT

Treatment of C57BL/6 mice with Bacillus Calmette-Guérin i.p. 8 days prior to the induction of leukopenia by cyclophosphamide (300 mg/kg i.p.) significantly (p < 0.002) increased peripheral granulocyte counts on each day during the recovery from leukopenia. The recovery of lymphocyte counts was unaffected by Bacillus Calmette-Guérin treatment. Further experiments indicated that the accelerated granulocyte recovery was the result of an earlier initiation of the recovery process rather than of the release of stored granulocytes. Bacillus Calmette-Guérin may have clinical value as a stimulator of myelopoiesis in patients rendered leukopenic by antineoplastic chemotherapy.

Introduction

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Experimental Design. Experimental groups were composed of 15 mice. Mice received either 0.1 ml of reconstituted BCG (1 × 10^8 organisms) or 0.1 ml of 0.9% NaCl solution (control) i.p. at the beginning of the experiment. Eight days later, animals in both groups were given a single dose of CTX (300 mg/kg i.p.). WBC were determined by hemocytometer on tail vein blood. Absolute granulocyte and lymphocyte counts were calculated from the total WBC and the differential count, determined on Wright-stained blood films.

Assessment of Granulocyte Stores. Mice were treated according to the experimental design described above and challenged with endotoxin (500 µg i.p.) 3 days after receiving CTX (i.e., at the time of the WBC nadir). For determination of the extent of granulocyte storage, WBC were determined before and 12 hr after endotoxin administration (9).

In addition, mice were examined for the presence of granulocytes in the spleen. Animals were sacrificed 3 days after treatment with CTX. The spleens were removed, fixed in 10% formalin, embedded, cut, and stained with hematoxylin-eosin and nonspecific esterase.

Statistics. The significance of the difference between the leukocyte counts of the experimental and control groups was determined by means of the Wilcoxon signed-rank test, with a modification for the analysis of the combined results of several experiments (6).

Results

Effect of BCG Pretreatment on WBC Recovery. The combined results of 3 experiments in which mice were pretreated with either BCG or 0.9% NaCl solution 8 days prior to the administration of CTX are shown in Chart 1. Prior to the administration of CTX, there was no difference between the WBC counts of the BCG-pretreated mice (mean ± S.E., 17.1 ± 1.0 × 10^3 WBC/cu mm) and control mice pretreated with 0.9% NaCl solution (16.6 ± 1.2 × 10^3 WBC/cu mm). Animals in both groups became leukopenic at the same rate and to the same degree following treatment with CTX, reaching a WBC nadir on Day 4 of 0.9 ± 0.1 and 0.7 ± 0.1 × 10^3 WBC/cu mm (BCG- and 0.9% NaCl solution-pretreated groups, respectively; p > 0.05). However, during the recovery period, the WBC of the BCG-pretreated mice were significantly higher (p < 0.0001) than those of the control mice at each time point tested. Similar results (data not shown) were obtained when the dosage of CTX was varied (500 mg/kg, single dose; 400 mg/kg in 4 divided daily doses).

Effect of BCG Pretreatment on Granulocyte Recovery. The absolute granulocyte and lymphocyte counts during the recovery period in the 2 groups of 1

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3 The abbreviations used are: BCG, Bacillus Calmette-Guérin; CTX, cyclophosphamide.

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experiment are shown in Table 1. While the difference in the granulocyte counts on each day was highly significant ($p < 0.002$ to $p < 0.0001$), there was no difference in the lymphocyte counts between the 2 groups on any day during the recovery period, which indicates that the accelerated recovery of the total WBC count was solely due to enhanced granulocyte recovery.

**WBC Mobilization by Endotoxin.** Endotoxin administration to mice pretreated with either BCG or 0.9% NaCl solution alone (not followed by CTX) resulted in a significant leukocytosis (Table 2). This phenomenon, which has been well described previously (9), is the result of granulocyte mobilization. Endotoxin administration to either control or BCG-pretreated mice that had also received CTX did not result in leukocytosis. This indicates the absence of stored granulocytes, which presumably were destroyed by the CTX treatment.

**Splenic Granulocyte Stores.** Histological examination of the spleens taken from mice 3 days after they had received CTX revealed the absence of granulocytes, whether or not the mice had been pretreated with BCG.

**Kinetics of Granulocyte Recovery.** For assessment of the kinetics of recovery, the absolute granulocyte counts during the phase of rapid recovery from CTX-induced leuko-

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### Chart 1. Effect of BCG pretreatment on recovery of the peripheral leukocyte count after administration of cyclophosphamide. Mice were treated i.p. with either BCG (•) or 0.9% NaCl solution (O) 8 days prior to receiving cyclophosphamide (300 mg/kg i.p.) on Day 0. Bar, mean of 45 determinations.

#### Table 1

<table>
<thead>
<tr>
<th>Days post-CTX</th>
<th>$10^5$ x granulocytes/cu mm BCG</th>
<th>$10^5$ x granulocytes/cu mm 0.9% NaCl solution</th>
<th>$10^5$ x lymphocytes/cu mm BCG</th>
<th>$10^5$ x lymphocytes/cu mm 0.9% NaCl solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>$0.3 \pm 0.1^{a,b}$</td>
<td>$0.1 \pm 0.1$</td>
<td>$0.6 \pm 0.1^{c}$</td>
<td>$0.4 \pm 0.1$</td>
</tr>
<tr>
<td>5</td>
<td>$1.9 \pm 0.3^{d}$</td>
<td>$0.4 \pm 0.1$</td>
<td>$1.2 \pm 0.1^{e}$</td>
<td>$1.1 \pm 0.1$</td>
</tr>
<tr>
<td>6</td>
<td>$10.8 \pm 0.4^{f}$</td>
<td>$4.1 \pm 0.4$</td>
<td>$2.5 \pm 0.3^{g}$</td>
<td>$2.4 \pm 0.7$</td>
</tr>
<tr>
<td>7</td>
<td>$20.4 \pm 1.1^{i}$</td>
<td>$13.1 \pm 1.3$</td>
<td>$3.3 \pm 0.7^{f}$</td>
<td>$3.3 \pm 0.3$</td>
</tr>
</tbody>
</table>

*Mean ± S.E., 15 mice/group.

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### Chart 2. Semilogarithmic plot of sequential granulocyte counts during the period of recovery from cyclophosphamide-induced leukopenia (15 mice/group). Pretreatment: BCG (•), 0.9% NaCl solution (O).

#### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$10^5$ x WBC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day -8</strong></td>
<td>Day 0</td>
</tr>
<tr>
<td>0.9% NaCl solution</td>
<td>0.9% NaCl solution</td>
</tr>
<tr>
<td>9.6 ± 0.8$^b$</td>
<td>12.9 ± 2.3</td>
</tr>
<tr>
<td>BCG</td>
<td>0.9% NaCl solution</td>
</tr>
<tr>
<td>9.0 ± 1.4</td>
<td>15.8 ± 3.3</td>
</tr>
<tr>
<td>0.9% NaCl solution</td>
<td>CTX</td>
</tr>
<tr>
<td>0.8 ± 0.2</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>BCG</td>
<td>CTX</td>
</tr>
<tr>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.2</td>
</tr>
</tbody>
</table>

*a Day 3, post-cyclophosphamide.

*b Mean ± S.E., 5 mice/group.
Acceleration of Myeloid Recovery by BCG

Plots for both the BCG- and 0.9% NaCl solution-pretreated mice are linear, which indicates that the recovery is an exponential process in each case. Furthermore, the fact that the plots are parallel shows that the actual rate of recovery is the same for both groups. The characteristic that differentiates between the 2 plots is the shift to the left of the BCG-pretreated group. This signifies an earlier initiation of the recovery process in the latter group.

DISCUSSION

In this study we have shown that i.p. treatment with BCG accelerates the recovery of the total WBC count from CTX-induced leukopenia. Moreover, we have established that this effect is due solely to an acceleration of granulocyte count recovery. Analysis revealed that the accelerated recovery was due to an earlier initiation of the recovery process rather than to an alteration of the kinetics of granulocyte production.

We considered several possible explanations for this phenomenon. The identical timing and depth of the WBC nadirs, indicating similar kinetics of WBC destruction, appear to exclude the possibility that the accelerated granulocyte count recovery is related to a BCG-induced alteration of CTX metabolism. We have also demonstrated that the accelerated recovery was not the result of demargination or mobilization of granulocytes from storage pools such as the bone marrow or spleen. If BCG had acted by causing the release of stored granulocytes resistant to the toxic effect of CTX, one would have expected a significant elevation of the WBC following endotoxin administration in mice that had been pretreated with BCG before receiving CTX. This was not observed. Evidence confirming these latter findings was obtained by histological documentation of the absence of granulocytes on examination of spleens removed 3 days after CTX treatment.

The precise mechanism responsible for the accelerated granulocyte recovery is not completely understood. The present studies suggest that the acceleration may be the result of an effect of BCG pretreatment on granulopoiesis. In the continuously irradiated mouse, BCG treatment has been shown to increase the number of committed granulocytic stem cells in the bone marrow and spleen (4). BCG stimulation of the production or release of granulocyte colony-stimulating factor (3, 7) is another mechanism that could account for the enhanced granulocyte recovery following marrow-suppressive chemotherapy.

Our experiments demonstrate that the i.p. pretreatment of mice with BCG stimulates recovery of the peripheral granulocyte count following CTX-induced leukopenia. Confirmation in other animal models and elucidation of the mechanism of this phenomenon may permit its exploitation in patients receiving antineoplastic chemotherapy.

ACKNOWLEDGMENTS

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REFERENCES

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