CONCISE REPORT

Effect of Human Recombinant Granulocyte Colony-Stimulating Factor on Hematopoietic Injury in Mice Induced by 5-Fluorouracil

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Intravenous (IV) administration of 5-fluorouracil (5-FU) caused severe hematopoietic injury in mice. Daily administration of purified human recombinant granulocyte colony-stimulating factor (rG-CSF) accelerated recovery from neutropenia in these injured mice. Granulocyte-macrophage progenitors (CFU-GM) in spleen were markedly increased by rG-CSF, while rG-CSF was less effective on the regeneration of CFU-GM in femoral marrow. The accelerated recovery from neutropenia in the mice might be mainly due to the stimulation of granulopoiesis in spleen by rG-CSF.

IT IS WELL KNOWN that the proliferation and differentiation of hematopoietic progenitors are directly regulated by the stimulating factors specific to each lineage of the progenitors, respectively. Recently the genes encoding some hematopoietic factors, such as erythropoietin, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF), have been cloned. Our laboratories have also succeeded in producing human recombinant G-CSF (rG-CSF) independently. In vivo activities of the preparations of rG-CSF have been already demonstrated. In consideration of clinical applications of r-G-CSF, we have been trying to estimate the therapeutic effect of rG-CSF on an animal model of neutropenia induced by anti-cancer drugs. In this paper we report an enhancing effect of rG-CSF on the granulopoietic recovery in mice injured hematopoietically by 5-fluorouracil (5-FU).

MATERIALS AND METHODS

Human G-CSF was purified to homogeneity from a protein-free culture cell line, Balanche-I, and the amino-terminal sequence of G-CSF was determined by a gas-phase sequencer (unpublished results). Using poly (a) RNA prepared from human macrophages, a cDNA library was constructed by the methods of Okayama and Berg. The resulting cDNA library was screened by the synthetic oligonucleotide probes, which were predicted by the amino-terminal sequence. The amino acid sequence deduced from the cloned cDNA was identical to that reported by Souza et al. The cDNA was expressed in Escherichia coli. K12 MM294 by construction of an expression plasmid for G-CSF using pGEL-I containing the trp promoter, and the mature protein was purified to homogeneity as previously reported. Details on cloning and expression of the cloned cDNA will be described elsewhere (unpublished results).

Units of r-G-CSF activity were defined according to the method of Nicola et al., i.e., the r-G-CSF concentration required for half-maximal stimulation of colony formation by 75,000 BDF mouse bone marrow cells in a semisolid culture medium was defined as 50 U of activity. As a measurement of purity, the purified r-G-CSF was analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis according to the method of Laemmli and was tested for endotoxin contaminations using a limulus amebocyte lysate assay. The purified r-G-CSF was dissolved in endotoxin-free normal saline for subcutaneous (SC) injection to mice in doses of 5 x 10⁴ to 2 x 10⁵ U/mouse/day.

Female BDF, mice (7 weeks old) were injected intravenously (IV) with 5-FU (200 mg/kg body weight; Sigma Chemical, St Louis) on day 0. Daily SC administration of r-G-CSF was carried out from day 0. The 5-FU-treated control mice received only saline. In one experiment mice were sampled randomly from each group on days 5, 7, 9, 11, and 13, and blood was collected from the orbital sinus for neutrophil counts. Nucleated cells in blood were counted using a Coulter counter (model ZF; Coulter Electronics, Hialeah, FL). Blood smears were stained with Wright-Giemsa for differential counts of leukocytes. A femur and spleen were collected from each mouse under sterile conditions. Using 23-gauge needles, femoral marrow cells or spleen cells were harvested in 2 or 5 mL of α-medium (Flow Laboratories, McLean, VA). The cells were counted using a Coulter counter and were washed by centrifugation at 400 x g. Granulocyte-macrophage progenitors (CFU-GM) in femoral marrow cells or in spleen cells were assayed in the semisolid agar culture system as follows: The cells (5 x 10⁴/plate) were plated in 0.5-M aliquots of α-medium containing 20% fetal bovine serum (Flow Laboratories, North Ryde, NSW, Australia), 0.3% agar, and 10% WEHI-3 conditioned medium as a source of colony-stimulating factor. These cultures were carried out in 24-multiwell tissue-culture plates. By day 7, CFU-GM were scored when they had formed a visible colony under a phase-contrast microscope.
culture plates (Corning 25820; Iwaki Glass, Japan). Duplicate cultures were made from each murine femur or spleen and were incubated at 37 °C in a humidified atmosphere of 5% CO₂ in air. After seven days of incubation, clonal colonies consisting of more than 50 cells were scored using an inverted microscope. In another experiment, blood (less than 30 μL) was collected successively on days 9, 11, and 13 from the tail vein of each mouse for neutrophil counts.

RESULTS

The purified rG-CSF was found to be homogenous by SDS-polyacrylamide gel electrophoresis (Fig 1) and to have an apparent molecular weight of 17,400, whereas human G-CSF purified from cell culture migrates with an apparent molecular weight of 18,000. The purified rG-CSF had a specific activity of 1 x 10⁴ U/mg of protein and contained less than 2.0 ng of endotoxin/mg of protein. This means that the in vivo studies were carried out in an almost endotoxin-free system.

Quantitative changes of neutrophils in blood and those of CFU-GM in femur or spleen of the mice injected with 5-FU are summarized in Fig 2. 5-FU caused severe neutropenia and a significant decrease of CFU-GM. More than 13 days were required for the full recovery from neutropenia in the 5-FU-treated mice. On the contrary, the daily administration of rG-CSF (1 x 10⁴ U/d) to the 5-FU-treated mice caused a marked elevation in neutrophil counts on days 11 and 13 (313% and 1,019% of each paired control, respectively) as shown in Fig 2A. In comparison with the mild enhancing effect of rG-CSF on CFU-GM in femur (Fig 2B), rG-CSF administration showed a marked increase in CFU-GM in spleens on days 11 and 13 (498% and 278% of each paired control, respectively) as shown in Fig 2C. The recovery from neutropenia in 5-FU-treated mice was accelerated by 5 x 10⁴ to 2 x 10⁵ units of rG-CSF in a dose-dependent manner (Fig 3).

DISCUSSION

Several laboratories have succeeded in large-scale production of hematopoietic factors from their cloned genes. Some of these recombinant factors are expected to be used as stimulators of hematopoietic restoration from anti-cancer drugs or irradiation-induced hematopoietic injury. Our observations described here support the possibility of therapeutic applications of rG-CSF in neutropenia caused by...
cancer chemotherapy. 5-FU in a dose of 200 mg/kg body weight caused severe neutropenia in mice. As shown in the results, it seemed that there were two stages in the process of granulopoietic recovery after being injured by 5-FU: The stage of regeneration of CFU-GM in bone marrow preceded that of a CFU-GM increase in spleen. The administration of rG-CSF was much more effective in this second stage than in the first one. Thus, rG-CSF stimulated in vivo granulopoiesis to accelerate recovery from neutropenia in an endotoxin-free system.

REFERENCES

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