AFRRI SCIENTIFIC REPORT

Use of glucan to enhance hemopoietic recovery after exposure to cobalt-60 irradiation

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The proliferation of murine bone marrow and splenic pluripotent hemopoietic stem cells (CFU-s), granulocyte-macrophage colony-forming cells (GM-CFC), and erythroid burst- and colony-forming cells (BFU-e, CFU-e) has been shown to be altered following administration of the immunomodulating agent glucan. Because of glucan's ability to modulate hemopoiesis, we examined the feasibility of using this agent to enhance hemopoietic recovery following exposure to a hemopoietically damaging dose of cobalt-60 irradiation. Unless otherwise stated, female C3H/HeN mice received a single

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20. ABSTRACT (continued)

intravenous injection of either 0.4 or 1.5 mg of particulate glucan at times ranging from 1 hour to 17 days either before or after exposure to 650 R of cobalt-60 irradiation. Eight days later, mice were sacrificed, the spleens removed, and the number of endogenous hemopoietic spleen colony-forming units (E-CFU) counted. The effects of glucan were dose-dependent, with greater and more prolonged effects observed with the 1.5-mg glucan treatment. E-CFU numbers increased with both glucan doses injected at all times before irradiation. Maximum responses were seen in mice injected at 1 hour and at 1 day before irradiation. At these respective times, compared to the 3 ± 1 E-CFU observed in radiation controls, 47 ± 5 and 45 ± 2 E-CFU were observed in 1.5-mg glucan-treated mice, and 17 ± 2 and 17 ± 1 E-CFU were observed in 0.4-mg glucan-treated mice. The most dramatic hemopoietic responses in postirradiation glucan-treated mice occurred with glucan injected just 1 hour after irradiation. In magnitude, these responses were similar to those observed with glucan injected at 1 hour and 1 day before irradiation (i.e., 42 ± 4 E-CFU in 1.5-mg and 18 ± 3 E-CFU in 0.4-mg glucan-treated mice). However, by administering consecutive glucan injections at 1 hour, 1 day, and 2 days after irradiation, even greater enhancement of E-CFU numbers in postirradiation glucan-treated mice could be produced. Clearly, these data suggest that glucan may be useful in enhancing hemopoietic recovery following radiation exposure. Currently we are in the process of assaying bone marrow and splenic CFU-s, GM-CFC, BFU-e, and CFU-e contents in postirradiated glucan-treated mice.

From: MACROPHAGES AND NATURAL KILLER CELLS
Edited by Sigurd J. Normann and Zanvil Gorkin
(Plenum Publishing Corporation, 1982)

USE OF GLUCAN TO ENHANCE HEMOPOIETIC RECOVERY AFTER EXPOSURE TO COBALT-60 IRRADIATION

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INTRODUCTION

Glucan is a B-1,3 polyglucose isolated from the inner cell wall of the yeast Saccharomyces cerevisiae (1). Administration of glucan to rodents significantly enhances reticuloendothelial and immune responses (2-4) and represses tumor growth and experimental infections (5-7). Glucan also alters bone marrow and splenic hemopoietic proliferation and differentiation (8-13). Similar to glucan-induced immunomodulation, glucan-induced hemopoietic regulation depends on the route of glucan administration, the glucan dose administered, and the source of the glucan preparation (8,10,12). In general, following intravenous administration of currently available particulate glucan preparations in the dose range of 0.1 to 2.0 mg per mouse, the proliferation of bone marrow and splenic pluripotent stem cells (CFU-s), splenic macrophage and granulocyte-macrophage colony-forming cells (M-CFC, GM-CFC) and splenic erythroid colony and burst-forming cells (CFU-e, BFU-e) is stimulated in a direct, dose-dependent manner (12). The exact mechanisms through which glucan mediates its stimulatory effects on hemopoiesis are still largely unknown; however, both macrophages and T-lymphocytes have been reported to be involved (9,11,14).

Because of glucan's ability to stimulate hemopoiesis, and in particular, pluripotent hemopoietic stem cell proliferation, we have investigated the ability of this agent to enhance hemopoietic recovery following exposure to cobalt-60 irradiation.

METHODS AND MATERIALS

Ten-to-twelve-week-old female C3H/HeN mice were used in all

experiments. All mice were quarantined and acclimated to laboratory conditions for 2 weeks before experimentation, during which time they were examined and found to be free of murine pneumonia complex and oropharyngeal Pseudomonas. Particulate, endotoxin-free glucan was obtained from Accurate Chemical and Scientific Corporation (Westbury, NY), and was prepared according to DiLuzio's modification (15) of Hassid's original procedure (1). Glucan was diluted in sterile saline, and either 0.4 or 1.5 mg was injected intravenously into experimental mice. Control mice were injected with an equivalent volume of sterile saline.

At the indicated times either before or after glucan injection, 650 rads of total-body irradiation from the AFRRI cobalt-60 source was administered to mice at a dose rate of 150 rads per minute. The post-irradiation survival and proliferation of pluripotent hemopoietic stem cells were measured by the endogenous spleen colony assay (E-CFU) (16-17). The spleens of experimental and control mice were removed, fixed in Bouin's solution, and the number of visible spleen colonies counted. Spleens of post-irradiated glucan-treated mice were removed 8 days after irradiation. Spleens of pre-irradiated glucan-treated mice were removed 8 days after glucan injection.

RESULTS

Figure 1 illustrates the effects of pre-irradiation glucan treatment on E-CFU proliferation. Both 0.4 and 1.5 mg of glucan, injected into mice 1 hour or 1, 5, 11 or 17 days prior to irradiation, enhanced E-CFU proliferation. More dramatic effects were produced by the higher glucan dose. At either glucan dose, the greatest enhancement occurred with glucan administered 1 day and 1 hour prior to irradiation. As the interval between glucan administration and radiation exposure increased, less enhancement was observed.

The effects of post-irradiation glucan treatment on E-CFU proliferation are presented in Figure 2. Glucan injected 1 hour after irradiation was as effective in enhancing E-CFU proliferation as glucan injected 1 hour or 1 day before irradiation. Glucan administered 1, 5, 11 or 17 days after irradiation had progressively less effect. For example, in comparison to the respective radiation controls, E-CFU proliferation was enhanced approximately 1250% after 1.5 mg of glucan injected at 1 hour but only 350% and 250% at 1 and 5 days, respectively. By the time spleens were harvested from mice receiving glucan 11 and 17 days after irradiation, these spleens (as well as the spleens of the respective radiation controls) exhibited confluent colony growth, which precluded accurate quantitation of spleen colony numbers. However, spleens of glucan-treated mice were larger and weighed more than those of radiation controls.

In animals that had been irradiated before glucan treatment,

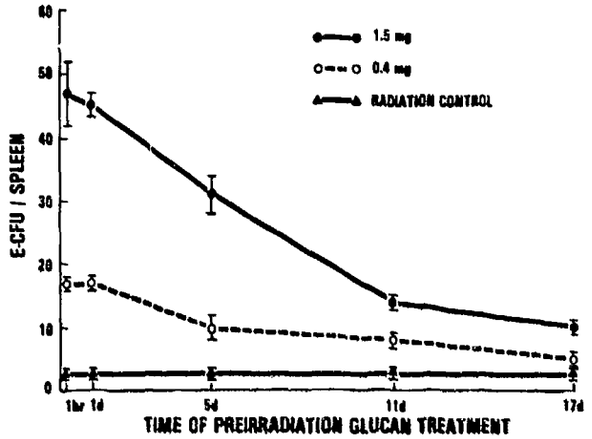

Fig. 1. Effect of pre-irradiation glucan treatment on E-CFU. Data points represent pooled mean values ± 1 standard error from 5 replicate experiments.

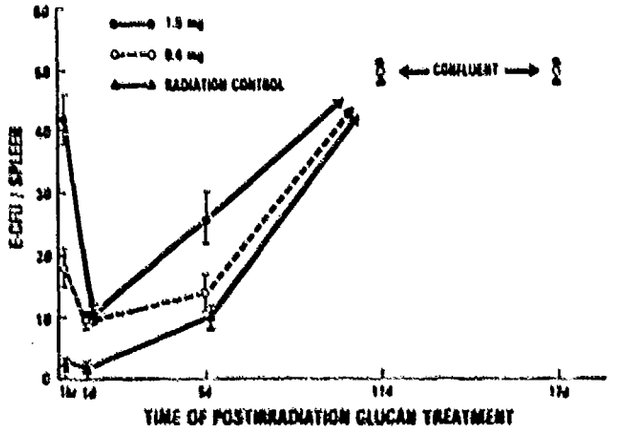

Fig. 2. Effect of post-irradiation glucan treatment on E-CFU. Data points represent pooled mean values ± 1 standard error from 4 replicate experiments.

further enhancement of E-CFU proliferation was attempted by administering multiple glucan treatments. Figure 3a illustrates that when mice received three consecutive 0.4 mg glucan injections at 1 hour, 1 day, and 2 days after irradiation (i.e., total cummulative dose of 1.2 mg), E-CFU proliferation was significantly enhanced above that observed with single 0.4 mg glucan injections. If mice also received 0.4 mg of glucan 1 day prior to irradiation (i.e., total commulative dose of 1.6 mg), an even greater enhancement of E-CFU proliferation was observed (Figure 3b).

DISCUSSION

These studies have shown that the immunomodulating agent glucan enhances the proliferation of pluripotent hemopoietic stem cells when administered either before or after exposure to a hemopoietically damaging dose of cobalt-60 irradiation. Enhancement depends on both the dose of glucan administered and the time of administration with respect to the time of irradiation. The timing of glucan administration after irradiation is more critical than before irradiation.

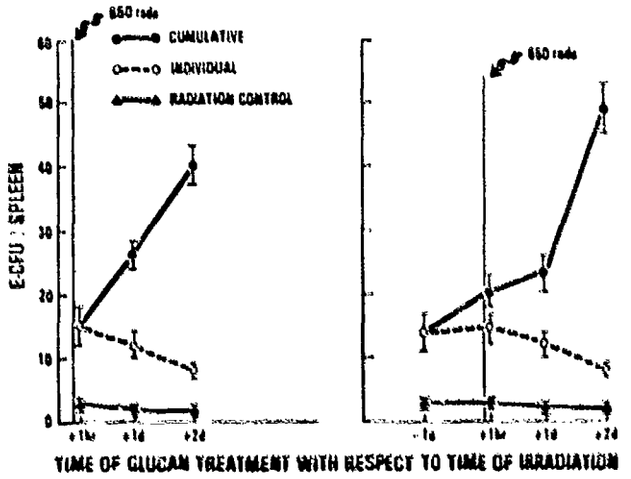

Fig. 3. Effect of multiple glucan injections on E-CFU. Data points represent pooled mean values ± 1 standard error from 3 replicate experiments. (3a) Three consecutive 0.4 mg glucan injections administered 1 hour, 1 day and 2 days after irradiation. (3b) Four consecutive 0.4 mg glucan injections administered 1 day before and 1 hour, 1 day and 2 days after irradiation.

Using a single glucan injection, maximum E-CFU proliferation was produced by glucan administered 1 day or 1 hour before or 1 hour after irradiation. Further enhancement could be produced by multiple glucan treatments.

The mechanism(s) by which glucan enhances proliferation of E-CFU in irradiated mice is not known. However, since macrophages are relatively radio-resistant cells (18-19) and since macrophages in glucan-treated animals have been shown to enhance at least some aspects of hemopoiesis (9,11,14), it is possible that these cells may contribute to the regulation of hemopoiesis in irradiated animals.

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DISCUSSION

FRIEDLANDER: What happens in the HeJ mouse?

PATCHEN: We have not used glucan in HeJ mice, but that would be an interesting experiment because the HeJ mouse does not respond to endotoxin. Whether or not it would respond to glucan, I'm not sure. I might add that our glucan has been tested for endotoxin and it's negative.

GORDON: How does glucan stimulate hemopoiesis? Is there any circulating substance?

PATCHEN: We used a particulate glucan preparation which is rapidly phagocytized by macrophages. Within 24 hours, it's definitely cleared from the blood. We've looked at the effect of glucan on GM-CSF and found that it is related to its macrophage activity. CSF is present in the sera of these mice.