Effect of Glucan, a Macrophage Activator, on Murine Hemopoietic Cell Proliferation in Diffusion Chambers in Mice

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ABSTRACT

Pretreatment of mice with glucan, a potent macrophage activator, resulted in enhanced myeloid cluster and colony formation by bone marrow cells in diffusion chambers implanted into the peritoneal cavity. Simultaneously, erythroid colony formation was also augmented. An increased yield of early proliferative cells, granulocytes, and macrophages was found in glucan-treated hosts. Concomitantly, higher leukocyte counts were noted in the peripheral blood of treated animals. These results suggest that glucan has a strong stimulatory effect on hemopoiesis. This stimulation is probably mediated by humoral factors of host animal origin rather than by direct interaction with proliferating hematopoietic precursors enclosed within the chambers.

INTRODUCTION

Glucan is a polysaccharide isolated from zymosan and is a potent stimulator of the mononuclear phagocyte system (3, 17). When glucan is administered to rodents, there is increased macrophage activity in the spleen, liver, and lungs (4–6). The macrophage-activating property of glucan has been related to tumor repression seen in experimental animals (5, 13). Glucan has also been used as an immunotherapeutic agent in the treatment of human neoplasms (13, 14).

The effects on hemopoiesis of agents used in immuno-therapy are largely unknown. Animal studies suggest that Corynebacterium parvum (7, 11, 19, 20), Bacillus Calmette-Guérin (9, 11), and glucan (2) increase the concentration of colonies, or increases their plating efficiency of another humoral factor, affecting the mobilization of colony-forming units, or increases their plating efficiency must also be considered.

This report describes the effect of glucan on hemopoiesis in diffusion chambers in mice. The results suggest that increased cell production is mediated by humoral factors elaborated as a consequence of glucan administration.

MATERIALS AND METHODS

Mice. Female Swiss-Webster mice weighing 20 to 30 g were used throughout the study. Hematocrit determinations and WBC, differential, and reticulocyte counts were routinely monitored.

Glucan. Glucan, a gift from Dr. N. DiLuzio, was prepared according to the technique of Hassid et al. (12). The stock solution was diluted in dextran. There was no endotoxin contamination of the preparation as measured by the Limulus assay. The mice were given i.p. injections of 2 mg of glucan 7 and 6 days before diffusion chamber implantation. Control mice were given injections of equal volumes of dextran. Microscopic examinations of the diffusion chamber contents did not show glucan particles, which are easily recognized under low-power magnification.

Diffusion Chamber Technique. Diffusion chambers were constructed by gluing 13-mm Millipore filters (pore size, 0.22 μm) to plastic rings and were sterilized under UV irradiation for 2 to 4 hr. Each chamber was filled with 1 x 10⁶ normal mouse cells (0.12 ml) and with 0.02 ml of citrated bovine plasma. After sealing, 2 diffusion chambers were implanted into the peritoneal cavity of either a glucan- or a dextran-pretreated mouse under pentobarbital anesthesia. Hosts (3 to 6) were sacrificed at varying intervals, and the chambers were removed. In some experiments the chambers were shaken unopened for 60 to 80 min in a solution containing 0.5% pronase (Calbiochem, Los Angeles, Calif.) and 5% Ficol (Sigma Chemical Co., St. Louis, Mo.) to dissolve the chamber clot. The cells were then retrieved for total and differential counting. Five hundred cells on each slide were scored and classified as early proliferative cells, granulocytes, or macrophages. The early proliferative cells lacked definite granulocyte or macrophage characteristics. The other chambers were processed for evaluation of myeloid and erythroid colony growth in situ in the diffusion chamber clot. One of the filters was removed from the plastic ring with a sharp microtome blade, and the plasma clot attached to the remaining filter was removed with a scalpel, fixed for 6 min with phosphate-buffered glutaraldehyde (pH 7.2), and washed with distilled water for 8 min. This wet preparation was stained with benzidine-hematoxylin, dried between 2 wire meshes, and made transparent in immersion oil (Transparency Medium 1.506; R. P. Cargille Laboratories, Inc.). At x100 magnification granuloid cell aggregates containing 4 to 19 cells were scored as clusters, and those larger than 19 cells were scored as colonies. Aggregates of 8 or more normoblasts were counted as erythroid colonies.

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4 The abbreviations used are: CFU-C, myeloid colony-forming unit (culture), assayed in agar; CFU-S, colony-forming unit (spleen), assayed in irradiated mice; CSA, colony-stimulating activity; CFU-DE, erythroid colony-forming units grown in diffusion chambers; CFU-DG, granulocyte colony-forming units grown in diffusion chambers.

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**Statistical Analysis.** Probability figures were calculated with Wilcoxon’s nonparametric rank test or Student’s 2-tailed t test.

**RESULTS**

In 3 experiments the peripheral leukocyte counts were determined and were found to be consistently higher in the glucan-treated mice, as in previous experiments (2). In 2 experiments hematocrit, reticulocyte, and platelet numbers in peripheral blood of Swiss-Webster mice were unchanged 6, 8, and 11 days after glucan administration.

In 4 experiments diffusion chambers were used for cell determination. Clearly higher cell numbers were obtained from chambers implanted into mice that received glucan treatment 7 and 6 days prior to implantation. The difference was statistically significant ($p < 0.01$) after 3 and 4 days of culture (Chart 1). Three days after the implantation, the number of early proliferative cells was consistently higher in the chambers in the glucan-treated mice. The difference was statistically significant ($p < 0.01$) after 3 and 4 days of culture (Chart 2). The numbers of mature granulocytes were also elevated in diffusion chambers of glucan-treated hosts. A statistically significant difference was observed on Day 3 ($p < 0.05$) and on Day 4 ($p < 0.01$) (Chart 3). The effect of glucan on macrophage production in diffusion chambers was not obvious before the seventh day ($p < 0.05$) (Chart 4). Very few RBC precursors were seen in either glucan-treated or control host animals.

Cultivation of normal murine marrow in diffusion chambers implanted into glucan-treated hosts also resulted in increased myeloid colony formation within the clot inside the chamber (Chart 5). The difference was statistically significant ($p < 0.01$) on Days 3, 4, and 5. Only a few colonies were seen during the first 2 days of culture; however, a number of granulocyte aggregates were observed at these times. On Day 2, 213 ± 35 (S.E.) and 135 ± 34 clusters were observed in the glucan and dextran groups, respectively ($p < 0.05$). These clusters were obvious predecessors of the colonies seen subsequently.

Usually, very few erythroid colonies are formed within the clot inside the diffusion chambers implanted into normal mice. Surprisingly, glucan treatment of the host resulted in markedly increased erythroid colony numbers (Chart 6).
Chart 4. Growth of macrophages in diffusion chambers implanted into mice pretreated with glucan or dextran. The number of replications was as in Chart 1.

Chart 5. Growth of CFU-DG's from mouse marrow cultured in mice. Values are means ± S.E. for 2 to 3 experiments (8 to 18 chambers)/point.

Chart 6. Growth of CFU-DE's from mouse marrow cultured in mice. The number of replications was as in Chart 5.
The difference was statistically significant ($p < 0.01$) on Days 4 and 5. Seemingly, there is a discrepancy between erythroid colony count/plasma clot within the chambers and the yield of RBC precursors per chamber. However, the individual erythroid colonies contained only 8 to 16 cells, in contrast to myeloid colonies consisting of up to several hundred cells; total numbers of erythroid cells would therefore be very low.

**DISCUSSION**

Profound changes in granulopoiesis have been noticed in mice treated with glucan (2). Granulocyte-monocyte progenitor cell (CFU-C) content in spleen and bone marrow increased markedly, and distinctly higher WBC and granulocyte numbers were observed in the peripheral blood of treated animals. More macrophage colonies were obtained from culture of peritoneal cells of glucan-treated mice, and there were elevated amounts of CSA in the serum. Macrophages from mice given glucan also elaborated more CSA in vitro. On this basis Burgaleta and Golde (2) suggested that glucan may stimulate granulopoiesis via increased CSA production. This or some other humoral factor is obviously responsible for increased granulopoiesis in the diffusion chamber. Glucan itself does not enter the chamber in particulate form. Also, the supernatants obtained after Millipore filtering of the glucan preparations lacked stimulatory effect when injected daily for 3 days in mice bearing diffusion chambers. Therefore, the direct effect on colony-forming cells seems unlikely.

In addition to increased granulopoiesis, augmented erythroid colony formation was also observed in diffusion chambers implanted in glucan-pretreated mice. Knowing that zymosan, from which glucan is derived, stimulated hepatic erythropoietin production (16), this response was not unexpected. However, it is impossible to show that this hormone increased CFU-DE numbers. Also, other humoral factors could be responsible for enhanced erythropoiesis. The observations by Aye (1) suggest that factors associated with CSA present in leukocyte-conditioned medium augment plating efficiency of erythroid progenitor cells. Of course, other factors, such as prostaglandins, known to affect erythropoiesis (8) must also be considered.

An additional possibility is that simultaneous increases in CFU-DG and CFU-DE concentrations reflect a humoral effect on the pluripotent stem cell. In earlier works higher CFU-DG, CFU-DE, and CFU-S numbers have been obtained about the same time from diffusion chambers implanted into hosts rendered neutropenic with cyclophosphamide (Refs. 15 and 18 and Footnote 5).

This study shows that glucan administration leads to formation of humoral factor(s) that stimulate myeloid and erythroid proliferation in diffusion chamber cultures in mice.

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**REFERENCES**

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