Glucan-Induced Modification of Murine Viral Hepatitis

Abstract. *Glucan, a macrophage stimulant, was evaluated for its ability to alter survival and phagocytic dysfunction in mice challenged with mouse hepatitis virus strain MHV-A59. Administration of glucan before the mice were challenged with the virus significantly prolonged median survival time but did not modify overall mortality compared with control mice given dextrose. Maximal effectiveness was achieved when glucan was administered both before and after the viral challenge. In contrast to the marked hepatic parenchymal cell necrosis observed in the control mice, glucan-treated mice exhibited reduced pathology. Intraperitoneal administration of MHV-A59 resulted in a significant depression of phagocytic activity compared with controls that were not exposed to the virus. The enhancement in phagocytic function in glucan-treated control mice was unaltered in virus-challenged, glucan-treated mice. Thus glucan is capable of increasing survival, inhibiting hepatic necrosis, and maintaining an activated state of phagocytic activity in mice challenged with MHV-A59. Macrophage stimulants may have a significant role in the modification of virally induced hepatic lesions.*

Glucan, a β-1,3-polyglucose isolated from the cell wall of the yeast *Saccharomyces cerevisiae*, has been shown to strongly activate the reticuloendothelial (RE) system (*1*). Glucan administration results in hypertrophy of the major RE organs and in a concomitant activation and proliferation of macrophages (*2, 3*). This hyperfunctional state is characterized by a marked enhancement of humoral and cellular immunity (*1*). Furthermore, glucan has been shown to be effective in enhancing host response against a variety of experimental syngeneic murine tumors (*4, 5*). Preliminary clinical studies indicated that the administration of glucan into subcutaneous metastatic lesions in humans results in prompt tumor necrosis with a concomitant monocytic infiltrate (*6–8*). Additionally, it was shown that glucan modifies the course of disease in experimental animals infected with *Staphylococcus aureus* (*9*), *Candida albicans* (*10*), *Mycobacterium leprae* (*11*), *Francisella tularensis* (*12*), or *Pseudomonas pseudomallei* (*12*).

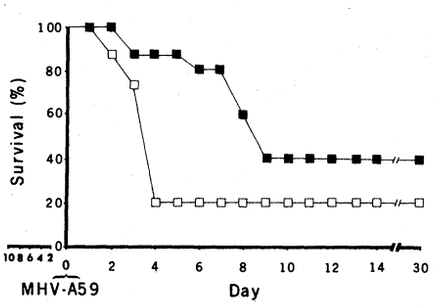

Fig. 1. Effect of glucan on survival of mice when administered before injection of MHV-A59. Glucan (0.45 mg per mouse) (■) or isovolumetric dextrose (□) was administered intravenously 10, 8, 6, 4, 2, and 0 days before the viral challenge. Survival of glucan-treated mice was significantly enhanced at days 4 to 8 ($.02 < P < .05$), but overall survival (days 9 to 30) was not significantly changed ($N = 15$ mice per group).

In view of (i) the ability of glucan to enhance macrophage-mediated host defense mechanisms against certain bacterial and fungal infections and neoplastic cells and (ii) the emerging understanding of the importance of macrophages in natural resistance to primary viral infections (*13*), we designed studies to ascertain whether glucan might enhance resistance to an experimentally induced viral disease.

Male C57B1/6 Tex mice (Timco) weighing 20 g were housed in metal cages and given unrestricted access to Purina laboratory chow and water. To ascertain whether the mice had previously been exposed to viruses, determinations were conducted for antibody to mouse hepatitis virus (MHV), mouse pneumonia virus, polyoma virus, ectromelia virus, Sendai virus, minute virus of mice, lymphochoriomeningitis virus, reovirus, and mouse adenovirus (Microbiological Associates). All serum samples were negative for antibodies to these viruses. Glucan was prepared by a modification of the method of Hassid *et al.* (*14*). In the glucan pretreatment experiments, intravenous injections of sterile pyrogen-free dextrose (5 percent, weight to volume) and water or glucan (0.45 mg per mouse) were given 10, 8, 6, 4, 2, and 0 days before intraperitoneal challenge with a 1:2.5 dilution of 16 complement-fixing units of MHV strain A59. In the glucan pre- and post-treatment groups, glucose or glucan (0.45 mg per mouse) was administered in the same way as for the pretreatment groups, except that it was given 3, 6, 9, and 12 days after the mice were exposed to the virus. Statistical comparisons between survival curves were based on chi-square analysis with 1 degree of freedom. A 95 percent level of confidence was considered significant.

Phagocytic activity was evaluated on day 1 by determining the rate of intravascular clearance of gelatinized [^{131}I]-triolein-labeled RE lipid emulsion (*15*). The RE lipid emulsion was administered intravenously (50 mg per 100 g of body weight), and blood samples from the tails were taken at intervals of 1, 3, 5, 7, and 9 minutes. The radioactivity of each sample was measured with a gamma counter, and the clearance time was determined from semilogarithmic graphs. Differences in clearance values between the glucan and control groups were then evaluated with Student's *t*-test; a difference was considered significant if P was $< .05$. Liver sections for histological studies were taken on days 1 and 4 from mice selected randomly from each group. All samples were fixed in Formalin (10 percent by volume) and were stained with hematoxylin and eosin.

A significant ($P < .05$) prolongation of survival resulted when glucan was administered to mice that were subsequently injected with MHV-A59. The median survival time of the glucan-treated, virus-challenged group was ~ 8.5 days; in contrast, the dextrose-treated control group had a median survival time of 3.5 days (Fig. 1). Seven days after the administration of MHV-A59, mortality was 80 percent in the control group and 20 percent in the glucan-treated group. Mortality in the glucan-treated groups increased on days 8 and 9, after which no additional deaths were observed. Therefore, the overall mortality was not significantly different from that of the control group.

Since the onset of mortality in the glucan-treated group coincided with the recovery from the glucan-induced activa-

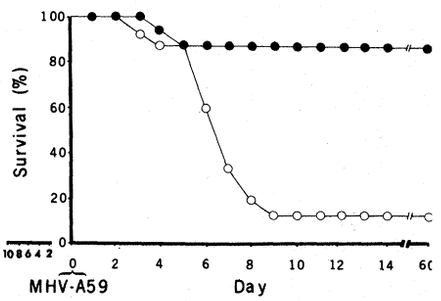

Fig. 2. Effect of glucan on survival of mice when administered before and after injection of MHV-A59. Glucan (0.45 mg per mouse) (●) or isovolumetric dextrose (○) was administered intravenously 10, 8, 6, 4, 2, and 0 days before the viral challenge and on days 3, 6, 9, and 12 after the challenge. Survival was significantly enhanced in the group that received glucan both before and after exposure to the virus (for day 7, $P < .05$; for day 8, $P < .02$; and for day 9 and beyond, $P < .0005$) ($N = 15$ mice per group).

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tion of macrophages (*2*), we conducted an experiment in which glucan was administered once every third day for 12 days after the injection of MHV-A59. Survival in the control group was 12 percent, with all deaths occurring in the first 9 days. In contrast, a long-term (60 day) survival was observed in 87 percent of the group that received glucan both before and after exposure to MHV-A59 (Fig. 2).

Histological examination of the livers of the control mice 1 day after injection of the virus revealed mild structural alterations of parenchymal cells but no necrosis or inflammation. Similarly, glucan-treated mice showed no notable hepatic pathology except for the typical glucan-induced granulomas. By day 4 there was marked necrosis of hepatic parenchymal cells in the dextrose-treated group; the necrotic cells were frequently surrounded by amorphous material, and a moderate inflammatory response was noted (Fig. 3a). In contrast, there was marked inhibition of hepatic necrosis in the glucan-treated mice (Fig. 3b). However, granulomas were prominently distributed.

Significant phagocytic impairment was observed in mice challenged with MHV-A59. The intraperitoneal administration of the virus to control mice induced a mean decrease of 51 percent in the intravascular clearance of the RE lipid emulsion compared to normal values (Table 1). Administration of glucan before exposure to MHV-A59 resulted in a maintenance of enhanced phagocytic activity ($P < .001$) compared to the dextrose-treated, virus-challenged controls (Table 1). The administration of glucan to mice not exposed to MHV-A59 also resulted in a significant ($P < .001$) increase in intravascular clearance relative to normal values (Table 1).

Table 1. Suppression of phagocytosis of the RE lipid emulsion in MHV-A59-challenged mice: modification by pretreatment with glucan. Values are expressed as means ± standard errors and were derived from ten mice per group. Vascular clearance of the gelatinized RE lipid emulsion was measured 24 hours after exposing the mice to the virus. Values for experimental groups are compared to the values for the control group that was given dextrose but not exposed to the virus.

Treatment	MHV-A59 challenge	Clearance $t_{1/2}$ (min)
Dextrose	No	12.1 ± 1.4
Dextrose	Yes	24.6 ± 3.6*
Glucan	No	3.1 ± 0.4†
Glucan	Yes	3.9 ± 0.3†

*$.01 > P > .001$. †$P < .001$.

Glucan was effective in modifying morbidity and mortality as well as in preventing the suppression of macrophage phagocytic function in mice with fulminant viral hepatitis. The loss of protection in the later stages of the disease, as reflected by the late onset of mortality in the group given glucan before but not after their exposure to MHV-A59, may reflect the reversible nature of the glucan-induced hyperfunctional state (*2*). The late onset of mortality was also observed in mice injected with *S. aureus* (*9*). Since the continued administration of glucan after challenge with MHV-A59 significantly extended survival, maintenance of enhanced macrophage number or function may have eliminated the virus.

The precise mechanism by which glucan protects against murine viral hepatitis is not known. However, we demonstrated previously that glucan enhances diverse immune factors such as serum lysozyme (*16, 17*), complement (*18*), and RE function (*1*). Burgaleta and Golde (*19*) have reported enhanced leukopoiesis after glucan administration (*19*). Also, preliminary data indicate that glucan administration enhances interferon levels in the host (*20*). The glucan-induced enhancement of host defense is mediated primarily by phagocytes such as Kupffer cells and other macrophage

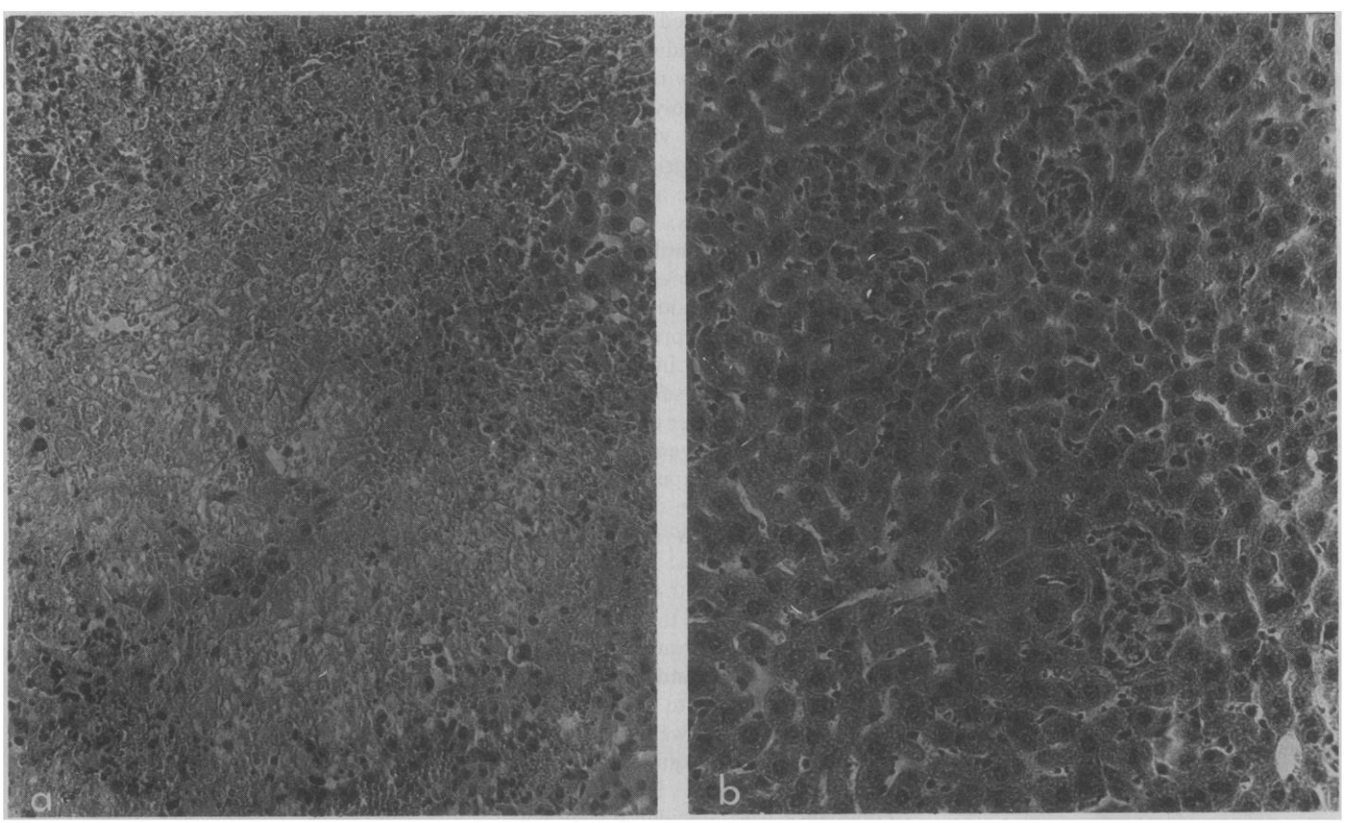

Fig. 3. Hepatic parenchymal cell necrosis in control mice (a) 4 days after intraperitoneal injection of MHV-A59 was not present in the livers of mice treated with glucan before being challenged with the virus (b). The characteristic glucan-induced granulomas are readily discernible. Stain: hematoxylin and eosin (×200).

populations (*9–12*). Since the littoral macrophage or Kupffer cell appears to be important in the pathogenesis of murine viral hepatitis (*21*), the action of glucan against MHV-A59 may be due to its ability to increase hepatic macrophage number (*3*) and function (*2*) and thus prevent initial viral replication, alter subsequent generations of viral progeny, or eliminate the virus. The present study supports the concept that the pathogenesis of viral infections is determined, in part, by macrophage-virus interactions (*13, 21–24*).

Murine infections with MHV 1 have been reported to result in primary involvement of the Kupffer cells (*21, 22*) and a subsequent lysis of these cells and release of virus particles (*22*). This impairment or destruction of liver macrophages results in a decrease of phagocytic function, with the degree of dysfunction related to the virulence of the virus (*23*). Previous studies also demonstrated that "activation" of peritoneal macrophages by a variety of immunomodulators resulted in increased antiviral and antitumor activity (*24*). Our observations with glucan are in agreement with these findings.

The phagocytic function of macrophages was enhanced when the mice were treated with glucan before being challenged with MHV-A59. Gledhill *et al.* (*23*) reported that MHV 3, another virulent murine hepatitis virus, causes a significant reduction in the intravascular clearance of colloidal carbon during the incubation period of the virus. This observation is in agreement with our data regarding the macrophage-suppressive activity of MHV-A59. Whether the decreased clearance of the RE lipid emulsion is due to impairment or destruction of Kupffer cells remains to be ascertained.

Histopathological examination of the liver on day 4 revealed that pretreating mice with glucan inhibited the viral destruction of hepatic tissue. Although some liver necrosis developed, the primary histological feature was the typical glucan-induced granulomatosis (*3*).

Since glucan has already been reported to have antitumor, antifungal, and antibacterial properties, it is apparent that this polyglucose is an immunotherapeutic agent with broad applications. Whether glucan can alter the course of human viral hepatitis remains to be determined.

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Bronchial Bifurcations and Respiratory Mass Transport

Abstract. *A new transport mechanism explains the importance of the shape of bronchial bifurcations in the transfer of gases and particles between the atmosphere and the alveoli. Photographs of flow visualization experiments illustrate the effect in models of bronchial branching. The mechanism provides a means of nondiffusional transport that helps to explain normal respiratory exchange of gases as well as successful ventilation with very low tidal volumes, as in some lung diseases and in the high-frequency panting of dogs.*

Measurements in vivo of purely convective aerosol and gas movement through the bronchial tree have shown that, during each breath cycle, 10 to 25 percent of the inspired tidal volume is exchanged with the functional residual volume (*1–5*). A new mechanism based on the different velocity profiles generated at the bronchial bifurcations during inspiration and expiration explains this exchange as a well-ordered consequence of lung design. Furthermore, the way in which the exchange occurs suggests that this is an important factor in the transport of particles and in the efficient transport of gases between the atmosphere and the alveoli deep in the lung.

The bulk exchange mechanism induced by the lung airways depends on the fact that velocity profiles—that is, intra-airway flow patterns—are different during inspiration and expiration. A hypothetical example with two different velocity profiles in a single tube serves to illustrate the effect. Consider a tracer aerosol (Fig. 1), whose particles have a negligible coefficient of diffusion and negligible settling due to gravity, in-

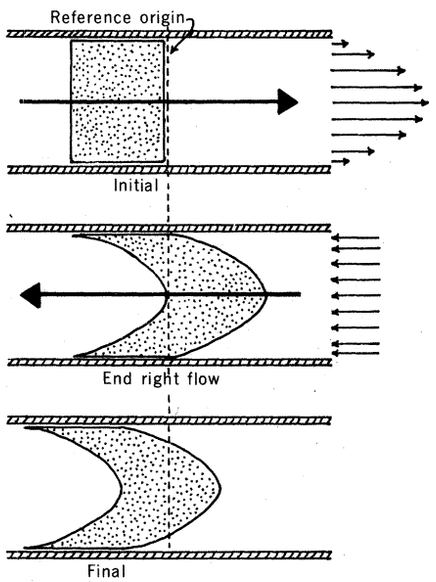

Fig. 1. Cross-sectional illustration of the convective exchange mechanism within a single tube. The position and shape of a tracer aerosol plug are shown at three times in a single flow cycle: initially, at the end of flow to the right and after an equal volume of flow to the left (final). Velocity profiles governing right and left flow are also shown. Net deformation of the fluid volume containing tracer aerosol results in an exchange of fluid across the fixed reference origin. At the end of the cycle the total net flow in the tube is zero, but aerosol particles near the center of the tube have a net movement to the right and those near the wall have a net movement to the left.

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