These observations suggest that the response against SSV-SSAV was mediated by T lymphocytes. Because of possible histocompatibility antigen-associated restriction of cell-mediated cytotoxicity (*20*), we may be underestimating the actual number of SSV-SSAV responders. A broader panel of target cells expressing a wider range of HLA antigens might be necessary to detect all reactors. Two of our four responders were also tested for RIP antibodies to SSV proteins and were found to have elevated levels (data not shown). Kurth *et al.* (*9*) had previously shown that approximately 50 percent of a healthy British population had RIP antibodies against SSV-SSAV-related proteins. Other investigators using different populations and differing serological techniques have obtained varying results (*10*). Thus, it is not yet possible to evaluate conclusively the level of immunity to SSV-SSAV-related antigens in humans.

MARTIN S. HIRSCH
ANN P. KELLY
DAVID S. CHAPIN
THOMAS C. FULLER
PAUL H. BLACK
Massachusetts General Hospital, Harvard Medical School, Boston 02114

REINHARD KURTH
Friederich-Miescher-Laboratorium, Max-Planck-Gesellschaft, Tubingen, West Germany

References and Notes

1. G. J. Todaro, *Am. J. Pathol.* **8**, 590 (1975); D. Gillespie and R. C. Gallo, *Science* **188**, 802 (1975).
2. M. S. Hirsch and P. H. Black, *Adv. Virus Res.* **19**, 265 (1974).
3. A. Hellman, A. K. Fowler, J. E. Strickland, N. M. Kouttab, *Bibl. Haematol. (Basel)* **43**, 161 (1976).
4. S. S. Kalter, R. J. Helmke, R. L. Heberling, M. Panigel, A. K. Fowler, J. E. Strickland, J. Hellman, *J. Natl. Cancer Inst.* **50**, 1081 (1973); M. L. Vernon, J. M. McMahon, J. J. Hackett, *ibid.* **52**, 987 (1974); M. Imamura, P. E. Phillips, R. C. Mellors, *Am. J. Pathol.* **83**, 383 (1976).
5. S. S. Kalter and R. C. Heberling, *Abstr. Annu. Meet. Am. Soc. Microbiol.* **194**, 233 (1974); R. E. Benveniste, M. M. Lieber, D. M. Livingston, C. J. Sherr, G. J. Todaro, S. S. Kalter, *Nature (London)* **248**, 17 (1974).
6. J. N. Ihle, M. G. Hanna, Jr., L. E. Roberson, F. T. Kenney, *J. Exp. Med.* **139**, 1568 (1974); V. W. Hollis, Jr., T. Aoki, O. Barrera, M. B. A. Oldstone, F. J. Dixon, *J. Virol.* **13**, 448 (1974); S. A. Aaronson and J. R. Stephenson, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1957 (1974); R. C. Nowinski and S. L. Kaehler, *Science* **185**, 869 (1974); S. E. Martin and W. J. Martin, *Nature (London)* **256**, 498 (1975).
7. M. S. Hirsch, A. P. Kelly, M. R. Proffitt, P. H. Black, *Science* **187**, 959 (1975); J. C. Lee and J. N. Ihle, *J. Immunol.* **118**, 928 (1977).
8. G. R. Shellam and N. Hogg, *Int. J. Cancer* **19**, 212 (1977).
9. R. Kurth, N. M. Teich, R. Weiss, R. T. D. Oliver, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1237 (1977).
10. H. W. Synder, Jr., T. Pincus, E. Fleissner, *Virology* **75**, 60 (1976); T. Aoki, M. J. Walling, G. S. Bushar, M. Liu, K. C. Hsu, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2491 (1976).
11. J. R. Stephenson and S. A. Aaronson, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1725 (1976); H. P. Charman, N. Kim, M. White, R. V. Gilden, *J. Natl. Cancer Inst.* **52**, 1409 (1974).
12. M. Lieber, C. J. Sherr, G. J. Todaro, *Int. J. Cancer* **13**, 587 (1974).
13. A. Boyum, *Scand. J. Clin. Lab. Invest.* **13**, 587 (1974).
14. L. Chess, R. P. MacDermott, S. F. Schlossman, *J. Immunol.* **113**, 1113 (1974); J. Porath, R. Axen, S. Ernback, *Nature (London)* **215**, 1491 (1967).
15. M. Jondal, *Scand. J. Immunol.* **3**, 739 (1974).
16. T. C. Fuller, F. L. Delmonico, A. B. Cosimi, C. E. Huggins, M. King, P. S. Russell, *Ann. Surg.*, in press.
17. WHO-IUIS Nomenclature Committee, *Transplantation* **21**, 353 (1976).
18. Y. H. Thong, R. W. Steele, M. M. Vincent, S. A. Hensen, J. A. Bellanti, *N. Engl. J. Med.* **289**, 604 (1973); O. M. Petrucco, R. F. Seamark, K. Holmes, I. J. Forbes, P. G. Symons, *J. Obst. Gynaecol. Br. Commonw.* **83**, 245 (1976); W. H. Stimson, *Clin. Exp. Immunol.* **25**, 199 (1976).
19. B. M. Vose and M. Moore, *Int. J. Cancer* **19**, 34 (1977); *Eur. J. Cancer* **12**, 639 (1976).
20. P. C. Doherty, R. V. Blanden, R. M. Zinkernagel, *Transplant. Rev.* **29**, 89 (1976); P. C. Doherty and R. M. Zinkernagel, *Lancet* **1975-I**, 1406 (1975).
21. Purified BEV was kindly provided by R. C. Gallo, National Cancer Institute; the SSV-SSAV complex was obtained from D. Livingston, Sidney Farber Cancer Center. These studies were supported in part by PHS grant CA 12464-07 and by contract NO 1-CP 43222 within the Virus Cancer Program of the National Cancer Institute.

15 August 1977; revised 25 October 1977

Increased Resistance to *Staphylococcus aureus* Infection and Enhancement in Serum Lysozyme Activity by Glucan

Abstract. *Glucan is a potent reticuloendothelial stimulant whose immunobiological activity is mediated, in part, by an increase in the number and function of macrophages. In studying the role of glucan as a mediator of antibacterial activity, we attempted to ascertain the ability of glucan to modify the mortality of mice with experimentally induced Gram-positive bacteremia, and to enhance antibacterial defenses in rats as denoted by serum lysozyme and phagocytic activity. After intravenous administration of glucan, serum lysozyme concentrations were increased approximately sevenfold over control concentrations. The increase in serum lysozyme appeared to parallel the glucan-induced increase in phagocytosis and induced hyperplasia of macrophages. Prior treatment of mice with glucan significantly enhanced their survival when they were challenged systemically with* Staphylococcus aureus. *These studies indicate that glucan confers an enhanced state of host defense against bacterial infections.*

Glucan, a β-1,3-polyglucose component isolated from the cell wall of *Saccharomyces cerevisiae*, is a potent reticuloendothelial (RE) stimulant as well as a modulator of cellular and humoral immunity (*1*). The administration of glucan to rats or mice is associated with an increase in weight and size of the major RE organs as a result of an increased number of activated macrophages (*2, 3*). The enhanced state of host resistance induced by glucan is characterized by a hyperphagocytic state as indicated by the increased rate of clearance of a variety of particulate agents (*2, 4*). The effectiveness of glucan in promoting increased resistance to tumor growth and dissemination has been demonstrated in rats with Shay choloroleukemia (*5*) and in syngeneic mouse melanoma B16 and adenocarcinoma BW 10232 tumor models (*6*). In studies of three types of metastatic lesions, intralesional administration of glucan caused a marked reduction in tumor size and tumor cell necrosis associated with an infiltration of macrophages containing glucan (*7*). In view of glucan's diverse immunostimulant properties, we evaluated the possible role of glucan as a mediator of antibacterial activity, as ascertained by survival following intravenously administered *Staphylococcus aureus* and by the status of certain antibacterial defenses, namely, phagocytic activity and serum lysozyme concentrations.

Male Long-Evans rats weighing 200 to 225 g were given free access to tap water and Purina Lab Chow. Glucan was prepared by a modification of previously described procedures (*8*). Intravenous injections of isotonic saline or glucan (1 mg/100 g) were given on days 0, 2, and 4, and the rats were killed on day 5 for as-

Table 1. Effect of intravenous glucan on serum lysozyme concentration and clearance of colloidal carbon.

Group	Serum lysozyme		Colloidal carbon	
	Concentration (μg/ml)	N	Clearance ($t_{1/2}$, min)	N
Saline	2.68 ± 0.68	12	10.6 ± 0.55	8
Glucan	18.14 ± 1.78	14	3.6 ± 0.23	8

0036-8075/78/0324-1340$00.50/0

say of serum lysozyme activity. Blood was drawn from the vena cava and allowed to clot for 1 hour. Lysozyme activity of the serum samples was ascertained by using *Micrococcus lysodeikticus* as a substrate (Lysozyme Reagent set, Worthington Biochemical). The change in percentage of transmission per minute was recorded and compared to a standard curve prepared with known concentrations of egg white lysozyme. Phagocytic activity was evaluated by the rate of intravascular clearance of colloidal carbon (*9*). Colloidal carbon (16 mg/100 g) was administered intravenously, and tail blood samples were obtained at 1, 3, 5, 7, and 9 minutes after the injection of the colloid. The amount of carbon in each sample was determined spectrophotometrically (*9*) and the intravascular half-time ($t_{1/2}$) for the clearance of colloidal carbon was determined by semilogarithmic plots.

In studies to evaluate the effectiveness of glucan as an agent promoting nonspecific bacterial resistance, an 18-hour tryptic broth culture of *S. aureus* was used to prepare the inocula. The experimental group, composed of 15 AKR mice weighing approximately 20 g each, received intravenous glucan (1 mg per mouse) 7 and 4 days prior to bacterial challenge. The control group received isovolumetric saline. On day 0 all mice were injected intravenously with 1×10^9 viable *S. aureus* and mortality patterns were ascertained. Differences in mortality between the glucan- and saline-treated groups were compared by means of the chi-square test.

The intravenous administration of glucan caused a sevenfold increase in serum lysozyme activity ($P < .001$) over controls (Table 1). This increase was associated with an increase in RE phagocytic function as indicated by an enhanced rate of colloidal carbon clearance in glucan-treated rats (Table 1). The administration of glucan caused a 66 percent decrease in $t_{1/2}$ compared to control clearance rates.

That glucan provided protection against *S. aureus* is shown by the data in Fig. 1. On day 3, only 10 percent of the control mice survived as opposed to 80 percent survival in the glucan-treated group ($P < .01$). By day 7 all of the saline controls succumbed to *S. aureus* infection, whereas 40 percent of the group first treated with glucan survived ($P < .05$).

Numerous studies have demonstrated that macrophages have an important role in the host defense against bacterial infections. The ability of glucan to increase the number (*2, 3*) and phagocytic function (*1–4*) of macrophages appears to provide the host with an enhanced protection against invading microorganisms. The increase in serum lysozyme by glucan also provides a means of reducing the number of viable bacteria. Lysozyme, a bacteriolytic enzyme first characterized by Fleming (*10*), is particularly effective against Gram-positive organisms because they lack an outer membrane to hinder the action of lysozyme on peptidoglycan (*11*). Although polymorphonuclear leukocytes (PMN's) are known to release large amounts of lysozyme after degranulation (*12*), the major cell type responsible for the secretion of lysozyme is the monocyte (*13*). In support of this concept, de novo synthesis of lysozyme by alveolar macrophages has been demonstrated (*14*). The addition of puromycin or actinomycin D to the culture medium reduced the release of lysozyme from the macrophage as well as cell-associated lysozyme (*14*). Gordon *et al.* (*13*) have also shown that peritoneal macrophages will secrete lysozyme for as long as 11 days in culture. These studies indicate that lysozyme may be used as a measure of macrophage activity in vitro.

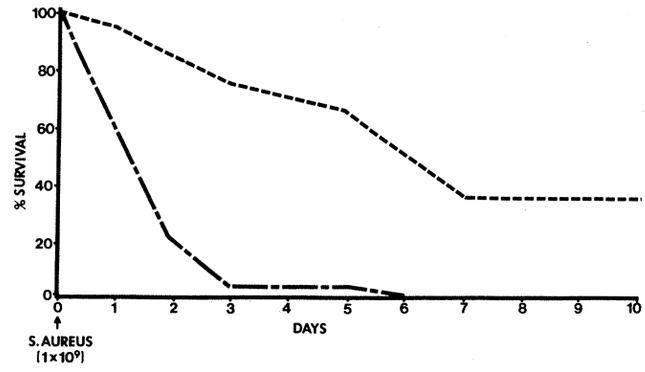

Fig. 1. Effect of glucan treatment on the survival of AKR mice with systemic *Staphylococcus aureus* infection (1×10^9 cells per mouse). Glucan (---) (1 mg per mouse) or isovolumetric saline (- — -) was administered intravenously on days 4 and 7 prior to bacterial challenge ($N = 15$).

Currie and Eccles (*15*) reported that bacillus Calmette-Guérin (BCG) and *Corynebacterium parvum* caused a significant increase in serum lysozyme activity and attributed this to an increase in total number of macrophages. Aside from serum lysozyme, Cappuccino *et al.* (*16*) reported that a variety of RE stimulants such as BCG, zymosan, or bacterial endotoxins increased splenic lysozyme activity. The increase in tissue lysozyme was associated with and paralleled the induced splenic hyperplasia. Cappuccino suggested that the augmentation of lysozyme activity may be the result of RE stimulation of the host (*16*). The present findings are in accord with this concept. The glucan-induced elevation in serum lysozyme, which has been repeatedly demonstrated in our laboratory, closely paralleled the hyperfunctional state of the reticuloendothelium as reflected by more rapid removal of colloidal carbon.

In addition to the increase in serum lysozyme activity, glucan also modified lethal septicemia induced by injection of 1×10^9 *S. aureus* intravenously. An enhanced state of nonspecific host resistance to a variety of pathogens has been observed after treatment with other immunostimulants (*17*). Adlam *et al.* (*18*) reported a significant protective effect when *C. parvum* was given intraperitoneally to mice prior to intraperitoneal challenge with 7.1×10^7 viable *S. aureus*. However, those authors reported no protection against intravenous challenge with *S. aureus*. It is of interest in this regard that glucan significantly enhanced the survival of mice challenged intravenously with a dose of *S. aureus* an order of magnitude higher than mice injected intraperitoneally with *C. parvum*.

The role of lysozyme as an antibacterial agent appears to be mediated through its direct bacteriolytic action (*19*), as well as via stimulatory effects on macrophage phagocytic function (*20*). Biggar and Sturgess (*19*) have demonstrated the significant role of lysozyme in mediating the microbicidal action of rat alveolar macrophages. They reported that phagocytosis of intact bacteria did not occur, whereas lysozyme-degraded bacteria were rapidly phagocytized. It has also been demonstrated that lysozyme may directly enhance phagocytic activity of PMN's (*21*) and macrophages (*20*). Although yeast cells are not degraded by lysozyme, there was a significant increase in the ingestion of yeast cells by human PMN's that were subjected to physiologic concentrations of human lysozyme (*21*). In a similar manner, studies with cultured monocytic cells showed that the addition of high concentrations of lysozyme to the media resulted in an enhanced phagocytosis of tubercle bacilli (*20*). In view of these findings, the glucan-induced increase in serum lysozyme activity may contribute to the removal of foreign entities from

the organism via direct stimulation of the phagocytic mechanisms. Lysozyme, therefore, may function as a self-stimulating secretory product of the macrophage.

These composite studies denote that the glucan-induced hyperfunctional state of the reticuloendothelium is associated with a significant increase in serum lysozyme activity. This increased activity may contribute to bactericidal properties of glucan by providing the host with a nonspecific mechanism whereby certain invading microorganisms may be reduced to less virulent forms. Since infectious complications are common in malignant conditions (*22*) and glucan possesses marked antitumor activity (*5, 6*), the antibacterial properties of glucan that we have now demonstrated make this compound an attractive immunotherapeutic agent.

P. L. KOKOSHIS
D. L. WILLIAMS
J. A. COOK, N. R. DI LUZIO
Department of Physiology, Tulane University School of Medicine, New Orleans, Louisiana 70112

References and Notes

1. W. R. Wooles and N. R. Di Luzio, *Science* **142**, 1078 (1963); S. H. Morrow and N. R. Di Luzio, *Proc. Soc. Exp. Biol. Med.* **119**, 647 (1965).
2. W. R. Wooles and N. R. Di Luzio, *J. Reticuloendothel. Soc.* **1**, 160 (1964).
3. C. T. Ashworth, N. R. Di Luzio, S. J. Riggi, *Exp. Mol. Pathol. Suppl.* **1**, 83 (1963).
4. N. R. Di Luzio and S. H. Morrow, III, *J. Reticuloendothel. Soc.* **9**, 273 (1971).
5. N. R. Di Luzio, R. McNamee, E. Jones, J. A. Cook, E. O. Hoffmann, in *The Macrophage in Neoplasia*, M. A. Fink, Ed. (Academic Press, New York 1976), pp. 181–198.
6. P. W. A. Mansell, N. R. Di Luzio, R. McNamee, G. Rowden, J. W. Proctor, *Ann. N.Y. Acad. Sci.* **277**, 20 (1976).
7. P. W. A. Mansell, H. Ichinose, R. J. Reed, E. T. Krementz, R. McNamee, N. R. Di Luzio, *J. Natl. Cancer Inst.* **54**, 571 (1975).
8. W. Hassid, M. A. Joslyn, R. M. McCready, *J. Am. Chem. Soc.* **63**, 295 (1941).
9. S. J. Riggi and N. R. Di Luzio, *Am. J. Physiol.* **200**, 297 (1961).
10. A. Fleming, *Proc. R. Soc. London* **93**, 306 (1927).
11. B. Witholt, H. Van Heerikhuizen, L. De Leij, *Biochim. Biophys. Acta* **443**, 534 (1976).
12. D. B. L. McLelland and P. VanFurth, *Immunology* **28**, 1099 (1975).
13. S. Gordon, J. Todd, Z. A. Cohn, *J. Exp. Med.* **139**, 1228 (1974).
14. E. P. Heise and Q. Myrvik, *J. Reticuloendothel. Soc.* **4**, 510 (1967).
15. G. A. Currie and S. A. Eccles, *Br. J. Cancer* **33**, 51 (1976).
16. J. G. Cappuccino, S. Winston, G. C. Perrie, *Proc. Soc. Exp. Biol. Med.* **116**, 869 (1964).
17. N. A. Sher, S. D. Chaparras, L. E. Greenberg, S. Bernard, *Infect. Immun.* **12** (6), 1325 (1975); V. C. Senterfitt and J. W. Shands, *ibid.* **1**, 583 (1970).
18. C. Adlam, E. S. Broughton, M. T. Scott, *Nature (London) New Biol.* **235**, 219 (1972).
19. W. D. Biggar and J. M. Sturgess, *Infect. Immun.* **16**, 974 (1977).
20. H. Thacore and H. P. Willet, *Am. Rev. Resp. Dis.* **93**, 786 (1966).
21. M. I. Klockars and P. Roberts, *Acta Haematol.* **55**, 289 (1976).
22. A. S. Levine, R. G. Graw, P. E. Young, *Semin. Hematol.* **9**, 141 (1972).
23. These studies were supported, in part, by the Cancer Research Institute, the National Institutes of Health, and the MECO Cancer Research Fund.

26 September 1977; revised 29 November 1977

Inhibition of Bone Resorption in vitro by a Cartilage-Derived Anticollagenase Factor

Abstract. *A cartilage-derived factor containing a specific collagenous inhibitor was found to block reversibly parathyroid hormone–stimulated ^{45}Ca release from fetal rat bone in vitro. Morphologic and quantitative histometric examination revealed that this factor modulates osteoclastic activities.*

Elucidation of the relation between osteoclasts and bone resorption has been investigated in systematically accelerated bone resorptive states induced by parathyroid hormone (PTH) stimulation both in vivo and in vitro (*1*). However, with the exception of the effects of specific circulating hormones on osteoclastic activities, little attention has been directed toward local regulatory mechanisms on these cells.

Proteoglycan-free cartilage extract which contains protease inhibitors prevents the proliferation of endothelial cells in culture (*2*). High activity of protease inhibitor also occurs in other poorly vascularized tissues, such as blood vessel walls, cornea, and dentin (*3*). Since endothelial cells penetrate extracellular matrices, presumably by enzymatic mechanisms, their control may be due to inhibition of proteolysis. The protease inhibitors from cartilage and aortic wall have been isolated and found to be low-molecular-weight cationic proteins capable of specifically inhibiting collagenolytic activity isolated from normal and pathological tissues (*2*). Since collagenase and vascular elements have been implicated in resorptive events of bone matrix (*4*), we have now evaluated the effect of this cartilage-derived material containing a specific collagenase-inhibitor and found that it reversibly inhibits osteoclastic activity in fetal rat bone in organ culture.

The organ culture technique which measures the resorption of bone has been described (*5*). Paired shafts of the radius and ulna from 19-day-old rat fetuses were radioactively labeled by injection of the mother with ^{45}Ca on the previous day. These shafts were cultured either in BGJ_b medium (Gibco) supplemented with 1 mg of bovine serum albumin (Pentex) per milliliter or in such medium containing additions of the cartilage-derived inhibitor (*6*). Bone resorption was stimulated in one of the shafts by the addition of 2.8 international

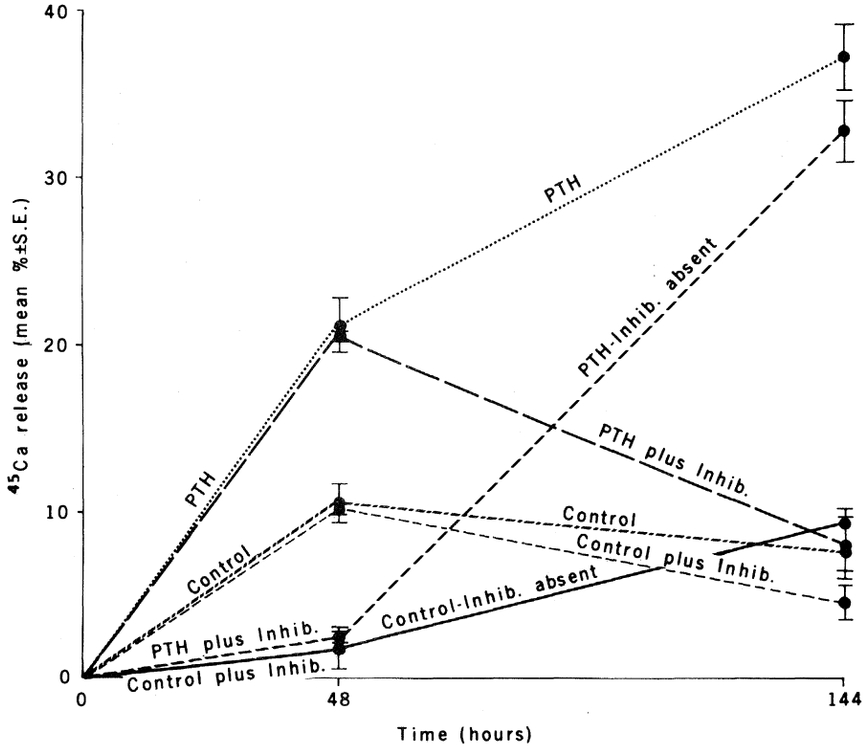

Fig. 1. Effects of the presence in and absence from the culture medium of the cartilage-derived inhibitor (*Inhib.*) on PTH-stimulated bone resorption in organ culture. Values are expressed as the mean percentage (± standard error) of ^{45}Ca released from four pairs of cultured bones after 48 and 144 hours of incubation.

0036-8075/78/0324-1342$00.50/0