tus eggs was 2.45 ± 1.69 percent; for *S. purpuratus* eggs it was 2.21 ± 0.685 percent. Thus, the decrease in order parameter appears to be a late event.

Fertilized eggs grown to plutei, an advanced stage of development, in the presence of BSA-solubilized 5-doxylstearate were indistinguishable from controls. Unfertilized eggs, exposed to the solubilized fatty acid and washed, showed some inability to raise normal fertilization membranes: 20 to 30 percent of the eggs raised blebby fertilization membranes after insemination. By phase-contrast microscopy these abnormal fertilization membranes resembled the membranes raised after eggs were treated with soybean trypsin inhibitor (*21*). This effect did not depend on the presence of the spin label fatty acid and may be due to the titration by BSA of the protease necessary for detaching the vitelline layer from the plasma membrane (*22*). Partially activated eggs do not raise fertilization membranes, but under the labeling conditions they showed characteristic chromosome condensation. By these criteria, the presence of spin label fatty acid perturbs the membrane little if at all.

Taken together, these results indicate that metabolic activation of the eggs of these two echinoderms is accompanied by an increase in membrane fluidity. However, since spin label fatty acids have been shown to equilibrate among all the subcellular membrane fractions (*23*), our results may be interpreted only in terms of bulk membrane fluidity. There are two possible interpretations of the data. One is that activation of the ovum is accompanied by a change in the total cellular membranes to a more fluid state. Alternatively, one or more specialized membranes (such as the plasma membrane) enters a more fluid state on activation and the probe is showing the average change experienced by the altered plus the unaltered membranes.

JUDITH CAMPISI
CARL J. SCANDELLA

Department of Biochemistry, State University of New York, Stony Brook 11794

References and Notes

1. D. Epel, *Am. Zool.* **15**, 507 (1975).
2. R. A. Steinhardt, L. Lundin, D. Mazia, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2426 (1971).
3. L. W. Stearns, *Sea Urchin Development: Cellular and Molecular Aspects* (Dowden, Hutchinson & Ross, Stroudsburg, Pa., 1974), p. 66.
4. D. Epel, R. Steinhardt, T. Humphreys, D. Mazia, *Dev. Biol.* **40**, 245 (1974).
5. V. D. Vacquier and B. Brandriff, *ibid.* **47**, 12 (1975).
6. A. B. Pardee, L. Jiménez de Astía, E. Rosengurt, in *Controls of Proliferation in Animal Cells*, B. Clarkson and R. Baserga, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1974), p. 547.
7. J. Seelig, in *Spin Labeling, Theory and Applications*, L. J. Berliner, Ed. (Academic Press, New York, 1976), p. 373.
8. B. J. Gaffney, *Methods Enzymol.* **32**, 161 (1974).
9. P. G. Kury, P. W. Ramwell, H. M. McConnell, *Biochem. Biophys. Res. Commun.* **56**, 478 (1974).
10. W. H. Huestis and H. M. McConnell, *ibid.* **57**, 726 (1974).
11. P. R. Gross, D. E. Philpott, S. Nass, *J. Biophys. Biochem. Cytol.* **7**, 135 (1965).
12. H. Mohri, *Biol. Bull.* **126**, 440 (1964).
13. J. Runnstrom, *Adv. Morphog.* **5**, 221 (1966).
14. D. Epel, *Biochem. Biophys. Res. Commun.* **17**, 69 (1964).
15. ———, *Proc. Natl. Acad. Sci. U.S.A.* **57**, 899 (1967).
16. A. H. Whiteley and E. L. Chambers, *J. Cell. Physiol.* **68**, 309 (1966).
17. J. Piatigorsky and A. H. Whiteley, *Biochim. Biophys. Acta* **108**, 404 (1965).
18. D. Epel, *Exp. Cell Res.* **72**, 74 (1972).
19. R. T. Hinegardner, B. Rao, D. E. Feldman, *ibid.* **36**, 53 (1964).
20. D. Mazia, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 690 (1974).
21. V. Vacquier and D. Epel, *Nature (London)* **237**, 34 (1972).
22. E. J. Carroll, Jr., and D. Epel, *Dev. Biol.* **44**, 22 (1975).
23. J. Kaplan, P. G. Canonico, W. J. Caspary, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 66 (1973).
24. We thank D. Poccia for his assistance and for the use of his facility to maintain sea urchins. Supported by NIH grant GM23218 to C.J.S. and NIH training grant CA 09176-03.

13 October 1977; revised 12 December 1977

Immunity to Antigens Associated with Primate C-Type Oncoviruses in Pregnant Women

Abstract. *Cell-mediated and humoral immune responses against antigens associated with primate C-type oncoviruses were evaluated in humans by microcytotoxicity and radioimmunoprecipitation assays. Five of six women tested sequentially during pregnancy developed selective cell-mediated reactivity against baboon endogenous virus (BEV)–infected human fibroblasts. Responsiveness peaked during the second and third trimesters and corresponded temporally with elevated antibody levels to BEV antigens. Similar cell-mediated reactivity was not observed in nonpregnant individuals. Selective cell-mediated reactivity directed against cells infected with the simian sarcoma virus–simian sarcoma associated virus complex (SSV-SSAV) was observed in four of 20 healthy adults (three of 14 nonpregnant, one of six pregnant). These observations suggest that cell-mediated reactivity against primate C-type oncoviruses is occasionally detected in healthy nonpregnant adults, but that during pregnancy both cell-mediated and humoral reactivity against BEV may become selectively expressed.*

C-type oncoviruses are widely distributed in mammals and birds (*1*). They may be horizontally transmitted and induce neoplastic disorders; in some species they persist in latent form within the host's cells and are vertically transmitted from generation to generation. Such latent viral genetic material may become activated by treatment with certain chemicals or hormones, during aging, or during periods of immunological stress (*2*). Periods of rapid cell proliferation, such as occur during neoplastic growth or during gestation, may also be accompanied by the preferential expression of C-type oncoviruses (*3*).

Many investigators have detected C-type viruses by electron microscopy in placentas of human and nonhuman primates as well as other mammalian species (*4*). Although no human oncoviruses have been isolated from placental tissue, Kalter and co-workers (*5*) have isolated an endogenous C-type virus from a baboon placenta. The physiological function, if any, of these placental viruses is obscure. Whether or not the pregnant host responds immunologically to the placental viruses is also unclear.

In mice, both humoral (*6*) and cell-mediated (*7*) immune responses to endogenous C-type oncoviruses have been demonstrated. Cell-mediated reactivity against endogenous viruses has also been observed in rats (*8*). In man, humoral responses to primate C-type viruses have been observed by some investigators (*9, 10*), but not by others (*11*). We have studied the cell-mediated responses of peripheral blood lymphocytes against cells infected with primate C-type oncoviruses in both pregnant and nonpregnant humans, using a microcytotoxicity assay. Selective responsiveness developed during pregnancy against antigens associated with the baboon endogenous virus (BEV). Serological studies, in which a modified radioimmunoprecipitation (RIP) assay was used, demonstrated a concomitant increase in antibody production against BEV-associated antigens during gestation.

Human embryonic lung (HEL) fibroblast cultures were obtained from Flow Laboratories and maintained in our laboratory in Eagle's minimal essential medium (MEM) containing glutamine (2 m*M*) and a fourfold concentration of other essential amino acids and vitamins, 10 percent gamma-irradiated fetal bovine serum (FBS), penicillin (250 U/ml), and streptomycin (250 μg/ml) (complete MEM 4×). The HEL fibroblasts (passage 7) were infected with either the M7

0036-8075/78/0324-1337$00.50/0

strain of BEV or the simian sarcoma virus–simian sarcoma associated virus complex (SSV-SSAV). These viruses had been maintained on human A-204 rhabdomyosarcoma cell cultures. Uninfected HEL cells were cultured in parallel. The virus-infected and uninfected cells were tested every 2 weeks for the presence of virus by determination of RNA-dependent DNA polymerase activity in supernatant fluid according to established techniques (*12*). The infected lines were consistently virus-positive and the uninfected lines were always virus-negative by this assay. Lymphocyte donors were 14 healthy, nonpregnant adults (five female, nine male) and six pregnant women tested at approximately 3-month intervals during gestation and following delivery. Peripheral blood (20 ml) was withdrawn by venipuncture and defibrinated by agitation with glass beads. Leukocyte fractions were separated by established buoyant density centrifugation techniques employing Ficoll-Hypaque solutions (*13*). To remove adherent and nonspecifically cytotoxic cells, we subjected the mononuclear cell fraction to passage in plastic tissue culture dishes for 17 hours at 37°C. The lymphocyte-enriched population was resuspended in RPMI 1640 medium containing 10 percent gamma-irradiated FBS.

Microcytotoxicity assays were performed as described for murine systems (*7*). The HEL fibroblast target cells were seeded in Falcon Microtest II plates containing complete MEM 4×, at a concentration of 100 to 150 cells per well. After 18 hours of incubation at 37°C, medium was removed and replaced with 10^4 to 10^5 lymphocytes in RPMI 1640 medium containing 10 percent gamma-irradiated FBS. Plates were then incubated at 37°C for 38 hours, after which lymphocytes were removed by aspiration. Target cells were washed with phosphate-buffered saline, fixed with 95 percent methanol, stained with Giemsa's stain, and the total number of cells remaining in each well was determined. Mean values were derived from at least ten replicate observations. These microcytotoxicity assays measure both inhibition of target cell replication and actual cytotoxicity.

Cell separation techniques were used to evaluate the reactive cell populations. Columns containing rabbit antibody to human Fab were prepared by conjugating the antibody to cyanogen bromide–activated Sepharose or Sephadex G-200 (*14*). Mononuclear cells were separated over these columns into nonimmunoglobulin-bearing and immunoglobulin-bearing lymphocytes. Recovery of the cells bound to the column was achieved by competitive inhibition with 1 percent immunoglobulin and subsequent elution. Further separation of the nonimmunoglobulin-bearing cell population was achieved by E-rosetting with sheep erythrocytes followed by Ficoll-Hypaque sedimentation (*15*). These procedures resulted in three populations defined as immunoglobulin-bearing (B) cells, E-rosette-forming (T) cells, and nonimmunoglobulin, non-E-rosette-forming (null and K) cells.

Table 1. Sequential cell-mediated cytotoxic responses against BEV-associated antigens during pregnancy. Postive and negative responses are indicated by plus and minus signs, respectively.

Patient	Pregnancies	Selective cytotoxic response (trimester)			
		First	Second	Third	Postpartum
1	1	–	+(23)*	+(41)	–
2	3	–	+(19)	–	NT†
3	2	–	–	+(36)	+(22)
4	2	NT	NT	+(24)	–
5	1	+(16)	+(35)	NT	–
6	1	–	–	NT	–

*Figures in parentheses represent percentage reduction of BEV-infected target cells minus the reduction of uninfected target cells. Each mean value was derived from at least ten replicate observations. †Not tested.

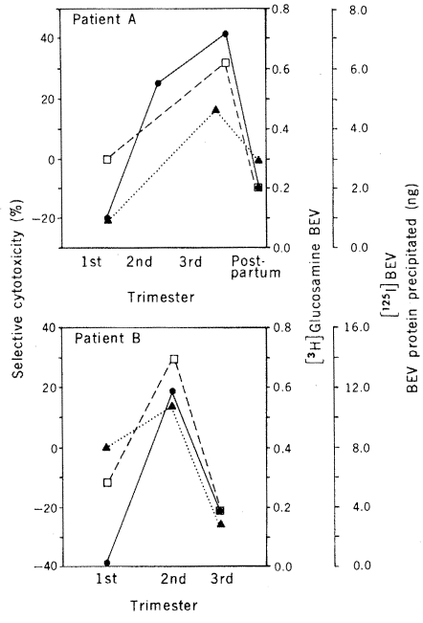

Fig. 1. Comparative cell-mediated cytotoxic and humoral immune responses of two pregnant women against antigens associated with BEV. (●) Selected cell-mediated reduction of BEV-infected target cells when compared with uninfected cells. (□) Precipitation of viral protein from [³H]glucosamine-labeled BEV derived from dog thymus cells. (▲) Precipitation of viral protein from ¹²⁵I-labeled BEV grown in dog thymus cells.

Serologic determination of the HLA-ABC antigens of the 20 lymphocyte donors was performed according to the National Institutes of Health lymphocytotoxicity technique as detailed previously (*16*). All HLA alloantigens officially or provisionally recognized by the World Health Organization–International Union of Immunological Societies (WHO-IUIS) (*17*) could be defined with the exception of HLA-Aw34, Aw36, Aw43, Bw37, and Cw5.

The HLA-ABC serotype of the HEL fibroblasts was determined serologically by adsorption-inhibition assays. Fifty microliters of 27 different HLA alloantiserums [specific for HLA-A1, A2, A3, A9 (W23 + W24), A10, A28, B7, B8, B12, Bw17, Bw22, B27, Bw35, Cw3, and Cw4] were each added to 5×10^6 to 10×10^6 packed HEL fibroblasts and mixed occasionally for 90 minutes at 37°C. After removal of the adsorbed antiserum by centrifugation (15000*g*, 5 minutes), back-titration of each adsorbed serum was performed in parallel with its unadsorbed counterpart against a number of unrelated lymphocyte donors known to possess the respective alloantigen. The HEL fibroblasts were assumed to express a particular HLA antigen if the antibody titer was reduced by at least two log dilutions over the control serum. The HLA phenotype of the HEL fibroblasts was HLA-A2,–;Bw17,27;Cw3,–, with each assigned antigen identified by two different alloantiserums.

Humoral antibodies that reacted in RIP assays with proteins in preparation of BEV were detected by assays previously described (*9, 10*). Radioactively labeled (^{125}I) proteins from detergent-disrupted BEV grown in dog thymus cells (*5*) were used as antigens. To check specifically for precipitation of viral glycoproteins, we cultured BEV-producing dog thymus cells overnight in MEM 4× without glucose and supplemented with [^{3}H]glucosamine (10 μCi/ml) and 10 m*M* fucose. Virus was purified from supernatant medium by two sedimentations (50,000*g*, 120 minutes each) and disrupted for use in RIP assays.

Selective cell-mediated reactivity against BEV was observed only in pregnant women. Five of six women tested at intervals during gestation and following delivery developed responses against BEV-infected fibroblasts (Table 1). All three multiparous women (two to three pregnancies) tested were reactive and two of three primigravid women were reactive. Responsiveness was low or ab-

sent during first trimesters but increased thereafter. Peak reactivity occurred during late second and early third trimesters. During the late third trimesters or following delivery, four of five women lost their selective responsiveness against BEV-infected fibroblasts. Reactivity against BEV-infected cells was not observed in any of ten healthy, nonpregnant adults (four females, six males). The type of cell reactive in this assay has not been clearly defined, although preliminary studies suggest that both T cell–mediated and antibody-dependent cellular cytotoxic mechanisms may occur. Responding cells and target cells did not share HLA antigens in all cases.

To evaluate the specificity of the observed cytotoxicity reactions among pregnant women, we added varying concentrations of cesium chloride–banded BEV to lymphocyte–target cell mixtures (Table 2). Concentrations of BEV ranging from 1 to 100 μg/ml blocked the cytotoxic response, indicating that the reactivity was specific for BEV antigens.

Selected, coded plasma specimens from pregnant individuals were evaluated for antibodies that reacted in RIP assays with BEV proteins. Antibody concentrations corresponded with cell-mediated responsiveness to BEV-associated antigens. Concentrations were low early in pregnancy, increased in midpregnancy, and decreased thereafter. Results of sequential antibody determinations were comparable for both glucosamine and iodine-labeled antigen preparations. Absorption of serum antibody activity was accomplished only with BEV-infected dog thymus cells, but not with uninfected cells (data not shown). Comparative cell-mediated and humoral immunity data from two pregnant individuals are shown in Fig. 1.

Selective reactivity directed against SSV-SSAV–infected fibroblasts was repeatedly observed in three of 14 nonpregnant and one of six pregnant healthy adults. In the nonpregnant group, two of nine males and one of five females were consistent reactors. In contrast to the reactors against BEV, all nonpregnant reactors against SSV-SSAV shared at least one histocompatibility antigen with target fibroblasts. Cells adhering to columns prepared with antibody to human Fab (predominantly B lymphocytes) were unreactive, as were nonadherent cells that did not form E rosettes (null and K cells). In contrast, nonadherent, E-rosetting T lymphocytes showed marked specific reactivity against SSV-SSAV–infected fibroblasts (Table 3).

Table 2. Blocking of cytotoxicity observed during pregnancy by purified BEV.

Blocking virus* (μg/ml)	Selective cytotoxicity (%)†
Patient 1	
0	22
1	9
10	−51
100	−54
Patient 2	
0	27
1	2
10	−27
100	−20

*Virus preparations and lymphocytes were added simultaneously to target cells; lymphocyte–target cell ratios were approximately 100 : 1. †Percentage reduction of BEV-infected target cells minus reduction of uninfected target cells. Each mean value was derived from at least ten replicate observations.

These studies indicate that cell-mediated cytotoxic responsiveness against primate C-type oncoviruses is not frequently observed among healthy nonpregnant adults or is below the threshold levels of detection of our assays. During pregnancy, however, it appears that antigens related to baboon endogenous oncoviruses may become selectively expressed resulting in the development of both specific cell-mediated and humoral immune responses. The oncovirus-specific cell-mediated reactivity during pregnancy contrasts with many other lymphocyte functions, including certain antiviral cell-mediated responses, which become diminished during gestation (*18*). This suggests that the observed changes do not reflect a nonspecific lymphoreticular stimulatory effect of pregnancy. The specificity of the BEV response is further suggested by failure of these patients to respond to uninfected or SSV-SSAV–infected fibroblasts, the correspondence of the responses to antibody reactivity against BEV proteins, and the specific blocking by purified BEV.

Table 3. Cytotoxic reactivity of two normal subjects against SSV-SSAV–infected fibroblasts (HLA-A2,−;Bw17,27;Cw3,−). The histocompatibility type of subject 1 was A1,11;Bw17,−;Cw4,−; and that of subject 2 was A1,2;B8,−;C−,−.

Population of reactor cells*	Selective cytotoxicity (%)†
Subject 1	
Mononuclear nonadherent cells	21, 19, 21
T lymphocytes plus null cells	25, 27
T lymphocytes	31
Null cells	−5
B lymphocytes	−4
Subject 2	
Mononuclear nonadherent cells	28
B lymphocytes plus null cells	6
T lymphocytes	29

*Mononuclear cells were initially separated from peripheral blood by Ficoll-Hypaque sedimentation. Adherent cells were removed by incubation for 17 hours on plastic dishes. Subsequent fractionations were performed with combinations of immunoadsorbent columns and E-rosetting techniques as described in the text. †Percentage reduction of SSV-SSAV–infected target cells minus reduction of uninfected target cells. Each mean value was derived from at least ten replicate observations.

Peripheral blood leukocytes have previously been demonstrated to react against tissue culture–passaged "normal" human fetal cell monolayers (*19*). The reactivity was largely expressed by cells of the monocyte-macrophage series. We have also observed such nonspecific reactivity against fetal lung cells, but this could be largely abrogated by culturing the leukocytes for 17 hours on plastic dishes prior to assay. The mechanisms by which 17-hour cultures remove nonspecifically reactive cells are unclear; it is possible that this population is short-lived or belongs to the monocyte-macrophage adherent cell population.

Our observations are compatible with numerous observations that C-type particles can be visualized in human and nonhuman placental tissue (*4*). Although to date no C-type oncoviruses have been cultured from human placental tissue, our findings suggest that the observed particles may have antigens in common with BEV. Similar viruses have been isolated from baboon placentas (*5*).

The significance of C-type oncovirus activation during pregnancy is unclear. These viruses may play a fundamental role in inducing rapid growth of cells during embryogenesis, as some C-type viruses do during experimentally induced oncogenesis (*1, 2*). Alternatively, by inducing maternal humoral responses, they may help protect the fetus from effector cell-mediated rejection mechanisms (*3*). Other possibilities are that the C-type viruses are merely by-products of altered cell-regulatory functions that occur during periods of rapid or autonomous growth, are induced by hormonal changes, or result from host-versus-graft reactions (*2*).

The low incidence (4/20) of cell-mediated reactivity in normal pregnant and nonpregnant individuals against SSV-SSAV–infected monolayers is difficult to interpret. Responders shared histocompatibility types with infected fibroblast target cells, and the reactive cells were free of surface immunoglobulin and formed rosettes with sheep erythrocytes.

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