

Two Mechanisms of Inhibition of Human Lymphocyte Proliferation by Soluble Yeast Mannan Polysaccharide

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The literature on chronic mucocutaneous candidiasis contains multiple reports which suggest that loss of cell-mediated immunity in this disease may be related in part to the presence of an inhibitory factor(s) present in patient plasma. One such inhibitory factor has been suggested to be mannan polysaccharide released from the cell wall of the pathogen. The present report describes results of experiments to consider mechanisms by which yeast mannan influences proliferative responses of human lymphocytes. Mannan for these experiments was isolated from *Saccharomyces cerevisiae*. We observed that mannan-mediated inhibition of proliferative responses to a battery of stimuli (phytohemagglutinin, pokeweed mitogen, and *Candida*, mumps, streptococcus, cytomegalovirus, and herpes simplex virus antigens) was related in part to an effect of copper associated with the mannan and possibly to the superoxide dismutase activity of the mannan-copper complex. Mannan made deficient in copper by use of a copper-chelating resin appeared to inhibit only lymphoproliferation stimulated by the *Candida* antigen. These results suggest that inhibitory effects of yeast mannans on lymphoproliferative responses may involve at least two mechanisms, one related to hydrogen peroxide production augmented by mannan-copper complexes and another related to still unknown effects independent of the metal ligand. We propose that our results represent a significant novel observation which may be useful in understanding mechanisms of immunoinhibitory effects of *C. albicans* mannan.

We have previously reported that mannan polysaccharide isolated from *Saccharomyces cerevisiae* (baker's yeast) has a nonspecific inhibitory influence on antigen-stimulated proliferation of human lymphocytes in vitro (R. D. Nelson, R. T. McCormack, M. J. Herron, R. L. Simmons, Fed. Proc. 38:1000, 1979). A similar phenomenon has been reported by Fischer et al. (8) for mannan isolated from *Candida albicans*. Mannans from these sources may therefore share an immunoinhibitory property. Although the mechanism of this phenomenon is not fully understood, it is important to understand its basis because of the possibility that circulating mannan may contribute to immune dysfunction associated with chronic forms of candidiasis (8).

Experiments described in this report represent an attempt to understand the mechanism involved in mannan-mediated inhibition of antigen-stimulated lymphoproliferation in vitro. Results obtained suggest that two mechanisms may be involved, one providing for a nonspecific inhibition of lymphoproliferation induced by multiple stimuli and another providing for selective inhibition of lymphoproliferation stimulated by *Candida* antigen. The nonspecific inhibitory influence of mannan appears to be attributable to copper complexed with the polysaccharide and a superoxide dismutase (SOD) activity of this complex; the specific inhibitory influence is independent of copper.

MATERIALS AND METHODS

Isolation of MNL and polymorphonuclear neutrophils. Leukocytes were isolated from heparinized blood specimens (10 U/ml) collected by venipuncture from healthy control donors volunteering to participate in this study. Mononuclear leukocytes (MNL) were isolated by the method of Bøyum (3), using commercial lymphocyte separation medium (Bionetics Laboratory Products, Kensington, Md.). Neutrophils were

isolated by the method of Ferrante and Thong (9), and contaminating erythrocytes were eliminated by hypotonic lysis. The cell suspension medium used for culture of MNL was minimum essential medium supplemented to contain 2 mM glutamine, 100 U of penicillin per ml, 100 µl of streptomycin per ml, and 15% decomplexed pooled human serum. The cell suspension medium used for measurement of leukocyte respiratory function was Dulbecco phosphate-buffered saline (GIBCO Laboratories, Grand Island, N.Y.).

Measurement of lymphoproliferation. Cultures of 0.2 ml total volume were prepared in wells of flat-bottomed microtissue culture plates (Flow Laboratories, Inc., McLean, Va.). Cultures for mitogen-stimulated lymphoproliferation contained 10⁵ MNL and 0.033 mitogenic units of phytohemagglutinin (PHA) (PHA MR-69; Burroughs Wellcome Co., Research Triangle Park, N.C.) or a 1:160 dilution of stock pokeweed mitogen (PWM; GIBCO). Cultures for antigen-stimulated lymphoproliferation contained 10⁵ MNL and 30 protein nitrogen units of *Candida* antigen (*C. albicans* allergenic extract in glycerin [1:10]; Hollister-Stier Laboratories, Downers Grove, Ill.), a 1:100 dilution of mumps skin test antigen (Eli Lilly & Co., Indianapolis, Ind.), 20 units of streptokinase as streptokinase-streptodornase varidase (Lederle Laboratories, Pearl River, N.Y.), a 1:50 dilution of heat-inactivated cytomegalovirus (10), a 1:100 dilution of heat-inactivated herpes simplex type 1 virus (10), or a 1:800 dilution of aluminum phosphate-adsorbed tetanus toxoid (Wyeth Laboratories Inc., Philadelphia, Pa.). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air for 3 days for mitogen- and 7 days for antigen-stimulated lymphoproliferation. One microcurie of tritiated thymidine (specific activity, 1.9 or 6.7 Ci/mmol; New England Nuclear Corp., Boston, Mass.) in 50 µl of minimal essential medium was added to each well for the final 18 h of culture. Cells were collected onto glass fiber filter paper with a semiautomatic cell harvester (Otto Hiller Co., Madison, Wis.). Dried

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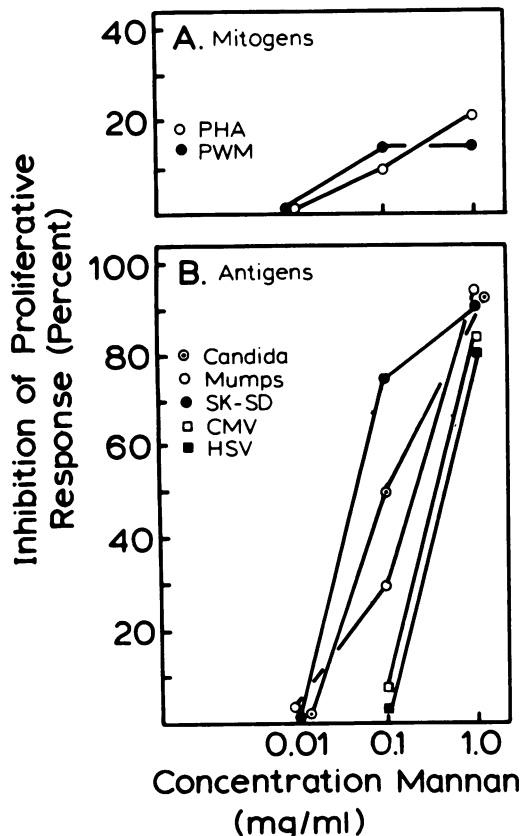


FIG. 1. Dose effect of *S. cerevisiae* mannan on proliferative responses of human lymphocytes stimulated by (A) the mitogens PHA and PWM and (B) the antigens *C. albicans* (Candida), mumps, streptokinase-streptodornase (SK-SD), cytomegalovirus (CMV) and herpes simplex virus (HSV). See the text for information on control and test levels of lymphoproliferation measured in terms of incorporation of tritiated thymidine.

filter segments were placed in toluene-based scintillation fluid, and radioactivity was measured by liquid scintillation spectrometry. Results were expressed as a percentage of the control response. Levels of spontaneous incorporation of the label were 1,000 to 2,500 cpm. Net levels of PHA-stimulated lymphoproliferation averaged approximately 100,000 cpm, PWM-stimulated lymphoproliferation averaged approximately 35,000 cpm, and antigen-stimulated lymphoproliferation fell in the range of 10,000 to 70,000 cpm. All tests were conducted with a minimum of three replicates. Standard deviation values reflecting variability between replicates within an experiment were consistently $\leq 10\%$ of the mean.

Preparation of mannan. *S. cerevisiae* wild-type strain X2180 was kindly provided by Clinton Ballou, Department of Biochemistry, University of California at Berkeley. The yeast was cultured in medium containing 1.7 g of yeast nitrogen base (Difco Laboratories, Detroit, Mich.) per liter supplemented to contain 5 g of ammonium sulfate and 20 g of glucose per liter (7). Mannan was isolated by the method of Peat et al. (16). Initial extraction of mannan involved autoclaving the yeast in neutral citrate buffer. Fehling solution was then added to precipitate the mannan in the form of coordinated complexes with copper. The mannan-copper complexes were subsequently decomposed by addition of HCl. The free mannan was precipitated by addition of

methanol-acetic acid, washed, dialyzed against deionized water, and lyophilized. Preparation of copper-deficient mannan involved passage of the mannan over Dowex chelating resin (Sigma Chemical Co., St. Louis, Mo.), using deionized water as eluant, and the mannan was recovered by lyophilization. Mannans were analyzed for the presence of copper by atomic absorption spectrophotometry with a Varian Techtron AA375.

Measurement of leukocyte respiratory functions. Stimuli for measurement of neutrophil respiratory functions included the synthetic chemotactic tripeptide *N*-formyl-methionyl-leucyl-phenylalanine (FMLP; Calbiochem-Behring, La Jolla, Calif.) and zymosan (Sigma) opsonized by incubation with fresh serum. Production of hydrogen peroxide was measured with phenol red (Allied Chemical Co., Morristown, N.J.) and horseradish peroxidase (Sigma) as described by Pick and Keisari (17). Production of superoxide was measured as superoxide-mediated reduction of cytochrome *c* (Sigma) as described by DeChatelet et al. (5). Oxygen consumption was quantified by using a biological oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio). Measurement of neutrophil hexose monophosphate shunt activity was accomplished as previously described (14).

RESULTS

Influence of mannan on lymphoproliferation. Data in Fig. 1A and B illustrate the dose-dependent influence of *S. cerevisiae* mannan on proliferative responses of human lymphocytes to mitogens and to microbial and viral antigens, respectively. Mannan was added to the test cultures to provide final concentrations of 0.01, 0.1, or 1.0 mg/ml. These data are presented as mean values derived from one or more experiments for the purpose of illustrating the gross difference in susceptibility of lymphoproliferative responses induced by mitogens and antigens to inhibition by mannan.

In three independent experiments involving leukocytes from three different donors, lymphoproliferation in response to PHA or PWM was inhibited by approximately 20% on the average in the presence of 1.0 mg of mannan per ml. Dilution of mannan to 0.1 mg/ml reduced the level of inhibition to approximately 10%, and further dilution of mannan to 0.01 mg/ml eliminated the inhibitory influence. The following information is provided to illustrate that mannan-mediated inhibition of proliferative responses to mitogens at levels of 10 to 20% represents a real phenomenon. In a representative experiment, control responses to PHA and PWM, expressed as $\bar{x} \pm$ standard deviation were $94,983 \pm 2,567$ and $16,502 \pm 817$ cpm, respectively. In the presence of 1.0 mg/ml, these responses were reduced to $75,726 \pm 5,294$ ($P < 0.01$) and $13,892 \pm 714$ cpm ($P < 0.01$), respectively. In the presence of 0.1 mg of mannan per ml, these responses were $84,506 \pm 3,667$ ($P < 0.05$) and $14,348 \pm 166$ ($P < 0.05$), respectively.

The effect of mannan on lymphoproliferation in response to various antigens has been tested on numerous occasions with leukocytes from multiple donors. Data presented have been drawn from such experiments to illustrate representative dose-related effects of mannan on lymphoproliferation in response to *C. albicans*, mumps, streptokinase-streptodornase, cytomegalovirus, and herpes simplex virus. Lymphoproliferation in response to all antigens was inhibited at levels $\geq 80\%$ in the presence of 1.0 mg of mannan per ml. The level of inhibition observed at the intermediate dose of mannan was variable. Responses to cytomegalovirus and herpes simplex virus were not inhibited by 0.1 mg of mannan per ml; responses to the other antigens were inhibited at

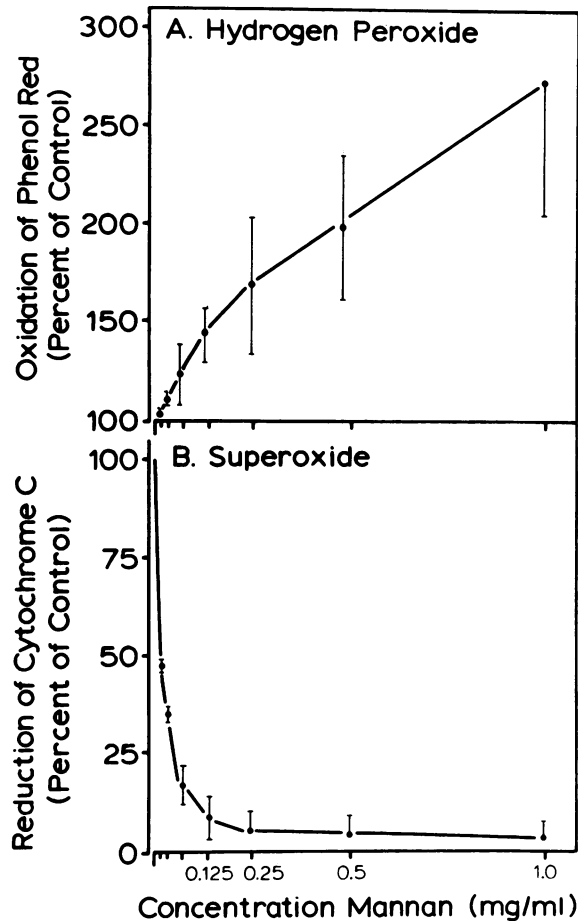


FIG. 2. Dose effect of *S. cerevisiae* mannan on production of hydrogen peroxide (A) and superoxide (B) by human neutrophils stimulated with the synthetic chemotactic tripeptide FMLP at a final concentration of 10^{-4} M. Data points represent the mean \pm standard error values derived from three experiments to evaluate each aspect of the respiratory burst. The average concentration of hydrogen peroxide produced by 10^6 stimulated neutrophils over a 30-min time period was 0.8 nmol; the average concentration of superoxide produced by 10^6 stimulated neutrophils over a 20-min time period was 0.7 nmol.

levels between 30 and 80% under this condition. Dilution of mannan to 0.01 mg/ml eliminated this inhibitory influence.

The inhibitory effect of mannan on these lymphoproliferative responses appeared to be due to a mechanism which did not involve cytotoxicity or measurement of lymphoproliferation. Cell viability monitored in terms of release of lactate dehydrogenase was found to be unaffected by exposure of leukocytes to 1.0 mg of mannan per ml for a 7-day period. The inhibitory influence could not be attributed to a competing stimulatory effect of mannan because the polysaccharide was never observed to stimulate lymphoproliferation when incubated alone with leukocytes isolated from multiple donors over incubation periods extending from 3 to 7 days. Inhibition of lymphoproliferation could also not be attributed to a nonspecific effect on cell division or incorporation of tritiated thymidine because mannan added to antigen-stimulated cultures for the final 3 days of incubation did not produce an inhibitory effect. The inhibitory property of mannan was heat stable because isolation of the polysaccharide involved an autoclaving step. Finally, other polysaccha-

rides (dextran and insulin) and sugars (D-mannose, D-galactose, D-glucose, and D-fructose) did not inhibit antigen-stimulated lymphoproliferative responses when tested under the same conditions used to assess the inhibitory effects of mannan. Based upon these observations, we concluded that mannan-mediated inhibition of lymphoproliferation was not artifactual and therefore worthy of further study.

SOD activity of mannan. Subsequent experiments to define the mechanism of mannan-mediated inhibition of lymphoproliferation were aided in part by two pieces of information. One was the preliminary observation made in our laboratory that mannan augmented hydrogen peroxide production by stimulated neutrophils; another derived from reports from other laboratories that hydrogen peroxide might suppress immune function, including lymphoproliferation (1, 6, 13, 15).

Data summarized in Fig. 2A and B illustrate the SOD activity of mannan in terms of its dose-related influence on hydrogen peroxide and superoxide production by neutrophils stimulated with the chemotactic tripeptide FMLP. The data were derived from three independent experiments to evaluate this influence. At mannan concentrations ≥ 0.03 mg/ml, hydrogen peroxide production was augmented by 10 to more than 100%; superoxide production was reduced by 50 to 95%. Under the same test conditions, mannan neither augmented nor inhibited neutrophil respiratory activity measured in terms of oxygen consumption or hexose monophosphate shunt activity.

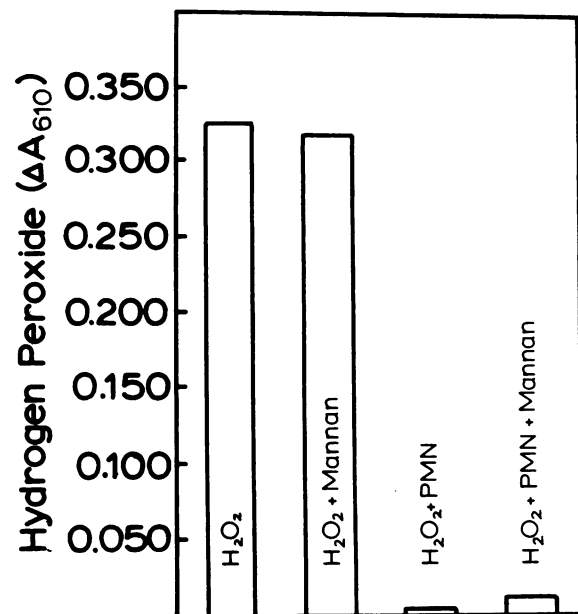


FIG. 3. Influence of *S. cerevisiae* mannan on spontaneous and human polymorphonuclear neutrophil-mediated (PMN) decomposition of exogenous hydrogen peroxide. Hydrogen peroxide was measured by a method using phenol red and horseradish peroxidase (17). Loss of optical density at 610 nm reflects decomposition of hydrogen peroxide. Test conditions: reaction volume, 1.0 ml; hydrogen peroxide concentration, 10 μ M; mannan concentration, 1 mg/ml; number of polymorphonuclear neutrophils, 10^6 ; time of incubation before addition of substrate and enzyme, 30 min at 37°C. The (ΔA_{610}) notation reflects subtraction of the optical density value for phosphate-buffered saline. Results derive from a single experiment with duplicate tests of each experimental condition.

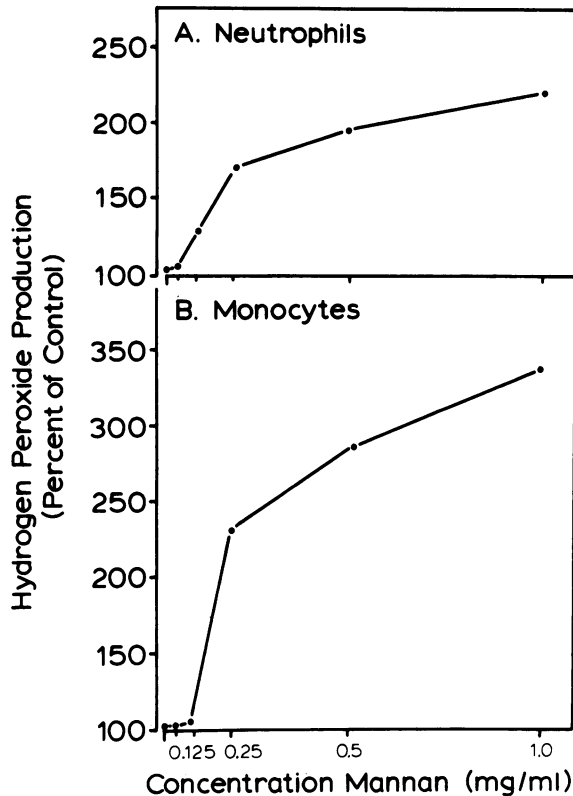


FIG. 4. Dose effect of *S. cerevisiae* mannan on production of hydrogen peroxide by human neutrophils (A) and monocytes (B) stimulated by phagocytosis of serum-opsonized zymosan. Test conditions: reaction volume, 1.0 ml; number of leukocytes, 10^6 neutrophils or 5×10^6 mononuclear leukocytes (representing approximately 10^6 monocytes); zymosan, 100 μ l; incubation time, 30 min at 37°C. The average concentration of hydrogen peroxide produced by 10^6 stimulated neutrophils over a 30-min period was 2 nmol; the average concentration of hydrogen peroxide produced by 10^6 monocytes was 0.1 nmol under the same conditions.

The augmentation of hydrogen peroxide production by mannan, at the apparent expense of superoxide production, suggested that mannan may possess SOD activity. However, hydrogen peroxide production would also appear to increase due to an inhibitory influence of mannan on cellular reactions providing for decomposition of hydrogen peroxide—reactions catalyzed by myeloperoxidase, catalase, and glutathione peroxidase. We therefore tested the effect of mannan on spontaneous and neutrophil-mediated decomposition of exogenous hydrogen peroxide. The results (Fig. 3) demonstrated that mannan had no effect on either the spontaneous decomposition of hydrogen peroxide or the ability of neutrophils to decompose the reagent. Thus, mannan appeared to possess SOD activity. It has an ability to augment hydrogen peroxide production by stimulated neutrophils in association with inhibition of superoxide production, independent of an effect on either cellular respiration or decomposition of hydrogen peroxide.

Data summarized in Fig. 4 derive from two experiments to test the influence of mannan on hydrogen peroxide production by monocytes. For these experiments, monocytes (as MNL) and neutrophils were incubated with serum-opsonized zymosan in the absence or presence of mannan at concentrations of 0.06 to 1.0 mg/ml. The change of stimulus

from FMLP to zymosan was made to provide a greater level of monocyte respiratory activity. We observed that mannan over the concentration range of 0.125 to 1.0 mg/ml increased the amount of hydrogen peroxide produced by neutrophils from 125 to approximately 225% of the control. Mannan over the concentration range of 0.25 to 1.0 mg/ml increased the amount of hydrogen peroxide produced by monocytes from 225 to 325% of the control. Because the numbers of neutrophils and monocytes were not precisely matched in these experiments, direct comparison of the influence of mannan on hydrogen peroxide production by these phagocytic leukocytes is not appropriate. The conclusion drawn is only that the ability of mannan to augment hydrogen peroxide production by phagocytosis-stimulated neutrophils applies as well to phagocytosis-stimulated monocytes.

The SOD activity associated with mannan in these experiments was considered to reflect either contaminating yeast enzyme or a catalytic activity of the polysaccharide itself. The low protein content of the mannan (<1% by weight) and survival of the catalytic activity in spite of an autoclaving step in its preparation, however, make it unlikely that SOD activity was due to contaminating yeast enzyme. We therefore considered the possibility that the mannan SOD activity might involve associated metal ions, since enzymes with SOD activity contain copper (21) and since copper is known to bind to polyhydroxyl compounds, including carbohydrate (4). Analysis of mannan for the presence of copper by atomic absorption spectrophotometry gave a copper concentration of 1.3 μ g/mg of polysaccharide.

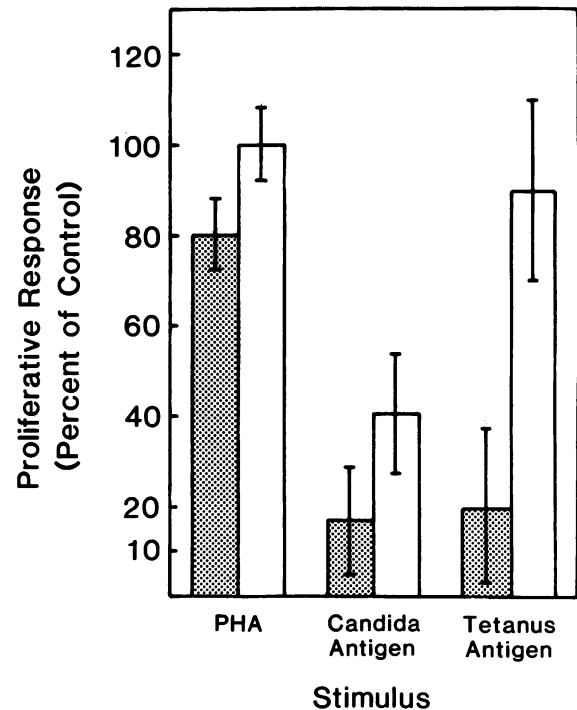


FIG. 5. Influence of *S. cerevisiae* mannan on proliferation of human lymphocytes stimulated by PHA, *C. albicans* antigen, or tetanus toxoid antigen. The net control responses to PHA, *C. albicans* antigen, and tetanus toxoid antigen were approximately 10^5 , 4×10^4 , and 4×10^4 cpm, respectively. Data expressed as $\bar{x} \pm$ standard error reflect the results of two independent experiments involving each of the stimuli.

To assess the role of copper in the SOD activity of mannan, we prepared mannan deficient in copper by passage of the mannan over Dowex chelating resin. The mannan eluted from the column was determined to be free of copper by atomic absorption spectrophotometry. The copper-deficient mannan was tested for its influence on superoxide production by neutrophils incubated with FMLP and was observed to have lost all SOD activity.

Role of copper in mannan-mediated inhibition of lymphoproliferation. Data in Fig. 5 compare the influence of copper-sufficient and copper-deficient mannans on proliferative responses of lymphocytes to PHA, *C. albicans* antigen, and tetanus toxoid antigen. Copper-sufficient mannan at a concentration of 1 mg/ml inhibited these responses by 20, 60, and 45%, respectively. Copper-deficient mannan at the same concentration did not inhibit either PHA- or tetanus toxoid antigen-stimulated lymphoproliferation ($P \leq 0.05$) but inhibited *C. albicans* antigen-stimulated lymphoproliferation by 55% ($P > 0.05$).

DISCUSSION

Data presented in this report illustrate that *S. cerevisiae* cell wall mannan can have a nonspecific or specific inhibitory influence on stimulated proliferative responses of human lymphocytes in vitro and that the presence or absence of copper determines the specificity of inhibition. Mannan isolated from baker's yeast by precipitation with Fehling reagent was observed to inhibit lymphoproliferation stimulated by all mitogens and antigens tested (Fig. 1A and B). In general, lymphoproliferation induced by the mitogens PHA and PWM was least affected by the addition of mannan (ca. 20% inhibition at 1 mg/ml). Lymphoproliferation induced by a battery of microbial and viral antigens was affected to a much greater degree by addition of mannan ($\geq 85\%$ inhibition at 1 mg of mannan per ml). We also observed that responses to different antigens were differentially sensitive to mannan at lower concentrations, but no attempt was made to further detail this phenomenon.

The inhibitory influence of baker's yeast mannan on lymphoproliferation stimulated by antigens described in this report mimics that described for *C. albicans* mannan by Fischer et al. (8). Although these investigators emphasized that isolated *C. albicans* mannan selectively inhibited lymphoproliferation stimulated by *C. albicans* metabolic antigen, they noted that higher concentrations (1 versus 0.5 mg/ml) of the mannan also reduced lymphoproliferation stimulated by tuberculin purified protein derivative. We suggest, therefore, that cell wall mannan polysaccharides isolated from these fungal species share an ability to nonspecifically inhibit proliferative responses of human lymphocytes. It is not completely unexpected that mannans from these sources should share this quality because they are known to be similar in terms of structure (2, 11, 10) and antigenicity (18).

Mechanisms considered to explain the nonspecific immunoinhibitory influence of the mannan included cytotoxicity, interference with cell division or measurement of proliferation, and interference with interaction of the stimuli with the lymphocyte or monocyte. For reasons cited earlier, we concluded that the mannan was not cytotoxic and that it did not inhibit either cell division or incorporation of the tritiated label. Although no experiments were conducted to assess the effect of mannan on binding of the various stimuli, we suggest that the mitogens and antigens used were too varied chemically to support the possibility that mannan might compete with all of them for binding to their respective

target cells. Whatever the mechanism of this phenomenon, it appears to be related to copper associated with the mannan and possibly to the SOD activity of the mannan-copper complex.

The SOD activity of mannan provided our initial clue to the association of copper with the isolated mannan. This activity was first recognized in studies to assess the influence of baker's yeast mannan on hydrogen peroxide and superoxide production by stimulated neutrophils (Fig. 2). That the ability of mannan to increase hydrogen peroxide production by neutrophils was due to SOD activity is supported by the observations that hydrogen peroxide production was increased at the expense of superoxide production, that the increase in hydrogen peroxide production was not associated with an increase in cellular respiratory activity, and that mannan did not inhibit cellular decomposition of hydrogen peroxide (Fig. 3). An SOD activity of mannan is also supported by our finding that the polysaccharide contained $>0.01\%$ copper, a metal active in electron transfer reactions performed by enzymes with this catalytic function, i.e., cupreins (20). Formation of mannan-copper complexes most likely involves conjugation of the metal ligand with hydroxyl groups of the component mannose residues (4). In other experiments, we have recently determined that mannan isolated from *C. albicans* also possesses SOD activity by the same criteria.

The role of copper in the nonspecific immunoinhibitory influence of mannan observed in these experiments remains to be explained. Lipsky (12) has reported that copper as CuSO_4 together with penicillamine inhibits the capacity of human lymphocytes to proliferate in response to mitogens due to an inhibitory effect on helper T cell function. Alternatively, a number of other investigators have reported that hydrogen peroxide is suppressive for lymphoproliferation. For example, Metzger et al. (13) observed an anti-inhibitory effect of catalase on macrophage-mediated suppression of mitogen-induced proliferation of murine splenic lymphocytes. The protective effect of catalase was presumed to involve reduction of toxic levels of hydrogen peroxide and prostaglandin production augmented by accumulating peroxide. Deshazo et al. (6) determined that the abnormal lymphoproliferative response seen in Hodgkin's disease may result in part from the excessive production of toxic oxygen metabolites and prostaglandins by adherent cell populations. Aune and Pierce (1) reported that hydrogen peroxide produced by murine macrophages converts T cell-derived soluble immune response suppressor to macrophage-derived suppressor factor, which in turn oxidizes cellular components essential for cell division. Finally, Nishida et al. (15) observed that catalase was able to prevent the suppressive effect of products of the reaction of xanthine oxidase and hypoxanthine on mitogen-stimulated proliferation of human lymphocytes. They concluded also that T cells were more sensitive than B cells to the suppressive effects of products of the oxidase system. Collectively, these reports strongly support an inhibitory role for hydrogen peroxide in reactions which affect proliferation of T lymphocytes and suggest that the nonspecific immunoinhibitory influence of mannan in our experiments may depend upon the SOD activity of the mannan-copper complex. The common finding of these investigators that macrophages, monocytes, or an adherent cell population was the source of hydrogen peroxide, together with our finding that mannan augments the production of hydrogen peroxide by monocytes (Fig. 4), suggests that the primary effect of mannan in our experiments may involve monocytes.

The mechanism by which copper-deficient mannan selectively inhibited *C. albicans* antigen-stimulated lymphoproliferation (Fig. 5) also remains unknown for the moment. Although we have identified this as a "specific" influence of the mannan, we recognize that the *C. albicans* antigen preparation used is a mixture of extractable components of the whole organism and that multiple antigenic specificities may have stimulated the lymphoproliferation measured. However, if a polysaccharidic antigen were the exclusive or primary stimulus for lymphoproliferation in our experiments, then simple competition for binding to a target cell could explain the apparent specificity of this phenomenon. Proof of specificity will require fractionation of the *C. albicans* "antigen" to isolate its separate stimulatory components and to compare the sensitivity of proliferation induced by each component to the inhibitory effects of copper-sufficient and copper-deficient mannans.

It is tempting to speculate that our observations may provide information useful to understanding the mechanism(s) of loss of cell-mediated immunity associated with chronic mucocutaneous candidiasis (19). There is reasonable support for the possibility that circulating yeast mannan is in part responsible for suppression of immune function in this disease (18). Certainly much more work is necessary to characterize and evaluate the phenomena we have described, but we suggest that the role identified for copper in these experiments represents a significant novel observation which may be useful in understanding the mechanisms of immunoinhibitory effects of *C. albicans* mannan.

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