Inflammation, Vol. 13, No. 4, 1989

FORMATION AND USE OF POLY-L-HISTIDINE-CATALASE COMPLEXES: Protection of Cells from Hydrogen Peroxide-Mediated Injury

DOUGLAS GIBBS,[1] JAMES VARANI,[1] and ISAAC GINSBURG[2,3]

[1]*Department of Pathology*
University of Michigan Medical School
Ann Arbor, Michigan 48109

[2]*Department of Oral Biology*
Hebrew University-Hadassah School of Dental Medicine
Jerusalem, Israel

[3]*Dr. Isaac Ginsburg was a visiting professor in the Department of Pathology, University of Michigan, when this study was done.*

Abstract—Insoluble complexes of poly-L-histidine (polyhistidine) and catalase were prepared by mixing the two reactants together in solution at pH 5.5 and subsequently elevating the pH to approximately 7.0, at which point they precipitated. Complexes formed at optimal ratios of polyhistidine to catalase contained essentially all of the catalase present in the original solution. The catalase present in such complexes contained greater than 50% of the H_2O_2-inhibiting activity of the native catalase used to prepare the complexes. The insoluble complexes rapidly bound to viable endothelial cells and were resistant to removal by extensive washing. The presence of polyhistidine-catalase complexes on the cell surface protected the cells against injury mediated by H_2O_2 or activated polymorphonuclear leukocytes. These data show that polyhistidine-catalase complexes can be prepared that have a high affinity for cells and that retain catalase activity. These complexes may be useful in treating inflammatory conditions in which it is necessary to maintain a high local concentration of inhibitor.

INTRODUCTION

Reactive oxygen metabolites play a central role in a number of inflammatory disease processes. In disease processes mediated by polymorphonuclear leukocytes (PMNs), the inflammatory stimuli trigger the generation of superoxide anion ($O\cdot_2^-$) through activation of the leukocyte membrane NADPH oxidase. The $O\cdot_2^-$ is spontaneously dismutated to hydrogen peroxide (H_2O_2), which is

0360-3997/89/0800-0465$06.00/0

then converted to a variety of metabolites. These metabolites, which include myeloperoxidase products such as hypochlorous and hypobromous acids, and hydroxyl radicals (HO·) generated by ferrous iron reduction of H_2O_2 in the Fenton reaction, are directly responsible for the tissue injury (reviewed in reference 1).

The importance of H_2O_2 as the central reactant in the generation of these injurious agents can be deduced from the protective effects afforded by the enzyme catalase. Catalase inhibits leukocyte killing of cells in vitro (2–5) and protects animals against injury mediated by leukocytes in a number of models (6, 7). In both in vitro and in vivo systems, the effectiveness of the treatment is limited by the ability of the catalase to localize at the target site. The close contact between the effector cells and the target cell is partially responsible for this (5, 8). In vivo, this is further complicated by the short circulatory half-life of exogenous catalase (9).

In order to overcome these limitations, chemically modified forms of catalase have been produced. It has recently been shown that chemical coupling of catalase to polyethylene glycol (PEG) allows it to move across the membranes of cells and thereby increases its internal concentration (10). While modification with PEG may be useful for moving catalase across plasma membranes, the critical task is to modify the enzyme in such a manner that it can bind to cells in the first place. Previous studies from our laboratories have shown that polycationic substances can be used to coat a variety of particles and facilitate their binding to anionic surface moieties of cells (11, 12). It is possible that a polycationic substance would interact with the anionic portions of catalase to form complexes, and in the proper ratio promote the binding of the enzyme to the cell surface. In the present report we describe the preparation of complexes of catalase with the cationic substance poly-L-histidine (polyhistidine). Due to their high cationic charge, the complexes readily attach to cell surfaces and resist removal by repeated washing. The complexes retain their catalase activity and afford complete protection to the cell against H_2O_2-mediated killing even after prolonged incubation and extensive washing.

MATERIALS AND METHODS

Reagents. Minimal essential medium with Earle's salts (MEM), and Hanks' balanced salt solution (HBSS) were obtained from Gibco (Grand Island, New York). Fetal bovine serum (FBS), penicillin, streptomycin, and fungizone were obtained from K.C. Biological (Lenexa, Kansas). The MEM was supplemented with 10% FBS, 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 0.25 μg/ml of fungizone. Potassium thiocyanate, trypsin, polyhistidine (10,800 mol wt), H_2O_2 (30% solution), and catalase (2800 units/mg) were obtained from Sigma (St. Louis, Missouri). Ferrous ammonium sulfate was obtained from Mallinckrodt (Paris, Kentucky). Trichlo-

roacetic acid was obtained from Columbus Chemical Industries (Columbus, Wisconsin). Culture dishes and flasks were obtained from Costar (Cambridge, Massachusetts).

Preparation of Polyhistidine–Catalase Complexes. Complexes consisting of polyhistidine and catalase were prepared utilizing four stock solutions: 0.9% saline at pH 5.5–6.0; catalase at a concentration of 10 mg/ml in 0.9% saline; polyhistidine at a concentration of 10 mg/ml in 0.9% saline, and HBSS buffered with 0.2 M HEPES buffer at pH 7.35. To prepare the complexes, 0.1 ml of the stock catalase solution was first added to 0.4 ml of the saline solution. While vortexing, various amounts of the polyhistidine solution were added. Upon addition of the polyhistidine, the solution became opalescent, although a true precipitate did not form. While still vortexing, enough of the HEPES-buffered HBSS was added to bring the final volume to 1.0 ml. The addition of the HEPES-buffered HBSS brought the pH of the solution to approximately 7.0, at which point a true precipitate formed. The mixture was allowed to stand for 5 min, after which the polyhistidine–catalase complexes were pelleted by centrifugation and washed two times. After the second wash, the pellet was resuspended in 1.0 ml of HBSS and tested for catalase activity as described below.

Endothelial Cells. Pulmonary artery endothelial cells were obtained from rat lungs as described elsewhere (13). They were identified as endothelial cells by the presence of angiotensin-converting enzyme activity, reactivity with antibodies to factor VIII and α_2-macroglobulin, and by typical electron microscopic features. The cells were cultured as monolayers in MEM with 10% FBS and antibiotics in 150-cm^2 flasks at 37°C and 5% CO_2. When confluency was reached, the cells were subcultured after retrieval with a rubber policeman. The cells were routinely used through passage 35.

Quantitation of H_2O_2 and Catalase Activity. H_2O_2 was measured using reduction of ferrous ammonium sulfate as indicator (14). A standard curve was established for each experiment and catalase activity was measured by dismutation of known quantities of H_2O_2. Catalase activity associated with the polyhistidine–catalase complexes was assessed by comparison to a standard curve generated with native catalase.

Cytotoxicity Assay. Endothelial cells were seeded into wells of a 24-well plate in 1 ml of MEM containing 10% FBS at a density of 1×10^5 cells/well. The plate was then incubated overnight at 37°C in 5% CO_2, during which time the cells formed confluent monolayers. At the start of the assay, the cells were washed three times in MEM and then appropriate reactants, i.e., native catalase or polyhistidine–catalase complexes, were added in a final volume of 1.0 ml/well. In certain experiments the plates were centrifuged at 400*g* for 5 min to promote interaction of the complexes with the cells. This was not essential, however, as the polyhistidine–catalase complexes would bind to the cells without centrifugation. Except where noted, the cells were then washed five times with MEM. Following this, various amounts of H_2O_2 in MEM were added to give a final volume of 1.0 ml/well. The H_2O_2-treated cells were incubated at 37°C and 5% CO_2 for 1 h. At the end of 1 h, residual H_2O_2 was inactivated by addition of excess catalase (1800 units/ml), and the cells were harvested with 0.2% trypsin. The harvested cells were centrifuged, resuspended in MEM with 10% FBS, and reseeded into the larger six-well (35 mm diameter) culture dishes. After overnight incubation at 37°C and 5% CO_2, the cells were washed once with 0.9% saline to remove debris, harvested in 0.5 ml of trypsin (0.2%), and counted with a Coulter counter. By comparing the number of cells harvested after the final incubation with the number originally exposed to H_2O_2, we were able to obtain a measure of the percentage killed.

A ^{51}Cr-release assay was used to measure neutrophil-mediated killing of endothelial cells. Human peripheral blood leukocytes were obtained from healthy adult volunteers, and the polymorphonuclear leukocytes were isolated by dextran sedimentation followed by density gradient centrifugation through Ficoll-Hypaque medium. The cells obtained were approximately 98% polymorphonuclear granulocytes. Endothelial cells were seeded into wells of a 24-well plate as described above and labeled with 2 μCi of ^{51}Cr per well. One day later, the cells were washed to remove unincorporated ^{51}Cr and incubated with polyhistidine–catalase complexes as described above. After

removal of nonbound complexes, treated and control cells were exposed to neutrophils at a 10:1 effector-to-target ratio. The neutrophils were activated with phorbol myristate acetate (PMA) at a concentration of 100 ng/ml. Cytotoxicity (^{51}Cr release) was assessed after 4 or 18 h. The ^{51}Cr-release assay is described in detail in our previous reports (5, 15).

RESULTS

Preparation and Assay of Polyhistidine–Catalase Complexes. Complexes of polyhistidine and catalase were prepared by varying the amount of polyhistidine while keeping the catalase constant. After formation, the complexes were removed by centrifugation and the amount of catalase incorporated into the complexes determined. Concomitantly, the amount of catalase remaining in solution was also determined. Table 1 shows the distribution of catalase after formation of polyhistidine–catalase complexes at three ratios of polyhistidine to catalase. When 0.1 mg of polyhistidine was used with 1.0 mg of catalase, the complexes contained approximately 17% of the catalase originally present with approximately 83% remaining in solution. When complexes were formed with 0.5 or 1.0 mg of polyhistidine per milligram of catalase, the complexes contained 50–58% of the catalase originally present. However, under these conditions, there was essentially no residual catalase remaining in solution. This suggests that all of the catalase was incorporated into the complexes but that some of the catalase was inactivated by the high concentration of polyhistidine. Figure 1 compares the H_2O_2-inhibiting activity of native catalase and catalase complexed with polyhistidine.

In additional experiments, higher-molecular-weight polyhistidine preparations (e.g., 15,000 and 20,000 mol wt) were used. When these were used, it

Table 1. Incorporation of Catalase into Complexes Formed with Polyhistidine

Reactants used[a]		Amount (μg) of catalase recovered[b]	
Polyhistidine	Catalase	Complex	Solution
100	1000	174	889
500	1000	579	0
1000	1000	505	0

[a] Complexes of polyhistidine and catalase were formed by mixing increasing amounts of polyhistidine with a constant amount of catalase as described in the Methods section.
[b] The amount of catalase recovered was determined based on activity (H_2O_2 inhibition), comparing the amount of activity in the complexes and in solution with the amount of activity present in known quantities of native catalase. The values are from a single experiment. The experiment was repeated three times with similar results.

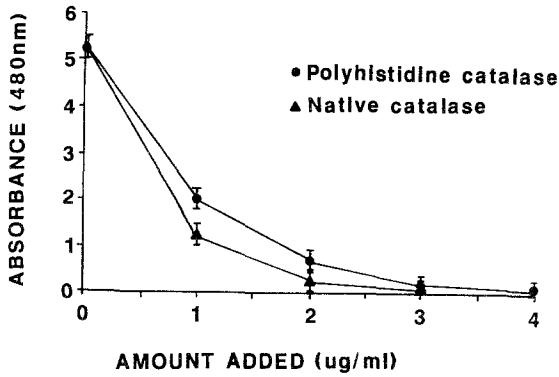

Fig. 1. Assessment of catalase activity in polyhistidine-catalase complexes. A 30% stock solution of H_2O_2 was diluted and assayed using reduction of ferrous ammonium sulfate as the indicator. Based on this, a stock solution containing 2000 nmol H_2O_2/ml was prepared. The stock H_2O_2 solution was incubated for 10 min with various amounts of native catalase or polyhistidine-catalase complexes and residual H_2O_2 determined. Values shown represent inhibition of ferrous ammonium sulfate reduction by H_2O_2 after incubation with native or complexed catalase. Values are means and standard deviations based on triplicate samples in a single experiment. The experiment was repeated five times with similar results.

required a correspondingly greater amount of polyhistidine (on a per microgram basis) to produce complexes containing comparable amounts of catalase. The maximal catalase activity incorporated into these complexes was always less than the amount incorporated into complexes formed with 10,800 mol wt polyhistidine (not shown). This suggests greater inactivation of the catalase when complexed to the higher-molecular-weight polyhistidine preparations.

Experiments were also conducted with catalase preparations containing greater amounts of catalase activity per milligram of protein. Polyhistidine-catalase complexes formed with these catalase preparations contained correspondingly greater amounts of catalase activity (not shown).

Binding of Polyhistidine-Catalase Complexes to Surface of Endothelial Cells. When monolayers of endothelial cells were treated in culture with the polyhistidine-catalase complexes, the complexes rapidly bound to the cell surface. This could be assessed visually under phase-contrast microscopy. The interaction of the complexes with cells could be accelerated by centrifuging the monolayers after addition of the complexes, and this was routinely done. However, it was not essential, as the precipitated complexes would settle onto the cells and bind to them without centrifugation. To quantitate the amount of catalase bound to the cell surface, the cells were treated with the complexes and centrifuged. Following this, the monolayers were washed five times and each of the washes assessed for catalase activity. No detectable catalase activity was

present in any of the washes. This suggests that once bound, the polyhistidine–catalase complexes were not readily released by the cells. In addition to measuring catalase activity released from the cell surface by washing, the amount of activity remaining on the cell surface was also measured. The total amount of activity remaining after five washes was approximately 25% of the amount added to the cells. These data are summarized in Table 2. It may be inferred by the lack of activity in the washes that most of the loss in activity was a result of enzyme inactivation due to interaction with the cell membrane. Alternatively, the reduction in detectable activity on the cell surface may reflect internalization of the polyhistidine–catalase complexes. Although no experiments were done to determine if the complexes were internalized, previous studies from our laboratory have shown that a variety of cationic complexes are readily internalized by cells in culture (11, 16).

Protection of Endothelial Cells from H_2O_2-Mediated Killing by Polyhistidine–Catalase Complexes. Rat pulmonary artery endothelial cells were examined for sensitivity to killing by H_2O_2. Figure 2 indicates that under the conditions of the experiment, 100 nmol of H_2O_2 induced killing of approximately 50% of the endothelial cells in 1 h. In the presence of 400 nmol of H_2O_2, virtually 100% of the cells were killed. Figure 2 also demonstrates the ability of the polyhistidine–catalase complexes to protect the target cells from

Table 2. Binding of Polyhistidine–Catalase Complexes to Surface of Endothelial Cells

Group[a]	Amount of catalase recovered[b] (μg)	% of total
Total amount added	2.90	100
Amount recovered in wash		
1	<0.05	<2
2	<0.05	<2
3	<0.05	<2
4	<0.05	<2
5	<0.05	<2
Amount remaining on cells after five washes	0.70	25

[a] Monolayers of endothelial cells (1×10^5 cells in 1 ml) were incubated with polyhistidine-catalase complexes containing catalase activity equivalent to 2.9 μg of native catalase. The culture medium was then removed (wash 1) and the monolayer washed four additional times. Each of the washes and the monolayers was assessed for catalase activity.

[b] The amount of catalase recovered was determined based on activity, comparing the amount of activity in each fraction to the amount of activity in known quantities of native catalase. The values are from a single experiment. The experiment was repeated three times with similar results.

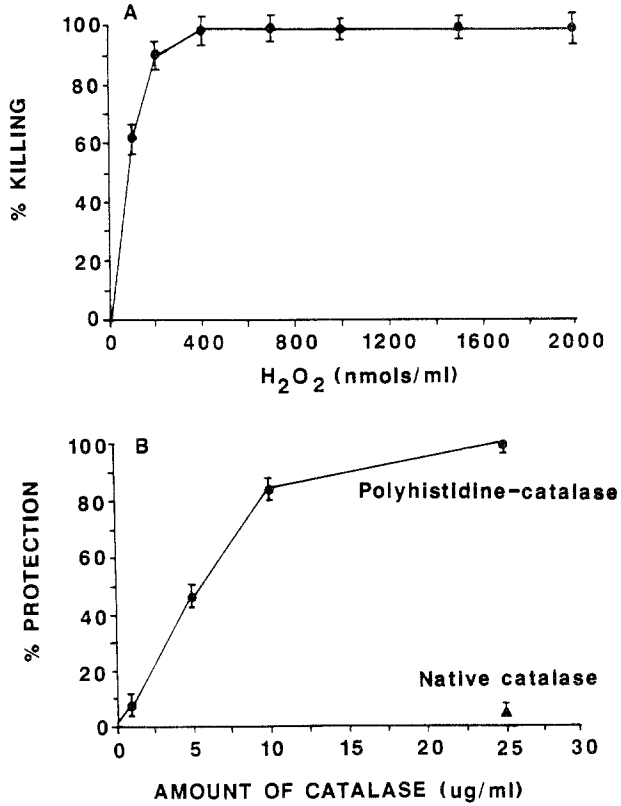

Fig. 2. (A) Concentration-dependent killing of endothelial cells by H_2O_2. Endothelial cells were exposed to varying amounts of H_2O_2 for 1 h as described in Materials and Methods. Following this, the percentage of cells that were incapable of replating was determined. (B) Protection of endothelial cells from H_2O_2-mediated killing by native catalase and polyhistidine–catalase complexes. Endothelial cells were treated with varying amounts of native catalase or polyhistidine–catalase complexes. After incubation for 10 min, the nonbound catalase was removed by washing five times. The cells were then exposed to 1000 nmol H_2O_2/ml and incubated for 1 h. Following this, the percentage of cells that were incapable of replating was determined. Values shown are means and standard deviations based on triplicate samples in a single experiment. The experiment was repeated five times with similar results.

H_2O_2-mediated killing after extensive washing. For this experiment, endothelial cells were incubated with various amounts of the complexes for 10 min. Cells treated with native catalase served as a control. After the initial incubation period, the endothelial cells were washed five times to remove catalase not bound to the cells and then exposed to 1000 nmol of H_2O_2. Under these conditions, protection was obtained with as little as 2 μg of the polyhistidine–

Table 3. Protection against PMN-Mediated Injury to Endothelial Cells by Polyhistidine–Catalase Complexes[a]

Treatment	4 hours		18 hours	
	CPM	Specific cytotoxicity (%)	CPM	Specific cytotoxicity (%)
None	915 ± 11	4	7885 ± 998	33
Polyhistidine–catalase	651 ± 8	0	5502 ± 650	24

[a] Endothelial cells were treated with polyhistidine–catalase complexes containing approximately 1 μg of catalase. After incubation with the complexes for 10 min, the monolayers were washed five times to remove nonbound complexes. Following this, treated and control monolayers were incubated with neutrophils (10:1 effector–target ratio), which were activated 15 min later by addition of 100 ng of PMA/well. ^{51}Cr release from control and treated monolayers was measured 4 h or 18 h later.

[b] Values shown represent the average specific ^{51}Cr released ± standard errors based on seven independent experiments for the 4-h assay and 11 independent experiments for the 18-h assay. The percent specific cytotoxicity was calculated based on the following formula:

Specific cytotoxicity (%) = experimental release − spontaneous release/total release − spontaneous release.

catalase complexes. Protection was dose-responsive over the range of 0–10 μg. In contrast, no protection was seen with 25 μg of native catalase under the same conditions.

Polyhistidine–catalase complexes were next examined for ability to protect endothelial cells from activated neutrophils. Past studies by several groups have shown that endothelial cell killing by activated neutrophils is H_2O_2-dependent (2–5). Table 3 summarizes ^{51}Cr-release data from several independent experiments. Neutrophils activated with PMA produced a net increase in ^{51}Cr release over the amount released by endothelial cells alone or endothelial cells incubated with nonactivated PMNs. Virtually 100% of the killing in the 4-h assay was inhibited by treatment of the cells with 2 μg of the polyhistidine–catalase complexes followed by extensive washing. Endothelial cell killing in the 18-h assay was also inhibited by the polyhistidine–catalase complexes. Although the degree of protection was much less after 18 h, this is probably attributable to the fact that killing in the 18-h assay is due, in part, to protease activity (unpublished observation).

DISCUSSION

This study describes the preparation of polyhistidine–catalase complexes and the ability of these complexes to bind to the surface of endothelial cells and protect them from H_2O_2-mediated killing. The chemistry of complex formation

involves electrostatic interaction between the highly cationic polyhistidine with anionic sites on the catalase molecule and precipitation of the complexes at neutral pH. Formation of the complexes is rapid and easy. The stock reagents used to prepare the complexes are stable for several days at 4°C, and the complexes themselves are also stable for several days at 4°C after preparation. Complexes formed to provide optimal amounts of catalase activity (determined by mixing various amounts of polyhistidine with a constant amount of catalase) incorporated virtually 100% of the available catalase under conditions in which a high degree of the catalase activity (greater than 50%) was retained. Preparation of complexes with a lower ratio of polyhistidine to catalase resulted in less of the catalase being incorporated into the complexes and complexes formed with a higher polyhistidine to catalase ratio had reduced enzymatic activity.

The polyhistidine–catalase complexes readily bound to the surface of endothelial cells and resisted removal by washing. The presence of complexes on the cell surface provided a high degree of protection against injury mediated by H_2O_2 directly or mediated by activated neutrophils. The ability to bind catalase to the surface of cells may provide a useful means for protecting cells from H_2O_2-mediated injury under a variety of conditions. Native catalase can protect cells in vitro from PMN-induced injury, but the degree of protection is limited by the fact that activated neutrophils adhere tightly to the target cell surface (5) and exclude fluid-phase inhibitors from the active site (8). Catalase can also protect animals against inflammatory processes mediated by neutrophils (6, 7). Protection against in vivo injury suffers from the same limitations as exist in vitro and is further limited by the short half-life of circulating catalase (9). By treating the target cells with a reagent that binds tightly to the cell surface, it may be possible to obtain a high specific activity of inhibitor at the site of attack. In this respect it is of note that cationization of catalase and horseradish peroxidase by the carbidiimide method was found to suppress the inflammatory response in two types of experimental arthritis in the mouse (17). Such cationized enzymes were also found to be retained in the joint tissues for much longer periods, as compared to native enzymes.

Finally, although all of the data presented in this report relate to the use of complexes formed with the enzyme catalase, it should be possible to prepare insoluble complexes of polyhistidine with other proteins as well. Using the same general approach as described here, we have prepared complexes of superoxide dismutase and polyhistidine and complexes of soybean trypsin inhibitor and polyhistidine. These complexes also retain their catalytic and inhibitory activities. The simplicity with which the complexes can be formed make this method potentially amenable to a variety of uses.

Acknowledgments—Supported in part by grants HL-28442, HL-31963, and GM-29507 from the USPHS, by grant IM-432 from the American Cancer Society, and by a grant from Dr. S. M. Robbins, Cleveland, Ohio.

REFERENCES

1. FANTONE, J. C., and P. A. WARD. 1982. Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. *Am. J. Pathol.* **107**:397–434.
2. SACKS, T., C. F. MOLDOW, P. R. CRADDOCK, T. K. BOWERS, and H. S. JACOB. 1978. Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes: An in vitro model of immune vascular damage. *J. Clin. Invest.* **61**:1161–1167.
3. WEISS, S. J., J. YOUNG, A. F. LOBUGLIO, A. SLIVKA, and N. F. NIMEH. 1981. Role of hydrogen peroxide in neutrophil-mediated destruction of cultured endothelial cells. *J. Clin. Invest.* **68**:714–720.
4. MARTIN, W. J. 1984. Neutrophils kill pulmonary endothelial cells by a hydrogen peroxide-dependent pathway: An in vitro model of neutrophil-mediated lung injury. *Am. Rev. Respir. Dis.* **130**:209–213.
5. VARANI, J., S. E. G. FLIGIEL, G. O. TILL, R. G. KUNKEL, U. S. RYAN, and P. A. WARD. 1985. Pulmonary endothelial cell killing by human neutrophils: Possible involvement of hydroxyl radical. *Lab. Invest.* **53**:656–663.
6. FLIGIEL, S. E. G., P. A. WARD, K. J. JOHNSON, and G. O. TILL. 1984. Evidence for a role of hydroxyl radical in immune complex-induced vasculitis. *Am. J. Pathol.* **115**:397–405.
7. WARD, P. A., G. O. TILL, R. KUNKEL, and C. BEAUCHAMP. 1983. Evidence for a role of hydroxyl radical in complement and neutrophil-dependent tissue injury. *J. Clin. Invest.* **72**:789–795.
8. CAMPBELL, E. J., R. M. SENIOR, J. A. MCDONALD, and D. L. COX. 1982. Proteolysis by neutrophils. Relative importance of cell-substrate contact and oxidative inactivation of protease inhibition in vitro. *J. Clin. Invest.* **70**:845–852.
9. TILL, G. O., C. BEAUCHAMP, D. MENAPACE, W. TOURTELLOTTE, JR., R. KUNKEL, K. J. JOHNSON, and P. A. WARD. 1983. Oxygen radical dependent lung damage following thermal injury of rat skin. *J. Trauma* **23**:269–276.
10. BECKMAN, J. S., R. L. MINOR, C. W. WHITE, J. E. REPINE, G. M. ROSEN, and B. A. FREEMAN. 1988. Superoxide dismutase and catalase conjugated to polethylene glycol increases endothelial enzyme activity and oxidant resistance. *J. Biol. Chem.* **263**: 6884–6892.
11. GINSBURG, I., M. N. SELA, A. MORAG, Z. RAVID, Z. DUCHAN, M. FERBE, S. RABINOWITZ-BERGNER, T. P. PAGE, P. DAVIS, J. MICCOLS, J. HUME, and R. BONNEY. 1981. Role of leukocyte factors and cationic polyelectrolytes in phagocytosis of *Candida albicans* by neutrophils, macrophages, fibroblasts and epithelial cells: Modulation by anionic polyelectrolytes in relation to pathogenesis of inflammation. *Inflammation* **5**:289–312.
12. GINSBURG, I. 1987. Cationic polyelectrolytes: A new look at their possible roles as opsonins, as stimulators of the respiratory burst in leukocytes, in bacteriolysis and as modulators of immune complex disease. *Inflammation* **11**:489–515.
13. RYAN, U. S., and L. WHITE. 1986. Microvascular endothelium, isolation with microcarriers: Arterial, venous. *J. Tissue Cult. Methods* **10**:9–13.
14. THURMAN, R. G., H. G. LEYLAND, and R. SCHOLZ. 1972. Hepatic microsomal ethanol oxidation, hydrogen peroxide formation and the role of catalase. *Eur. J. Biochem.* **25**:420–430.
15. GANNON, D. E., J. VARANI, S. H. PHAN, J. H. WARD, J. KAPLAN, G. O. TILL, R. H. SIMON, U. S. RYAN, and P. A. WARD. 1987. Source of iron in neutrophil-mediated killing of endothelial cells. *Lab. Invest.* **57**:37–44.
16. GINSBURG, I., S. E. G. FLIGIEL, R. G. KUNKEL, B. L. RISER, and J. VARANI. 1987. Phagocytosis of *Candida albicans* enhances malignant behavior of murine tumor cells. *Science* **238**:1573–1575.
17. SCHALKWIJK, J., W. B. VAN DEN BERG, L. B. A. VAN DE PUTTE, L. A. B. JOOSTEN, and L. VAN DER BERSSELAAR. 1985. Cationization of catalase, peroxidase, and superioxide dismutase: Effect of improved intraarticular retention on experimental arthritis in mice. *J. Clin. Invest.* **76**:198–205.