Antitumor Activity of an Immobilized Peroxidase System Against Murine Ehrlich Ascites Is Mediated by the Immune System

(43667)

STEVEN H. COMBS,* CLINTON D. MORGAN,† AND JOHANNES EVERSE*,[1]

Departments of Biochemistry and Molecular Biology and Microbiology and Immunology,† Texas Tech University Health Sciences Center, Lubbock, Texas 79430*

Abstract. **Earlier studies have shown that a mixture of glucose oxidase and a peroxidase exerts a tumoricidal effect on rats bearing Novikoff hepatomas when the enzyme mixture is injected intraperitoneally. The enzyme mixture was shown to be nontoxic when injected into healthy animals at levels up to 600 times the therapeutic dose. In the present study, we have evaluated the possibility that the host immune defense system may be involved in the antitumor activity of the peroxidase system, using the murine Ehrlich ascites tumor as the target. The results revealed that the antitumor activity of the peroxidase system is absent in tumor-bearing animals whose immune system has been compromised by whole body γ-irradiation or by an induced selenium deficiency. The peroxidase system was also found to be inactive in tumor-bearing mice whose immune system was suppressed by the administration of cyclosporin A as well as in athymic (*nu/nu*) mice. These results indicate that T lymphocytes may directly or indirectly be involved in the *in vivo* antitumor activity of the peroxidase system. This could explain the observed high selectivity toward tumor cells by the enzyme system *in vivo* and its lack of toxicity in healthy animals.** [P.S.E.B.M. 1993, Vol 204]

Some time ago we reported that a mixture of glucose oxidase and a peroxidase, either in solution or co-immobilized onto a solid support, exerts a tumoricidal effect when administered to tumor-bearing rats and mice (1). The enzyme mixture was shown to promote the regression of Novikoff hepatomas in rats and, to a lesser extent, of B16 melanomas in mice and of dimethylbenzanthracene-induced mammary tumors in rats. The most exciting observation was that the antitumor effect of the peroxidase system was highly specific for the tumor tissue; no toxic effects could be demonstrated even when very high levels of the enzyme mixture were administered to healthy animals. We were unable at that time, however, to obtain complete remission in all experimental animals and we also were unable to demonstrate that the antitumor effect of the peroxidase system was dose dependent. Based on these results, we suggested that an endogenous entity, presumably present in limited amounts in the body, may be involved in the anticancer effect of the peroxidases.

In a subsequent communication, we demonstrated that peroxidases are able to activate thioglycollate-induced murine peritoneal macrophages to the tumoricidal state *in vitro* (2). Activation of macrophages was observed with horseradish peroxidase as well as with lactoperoxidase and microperoxidase. The activation was observed at a very low concentration of the peroxidases and was shown to be dependent on the presence of hydrogen peroxide.

The latter results obviously raised the possibility that the observed antitumor activity of the peroxidase system *in vivo* could involve an activation of macrophages or perhaps of some other cell type of the immune system. If such a mechanism were operative in the tumor-bearing animal, then the absence of nonspecific toxicity of the peroxidase system could easily be explained.

[1] To whom requests for reprints should be addressed at Department of Biochemistry and Molecular Biology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Received March 29, 1993. [P.S.E.B.M. 1993, Vol 204]
Accepted July 2, 1993.

0037-9727/93/2043-0306$3.00/0

The experiments reported in this article were performed in an attempt to demonstrate whether or not the immune defense system of the host is in any way involved in promoting the antitumor activity of the oxidase-peroxidase mixture. The obtained results do indeed suggest that the antitumor activity of the peroxidase system *in vivo* is dependent on the presence of at least one of the immune cells, probably a T lymphocyte.

Materials and Methods

Materials. Horseradish peroxidase, type VI, inactive horseradish peroxidase, glucose oxidase from *Aspergillus niger*, bovine serum albumin, glutaraldehyde, NADPH, and glutathione reductase were purchased from Sigma Chemical Co., St. Louis, MO, and used without further purification. Cyclosporin A (Sandimmune) was obtained as a solution of 50 mg/ml from the University Medical Center pharmacy.

BALB/c mice and athymic (nude) mice were purchased from Sasco, Inc., Omaha, NE, and were housed in the Texas Tech University Health Sciences Center (TTUHSC) vivarium. The experiments reported here were reviewed and approved by the TTUHSC Animal Care and Use Committee (approval no. AR85069).

The Ehrlich ascites tumor was kindly supplied by Dr. L. M. Slater of the University of California at Irvine. The tumor was maintained by weekly intraperitoneal passage of 0.2 ml ascites fluid into 2–3 healthy mice.

Preparation of the Immobilized Peroxidase System. Glucose oxidase and horseradish peroxidase were immobilized by crosslinking the enzymes with an excess of bovine serum albumin (3). Ten milligrams of horseradish peroxidase, type VI, 2400 units of glucose oxidase, and 300 mg of bovine serum albumin were dissolved in 3 ml of 1 m*M* phosphate buffer, pH 7.2. Glutaraldehyde, 75 μl, 25%, was then added and the mixture was allowed to gel at 4°C overnight. The gel was mechanically broken into small pieces and extracted with buffer to remove any unbound enzyme. The gel was then lyophilized and converted into a fine powder with a mortar and pestle. The immobilized enzyme combination was stored at 4°C as a dry powder in the presence of a desiccant. In the control preparations, either the peroxidase or the oxidase was omitted during the preparation.

Experimental Protocol. Six- to 8-week-old mice were each inoculated intraperitoneally with 0.2 ml of ascites fluid that was freshly drawn from a donor mouse and that contained about $2–3 \times 10^8$ viable tumor cells/ml. The inoculated mice were then randomized into groups of 10. On the day after the administration of the tumor cells, each of the animals was injected with 0.5 ml saline solution (0.9% NaCl in water), containing 1.0 mg of the immobilized enzyme system. The first group of 10 received the complete enzyme system, the second group of 10 received the system in which the peroxidase was omitted, the third group received the system with the glucose oxidase omitted, and the fourth group received the system with both enzymes omitted (i.e., crosslinked albumin only). The same amount was administered again on the second and third day after tumor inoculation. On the 9th day, the animals were sacrificed by cervical dislocation and their peritoneal cavity was visually examined for the presence of tumor. Animals that did not have any ascitic fluid in their abdomens, in contrast to the control animals, which had up to 15 ml of ascites fluid in their abdomens, were considered to be "negative" in terms of tumor growth. In some cases, however, these negative animals did show one or two small solid skin tumors that were growing at the site of tumor injection.

Irradiation of Mice. For the purpose of destroying their stem cells, mice were irradiated with gamma rays, using a J. L. Shepherd Model 143 blood irradiator, containing a ^{137}Cs source. The animals were irradiated for 0.47 min in groups of 10, receiving a total of 500 rads of radiation in this time period (4).

Selenium-Deficient Mice. In order to induce a selenium deficiency, mice were given a selenium-free diet (Schwartz-Fredga Se-deficient diet, containing 50 IU/kg of vitamin E, cat. no. 116014), purchased from Dyet, Inc., Bethlehem, PA, for at least 4 weeks. Water was given *ad libitum* during this period.

Assay for Glutathione Peroxidase. Mice were sacrificed by cervical dislocation and their livers were removed and carefully blotted dry. Approximately 1 g of tissue was then excised from each liver and perfused in 0.15 *M* KCl for 10 to 20 min. The tissue was homogenized at 4°C in a total of 4 ml 0.25 *M* sucrose and centrifuged for 10 to 20 min at 20,000 rpm. Assays were done on the livers of three healthy mice and three Se-deficient mice.

Assays were done spectrophotometrically by coupling the glutathione peroxidase reaction to the oxidation of NADPH with glutathione reductase:

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

$$GSSG + 2NADPH \rightarrow 2GSH + 2NADP^+$$

Assay cuvettes contained the following ingredients: 2 m*M* reduced glutathione, 0.25 m*M* NADPH, 1 m*M* sodium azide (to inhibit any catalase activity), 1 unit/ml glutathione reductase, and 20–200 μl of the crude liver extract in a total of 2 ml 50 m*M* phosphate buffer, pH 7.0, containing 1.5 m*M* EDTA. The reaction was started with the addition of hydrogen peroxide to a final concentration of 0.25 m*M*. Activity was determined by measuring the decrease in absorbance of NADPH at

340 nm over a 5-min period, using a Model 8450A Hewlett Packard UV/VIS spectrophotometer.

Results

Activity of Peroxidases Against Ehrlich Ascites Tumor. The administration of an immobilized mixture of glucose oxidase and horseradish peroxidase to mice bearing Ehrlich ascites tumor resulted in a complete inhibition of growth of the injected tumor in most animals. The results of a typical experiment demonstrating this activity are shown in Table I. Similar to our earlier results with the rat Novikoff hepatomas, we typically observed inhibition of tumor growth in 60–80% of the experimental animals. No difference in response to the antitumor agents was observed between male and female mice.

The data presented in Table I also indicate clearly that a combination of the two enzymes is required for the antitumor activity; in fact, the antitumor effect observed with only one of the two enzymes was insignificant. We concluded from this observation that the peroxidase expresses its antitumor activity only when glucose oxidase is present, presumably serving as a source for hydrogen peroxide. This in turn could suggest that a product of the peroxidase rather than the enzyme system itself is responsible for the antitumor effect. Further experimental evidence for this possibility was obtained when the experiment was repeated using an inactivated peroxidase (horseradish peroxidase without the heme moiety). This compound was totally inactive as an antitumor agent (Table I).

Effect of Whole Body Irradiation. After establishing that the peroxidase system is active against the Ehrlich ascites tumor, we used this model to investigate whether or not the immune system of the host is involved in the action of the peroxidase system. For this purpose, we first investigated the effects of exposing the experimental animals to whole body γ-irradiation.

Table I. Effect of Co-Immobilized Glucose Oxidase and Horseradish Peroxidase in Ehrlich Ascites Tumor-Bearing Mice

Administered compound(s)	Tumor incidence[a]
Co-immobilized glucose oxidase and horseradish peroxidase	4/10
Glucose oxidase only	8/10
Horseradish peroxidase only	9/10
Co-immobilized glucose oxidase and inactivated peroxidase	8/9
Polymerized albumin carrier only	10/10

[a] Values represent number of animals bearing tumor/total number of animals in experiment. Comparisons of tumor incidence between treatment groups for statistical significance by chi-square analysis revealed the following: GO/HRP vs GO only, $P < 0.10$; GO/HRP vs HRP only, $P < 0.05$; GO/HRP vs GO/HRP$_{inact}$, $P < 0.05$; GO/HRP vs ALB carrier, $P < 0.005$.

Whole body irradiation at the dose used in our experiments (500 rad) destroys about 96% of the animal's stem cells (4), which results in a depletion of virtually all lymphocytes and granulocytes in the host. In our experiments, we inoculated the mice with tumor cells 1 day after they received the γ-radiation and then treated them with the peroxidase system according to our normal protocol. The results, presented in Table II, indicate that the antitumor effect of the peroxidase system became insignificant in the irradiated mice. These results indicate that some endogenous entity that is sensitive to γ-radiation plays an essential part in the antitumor effect of the peroxidase system.

Extreme care was taken to prevent any exposure of the irradiated mice to conditions that might cause infections. In this experiment, none of the 40 mice died before the end of the experiment.

Antitumor Effect of Peroxidases in Selenium-Deficient Mice. In a subsequent experiment, mice were fed a selenium-free diet for at least 4 weeks before they were inoculated with Ehrlich ascites cells. Studies by other investigators have firmly established that the normal functioning of granulocytes and lymphocytes is greatly impaired in selenium-deficient animals (liver glutathione peroxidase <5% of normal). Selenium deficiency leads to an impairment of the normal function of neutrophils (5) and of T cells (6). In some cases of selenium deficiency, the glutathione peroxidase content of neutrophils dropped to undetectable levels (7). Glutathione peroxidase, the enzyme that contains selenium, is also present in macrophages and lymphocytes (8, 9) and it is therefore quite conceivable that the normal functioning of these cells is also impaired in the absence of selenium. Indeed, Robinson *et al.* (9) have recently shown that depletion of reduced glutathione in rats leads to a severe impairment of T cell and macrophage immune function. Spallholz *et al.* (10), however, using specific monoclonal antibodies, reported little or no change in the population of cytotoxic T cells and of T helper cells from thymus as well as from spleen tissue in response to a decrease in dietary Se.

At the time of tumor inoculation, the liver glutathione peroxidase level of our experimental animals

Table II. Anticancer Effect of Immobilized Oxidase-Peroxidase in Mice Exposed to γ Radiation

Administered compound(s)	Tumor incidence[a]
Co-immobilized oxidase-peroxidase	10/10
Glucose oxidase only	10/10
Horseradish peroxidase only	8/10
Polymerized albumin only	10/10

[a] No significant differences ($P > 0.05$) were determined between the various treatment groups by chi-square analysis.

that the animals suffered from a considerable selenium deficiency. After tumor inoculation, the animals were treated with the immobilized peroxidase system in the usual manner. A parallel control experiment was performed at the same time using mice that had received normal chow.

The results, shown in Table III, revealed that the immobilized peroxidase system was totally ineffective as an anticancer agent in the selenium-deficient mice, in contrast to its activity in normal mice. No significant differences ($P > 0.05$) were demonstrated between the groups of selenium-deficient mice administered the various treatments. Significant differences in tumor incidence were found, however, in comparisons of normal mice administered glucose oxidase/peroxidase with those administered peroxidase only ($P < 0.010$), and of the group with glucose oxidase/peroxidase and those with albumin carrier only ($P < 0.005$).

These results add further support to the conclusion reached with the irradiated mice, namely that the antitumor activity of the peroxidase system may require the participation of one or more cell types of the animal's defense system.

Effect of Cyclosporin A. In the next series of experiments, we investigated the antitumor effect of the immobilized peroxidase system in immunosuppressed mice. Immunosuppression was achieved with periodic injections of cyclosporin A. Cyclosporin A was chosen because of its excellent properties in inhibiting tissue rejection following organ transplantations. Cyclosporin apparently prevents the host immune system from recognizing cells that are "non-self" and destroying them. We should point out, however, that mice are much less sensitive to cyclosporin A as an immunosuppressant than rats and other mammals. Thus, doses of 5–25 mg/kg/day have proved to be effective as an immunosuppressant in humans, rats, rabbits, pigs, and dogs, whereas mice require doses of up to 200 mg/kg/day to obtain the same effect (11). We nevertheless decided to cyclosporin A.

Cyclosporin A at 30 mg/kg was administered to the mice on Days 0, 2, 4, and 6 following the inoculation with tumor cells. Otherwise, the same protocol was followed as that described for the irradiated animals. The results, presented in Table IV, revealed that the immobilized peroxidase system completely lost its antitumor activity when the experimental animals were immunosuppressed with cyclosporin A. No significant difference ($P > 0.05$) was found between cyclosporin-treated mice administered oxidase/peroxidase and albumin carrier. Significant differences were found in comparisons of tumor incidence in normal mice administered oxidase/peroxidase versus oxidase only ($P < 0.010$), oxidase/peroxidase versus peroxidase only ($P < 0.010$), and oxidase/peroxidase versus albumin carrier only ($P < 0.005$). No adverse effects were observed that could be attributed to the cyclosporin treatment and none of the animals died before the end of the experiment.

Antitumor Activity of the Peroxidase System in Athymic Mice. In a final set of experiments, we tested the effectiveness of the system against Ehrlich ascites tumor in athymic (*nu/nu*) mice. The protocol was the same as that for normal mice. The results showed that all 10 nude mice in the test group developed tumors to the same extent and as rapidly as untreated normal mice did, indicating that the peroxidase-oxidase system is ineffective as an antitumor agent in nude mice. These results strongly suggest that the presence of some endogenous entity is essential for the peroxidase-oxidase system to express its antitumor activity and that this endogenous entity is lacking in the athymic mice.

Discussion

In an earlier communication, we described the antitumor activity of a mixture of glucose oxidase and a peroxidase against a Novikoff hepatoma in rats, a

Table III. Antitumor Effect of the Immobilized Peroxidase System in Selenium-Deficient Mice

Administered compound(s)	Tumor incidence[a]		*P*
	Normal	Se-deficient	
Co-immobilized oxidase-peroxidase	3/10	9/10	<0.010
Glucose oxidase only	—	9/10	—
Horseradish peroxidase only	9/10	9/10	>0.05
Polymerized albumin only	10/10	9/10	>0.05

[a] Significant differences in tumor incidence between normal and selenium-deficient mice administered the indicated compounds were assessed by chi-square analysis, as shown under *P*.

Table IV. Effect of Immobilized Oxidase-Peroxidase Against Ehrlich Ascites Tumor in Immunosuppressed Mice

Administered compound(s)	Tumor incidence[a]		*P*
	Normal	Cyclosporin treated	
Co-immobilized oxidase-peroxidase	3/10	9/10	<0.010
Glucose oxidase only	9/10	—	—
Horseradish peroxidase only	9/10	—	—
Polymerized albumin only	10/10	9/10	>0.05

[a] Significant differences in tumor incidence between normal and cyclosporin-treated mice administered the indicated compounds were assessed by chi-square analysis, as shown under *P*.

B16 melanoma in mice, and a dimethylbenzanthracene-induced mammary tumor in rats (1), all of which are solid tumors. We now report that this peroxidase system is also effective in inhibiting the growth of the liquid Ehrlich ascites tumor in mice. Thus it appears that this enzyme system can successfully inhibit the growth of several types of tumors *in vivo*; however, there is some variation in the degree of effectiveness from one tumor to another.

In considering possible mechanisms by which the peroxidase system could exert its antitumor effect, the following points are relevant. It is a well-established fact that peroxidases in the presence of hydrogen peroxide and a halide ion exert a potent cytocidal activity. Experiments *in vitro* have indicated that this toxic activity is directed against all kinds of cells, prokaryotic as well as eukaryotic, normal as well as neoplastic (12–15). In other words, the cytocidal activity of the peroxidase-peroxide-halide system is nondiscriminatory *in vitro*; it appears to kill any cell that it is exposed to. Hence, the specificity for tumor cells that the peroxidase system appears to display *in vivo* (1) cannot be explained simply by the cytocidal properties of the peroxidase-peroxide-halide system. Either the specificity of this system is dramatically different *in vivo* than *in vitro*, or the antitumor activity of the system is not due to the cytocidal activity of the triad, but some other mechanism is operating *in vivo*. The latter suggestion includes the possibility that, rather than exerting a direct toxic action toward neoplastic cells *in vivo*, the peroxidase system (or a product of the enzymes) may in some way activate or stimulate the body's defense system against the presence of transformed cells.

The suggestion that peroxidases may be involved in the activation of one or more cell types of the immune system is not unprecedented. Henderson *et al.* (16) and Chi and Henderson (17) have shown that mast cells can be stimulated into histamine release by eosinophil peroxidase in the presence of H_2O_2. This activation of mast cells was observed *in vitro* at peroxidase concentrations of 4–30 milliunits. The authors presented rather convincing evidence indicating that the activation was promoted by the cytotoxic triad, consisting of the peroxidase, H_2O_2, and a halide (chloride) ion.

Activation of macrophages by a peroxidase was first reported by Buchmueller and Mauel (18). These authors showed that small amounts of horseradish peroxidase (6 units/ml) could activate macrophages and stimulate their ability to kill parasites. The activating effect was not observed when the peroxidase inhibitor aminotriazole was added. The authors postulated that the activated macrophages produced abundant amounts of H_2O_2, which together with the peroxidase and chloride formed the cytotoxic triad that in turn killed the parasites.

We recently reported that horseradish peroxidase as well as other peroxidases are able to activate murine peritoneal macrophages to the tumoricidal state *in vitro* (2). The tumoricidal activity was specifically directed toward the tumor cells (3T12 cells); the macrophages did not kill each other unless macrophages from two different strains of mice were mixed together. The experimental results again indicated that the presence of H_2O_2 was required for this activation.

It is of considerable interest that in all cases cited above the peroxidase does not present its activating effects unless H_2O_2 or a source for H_2O_2 is present. This indicates that the activating effects are most likely not promoted by the enzyme itself, but by a reaction product of the enzyme. This conclusion was already advanced by Henderson *et al.* (16) as well as by Buchmueller and Mauel (18), who suggested that the activation of mast cells and macrophages may involve the action of the cytotoxic triad, consisting of the peroxidase, H_2O_2, and a halide ion. The cytotoxic product produced by the triad is a hypohalous acid, e.g., HOCl or HOI (19, 20).

A second possibility is that a normal product of the peroxidase reaction, such as a quinone-type compound or an oxidized sulfhydryl group, is responsible for the activating effect. In considering this possibility, it should be pointed out that radical species are often formed as intermediates in the peroxidase reaction. Thus, the peroxidase-mediated oxidation of substrates such as guaiacol, pyrogallol, hydroxyanisole, etc., leads to the formation of semiquinone-type radicals as intermediary products (21, 22 and references therein). In this context, it might be relevant to remind the reader of some experiments done several years ago by Szent-Gyorgyi and his colleagues. These investigators demonstrated that considerable anticancer activity is displayed by several semiquinone-type radicals when injected into tumor-bearing mice (23). The more stable semiquinone radicals appeared to be more effective as antitumor agents than the less stable radicals. It is thus possible that the peroxidases in the presence of H_2O_2 and some unknown endogenous compound could generate a radical intermediate that in turn exerts the observed antitumor activity. Whether such a link exists between the observations presented here and those of Szent-Gyorgyi's group is presently under investigation.

The results reported here do not allow a firm conclusion as to which cell type of the immune defense system is affected by the peroxidase. The mechanism of action of cyclosporin, however, has been quite firmly established as inhibiting the maturation of T cells to the cytotoxic state (24). The fact that cyclosporin administration inhibits the antitumor activity of the peroxidase system thus suggests that the T lymphocyte (or a product produced by this cell type) may play an important role in the inhibition of the tumor cell

growth. This conclusion is further supported by the results obtained with the nude mice. Both the athymic mice and the cyclosporin-treated mice have fully functional macrophages and mast cells; activation of these cells therefore seems to be irrelevant to the reported observations.

The results presented in this article strongly suggest that, with respect to its antitumor activity, the peroxidase system, rather than acting as a cytotoxic or cytostatic agent, acts at least in part as an activator of the host's immune defense system. This mechanism of action could explain our earlier observations concerning the high specificity of this system for tumor cells and its consequent low toxicity level *in vivo* (1). It also could explain why we were unable to establish a dose response: the higher doses of the enzyme mixture that we were using may have been higher than those needed to achieve a complete activation of the relevant parts of the immune system.

This work was supported in part by a grant from the American Institute for Cancer Research.

1. Everse KE, Lin H, Stuyt EL, Brady JC, Buddingh F, Everse J. Antitumour activity of peroxidases. Br J Cancer **51**:743–746, 1985.
2. Wei R-Q, Lefkowitz SS, Lefkowitz DL, Everse J. Activation of macrophages by peroxidases. Proc Soc Exp Biol Med **182**:515–521, 1986.
3. Kiel JL. The cytotoxic activity of peroxidases. PhD thesis, Texas Tech University Health Science Center, Lubbock, TX p241, 1981.
4. Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res **14**:213–222, 1961.
5. Baker SS, Lerman RH, Krey SH, Crocker KS, Hirsh EF, Cohen H. Selenium deficiency with total parenteral nutrition: Reversal of biochemical and functional abnormalities by selenium supplementation. Am J Clin Nutr **38**:769–774, 1983.
6. Fishbach M, Jenkinson S, Talal N, Lawrence R. Selenium deficient T-cells are unresponsive to antigenic stimulation. Clin Res **32**:232A, 1984.
7. Arthur JR, Boyne R. Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life Sci **36**:1569–1575, 1985.
8. Serfass RE, Ganther HE. Effects of dietary selenium and tocopherol on glutathione peroxidase and superoxide dismutase activities in rat phagocytes. Life Sci **19**:1139–1144, 1976.
9. Robinson MK, Rodrick ML, Jacobs DO, Rounds JD, Collins KH, Saporoschetz IB, Mannick JA, Wilmore DW. Glutathione depletion in rats impairs T-cell and macrophage immune function. Arch Surg **128**:29–35, 1993.
10. Spallholz JE, Boylan LM, Larsen HS. Advances in understanding selenium's role in the immune system. Ann NY Acad Sci **587**:123–139, 1990.
11. Attridge S, Eccles SA, Alexander P. Serum cyclosporin A levels in rats and mice. In: White DJG, Ed. Cyclosporin A. Amsterdam: Elsevier Biomed Press, pp 173–175, 1982.
12. Clark RA, Klebanoff SJ, Einstein AB, Fefer A. Peroxidase-H_2O_2-halide system: Cytotoxic effect on mammalian tumor cells. Blood **45**:161–170, 1975.
13. Clark RA, Klebanoff SJ. Myeloperoxidase-H_2O_2-halide system: Cytotoxic effect on human blood leukocytes. Blood **50**:65–70, 1977.
14. Reiter B. The lactoperoxidase-thiocyanate-hydrogen peroxide antibacterium system. In: Fitzsimons DW, Ed. Oxygen Free Radicals and Tissue Damage. CIBA Found Symp (new series) **65**:285–314, 1978.
15. Roos D, Weening RS. Defects in oxidative killing of microorganisms by phagocytic leukocytes. In: Fitzsimons DW, Ed. Oxygen Free Radicals and Tissue Damage. CIBA Found Symp (new series) **65**:225–262, 1978.
16. Henderson WR, Chi EY, Klebanoff SJ. Eosinophil peroxidase-induced mast cell secretion. J Exp Med **150**:265–279, 1980.
17. Chi EY, Henderson WR. Ultrastructure of mast cell degranulation induced by eosinophil peroxidase. J Histochem Cytochem **32**:337–341, 1980.
18. Buchmueller Y, Mauel J. Studies on the mechanisms of macrophage activation: Possible involvement of oxygen metabolites in killing of *Leishmania enriettii* by activated mouse macrophages. J Reticuloend Soc **29**:181–191, 1981.
19. Harrison JE, Watson BD, Schultz J. Myeloperoxidase and singlet oxygen: A reappraisal. FEBS Lett **92**:327–332, 1978.
20. Bakkenist ARJ, DeBoer JEG, Plat H, Wever R. The halide complexes of myeloperoxidase and the mechanism of the halogenation reaction. Biochim Biophys Acta **613**:337–348, 1980.
21. Job D, Dunford HB. Substituent effect on the oxidation of phenols and aromatic amines by horseradish peroxidase compound I. Eur J Biochem **66**:607–614, 1976.
22. Dunford HB, Adeniran AJ. Hammett $\rho\sigma$ correlation for reactions of horseradish peroxidase compound II with phenols. Arch Biochem Biophys **251**:536–542, 1986.
23. Pethig R, Gascoyne PRC, McLaughlin JA, Szent-Gyorgyi A. Ascorbate-quinone interactions: Electrochemical, free radical, and cytotoxic properties. Proc Natl Acad Sci USA **80**:129–132, 1983.
24. Hess AD, Tutschka PJ, Santos GW. The effect of cyclosporin A on T-lymphocyte subpopulations. In: White DJG, Ed. Cyclosporin A. Amsterdam: Elsevier Biomedical Press, pp209–231, 1982.