Relationship between the Susceptibility of Various Bacteria to Active Oxygen Species and to Intracellular Killing by Macrophages

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(Received 30 September 1986; revised 18 February 1987)

The susceptibilities of six micro-organisms to active oxygen species generated in the xanthine oxidase-mediated bactericidal system were as follows: *Escherichia coli* 81 $\geq$ *Listeria monocytogenes* EGD $\geq$ *Salmonella typhimurium* HKB-1 $\geq$ *Staphylococcus aureus* Smith $\gg$ *Mycobacterium tuberculosis* H₃₇Rv $\simeq$ *Candida albicans* NIH A207 (the last two organisms were essentially resistant to this system). The H₂O₂-Fe-mediated halogenation system exhibited a higher microbicidal activity. When the micro-organisms were compared for their sensitivity to bactericidal activity of resident mouse peritoneal macrophages (M ϕ s), *C. albicans*, *Staph. aureus* and *E. coli* were killed rapidly, whereas *M. tuberculosis*, *L. monocytogenes* and *S. typhimurium* were more resistant. In tests for the ability to trigger an oxidative burst in mouse peritoneal M ϕ s (as measured by chemiluminescence), *Staph. aureus* showed the highest activity followed by the other organisms in the following order: *C. albicans* $>$ *E. coli* $>$ *L. monocytogenes* $\simeq$ *M. tuberculosis*. *S. typhimurium* exhibited no triggering activity. The high susceptibility of *Staph. aureus* and *E. coli* to M ϕ bactericidal activity, and the partial resistance of *L. monocytogenes* and *M. tuberculosis*, correlated with their susceptibility to active oxygen and the H₂O₂-Fe-mediated halogenation reaction.

INTRODUCTION

In the foregoing paper (Yamada *et al.*, 1987), we reported that O₂⁻ and [•]OH play important roles in the expression of antimicrobial activity by the xanthine oxidase-acetaldehyde (XOA)-Fe-EDTA system, which enzymically generates active oxygen species. This antimicrobial system readily killed *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella typhimurium*, but *Candida albicans* and *Mycobacterium tuberculosis* were highly resistant to it. Antimicrobial activity of active oxygen species can be demonstrated directly, but also by H₂O₂-mediated halogenation reactions such as the myeloperoxidase-H₂O₂-halide-mediated bactericidal system in polymorphonuclear leucocytes (PMNs) (Klebanoff, 1968, 1974; Klebanoff & Shepard, 1984; Locksley *et al.*, 1982). Although macrophages (M ϕ s) have no myeloperoxidase (Klebanoff, 1980), iron at physiological concentrations (Flitter *et al.*, 1983; Gutteridge *et al.*, 1981; Halliwell & Gutteridge, 1984) or endogenous catalase can catalyse the halogenation system (Gee *et al.*, 1970; Klebanoff, 1969, 1982a; Klebanoff & Hamon, 1975; Levitz & Diamond, 1984). At the acid pH achieved within the phagolysosome, the iron-mediated halogenation system acts more efficiently and has a more potent antimicrobial activity than at neutral and alkaline pH (Klebanoff, 1982a; Levitz & Diamond, 1984). Therefore, H₂O₂-Fe-mediated halogenation is thought to be important in the microbicidal mechanism of M ϕ s. In this study, we examined the susceptibility of various organisms to the H₂O₂-Fe-mediated halogenation system and to the antimicrobial action of mouse peritoneal M ϕ s.

Abbreviations: i.p., intraperitoneal(ly); M ϕ , macrophage; PEC, peritoneal exudate cells; PMA, phorbol myristate acetate; PMN, polymorphonuclear leucocyte; XOA, xanthine oxidase-acetaldehyde.

METHODS

Animals. Four-week-old female ddY mice were purchased from the Shizuoka Laboratory Animal Center, Shizuoka, Japan. At the time of experiment, they were 6 to 8 weeks old.

Organisms. The test organisms and their sources were as follows: *Escherichia coli* 81 and *Staphylococcus aureus* Smith (A. Matsumae, Kitasato Institute, Tokyo, Japan), *Listeria monocytogenes* EGD (K. Takeya, Kyushu University, Fukuoka, Japan), *Salmonella typhimurium* HKB-1 and *Candida albicans* NIH A207 (M. Kuwabara, Hiroshima Prefectural Hospital, Hiroshima, Japan), bacillus Calmette-Guérin (BCG) (Japan BCG Laboratory, Tokyo). Organisms were cultured as described previously (Yamada *et al.*, 1987).

Chemicals. All reagents were purchased from Wako Pure Chemical.

Microbicidal activity of H_2O_2 -Fe-iodide system. Microbicidal activity of the H_2O_2 -Fe-iodide system (H_2O_2 -Fe-mediated halogenation system) was measured according to the method of Klebanoff (1982a). The reaction mixture (2 ml), consisting of 10 or 100 μ M-sodium iodide and H_2O_2 , various concentrations of ferrous sulphate, 20 mM-acetate buffer (pH 5.5), and test organisms (final concentration 5×10^5 c.f.u. ml⁻¹), was incubated in a shaking water bath at 37 °C for 60 min. The number of c.f.u. of the resultant microbial suspension was then determined on appropriate agar media after serial 10-fold dilutions with 0.15 M-saline (Yamada *et al.*, 1987).

Microbicidal activity of host peritoneal Mφs. Mice were given intraperitoneally (i.p.) viable (0.01 mg per mouse) and heat-killed (0.1 mg per mouse) BCG 21 d and 3 d before microbial challenge, respectively. Normal and BCG-treated mice were infected i.p. with 1×10^6 test organisms suspended in saline. Two hours later, micro-organisms present in the peritoneal cavity were harvested in Hanks' balanced salt solution (HBSS) and sonicated for 20 s with a Handy Sonic model UR-20P (Tomy Seiko Co., power 10) to free and disperse the phagocytosed micro-organisms, and the number of c.f.u. was determined. To determine the virulence of test organisms, mice were injected i.p. with 1×10^6 organisms per mouse and survival of the infected animals was observed for up to 4 weeks.

Measurement of Mφ oxidative burst. This was done by a chemiluminescence method. Mouse peritoneal exudate cells (PEC) were harvested with HBSS 4 d after i.p. injection of 1 mg zymosan A per mouse. The PEC contained more than 85% of Mφs, with 9% contamination by PMNs. The incubation mixture (1 ml), consisting of PEC (10^6 cells in 0.9 ml) and 0.1 ml 0.1 mM-luminol, was measured for background chemiluminescence at 37 °C in a Toyo Kagaku lumiscouter ATP-958. After addition of various concentrations of microbial suspension (0.1 ml), light emissions were counted for 10 s at 2 min intervals for up to 10 min. After the incubation, PEC were smeared on a glass slide, stained with Giemsa stain and the number of Mφ-associated organisms was counted microscopically.

RESULTS

Susceptibility of various micro-organisms to the H_2O_2 -Fe-mediated halogenation system

Table 1 shows the susceptibility of the test organisms to microbicidal activity of H_2O_2 -Fe-iodide-mediated halogenation system. In the presence of 10 μ M each of H_2O_2 and iodide but in the absence of Fe^{2+} , considerable killing of *L. monocytogenes* was noted, whereas the other organisms were not killed. Addition of 10 μ M- Fe^{2+} markedly augmented the microbicidal activity of this system, and killing of the test organisms was observed in the following order: *Staph. aureus* $\simeq$ *E. coli* $\simeq$ *S. typhimurium* $>$ *L. monocytogenes* $>$ *M. tuberculosis*. Although *C. albicans* was most resistant to this system, reductions of two orders of magnitude in the numbers of c.f.u. of the organisms was observed in the presence of equimolar amounts of Fe^{2+} and iodide. In the presence of 100 μ M each of H_2O_2 and iodide, rapid killing of *Staph. aureus*, *E. coli*, *S. typhimurium* and *L. monocytogenes* was noted, even in the absence of Fe^{2+} . In contrast, *M. tuberculosis* and *C. albicans* were killed in the halogenation system with 100 μ M each of H_2O_2 and iodide only when Fe^{2+} was added at concentrations over 10 μ M. In summary, the sensitivity of test micro-organisms to the H_2O_2 -Fe-mediated halogenation system was in the order *S. typhimurium* $\simeq$ *E. coli* $\simeq$ *Staph. aureus* $>$ *L. monocytogenes* $>$ *M. tuberculosis* $>$ *C. albicans*.

Susceptibility of various organisms to the microbicidal activity of Mφs

Table 2 shows the susceptibility of test organisms to mouse peritoneal Mφ-mediated killing *in vivo*. As indicated in experiment 1, *C. albicans*, *Staph. aureus* and *E. coli* were readily eliminated from the peritoneal cavity of the normal mice during the first 2 h after i.p. injection, whereas *S. typhimurium*, *L. monocytogenes* and *M. tuberculosis* were less readily killed. Since resident Mφs were dominant in the PEC populations of the normal mice [nearly 60% of the PEC were Mφs with a very low capacity to produce O_2^- and H_2O_2 in response to phorbol myristate acetate (PMA)], and PMN contamination was low, the elimination of *C. albicans*, *Staph. aureus* and *E.*

Table 1. Microbicidal effect of the H_2O_2 -iodide-Fe-mediated halogenation system (pH 5.5)

The results are means of two determinations $\pm$ the range. A value of 0 indicates that no survivors were detected. The initial values of log c.f.u. were as follows: *Staph. aureus*, 5.80 ± 0.02 ; *E. coli*, 5.74 ± 0 ; *L. monocytogenes*, 5.88 ± 0 ; *S. typhimurium*, 5.88 ± 0.01 ; *M. tuberculosis*, 5.39 ± 0.01 ; *C. albicans*, 5.85 ± 0.02 .

Addition (μ M)			Survival (log c.f.u.)					
H_2O_2	Iodide	Fe^{2+}	<i>Staph. aureus</i>	<i>E. coli</i>	<i>L. monocytogenes</i>	<i>S. typhimurium</i>	<i>M. tuberculosis</i>	<i>C. albicans</i>
0	0	0	5.66 ± 0.01	5.68 ± 0.02	5.89 ± 0.01	5.90 ± 0.01	5.53 ± 0.02	5.85 ± 0.03
10	0	0	5.74 ± 0.02	5.72 ± 0.02	5.88 ± 0.02	5.69 ± 0.04	5.34 ± 0.08	5.85 ± 0
0	10	0	5.29 ± 0.03	5.73 ± 0.01	5.84 ± 0.03	5.86 ± 0.03	5.26 ± 0.06	5.82 ± 0.03
10	10	0	5.48 ± 0.02	5.54 ± 0.09	3.42 ± 0.24	5.34 ± 0.30	5.49 ± 0.09	5.89 ± 0.01
10	10	1	0	0	2.40 ± 0.02	0	2.81 ± 0.14	5.85 ± 0.02
10	10	10	0	0	0	0	0	3.82 ± 0.18
10	10	100	0	0	0.55 ± 0.21	0	3.63 ± 0.07	4.67 ± 0.09
100	0	0	5.74 ± 0.18	5.71 ± 0.01	5.85 ± 0.06	5.74 ± 0.01	5.28 ± 0.08	5.82 ± 0.01
0	100	0	5.51 ± 0.31	5.69 ± 0.02	5.88 ± 0.03	5.87 ± 0.02	5.55 ± 0.03	5.78 ± 0.02
100	100	0	0	0	2.02 ± 0.17	0	4.52 ± 0.11	5.81 ± 0.06
100	100	1	0	0	0	0	0.43 ± 0.09	5.36 ± 0.08
100	100	10	0	0	0	0	0	0
100	100	100	0	0	0	0	0	0

Table 2. Susceptibility of the test organisms to microbicidal activity of $M\phi$

Mice were given i.p. 1×10^6 each of the indicated organisms, and the number of viable organisms in the peritoneal cavity was measured 2 h later. The number of organisms at 0 h is fixed at 100%. The results are means $\pm$ standard error ($n = 3$).

	Survival (%)					
	<i>Staph. aureus</i>	<i>E. coli</i>	<i>L. monocytogenes</i>	<i>S. typhimurium</i>	<i>M. tuberculosis</i>	<i>C. albicans</i>
Expt 1: normal mice (resident $M\phi$)	9.3 ± 0.2	10.5 ± 0.9	81.3 ± 23.3	55.0 ± 5.2	93.7 ± 6.0	4.2 ± 0.1
Expt 2: BCG-treated mice (activated $M\phi$)*	8.6 ± 1.0	1.5 ± 0.3	2.8 ± 1.4	72.1 ± 34.9	28.0 ± 3.5	0.3 ± 0.1

* Mice were injected i.p. with 0.01 and 0.1 mg viable and heat-killed BCG per mouse 21 and 3 d before bacterial challenge, respectively.

coli from the peritoneal cavity is thought to be attributable to resident $M\phi$ s. Experiment 1 (Table 2) shows that the susceptibility of the test organisms to the microbicidal action of resident $M\phi$ s is in the following order: *C. albicans* $\approx$ *Staph. aureus* $\approx$ *E. coli* $>$ *S. typhimurium* $\geq$ *L. monocytogenes* $\approx$ *M. tuberculosis*. In experiment 2 (Table 2), the resident $M\phi$ s in the peritoneal cavity were immunologically activated by double injections of viable and heat-killed BCG at 21 d and 3 d before microbial challenge, respectively [in this case, the PEC were mainly composed of $M\phi$ s (75%) and other cells such as lymphocytes; the resultant $M\phi$ s showed a high PMA response, releasing $430 \text{ nm-H}_2\text{O}_2$ in response to $10 \text{ ng PMA ml}^{-1}$ per 10^6 cells; unpublished data]. In this case, *C. albicans*, *E. coli* and *L. monocytogenes* were readily eliminated from the peritoneal cavity. Although *M. tuberculosis* was also more rapidly killed by activated $M\phi$ s than by resident $M\phi$ s, the organisms was still relatively resistant. On the other hand, *S. typhimurium* was highly resistant to both the resident and the activated $M\phi$ s. The high resistance of *S. typhimurium* HKB-1 to the microbicidal activity of host $M\phi$ s was consistent with its high virulence in mice: 1×10^6 organisms given i.p. to mice killed 90% of the host animals within 1 week, while the same number of the other micro-organisms killed only 20% or less of mice within 4 weeks.

Fig. 1. Chemiluminescence-triggering ability of *Staph. aureus* (a), *E. coli* (b), *L. monocytogenes* (c), *S. typhimurium* (d), *M. tuberculosis* (e) and *C. albicans* (f). Reaction mixture (1 ml), consisting of 1×10^6 zymosan A-induced M ϕ s ml $^{-1}$, 0.1 mM-luminol and various concentrations of microbial suspension (m.o.i. 40, solid line; m.o.i. 20, broken line), was measured for chemiluminescence at 37 °C.

Bacterial activation of the M ϕ oxidative burst

Fig. 1 shows chemiluminescence of zymosan A-induced M ϕ s in contact with test organisms at m.o.i. values of 20 and 40. The test organisms were examined for their ability to trigger an oxidative burst by zymosan A-induced mouse PEC (composed of 88% M ϕ s, with low PMN contamination). Over the first 10 min, *Staph. aureus* triggered the highest chemiluminescence, followed by the other micro-organisms in the following order; *Staph. aureus* $\geq$ *C. albicans* > *E. coli* > *L. monocytogenes* $\approx$ *M. tuberculosis*. *S. typhimurium* failed to trigger chemiluminescence (Fig. 1d). Three groups of test organisms could be recognized, according to the light emission patterns of M ϕ s induced by contact with organisms: (1) chemiluminescence continued to increase during the course of measurement for up to 12 min (*Staph. aureus* and *E. coli*); (2) chemiluminescence reached a maximum in the early phase and declined thereafter (*C. albicans*); (3) chemiluminescence reached a plateau in the early phase (*L. monocytogenes*, *M. tuberculosis* and *S. typhimurium*). Patterns (2) and (3) suggest the release from the test organisms of inhibitory factors against M ϕ chemiluminescence.

S. typhimurium, which lacked M ϕ -chemiluminescence-triggering activity, was associated with M ϕ cells to the same extent as the other organisms (data not shown), indicating that deficiency in the chemiluminescence-triggering ability of the organisms is not due to a lack of ability to contact the M ϕ cell surface.

DISCUSSION

Although it is reported that M ϕ s have no myeloperoxidase (Klebanoff, 1980), there is evidence that the halogenation reaction is important for the bactericidal activity of these cells (Lehrer, 1975). There are at least three candidates for the mediator of the H₂O₂-dependent halogenation reaction in M ϕ s: first, myeloperoxidase molecules produced by PMN and taken up by M ϕ s (Hanker & Giammara, 1983); second, catalase, which exhibits peroxidase activity rather than H₂O₂-decomposing activity when H₂O₂ is maintained at a very low steady-state concentration, particularly at acid pH (Gee *et al.*, 1970; Klebanoff, 1969; Klebanoff & Hamon, 1975); third, iron ions in phago(lyso)somes of phagocytic cells which are at a sufficient concentration under physiological conditions to support the halogenation reaction, as described here (about 5 μ M; Flitter *et al.*, 1983; Gutteridge *et al.*, 1981; Halliwell & Gutteridge, 1984). Also, the H₂O₂-Fe-mediated halogenation reaction is thought to be important for mechanisms related to bacterial killing by phagocytes. When the microbicidal efficacy of the active oxygen-generating system (XOA-Fe-EDTA system), representing direct toxic effects of active oxygen species, is compared with the H₂O₂-Fe-mediated halogenation system, the latter seems to be more important for intracellular killing, particularly of *Mycobacterium* and *Candida*.

Because M ϕ s are dominant in the peritoneal cell populations in normal and in BCG-treated mice, the present results reflect essentially the susceptibility of test organisms to resident and activated M ϕ s. *S. typhimurium* and *M. tuberculosis*, and also *L. monocytogenes*, all of which are intracellular parasites, were highly or markedly resistant to M ϕ -mediated killing. In contrast, *C. albicans*, which is highly resistant to the XOA-Fe-EDTA system was rapidly killed by host M ϕ s. Also *C. albicans* was more resistant to the H₂O₂-Fe-mediated halogenation system than were the other microbes. Thus, the mechanisms of intracellular killing of *C. albicans* may involve other factors, such as cationic proteins, unsaturated fatty acids and lysosomal hydrolases (Martinez & Carroll, 1980; Miller, 1969) rather than oxygen-dependent mechanisms, as proposed by Lehrer (1972, 1975). Our group (Saito *et al.*, 1984) and Kanai & Kondo (1979) have suggested the importance of unsaturated free fatty acids (arachidonic, oleic, palmitoleic, etc.) in the intraphagolysosomal killing of mycobacteria, which are resistant to active oxygen species (Yamada *et al.*, 1987) and show considerable resistance to H₂O₂-Fe-mediated halogenation (Table 1). A similar proposal was made earlier by Hemsworth & Kochan (1978).

In the presence of luminol, chemiluminescence originates from $\cdot$ OH and 1 O₂ rather than from O $_2^-$ and H₂O₂ (Babior, 1978; Brummer *et al.*, 1985; Johnston, 1978), and thus we can recognize more complex intracellular events in M ϕ s after the onset of the respiratory burst by the chemiluminescence assay than by measurement of O $_2^-$ and H₂O₂ generation. In fact, we previously found that in infections due to *Mycobacterium intracellulare* or *L. monocytogenes*, the responsiveness of host M ϕ s to PMA in the form of the oxidative burst (which correlates well with the extent of M ϕ activation: Nathan & Root, 1977; Tomioka & Saito, 1980) was better represented by chemiluminescence than by H₂O₂ release (Saito *et al.*, 1986). In addition, $\cdot$ OH, which is detectable by the chemiluminescence assay, is very important for the expression of direct microbicidal activity of active oxygen species (Yamada *et al.*, 1987) and also for halogenation reaction system (Klebanoff, 1982*b*). Therefore, the chemiluminescence assay is a more reliable indicator of the O₂-dependent microbicidal function of a given M ϕ cell than the measurement of O $_2^-$ and H₂O₂ generation.

All the intracellular parasites tested here, except *C. albicans*, were less active than the extracellular parasites in triggering a M ϕ oxidative burst. This finding is consistent with the observations by other investigators that virulent species (or strains) of *Candida* (Sasada & Johnston, 1980), *Salmonella typhi* (Kossack *et al.*, 1981; Miller *et al.*, 1972) and *M. intracellulare* (Gangadharan & Edwards, 1984) trigger a reduced respiratory burst in M ϕ s and PMNs. In this study, *S. typhimurium* HKB-1 showed a high resistance to M ϕ -mediated bacterial killing, although it was highly sensitive to the XOA-Fe-EDTA system (Yamada *et al.*, 1987) and to the H₂O₂-Fe-mediated halogenation system (Table 1). The inability of this microbe to trigger a M ϕ oxidative burst (Fig. 1) may partly explain this enigmatic status. Alternatively, it is possible that this microbe inhibits phagolysosomal fusion of M ϕ s after phagocytosis as in the case of *M. tuberculosis* (Lowrie, 1983).

REFERENCES

- BABIOR, B. M. (1978). Oxygen-dependent microbial killing by phagocytes. *New England Journal of Medicine* **298**, 659-668.
- BRUMMER, E., SUGAR, A. M. & STEVENS, D. A. (1985). Enhanced oxidative burst in immunologically activated but not elicited polymorphonuclear leukocytes correlates with fungicidal activity. *Infection and Immunity* **49**, 396-401.
- FLITTER, W., ROWLEY, D. A. & HALLIWELL, B. (1983). Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts; what is the physiological iron chelator? *FEBS Letters* **158**, 310-312.
- GANGADHARAM, P. R. J. & EDWARDS, C. K., III (1984). Release of superoxide anion from resident and activated mouse peritoneal macrophages infected with *Mycobacterium intracellulare*. *American Review of Respiratory Diseases* **130**, 834-838.
- GEE, J. B. L., VASSALLO, C. L., BELL, P., KASKIN, J., BASFORD, R. E. & FIELD, J. B. (1970). Catalase-dependent peroxidative metabolism in the alveolar macrophage during phagocytosis. *Journal of Clinical Investigation* **49**, 1280-1287.
- GUTTERIDGE, J. M. C., ROWLEY, D. A. & HALLIWELL, B. (1981). Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts; detection of 'free' iron in biological systems by using bleomycin-dependent degradation of DNA. *Biochemical Journal* **99**, 263-265.
- HALLIWELL, B. & GUTTERIDGE, J. M. C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal* **219**, 1-14.
- HANKER, J. S. & GIAMMARA, B. L. (1983). Neutrophil pseudoplatelets: their discrimination by myeloperoxidase demonstration. *Science* **220**, 415-417.
- HEMSWORTH, G. R. & KOCHAN, I. (1978). Secretion of anti-mycobacterial fatty acids by normal and activated macrophages. *Infection and Immunity* **19**, 170-177.
- JOHNSTON, R. B., JR (1978). Oxygen metabolism and the microbicidal activity of macrophages. *Federation Proceedings* **37**, 2759-2764.
- KANAI, K. & KONDO, E. (1979). Antibacterial and cytotoxic aspects of long-chain fatty acids as cell surface events: selected topics. *Japanese Journal of Medical Science and Biology* **32**, 135-174.
- KLEBANOFF, S. J. (1968). Myeloperoxidase-halide-hydrogen peroxide antibacterial system. *Journal of Bacteriology* **95**, 2131-2138.
- KLEBANOFF, S. J. (1969). Antimicrobial activity of catalase at acid pH. *Proceedings of the Society for Experimental Biology and Medicine* **132**, 571-574.
- KLEBANOFF, S. J. (1974). Role of the superoxide anion in the myeloperoxidase-mediated antimicrobial system. *Journal of Biological Chemistry* **249**, 3724-3728.
- KLEBANOFF, S. J. (1980). Oxygen metabolism and the toxic properties of phagocytes. *Annals of Internal Medicine* **93**, 480-489.
- KLEBANOFF, S. J. (1982a). The iron- H_2O_2 -iodide cytotoxic system. *Journal of Experimental Medicine* **156**, 1262-1267.
- KLEBANOFF, S. J. (1982b). Iodination catalyzed by the xanthine oxidase system: Role of hydroxyl radicals. *Biochemistry* **21**, 4110-4116.
- KLEBANOFF, S. J. & HAMON, C. B. (1975). Antimicrobial systems of mononuclear phagocytes. In *Mononuclear Phagocytes in Immunity, Infection and Pathology*, pp. 507-529. Edited by R. van Furth. Oxford: Blackwell Scientific Publications.
- KLEBANOFF, S. J. & SHEPARD, C. C. (1984). Toxic effect of the peroxidase-hydrogen peroxide-halide antimicrobial system on *Mycobacterium leprae*. *Infection and Immunity* **44**, 534-536.
- KOSSACK, R. E., GUERRANT, R. L., DENSEN, P., SCHADELIN, J. & MANDELL, G. L. (1981). Diminished neutrophil oxidative metabolism after phagocytosis of virulent *Salmonella typhi*. *Infection and Immunity* **31**, 674-678.
- LEHRER, R. I. (1972). Functional aspects of a second mechanism of candidacidal activity by human neutrophils. *Journal of Clinical Investigation* **51**, 2566-2572.
- LEHRER, R. I. (1975). The fungicidal mechanisms of human monocytes. I. Evidence for myeloperoxidase-linked and myeloperoxidase-independent candidacidal mechanisms. *Journal of Clinical Investigation* **55**, 338-346.
- LEVITZ, S. & DIAMOND, R. D. (1984). Killing of *Aspergillus fumigatus* spores and *Candida albicans* yeast phase by the iron-hydrogen peroxide-iodide cytotoxic system: comparison with the myeloperoxidase-hydrogen peroxide-halide system. *Infection and Immunity* **43**, 1100-1102.
- LOCKSLEY, R. M., JACOBS, R. F., WILSON, C. B., WEAVER, W. M. & KLEBANOFF, S. J. (1982). Susceptibility of *Legionella pneumophila* to oxygen-dependent microbicidal system. *Journal of Immunology* **129**, 2192-2197.
- LOWRIE, D. B. (1983). How macrophages kill tubercle bacilli. *Journal of Medical Microbiology* **16**, 1-12.
- MARTINEZ, R. J. & CARROLL, S. F. (1980). Sequential metabolic expressions of the lethal process in human serum-treated *Escherichia coli*; role of lysozyme. *Infection and Immunity* **28**, 735-745.
- MILLER, T. E. (1969). Killing and lysis of Gram-negative bacteria through the synergistic effect of hydrogen peroxide, ascorbic acid, and lysozyme. *Journal of Bacteriology* **98**, 949-955.
- MILLER, R. M., GARBUS, J. & HORNICK, R. B. (1972). Lack of enhanced oxygen consumption by polymorphonuclear leukocytes on phagocytosis of virulent *Salmonella typhi*. *Science* **175**, 1010-1011.
- NATHAN, C. F. & ROOT, R. K. (1977). Hydrogen peroxide release from mouse peritoneal macrophages: dependence on sequential activation and triggering. *Journal of Experimental Medicine* **146**, 1648-1662.
- SAITO, H., TOMIOKA, H. & YONEYAMA, T. (1984). Growth of group IV mycobacteria on medium containing various saturated and unsaturated fatty acids. *Antimicrobial Agents and Chemotherapy* **26**, 164-169.
- SAITO, H., TOMIOKA, H., YAMADA, Y. & JIDOI, J. (1986). Oxidative and phagocytic functions of macrophages during infections induced in mice by *Mycobacterium intracellulare* and *Listeria monocytogenes*. *Journal of General Microbiology* **132**, 1117-1125.
- SASADA, M. & JOHNSTON, R. B., JR (1980). Macrophage microbicidal activity: correlation between phagocytosis-associated oxidative metabolism and the

- killing of *Candida* by macrophages. *Journal of Experimental Medicine* **152**, 85–98.
- TOMIOKA, H. & SAITO, H. (1980). Hydrogen peroxide-releasing function of chemically elicited and immunologically activated macrophages: differential response to wheat germ lectin and concanavalin A. *Infection and Immunity* **29**, 469–476.
- YAMADA, Y., SAITO, H., TOMIOKA, H. & JIDOI, J. (1987). Susceptibility of micro-organisms to active oxygen species: sensitivity to the xanthine-oxidase-mediated antimicrobial system. *Journal of General Microbiology* **133**, 2007–2014.