Journal of Cellular Biochemistry 22:173–185 (1983)
Molecular Biology of Host–Parasite Interactions 17–29

Oxygen-Dependent Microbicidal Systems of Phagocytes and Host Defense Against Intracellular Protozoa

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The role of oxygen-dependent microbicidal systems of leukocytes in the host defense against the major nonerythrocytic intracellular protozoa which infect man—Toxoplasma gondii, Trypanosoma cruzi, and the Leishmania species—is reviewed. The hydrogen peroxide-halide-peroxidase microbicidal system is uniformly cidal to these organisms in vitro. Peroxidase-independent oxygen product(s) toxicity is more variable. Studies to date indicate that phagocytes which contain granule peroxidase and which have the capacity to generate a vigorous respiratory burst; eg, neutrophils and monocytes, possess substantial activity against these protozoa. The absence of granule peroxidase together with the markedly attenuated respiratory burst of resident macrophages leaves these cells with a severe microbicidal defect. These protozoa can enter resident macrophages in the absence of antibody and survive and replicate within the intracellular environment. Enhancement of the antiparasite activity of resident macrophages can be accomplished either by activation of these cells by exposure to sensitized T-cell products, or by the introduction of exogenous peroxidase into the vacuole. Other factors influencing the ability of protozoa to survive intracellularly include the capacity of these organisms to avoid effective triggering of the macrophage respiratory burst and the levels of endogenous scavengers of oxygen products within the parasite.

Key words: Toxoplasma gondii, Leishmania, Trypanosoma cruzi, peroxidase, phagocytes, protozoa, respiratory burst, myeloperoxidase

The principal host defense against the major nonerythrocytic intracellular protozoa which infect man—Toxoplasma gondii, Trypanosoma cruzi, and the Leishmania species—is the development of cell-mediated immunity. Thus, through a complex series of signals, including recognition of antigen and Ia (or Ia-like antigens) on macrophages and the subsequent interaction with interleukins, interferons, and other lymphokines, T-lymphocytes confer upon macrophages the ability to restrict intracellular replication and, in some cases, to kill these parasites. The reasons why tissue macrophages permit the establishment and unrestricted intracellular growth of these pathogens, and why exposure to sensitized T-lymphocytes or their products confers

Received February 15, 1983; revised and accepted July 25, 1983.

upon the macrophages the ability to control these organisms, have been only recently explored. This review will summarize data from the recent literature and from this laboratory that have implicated oxgyen-dependent microbicidal systems of phagocytes as an important effector mechanism against intracellular protozoa.

OXYGEN-DEPENDENT MICROBICIDAL SYSTEMS OF PHAGOCYTES

Perturbation of the membrane of phagocytes with particulate or soluble stimuli results in a rapid increase in oxygen consumption by the cell termed the respiratory burst. Evidence suggests that a membrane associated, cyanide-insensitive flavoprotein oxidase(s), which is dormant at rest, is activated by stimulation to reduce molecular oxygen, which in turn is linked via an incompletely understood transmembrane electron transport system to the oxidation of an intracellular reduced pyridine nucleotide, NAD(P)H (for review see [1–3]). Presumably the reduction of oxygen occurs initially at the site of membrane stimulation in the extracellular fluid and subsequently, in the case of an ingestable particle (with invagination of the plasma membrane), within the phagosome. An important role for the respiratory burst in the microbicidal activity of phagocytes was suggested by the occurrence of severe, repetitive infections in patients whose neutrophils and monocytes have major deficiencies in oxygen consumption. The etiologies of these respiratory burst defects may differ, as in patients with chronic granulomatous disease or severe leukocyte glucose-6-phosphate-dehydrogenase deficiency, but in each the impaired respiratory burst is associated with a pronounced leukocyte microbicidal defect. Extensive studies of the in vitro microbicidal capacity of these patients' cells and of normal phagocytes under hypoxic conditions have corroborated the clinical experience and confirmed the importance of an intact respiratory burst for optimal microbicidal activity.

In polymorphonuclear leukocytes (PMN), almost all of the increased oxygen consumed during phagocytosis results in the formation of the one-electron reduction product, superoxide anion (O_2^-) [4]. In monocytes, over half of the total oxygen consumed is due to the increased mitochondrial respiration which accompanies phagocytosis; almost all of the remainder, as in PMN, can be recovered as products of the respiratory burst [5].

Hydrogen peroxide, the two-electron reduction product of oxygen, may be formed from O_2^- by dismutation:

$$O_2^- + HO_2^\bullet + H^+ \rightarrow\!\!\!\!\rightarrow H_2O_2 + O_2$$

Dismutation may be either spontaneous or catalyzed by a broad class of metalloproteins designated superoxide dismutases. Although the spontaneous dismutation of O_2^- is relatively slow at neutral pH ($4.5 \times 10^5\ M^{-1}sec^{-1}$), it is significantly more rapid ($8.5 \times 10^7\ M^{-1}sec^{-1}$) [6] at the acid pH presumed to exist in the phagosome [7], where a significant portion of the anion would exist as the perhydroxyl radical ($HO_2^\bullet$). In PMN over 80% of the O_2^- formed during the respiratory burst undergoes dismutation to H_2O_2 [4]. Whereas H_2O_2 itself requires fairly high concentrations to function as a toxic oxidant, there exist two known mechanisms by which its toxicity may be amplified. The first is the ability of peroxidases to catalyze the oxidation and halogenation of target substances by H_2O_2 and a halide [8]. The second is the metal-catalyzed interaction of H_2O_2 and O_2^- to form highly reactive radicals of oxygen, such

as hydroxyl radical ($OH^{\cdot}$) and possibly, singlet molecular oxygen ($^1O_2$) (for review see [2]).

Peroxidase-Catalyzed Systems

Neutrophils and monocytes contain in primary granules the hemeprotein myeloperoxidase (MPO); eosinophils contain a similar but distinct peroxidase (EPO) in the matrix of large cytoplasmic granules surrounding the central crystalloid body. Phagocytosis by these cells is accompanied by the discharge into the phagosome of MPO (or EPO) where, in the presence of H_2O_2 (generated in the respiratory burst) and a halide, a potent microbicidal system is constituted [8]. Either chloride, iodide, or bromide can meet the halide requirement for MPO; of these, chloride is present in phagocytes at concentrations greater than those required for microbicidal activity. A thorough discussion of the halide requirement is beyond the scope of this review and is detailed elsewhere [9]. Eosinophil peroxidase is somewhat less effective with chloride as compared to iodide or bromide than is MPO [10]. The toxicity of the MPO (EPO)-H_2O_2-halide system involves both halogenation and oxidation of the target cell surface [11]. It is extremely potent against a wide variety of microorganisms and to both normal and malignant cells. The concentration of H_2O_2 necessary for microbicidal activity is typically reduced over 100-fold by the addition of MPO and chloride to in vitro antibacterial systems [12].

Hydroxyl Radical

A second mechanism by which H_2O_2-mediated toxicity might be augmented is the formation of highly reactive radicals, such as hydroxyl radical, and possibly singlet molecular oxygen (reviewed in [2]). The first part of the reaction involves the generation of $OH^{\cdot}$ by the oxidation of ferrous iron (Fe^{2+}) by H_2O_2 (Fenton's reagent). The limiting reagent, Fe^{2+}, is subsequently generated by the interaction of O_2^- with Fe^{3+}, and the complete reaction has been termed the metal-catalyzed Haber-Weiss reaction:

$$\begin{array}{c} H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^{\cdot} \\ O_2^- + Fe^{3+} \rightarrow Fe^{2+} + O_2 \\ \hline H_2O_2 + O_2^- \rightarrow O_2 + OH^- + OH^{\cdot} \end{array}$$

Evidence for $OH^{\cdot}$ production by phagocytes [13–18] and by enzyme systems which generate both H_2O_2 and O_2^-, ie, xanthine-xanthine oxidase or acetaldehyde-xanthine oxidase [12,19], has been reported. In general, involvement of $OH^{\cdot}$ in microbicidal activity has been proposed based on inhibition of toxicity by scavengers of O_2^- (superoxide dismutase), H_2O_2 (catalase), and $OH^{\cdot}$ (mannitol or ethanol).

Evidence for $^1O_2$ production by the Haber-Weiss reaction both by enzyme systems which generate both H_2O_2 and O_2^- and by stimulated phagocytes has also been sought based in general on production of characteristic chemiluminescence, product analysis (for $^1O_2$-dependent products), stimulation by D_2O, and inhibition by $^1O_2$ quenchers such as diazabicyclooctane (DABCO) or histidine (reviewed in [2]). The nonspecificity of the quenching agents and the problems of detecting a specific singlet oxygen product leaves the role of $^1O_2$ in phagocyte cidal activity less firmly established.

TOXICITY OF OXYGEN-DEPENDENT MICROBICIDAL SYSTEMS AGAINST PROTOZOA IN CELL-FREE SYSTEMS

Superoxide Anion

There is no evidence that O_2^- alone is protozoacidal. The radical is relatively slow reacting with most biologic compounds under physiologic conditions. Further, strains of T gondii [20], T cruzi [21], L donovani [22], and L tropica [22,23] contain endogenous superoxide dismutase which may be protective.

Hydrogen Peroxide

The two-electron reduction product of oxygen, H_2O_2, is a stable, diffusable oxidant which is important in the extracellular toxicity of macrophages against tumor cells [24] and helminths [25] under certain in vitro conditions. In cell-free systems employing H_2O_2 or enzyme systems which generate H_2O_2 but not O_2^- (glucose plus glucose oxidase), these protozoa display significant variability in their susceptibility. T gondii (tachyzoites) are quite resistant to H_2O_2; fluxes of 10 nmol H_2O_2/min are nontoxic [26]. Using both a fluorescent assay system with acridine orange and ethidium bromide and ^{3}H-2-deoxy-D-glucose uptake to measure parasite viability [27], we have measured an LD_{50} of 2×10^{-3}M H_2O_2 for the virulent RH strain of T gondii, a value in agreement with that reported by Murray and Cohn [26]. T cruzi trypomastigotes and epimastigotes were susceptible (LD_{50}) to fluxes of H_2O_2 of 9.4 and 6.5 nmol/min, respectively, corresponding to concentrations of 5.6×10^{-4} M and 3.9×10^{-4} M H_2O_2 [28]. Under different conditions, and using glucose oxidase covalently bound to zymosan particles rather than free in the media, Tanaka et al calculated that the H_2O_2 flux required to kill a single epimastigote was as low as 8.7×10^{-7} nmol/min [29]. In contrast, the related bat trypanosome, T dionisii, is resistant to concentrations of H_2O_2 of 10^{-3} M [30]. The amastigote form of the Leishmania species, which is the form which resides intracellularly within macrophages, the sole cell type infected, is consistently more resistant to H_2O_2 than the promastigote, the hemoflagellate form which initiates infection. Further, L donovani, the agent of visceral leishmaniasis, is more resistant to H_2O_2 than L tropica, the agent of Old World cutaneous leishmaniasis. For L donovani promastigotes, LD_{50}s of 1.8 nmol/min [22] and 4×10^{-5} M H_2O_2 [31] have been reported, and for amastigotes, 8.1 nmol/min and 10^{-4} M, respectively. Values for L tropica promastigotes of 0.5 nmol/min [22] and 2.5×10^{-5} M H_2O_2 [RM Locksley, unpublished observations], and amastigotes of 8×10^{-5} M H_2O_2 [RM Locksley, unpublished observations] have been obtained.

Although these reports indicate susceptibility of these protozoa to H_2O_2, it should be noted that under the assay conditions employed, organisms were exposed to H_2O_2 for periods ranging from 30 to 60 min. Whether such prolonged exposure to H_2O_2 in these concentrations occurs in the phagosome is unknown. Possible strain variations in susceptibility to H_2O_2 have not been investigated.

Peroxidase-H_2O_2-halide

T gondii [26,27], L donovani [31–33], and L tropica amastigotes [RM Locksley, CA Nacy; unpublished observations] and promastigotes [22,31,34], L braziliensis promastigotes [35] and T cruzi trypomastigotes [35,36] are highly susceptible to the peroxidase-H_2O_2-halide microbicidal system. In most instances iodide has been used

as the halide and a variety of peroxidases, including horseradish peroxidase, lactoperoxidase, purified human or canine MPO, purified equine EPO, and human neutrophil granules and sonicated neutrophil supernatants have been used. When it has been studied, either bromide or chloride could fulfill the halide requirement with MPO or EPO, although chloride is less effective when EPO is used [10]. Deletion of any of the three components, or the addition of catalase or hemeprotein inhibitors such as azide or aminotriazole effectively blocked the protozoacidal activity of the system, thus implicating a peroxidatic mechanism. It is noteworthy that the microbicidal levels of H_2O_2 against these organisms under a wide variety of assay conditions were reduced from five- to over 100-fold in the presence of peroxidase and a halide, emphasizing the potent augmentation of H_2O_2 toxicity by peroxidases. The report that trypomastigotes of T dionisii, the bat trypanosome, are resistant to MPO and halide plus 10^{-3} M H_2O_2, in contrast to the susceptibility of the epimastigotes, is unique [30].

Hydroxyl Radical

Using the xanthine plus xanthine oxidase generating system, Murray and Cohn reported that toxicity to T gondii was abolished by either superoxide dismutase, catalase, mannitol, benzoate, DABCO, or histidine [26]. Although mannitol and benzoate, and DABCO and histidine, are somewhat nonspecific scavengers of hydroxyl radicals and singlet oxygen, respectively, the requirement for both O_2^- (as indicated by inhibition with superoxide dismutase) and H_2O_2 (as indicated by inhibition with catalase) incriminated the more distal reactive oxygen products. Similar experiments with Leishmania species were unable to attribute toxicity to products other than H_2O_2 [22]. Other investigators have found some inhibition of xanthine plus xanthine oxidase mediated toxicity to L donovani by both catalase (50% inhibition) and superoxide dismutase (15% inhibition) [31], and to L braziliensis by superoxide dismutase (87% inhibition) [37] suggesting that some of the leishmanicidal activity might be due to hydroxyl radical. The extreme reactivity of these radicals requires that care be taken with the assay conditions (buffers, pH, etc) to avoid unintentional scavenging of radicals before they can interact with their intended targets.

In summary, the collected data from in vitro exposure of the protozoa to various products of the phagocyte respiratory burst suggest parasite susceptibility. The peroxidase-H_2O_2-halide system is almost uniformly cidal in vitro; peroxidase-independent oxygen-mediated toxicity is more variable. Resident macrophages, which are incapable of killing these protozoa and support their protected replication, lack granule peroxidase [2]. All of these organisms enter or are ingested by macrophages in the absence of antibody. Each interacts in a unique fashion within the host macrophage: Leishmania species thrive within phagolysosomes [38], T gondii inhibits phagolysosomal fusion [39], and T cruzi escapes from the phagosome shortly after ingestion to replicate undisturbed in the cytosol [40]. Leishmania parasitize only macrophages, whereas T gondii and T cruzi subsequently infect cells of different tissues. While much remains to be learned, identified factors which influence the outcome of parasite-phagocyte interactions in vitro include: 1) the presence or absence in the phagocyte of granule peroxidase; 2) the magnitude of the stimulated respiratory burst by the phagocyte; 3) the capacity of the phagocyte to respond to parasite ingestion with an effective respiratory burst; and 4) the levels of endogenous oxygen-product scavengers in the organism. Each of these will be considered in turn.

TOXICITY OF OXYGEN-DEPENDENT MICROBICIDAL SYSTEMS AGAINST PROTOZOA IN PHAGOCYTES

Granule peroxidase. Evidence supporting a role for granule peroxidase in phagocyte protozoacidal activity has been derived 1) from studies correlating the capacity of different phagocytes to kill protozoa with their granule MPO content, 2) from studies with cells from patients with MPO deficiency, and 3) from studies in which exogenous peroxidase is supplied to peroxidase-negative cells in an attempt to boost their cidal capacity.

Neutrophils, in which MPO comprises up to 5% of the dry weight of the cell, effectively ingest and kill T gondii [41], L donovani promastigotes [34] and amastigotes [32], T cruzi trypomastigotes [42], and T dionisii epimastigotes and trypomastigotes [43,44]. Antibody facilitates phagocytosis of T cruzi [42,45] and T dionisii [43], and may be important in extracellular killing of T cruzi by PMN [46,47]. MPO inhibitors (azide, cyanide, diethyldithiocarbamate) abolished the toxicity of PMN to L donovani [48] and T dionisii [30,44], supporting a role for MPO in mediating cidal activity. Electron microscopy demonstrated the release of granule material into the phagosome following ingestion of T cruzi trypomastigotes by rat neutrophils and eosinophils [42]. Histochemical evidence of MPO and EPO in the phagosome has been demonstrated for human PMN and eosinophils ingesting L donovani amastigotes [32], and for murine PMN [RM Locksley, unpublished observations] and eosinophils [49] ingesting T gondii. Presumably, H_2O_2 formed in the phagosome during the respiratory burst would be available with halide(s) in the media to complete the microbicidal system. Limited studies have demonstrated O_2^- production by human PMN ingesting L donovani promastigotes [34] and T dionisii [30].

Freshly isolated human blood monocytes contain approximately one-third of the MPO as neutrophils [50] and undergo a less vigorous respiratory burst as measured by oxygen consumption and generation of O_2^- and H_2O_2 [5]. When care has been taken to examine these cells within 2 hr of isolation, substantial cidal activity has been demonstrated against T gondii [27,41], L donovani [51], and L tropica [RM Locksley, unpublished observations]. Evidence of respiratory burst activity accompanied phagocytosis of these organisms. The trypomastigotes of T cruzi [52] and T dionisii [30] have been reported to survive and replicate as amastigotes within monocytes; however, the monocytes were cultured in vitro for at least 4 hr in the first study and for an unspecified time in the second study before challenge with the organisms. Monocytes rapidly lose granule peroxidase with in vitro culture; up to 50% is lost after 24 hr and virtually all by 72 hr [53]. After 72 hr in culture, human monocyte-derived macrophages displayed significantly less toxoplasmacidal and leishmanicidal activity than did monocytes studied immediately after isolation [RM Locksley, unpublished observations]. Monocytes cultured for 72 hr actually release greater quantities of H_2O_2 [53], and perhaps O_2^- [54,55], after stimulation with PMA than do freshly isolated monocytes, suggesting that the loss of granule peroxidase may significantly impair monocyte toxicity to protozoa. The enhanced recovery of oxygen products of the respiratory burst from these cells may reflect both decreased scavenging of H_2O_2 by MPO and the potential role of MPO in terminating the respiratory burst [56,57]. Finally, resident macrophages (mouse peritoneal) and 5–10 day explanted human monocyte-derived macrophages, which have lost all granule peroxidase, have minimal activity against protozoa; these cells also have a markedly

attenuated respiratory burst (see below).

A few studies have been performed with MPO-deficient leukocytes. MPO-deficient PMN and monocytes displayed early post-phagocytic cidal defects against L donovani promastigotes [48] and T gondii [27], respectively, despite the observation that both cell types generate an approximately 2- to 4-fold greater respiratory burst than do normal PMN [58] and monocytes [57].

Attempts have been made to supply peroxidase exogenously to phagocytes lacking this enzyme (mouse resident macrophages, human monocyte-derived macrophages and MPO-deficient monocytes) in order to boost protozoacidal activity. It had been previously shown that macrophages, which are incapable of iodination [59], acquire this capability after phagocytosis of neutrophil debris, presumably a source for MPO [60]. Fluid-phase peroxidase may also be pinocytosed and reach the intravacuolar space [61]. Buchmuller and Mauel [62] were able to markedly augment lymphokine-induced amastigocidal (L enriettii, a leishmanial species which infects guinea pigs) activity by adding exogenous horseradish peroxidase to 1-day explanted, elicited mouse peritoneal macrophages. The addition of the hemeprotein inhibitor aminotriazole abolished the effect. The strongly basic EPO adsorbs firmly to the negatively charged surface of many organisms in a nontoxic fashion with retention of enzyme activity [49]. Addition of H_2O_2 and a halide is toxic to EPO-coated bacteria [63,64] and parasites [65], including T gondii [27], T cruzi [36], and L tropica [RM Locksley, CA Nacy; unpublished observations]. Resident mouse peritoneal macrophages, which are incapable of killing ingested T gondii [27,66] or T cruzi trypomastigotes [28], acquire substantial protozoacidal activity if these organisms are first coated with EPO [27,36]. The peroxidase can be visualized on the surface of the organisms within the phagosome [27,36]. The requirements for both respiratory burst activity and participation by the acquired EPO were demonstrated by the inhibition of toxicity by catalase or hemeprotein inhibitors. Resident macrophages undergo only a minimal respiratory burst with phagocytosis which is not enhanced by the presence of EPO on the surface of the particle [27]. These studies emphasize the potency of peroxidase in augmenting H_2O_2 toxicity. The post-phagocytic toxoplasmacidal defect of MPO-deficient monocytes was also restored to normal by preincubation of T gondii with EPO [27].

Whether a correlate of these in vitro studies exists in vivo is unknown. Macrophages within inflammatory foci can ingest the cellular debris of neutrophils [67] and eosinophils [68] and acquire phagosomal peroxidase. Extracellular MPO has been identified in areas of pyogenic inflammation [69]. Foci of neutrophils and eosinophils have been described in patients with Chagas' disease [70] and toxoplasmosis [71]. Utilization of acquired peroxidase by macrophages in vivo, however, has not been shown.

The magnitude of the stimulated respiratory burst. The initial observations by Nathan et al [28] drew attention to the inverse correlation of intracellular survival by protozoa with the magnitude of the phorbol myristate acetate (PMA)–induced macrophage respiratory burst. Thus, in vivo (from T cruzi or Bacille Calmette-Guerin [BCG] infected mice) or in vitro activated mouse peritoneal macrophages both released substantial quantities of H_2O_2 after stimulation with PMA and killed ingested trypomastigotes of T cruzi, whereas resident or elicited macrophages accomplished neither. The elegant studies of Murray et al extended these observations to both T gondii [66,72] and Leishmania species [33,51,73]. The ability of a wide

variety of murine macrophage populations, including resident, elicited, in vivo and in vitro activated, and macrophage-like cell lines, to kill or inhibit the intracellular replication of these protozoa was directly related to the quantities of oxygen-derived products (H_2O_2 or O_2^-) recovered after stimulation with PMA. Further, the ability of macrophages with protozoacidal activity to kill these organisms could be blocked by scavengers of products of the respiratory burst [72,73]. The method usually involves preincubation of the cells with inhibitors (such as superoxide dismutase, catalase, or mannitol) at high concentrations, which results in intracellular sequestration in secondary lysosomes via pinocytosis. Although the use of inhibitors at high levels raises some questions regarding specificity, these experiments in general support the requirement for an oxygen-dependent respiratory burst for optimal activity against protozoa. Similarly, preincubation of the cells in glucose-free media [33,72,73] or pre-stimulation with PMA [33,74], both of which markedly attenuate the ability of the cells to mount a subsequent respiratory burst, leaves otherwise competent macrophages susceptible to intracellular parasitism. In the converse experiment, susceptible resident macrophages acquired toxoplasmacidal activity when supplied with an exogenous source (xanthine plus xanthine oxidase) of oxygen-derived products [66]. Also, a variant clone of the J774 murine macrophage-like tumor cell line, which was deficient in the ability to produce O_2^- or H_2O_2 and unable to kill T cruzi epimastigotes, acquired the capacity to kill intracellular T cruzi after ingestion of glucose oxidase covalently coupled to zymosan particles and subsequent incubation in glucose-containing media [29]. Studies with specific scavengers and exogenous oxygen-radical generating systems have supported a potential role in macrophages for H_2O_2 in mediating toxicity against Leishmania [22] and T cruzi [29], and for more distal radicals (ie, $OH^{\cdot}$) against T gondii [26,66].

These experiments suggest that the capacity to generate reactive oxygen products at the time of phagocytosis of the parasite influences subsequent intracellular survival. Several investigators have reported that addition of lymphokines *after* phagocytosis was completed can activate otherwise susceptible macrophage populations to kill ingested protozoa [31,62,75,76]. Although such activation may proceed by oxygen-independent pathways, in at least two studies the use of inhibitors such as catalase have suggested that killing was via an oxygen-dependent mechanism [31,62]. The mechanism by which such oxygen-dependent cidal activity might occur is unclear; there is no evidence that lymphokine induces an intraphagosomal respiratory burst in previously infected macrophages. Macrophages which had been infected with L donovani did not continue to generate a respiratory burst after phagocytosis was completed; however, they were capable of responding with a normal respiratory burst (chemiluminescence) after subsequent stimulation [77]. The studies suggesting oxygen-dependent, post-phagocytic killing both used elicited mouse peritoneal macrophages, which may contain granule peroxidase by virtue of their recent migration from the blood [78].

In agreement with studies using mouse macrophages, human monocytes have an easily quantitated respiratory burst after PMA stimulation and demonstrate significant protozoacidal activity, whereas human monocyte-derived macrophages (5–14 days in culture) have both an attenuated respiratory burst after stimulation with PMA and poor activity against these organisms [51,53,79–81]. The possible contribution of the loss of granule peroxidase to this loss in activity has been discussed.

When PMN or monocytes from patients with chronic granulomatous disease

have been studied, significant defects in toxicity to Leishmania [32,34] and T gondii [27] have been observed, although monocytes from these patients demonstrate evidence for substantial nonoxidative protozoacidal activity as well.

Macrophages activated by exposure to antigen- or mitogen-stimulated T-cell products acquire the ability to generate significantly greater quantities of oxygen-derived products after membrane perturbation [82]; recent reports have extended this observation to human monocyte-derived macrophages [55,83]. Further, human monocyte-derived macrophages exposed to lymphokines in vitro acquire substantial activity against T cruzi [84], T gondii [85,86], and L donovani [51]. Exposure to lymphokines does not seem to stimulate reappearance of granule MPO [83], thus implicating peroxidase-independent toxicity. It has been estimated that activated macrophages might achieve intraphagosomal H_2O_2 concentrations ranging from 7.6×10^{-2} M [28] to levels three times greater [29]; such concentrations are lethal to all of these protozoa in vitro. Both lymphokine-induced respiratory burst activity and antiprotozoa activity could be abolished by incubation of the macrophages with corticosteroids [83,87]. These observations suggest that oxygen-dependent macrophage cytotoxic activity may be an important effector arm in cell-mediated immunity against these protozoa [3].

The capacity to respond to parasite ingestion with an effective respiratory burst. The observation by Wilson et al [79] that resident murine and human monocyte-derived macrophages mounted a minimal respiratory burst upon phagocytosis of T gondii but an easily measured burst upon phagocytosis of heat-killed Candida or latex particles, underscored another important factor determining intracellular survival by protozoa; namely, the ability to elude effective triggering of the respiratory burst during phagocytosis. Similar studies have shown that the enhanced intramacrophage survival of the amastigotes of L donovani and L tropica compared to the promastigotes correlates with the minimal respiratory burst elicited by the former and the substantial burst by the latter [22,33]. Respiratory burst activity in these studies has been measured both quantitatively (H_2O_2 or O_2^- release, nitroblue tetrazolium [NBT] reduction, chemiluminescence) and qualitatively (slide NBT reduction). Despite eliciting a respiratory burst comparable (although delayed) to that of opsonized zymosan, however, a significant number of L donovani promastigotes do seem to survive phagocytosis by human monocyte-derived macrophages and to transform intracellularly to the more hardy amastigote form [77].

Mouse macrophages require *both* an enhanced capacity to generate oxygen products following membrane stimulation *and* the ability to respond to phagocytosis with an effective respiratory burst to achieve cidal activity against L donovani amastigotes [33]. Resident peritoneal macrophages respond to parasite ingestion (as measured by qualitative NBT reduction) but have a minimal PMA stimulated respiratory burst when measured quantitatively. Conversely, in vivo activated macrophages (following intraperitoneal BCG injection) have enhanced respiratory burst activity after PMA stimulation but do not respond to parasite ingestion with an effective burst. Both cell populations fail to kill amastigotes. In contrast, in vitro activated macrophages have both properties and efficiently kill ingested amastigotes.

Importantly, the response of a given cell type to each parasite or to different stages of the same parasite may be different. T gondii eludes effective triggering of the respiratory burst by resident macrophages whereas Leishmania promastigotes and T cruzi epi- and trypomastigotes do not [22,30,74]. Survival of the latter two

organisms is presumably due to the meager amplitude of the respiratory burst in these cells.

Endogenous parasite oxygen product scavenging systems. Protozoan parasites contain endogenous scavengers of oxygen-derived products which may be important in protozoan defense against the phagocyte respiratory burst. The greater resistance of T gondii to H_2O_2 and to macrophage killing compared to Leishmania species has been correlated with the greater levels of endogenous scavengers of H_2O_2 —catalase and glutathione peroxidase—in the former [22,26]. Similarly, the more resistant amastigotes of Leishmania contain more of these compounds than the promastigotes [33]. A role for these endogenous scavengers was suggested by the observation that inhibition of T gondii catalase by preincubation with aminotriazole enhanced the susceptibility of the organisms to H_2O_2 and to killing by resident mouse macrophages [20].

L tropica promastigotes contain little catalase or glutathione peroxidase, and although they contain substantial quantities of superoxide dismutase, the enzyme is different from the mammalian enzyme [23]; chemotherapy exploiting these differences might prove to be effective anti-Leishmania agents.

T cruzi lacks catalase [21] and glutathione peroxidase [88] and is unable to degrade its own endogenously produced H_2O_2. Up to 4% of the total oxygen consumed by epimastigotes results in H_2O_2 formation by mitochondrial, microsomal, and cytosolic enzymes [21]. Intracellular levels of H_2O_2 up to 100-fold higher than that reported in mammalian cells have been measured in T brucei brucei [89]. Chemotherapeutic strategies which increase the parasites' own production of H_2O_2 [90] or deplete endogenous intracellular glutathione (which with glutathione reductase comprises the organisms' major defense against H_2O_2 oxidant stress) [91] effectively kill the organisms. Also, exogenously supplied peroxidase (EPO) and a halide caused auto-toxicity and auto-iodination by T cruzi, which was abolished by catalase [36], thus implicating H_2O_2 produced by the parasite in its own demise. The ability of T cruzi to rapidly escape from the phagosome and invade the cytosol, where host cell scavenging systems are operative, may be important in the ability of the organism to elude killing by resident macrophages. Activated macrophages presumably overwhelm the protozoa with large quantities of oxygen metabolities which are toxic before escape from the phagosome can be completed.

In summary, extensive evidence supports a role for phagocyte oxygen-dependent cidal mechanisms in host defense against the intracellular protozoan parasites. Circulating neutrophils, and possibly eosinophils, which have a high granule peroxidase content and are capable of a vigorous respiratory burst, are protozoacidal and may be important in limiting the spread of these organisms through the bloodstream. Monocytes when examined early after harvesting also have quite substantial cidal capabilities, despite having less granule peroxidase and a less vigorous respiratory burst following stimulation. Tissue macrophages both lack granule peroxidase and have a comparatively attenuated respiratory burst following stimulation; this severe microbicidal deficit leaves these cells highly susceptible to the protozoa and is consistent with their role as important sites for the establishment of infection. Supplying these cells with exogenous peroxidase, or boosting their respiratory burst capability by exposure to sensitized T-cell products, enhances their protozoacidal activity. The latter may be an important in vivo effector arm of cell-mediated immunity, leading to control of or eradication of infection. Protozoan defense mechanisms against phago-

cyte oxygen-dependent systems may be important in their intracellular survival; high levels of endogenous scavengers of oxygen products of the respiratory burst and the ability to elude effective triggering of the respiratory burst during phagocytosis correlate with intracellular survival of these organisms.

Clearly the interaction of host and parasite is exceedingly complex and involves far more than the oxygen-dependent capabilities of host phagocytes. The important role for suppressor T-cells in perpetuating infection by protozoa, the microbicidal capabilities of other effector cells, such as T-cells and natural killer (NK)-cells, and the importance of nonoxidative killing mechanisms of leukocytes have not been considered here. Host antibodies do not seem to be protective in Leishmania infection, but do have a role in potentiating phagocyte activity against T cruzi [45–47] and T gondii [39,79,92], and perhaps in limiting extracellular spread of these organisms [93,94]. For Leishmania, where organisms replicate only within macrophages, macrophage oxygen-dependent cidal capacity may be the most important determinant in host control of infection. For T cruzi and T gondii, where infection disseminates from macrophages to other cell types, macrophages may still function to limit or control the extent of disease in concert with other of the above or as yet unidentified host defense mechanisms.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the advice and helpful comments of Henry Rosen, MD, and Christopher B. Wilson, MD, in review of this manuscript.

This work was supported in part by USPHS grant A107763 and by grants from the Rockefeller Foundation and the Edna McConnell Clark Foundation. R.M.L. was supported by PHS Training Grant A107044.

REFERENCES

1. Babior BM: N Engl J Med 298:721, 1978.
2. Klebanoff SJ: In Gallin JI, Fauci AS (eds): "Advances in Host Defense Mechanisms," vol 1, New York: Raven Press, 1982, pp 111-162.
3. Root RK, Cohen MS: Rev Infect Dis 3:565, 1981.
4. Root RK, Metcalf JA: J Clin Invest 60:1266, 1977.
5. Reiss M, Roos D: J Clin Invest 61:480, 1978.
6. Behar D, Czapski G, Rabani J, Dorfman LM, Schwarz HA: J Phys Chem 74:3209, 1970.
7. Jensen MS, Bainton DF: J Cell Biol 56:379, 1973.
8. Klebanoff SJ: In Sbarra AJ, Strauss R (eds): "The Reticuloendothelial System, 2. Biochemistry and Metabolism," New York: Plenum Publ, 1983, pp 279-308.
9. Klebanoff SJ, Clark RA: "The Neutrophil: Function and Clinical Disorders," New York: North-Holland Publ, 1978, pp 416-420.
10. Klebanoff SJ, Jong EC, Henderson WR: In Mahmoud AAF, Austen KF (eds): "The Eosinophil in Health and Disease," New York: Grune and Stratton, 1980, pp 99-111.
11. Klebanoff SJ, Clark RA: "The Neutrophil: Function and Clinical Disorders," New York: North-Holland Publ, 1978, pp 421-430.
12. Rosen H, Klebanoff SJ: J Exp Med 249:27, 1979.
13. Tauber AI, Babior BM: J Clin Invest 60:374, 1977.
14. Weiss SJ, King GW, LoBuglio AF: J Clin Invest 60:370, 1977.
15. Rosen H, Klebanoff SJ: J Clin Invest 64:1725, 1979.
16. Repine JE, Eaton JW, Anders MW, Hoidal JR, Fox RB: J Clin Invest 64:1642, 1979.

17. Drath DB, Karnovsky ML, Huber GL: J Appl Physiol 46:136, 1979.
18. Klebanoff SJ, Rosen H: J Exp Med 148:490, 1978.
19. Beauchamp C, Fridovich I: J Biol Chem 245:4641, 1970.
20. Murray HW, Nathan CF, Cohn ZA: J Exp Med 152:1610, 1980.
21. Boveris A, Stoppani AOM: Experientia 33:1306, 1977.
22. Murray HW: J Exp Med 153:1302, 1981.
23. Meshnick SR, Eaton JW: Biochem Biophys Res Commun 102:970, 1981.
24. Nathan CF, Silverstein SC, Brukner LH, Cohn ZA: J Exp Med 149:200, 1979.
25. Bass DA, Szejda P: J Clin Invest 64:1558, 1979.
26. Murray HW, Cohn ZA: J Exp Med 150:938, 1979.
27. Locksley RM, Wilson CB, Klebanoff SJ: J Clin Invest 69:1099, 1982.
28. Nathan CF, Nogueira N, Juangbhanich C, Ellis J, Cohn ZA: J Exp Med 149:1056, 1979.
29. Tanaka Y, Kiyotaki C, Tanowitz H, Bloom BR: Proc Natl Acad Sci USA 79:2584, 1982.
30. Thorne KJI, Glauert AM, Svvennsen RJ, Thomas H, Morris J, Franks D: Parasitol 83:115, 1981.
31. Haidaris CG, Bonventre PF: J Immunol 129:850, 1982.
32. Chang K-P: Am J Trop Med Hyg 30:322, 1981.
33. Murray HW: J Immunol 129:351, 1982.
34. Pearson RD, Steigbigel RT: J Immunol 127:1438, 1981.
35. Jacques PJ, Avila JL, Pinardi ME, Convit J: Arch Intern Physiol Biochim 83:976, 1975.
36. Nogueira NM, Klebanoff SJ, Cohn ZA: J Immunol 128:1705, 1982.
37. Avila JL, Bretana A, Jacques PJ, Casanova MA, Convit J: In Siegenthaler W, Luthy R (eds): "Current Chemotherapy Proceedings of the 10th International Congress of Chemotherapy," Washington: American Society of Microbiology, 1978, pp 153-155.
38. Chang K-P, Dwyer DM: Science 193:678, 1976.
39. Jones TC, Hirsch JG: J Exp Med 136:1173, 1972.
40. Nogueira NM, Cohn ZA: J Exp Med 143:1402, 1976.
41. Wilson CB, Remington JS: J Infect Dis 140:890, 1979.
42. Sanderson CJ, DeSouza W: J Cell Sci 37:275, 1979.
43. Thorne KJ, Glauert AM, Svvennsen RJ, Franks D: Parasitology 79:367, 1979.
44. Thorne KJ, Svvennsen RJ, Franks D: Infect Immun 21:798, 1978.
45. Nogueira NM, Chaplan S, Cohn ZA: J Exp Med 152:447, 1980.
46. Kierszenbaum F: Am J Trop Med Hyg 28:965, 1979.
47. Kipnis TL, James SL, Sher A, David JR: Am J Trop Hyg 30:47, 1981.
48. Rush T, Steigbigel RT: Intnatl Congr Antimicrob Ag Chemother, Washington: American Society of Microbiology, 29(abstr), 1982.
49. Klebanoff SJ, Henderson WR, Jong EC, Jorg A, Locksley RM: In Yoshida T, Torisu M (eds): "Immunobiology of the Eosinophil," Amsterdam: Elsevier, 1983, pp 261–282.
50. Box A, Wever R, Roos D: Biochim Biophys Acta 525:37, 1978.
51. Murray HW, Cartelli D: J Clin Invest 72:32, 1983.
52. Williams DM, Sawyer S, Remington JS: J Infect Dis 134:610, 1976.
53. Nakagawara A, Nathan CF, Cohn ZA: J Clin Invest 68:1243, 1981.
54. Musson RA, McPhail LC, Shafran H, Johnston RB Jr: J Reticuloendothel Soc 31:261, 1982.
55. Seim S: Acta Path Microbiol Immunol Scand (Section C) 90:179, 1982.
56. Jandl RC, Andre-Schwartz J, Borges-DuBois L, Kipnis RS, McMurrich BJ, Babior BM: J Clin Invest 61:1176, 1978.
57. Locksley RM, Wilson CB, Klebanoff SJ: Blood 62:902, 1983.
58. Rosen H, Klebanoff SJ: J Clin Invest 58:50, 1976.
59. Simmons SR, Karnovsky ML: J Exp Med 138:44, 1973.
60. Heifets L, Imai K, Goren MB: J Reticuloendothel Soc 28:391, 1980.
61. Steinman RM, Cohn ZA: J Cell Biol 55:186, 1972.
62. Buchmuller Y, Mauel J: J Reticuloendothel Soc 29:181, 1981.
63. Ramsey PG, Martin T, Chi E, Klebanoff SJ: J Immunol 128:415, 1982.
64. Locksley RM, Jacobs RF, Wilson CB, Weaver WM, Klebanoff SJ: J Immunol 129:2192, 1982.
65. Jong EC, Chi E, Klebanoff SJ: Am Fed Clin Res 30:370A (abstr), 1982.
66. Murray HW, Juangbhanich CW, Nathan CF, Cohn ZA: J Exp Med 150:950, 1979.
67. Atwal O: J Reticuloendothel Soc 10:163, 1971.
68. Ross R, Klebanoff SJ: J Exp Med 124:653, 1966.
69. Bradley PP, Christensen RD, Rothstein G: Blood 60:618, 1982.

70. Köberle F: Adv Parasitol 6:63, 1968.
71. Callahan WP Jr, Russell WO, Smith MC: Medicine 25:343, 1946.
72. Murray HW, Cohn ZA: J Exp Med 152:1596, 1980.
73. Murray HW: J Exp Med 153:1690, 1981.
74. Murray HW: J Reticuloendothel Soc 31:479, 1982.
75. Chang K-P, Chiao JW: Proc Natl Acad Sci USA 78:7083, 1981.
76. Nacy CA, Meltzer MS, Leonard EJ, Wyler DJ: J Immunol 127:2381, 1981.
77. Pearson RD, Harcus JL, Symes PH, Romito R, Donowitz GR: J Immunol 129:1282, 1982.
78. Beelen RHJ, Fluitsma DM: Immunobiol 161:266, 1982.
79. Wilson CB, Tsai V, Remington JS: J Exp Med 151:328, 1980.
80. Berman JD, Dwyer DM, Wyler DJ: Infect Immunol 26:375, 1979.
81. Pearson RD, Romito R, Symes PH, Harcus JL: Infect Immunol 32:1249, 1981.
82. Johnston RB Jr, Godzik CA, Cohn ZA: J Exp Med 148:115, 1978.
83. Nakagawara A, DeSantis NM, Nogueira NM, Nathan CF: J Clin Invest 70:1042, 1982.
84. Nogueira NM, Chaplan S, Reesink M, Tydings J, Cohn ZA: J Immunol 128:2142, 1982.
85. Borges JS, Johnson WD Jr: J Exp Med 141:483, 1975.
86. Anderson SE Jr, Remington JS: J Exp Med 139:1154, 1974.
87. Masur H, Murray HW, Jones TC: Infect Immunol 35:709, 1982.
88. Boveris A, Sies H, Martino EE, Docampo R, Turrens JF, Stoppani AOM: Biochem J 188:643, 1980.
89. Meshnick SR, Chang KP, Cerami A: Biochem Pharmacol 26:1923, 1977.
90. Cruz FS, Docampo R, DeSouza W: Acta Tropica 35:35, 1978.
91. Arrick BA, Griffith OW, Cerami A: J Exp Med 153:720, 1981.
92. Anderson SE Jr, Bautista SC, Remington JS: Clin Exp Immunol 26:375, 1976.
93. Schreiber RD, Feldman HA: J Infect Dis 141:366, 1980.
94. Krettli AM, Brener Z: J Immunol 128:2009, 1982.