INFECTION AND IMMUNITY, Oct. 1997, p. 4173–4178 Vol. 65, No. 10
0019-9567/97/$04.00+0
Copyright © 1997, American Society for Microbiology

Redundant Contribution of Myeloperoxidase-Dependent Systems to Neutrophil-Mediated Killing of *Escherichia coli*

HENRY ROSEN* AND BRYCE R. MICHEL

Department of Medicine, University of Washington, Seattle, Washington 98195

Received 1 May 1997/Returned for modification 19 June 1997/Accepted 25 July 1997

Neutrophil microbicidal activity is a consequence of overlapping antimicrobial systems that vary in prominence according to the conditions of the neutrophil-microbe interaction, the nature of the microbe, and its metabolic state. In this study, normal, myeloperoxidase-deficient, and respiratory burst-deficient (chronic granulomatous disease [CGD]) neutrophils killed *Escherichia coli* with equivalent, high efficiencies. Killing by CGD and myeloperoxidase-deficient neutrophils was not augmented by supplements, such as exogenous H_2O_2 and myeloperoxidase, directed at ameliorating their metabolic defects, suggesting that nonoxidative microbicidal systems were sufficient for a full microbicidal effect. Neutrophils with an intact myeloperoxidase antimicrobial system (normal or appropriately supplemented deficient cells) were capable of rapidly suppressing *E. coli* DNA synthesis, while unsupplemented CGD or myeloperoxidase-deficient cells were far less effective, indicating that the myeloperoxidase system was active in normal neutrophils. The degree of DNA synthesis inhibition by myeloperoxidase-sufficient neutrophils could account, in a cell-free system, for most of the observed microbicidal activity. While the myeloperoxidase system was active and probably bactericidal, it was not rate limiting for microbicidal activity and appears to have been redundant with other microbicidal systems in the cell. Rapid and extensive inhibition of bacterial DNA synthesis appears to be an indicator of myeloperoxidase activity in neutrophils.

Phagocytosis of bacteria by neutrophils is typically followed by loss of microbial viability as indicated by the inability to form colonies on nutrient agar. Phagocytosis-associated events introduce preformed microbicidal proteins, stored in cytoplasmic granules, and reactive oxygen species, such as hydrogen peroxide, generated by a membrane-associated NADPH oxidase. An analysis of neutrophil granule components has identified several antimicrobial proteins, including myeloperoxidase (MPO), bactericidal-permeability-increasing protein, defensins, serprocidins, cathelicidins, and others (22). These proteins optimally exert their antimicrobial effects alone, in combination, or, for MPO, in concert with products of the respiratory burst. Their antimicrobial activities vary with the nature of the microbe, its metabolic state, and the pH, osmolality, and protein composition of the medium in which they function. The assessment of the relative contributions of these possibly redundant systems to the aggregate of neutrophil-mediated events affecting a specific pathogen poses a challenge.

While characterizing the effects of an isolated, neutrophil-related, microbicidal system consisting of MPO, chloride, and H_2O_2, we observed a good correlation between loss of bacterial viability and inhibition of microbial DNA synthesis (32). Other oxidative and nonoxidative microbicidal systems evaluated failed to manifest a similarly close relationship, and the MPO system seemed to possess a measure of specificity for this effect. Model systems serve, at best, to approximate conditions believed to prevail in intact cells. We wished to ascertain whether the concordance between DNA synthesis inhibition and bactericidal effect was also a feature of the microbicidal activity of intact neutrophils and, if so, whether the MPO system was required. Thus, the effects on *Escherichia coli* viability and DNA synthesis of normal neutrophils were compared to those of neutrophils that lacked the capacity to activate the membrane-associated NADPH oxidase (chronic granulomatous disease [CGD] neutrophils) or that were devoid of MPO (hereditary MPO deficiency).

* Corresponding author. Mailing address: Box 356420, University of Washington, Seattle, WA 98195. Phone: (206) 543-3238. Fax: (206) 543-3947.

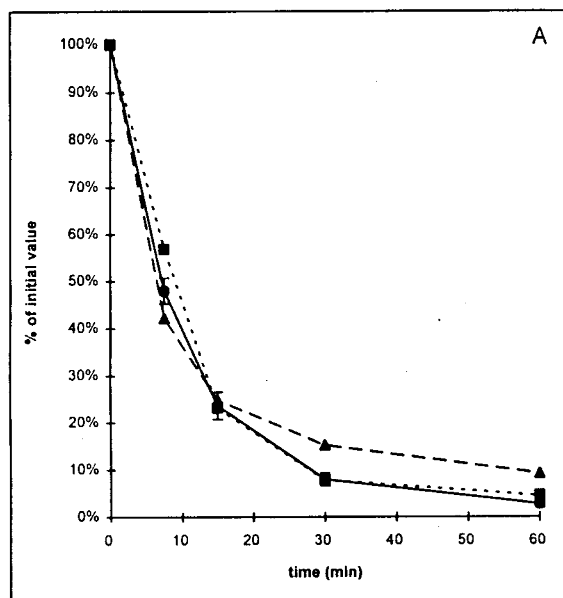
MATERIALS AND METHODS

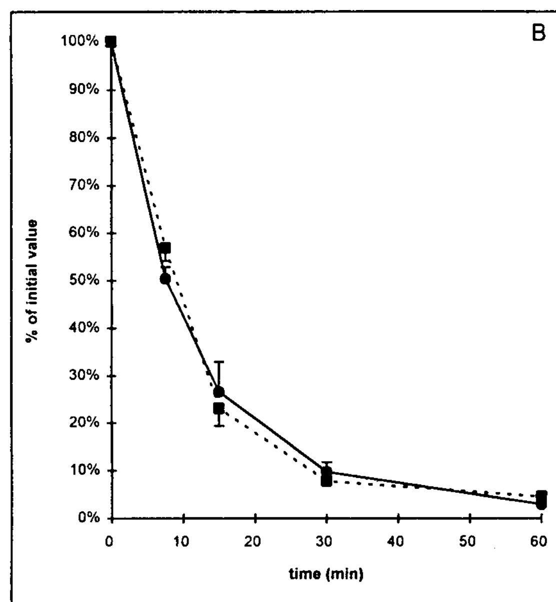
Special reagents. MPO was purified from leukapheresed blood cells of a patient with chronic myelogenous leukemia (28) and was assayed (17) as previously described. Glucose oxidase (1,130 U/ml, from *Aspergillus niger*; type V-S; Sigma) and [*methyl*-^{3}H]thymidine (85 Ci/mmol; Amersham) were used as received from the supplier.

Bacteria. *E. coli* ATCC 11775, a serum-resistant urinary isolate, was maintained as a frozen stock at −70°C in 50% Trypticase soy broth (BBL, Becton Dickinson and Co., Cockeysville, Md.) and 50% fetal calf serum. Periodically, scrapings from the frozen stock were streaked on nutrient agar, grown overnight at 37°C, and maintained at 4°C for up to 4 weeks. Colonies from agar were inoculated into Trypticase soy broth and grown overnight at 37°C. Bacteria were pelleted by centrifugation for 20 min at 3,300 × *g* and 4°C, washed once by centrifugation in HEPES-supplemented Hanks balanced salt solution without calcium or magnesium (HHBSS; 20 mM HEPES, 137 mM NaCl, 5.4 mM KCl, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 5.6 mM glucose; adjusted to pH 7.45 with NaOH) containing 1 mg of gelatin per ml, and suspended at 10^9 CFU per ml in the same medium. Washed bacteria were maintained on ice for up to 1 h prior to initiation of the bactericidal assay.

Neutrophils. Neutrophils were obtained from EDTA-anticoagulated blood by dextran sedimentation and centrifugation through Histopaque (Sigma) as described previously (31). Patients with complete hereditary MPO deficiency (30) and X-linked, cytochrome *b*-positive, CGD (3, 7, 30) have been characterized previously.

Microbicidal assay. HHBSS supplemented with calcium chloride (400 μM) and magnesium chloride (160 μM) was warmed to 37°C in 50-ml conical-bottom polypropylene tubes (Corning Glass Works, Corning, N.Y.). Bacteria and fresh autologous (healthy donor; 10% final concentration) serum were added. After a 2-min preincubation of these components at 37°C, neutrophils were added (t = 0; total volume, 3.0 ml). When exogenous MPO was used to supplement MPO-deficient neutrophils, *E. coli* cells in HHBSS-gelatin (1.4 ml of a 10^9-cell/ml concentration of HHBSS-gelatin) were preincubated with 540 mIU of MPO (17, 28) (0.04 ml) for 2 min at 4°C to permit firm binding of the active enzyme to the bacterial cell surface (33) prior to ingestion by neutrophils. When glucose oxidase was used to generate H_2O_2, the glucose concentration was increased to 15.6 mM and glucose oxidase was added immediately prior to the addition of neutrophils. H_2O_2 so generated in the suspension medium can diffuse to sites in the neutrophil where it can support, in CGD cells, the MPO-dependent chlorination





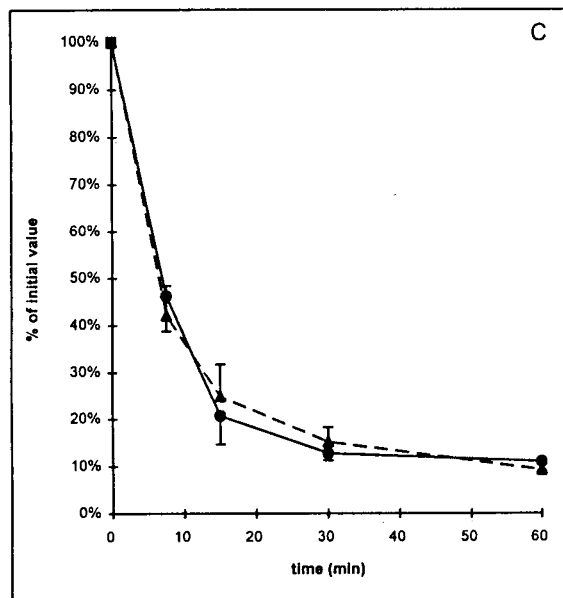


FIG. 1. Decline in *E. coli* viability following incubation with normal, CGD, and MPO-deficient neutrophils. Neutrophils (5×10^6 cells/ml) were incubated at 37°C with *E. coli* (10^8 cells/ml) in HHBSS supplemented with Ca^{2+}, Mg^{2+}, and 10% fresh autologous serum. At the indicated times, samples were lysed, diluted, and plated to determine residual bacterial viability as described in Materials and Methods. (A) Percent residual viability for bacteria incubated with normal (circles; $n = 19$), CGD (squares; $n = 6$), and MPO-deficient (triangles; $n = 5$) neutrophils. Error bars are drawn only for normal neutrophil data. (B) Effects of the glucose (15.6 mM) plus glucose oxidase (1.3 U/ml) H_2O_2-generating system on the microbicidal activity of CGD neutrophils (circles; $n = 6$). Data for unsupplemented CGD neutrophils (squares) are as in panel A. (C) Effects of supplemental human MPO on the microbicidal activity of MPO-deficient neutrophils (circles; $n = 2$). Data for unsupplemented MPO-deficient neutrophils (triangles) are as in panel A. Error bars represent the standard errors of the means.

of fluorescein covalently bound to ingested zymosan particles (11). Samples for the determination of microbial viability and incorporation of tritiated thymidine into macromolecular compounds were taken immediately and at 7.5, 15, 30, and 60 min after the addition of neutrophils. Between samplings, tubes were vortexed continuously at low speed in a 37°C chamber.

Bacterial viability. Phagocytosis and killing were terminated by the transfer of 20 μl of neutrophil-bacterium suspension to 180 μl of 0.1% (wt/vol) Triton X-100 detergent in a microtiter plate well, with mixing by repetitive pipetting. Neutrophil lysates were allowed to stand for 2 to 30 min; there was no discernible effect on bacterial viability. Subsequent serial 1:10 dilutions were made into M63 salts (34), and 0.1-ml samples of each dilution were plated in molten Trypicase soy agar. Microbial viability was assessed by enumeration of visible colonies after 18 to 36 h of incubation at 37°C.

Thymidine uptake. Five hundred microliters of neutrophil-bacterium suspension was combined with an equal volume of double-strength Trypticase soy broth containing 0.2% (wt/vol) Triton X-100 and 2 μCi of [^{3}H]thymidine in a 1.7-ml polypropylene microcentrifuge tube. Tubes were tumbled at 10 to 30 rpm in a 37°C chamber for 45 min. Thymidine incorporation was terminated by the addition of 0.5 ml of 30% (wt/vol) trichloroacetic acid (TCA). Precipitates were collected by centrifugation in a microcentrifuge, washed with 1 ml of 10% (wt/vol) TCA, and digested overnight with 25 μl of 5 N NaOH. Tritium content was determined by liquid scintillation after allowing at least a day for subsidence of the chemiluminescence induced by the NaOH. Background values from neutrophil suspensions without bacteria were determined for each experiment and were subtracted from experimental values obtained for neutrophils plus bacteria. Radioactivity associated with neutrophils in the absence of bacteria was indistinguishable from radioactivity precipitated from Trypticase soy medium alone and amounted to approximately 10% of that associated with fully viable bacteria.

Statistics. Viability and DNA synthesis (thymidine incorporation) for sequential samples were expressed as percentages of the initial values ($t = 0$) for that experimental tube. On days when experimental conditions were replicated, results were averaged and data were considered to represent a single experiment. Data points in the figures represent the means ± standard errors of the means for the numbers of experiments indicated in the legends. For comparison of the effect of neutrophil cell type on *E. coli* viability and DNA synthesis, data were grouped as described in each figure legend and arranged in a two-by-two table with cell type and enzyme supplement if any (normal, CGD with or without glucose oxidase, or MPO deficient with or without MPO) along one axis and time of incubation (7.5, 15, 30, or 60 min) with neutrophils along the other. Percentage data below 0% were set to 0, and data above 100% were set to 1.0 prior to transforming all data with the arcsine function. Two-way analysis of variance was used to calculate an F statistic (SigmaStat; Jandel Scientific, San Francisco, Calif.), and column comparisons (cell type with or without enzyme) were performed by using the Student-Neuman-Keuls test, with P values below 0.05 considered significant.

RESULTS

Figure 1A shows the decline in viability for *E. coli* incubated with normal human neutrophils in 10% fresh autologous serum. After an hour's incubation 3% ± 1% of the initial inoculum remained viable. *E. coli* ATCC 11775 is serum resistant, with a doubling time of approximately 1 h in control tubes containing 10% serum without neutrophils (data not shown). When neutrophils from patients with CGD or hereditary com-

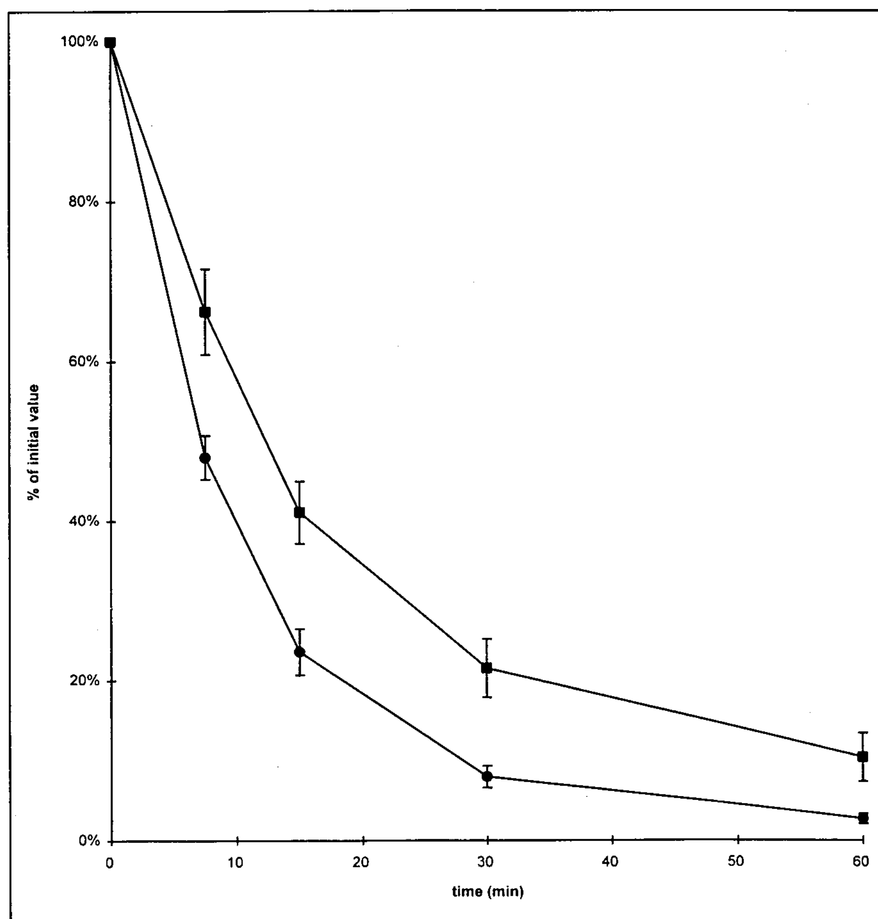


FIG. 2. Neutrophil-mediated inhibition of *E. coli* DNA synthesis compared to viability loss. Conditions were as described in the legend for Fig. 1 for normal neutrophils. The viability curve (Fig. 1A; circles) is repeated. Simultaneous samples were obtained for the determination of DNA synthesis (squares) as described in Materials and Methods. Initial thymidine incorporation into DNA (mean ± standard deviation) was 15.4 ± 11.8 pmol of [^{3}H]thymidine per ml of suspension per 45 min. Results are presented as the percentages of the initial value for each experiment. Error bars indicate the standard errors of the means of 19 experiments.

plete MPO deficiency were substituted for normal neutrophils, there was no significant alteration in either the rate or extent of neutrophil-mediated microbicidal effect. After an hour 5% ± 1% (for CGD neutrophils) and 9% ± 1% (for MPO-deficient neutrophils) *E. coli* cells remained viable (Fig. 1A). Supplementation of CGD neutrophils with the glucose plus glucose oxidase H_2O_2-generating system (Fig. 1B; viability at 1 h, 3% ± 1%) or of MPO-deficient neutrophils with exogenous MPO (Fig. 1C; viability at 1 h, 11% ± 2%) did not augment their bactericidal activities. Although under some conditions (rough *E. coli* strain with restricted complement activity during opsonization) lethal effects of CGD nonoxidative microbicidal systems can be reversed by incorporation of 0.1% bovine serum albumin in lysis or culture media (23), this phenomenon was not observed under the conditions employed for Fig. 1 (smooth serum-resistant strain, 10% fresh complement-replete serum; data not shown).

The ability of *E. coli* cells to incorporate radiolabeled thymidine into TCA-insoluble material was taken as an indicator of DNA synthesis. The decline in DNA synthesis was similar to the decline in viability (Fig. 2), although in comparison with the decline observed with the cell-free MPO system (32), the concordance was more approximate. *E. coli* DNA synthesis fell to 10% ± 3% of control during the 1-h incubation. In contrast to the normal effects of CGD and MPO-deficient phenotypes on microbicidal activity, there were readily discerned, statistically significant deficits in the capacity of these metabolically deficient neutrophils to inhibit *E. coli* DNA synthesis. After 60 min, CGD and MPO-deficient neutrophils reduced *E. coli* DNA synthesis to 49% ± 9% (Fig. 3) and 43% ± 10% (Fig. 4) of the initial values, respectively (corresponding value for normal neutrophils: 10% ± 3%).

Attempts to correct the metabolic defect of CGD cells by supplementing the incubation medium with the hydrogen peroxide-generating system glucose plus glucose oxidase resulted in a dose-dependent restoration of DNA synthesis suppression that approached normal levels (Fig. 3; low glucose oxidase supplement concentration, 1.3 U/ml; $P < 0.05$ versus normal neutrophils; $P < 0.05$ versus CGD neutrophils without supplementation). Higher concentrations of glucose oxidase (2.6 to 5.2 U per ml) produced almost complete normalization of CGD cells with respect to suppression of DNA synthesis. Similarly, precoating of bacteria with MPO allowed MPO-deficient neutrophils to manifest a nearly normal suppression of *E. coli* DNA synthesis (Fig. 4; $P < 0.05$ versus normal neutrophils; $P < 0.05$ versus MPO-deficient neutrophils without supplementation).

Neither supplementation of media with glucose plus glucose oxidase nor precoating of bacteria with MPO had discernible effects on bacterial viability or DNA synthesis in the absence of

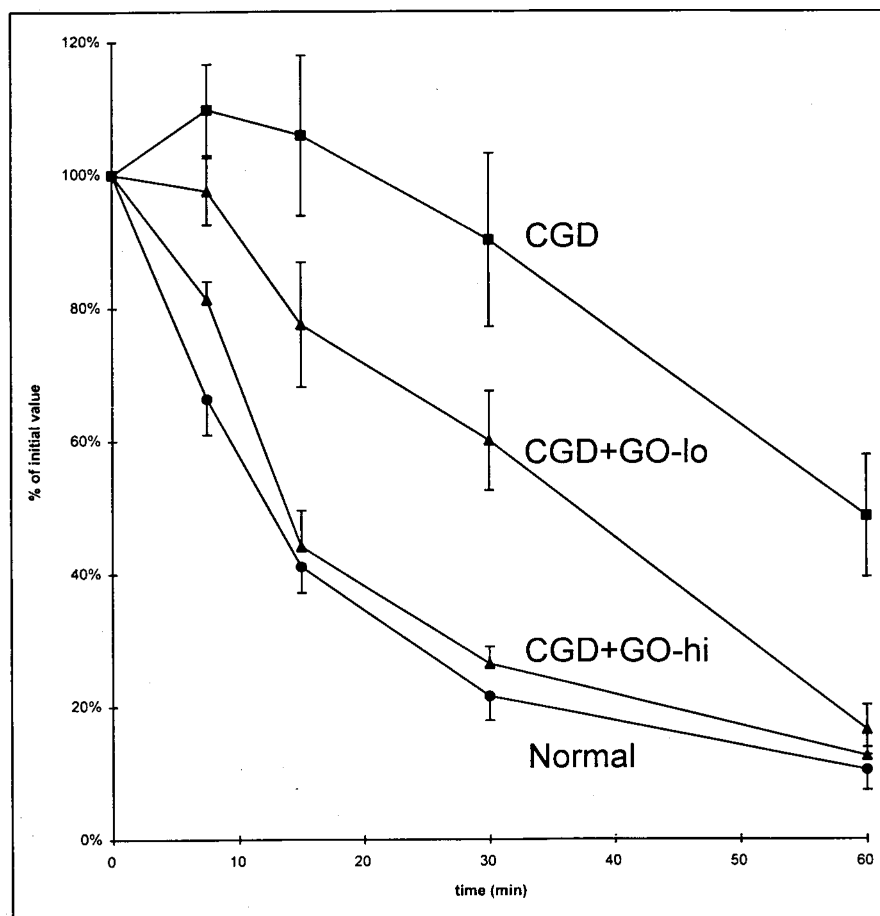


FIG. 3. Decline in *E. coli* DNA synthesis following incubation with CGD neutrophils. Conditions were as described in the legend for Fig. 1. CGD neutrophils from two individuals with CGD were evaluated on six occasions (CGD; $n = 6$). Where indicated, glucose oxidase and incremental glucose (GO; 15.6 mM) were present. The glucose oxidase concentrations were either 1.3 U/ml (CGD+GO-lo; $n = 6$) or 2.5, 3.8, and 5.0 U/ml (CGD+GO-hi; pooled data, two determinations at each concentration). Average values for initial thymidine incorporation into DNA ± standard deviations were 19.0 ± 14.2 (CGD) and 18.6 ± 15.2 (CGD supplemented with glucose oxidase) pmol of [^{3}H]thymidine per ml of suspension per 45 min. Results from Fig. 2 for normal neutrophils are included for comparison (circles).

neutrophils. Further, these supplements had no effect on microbicidal activity or inhibition of DNA synthesis by normal neutrophils (data not shown).

DISCUSSION

MPO-deficient neutrophils lack the enzyme that catalyzes the H_2O_2-mediated oxidation of chloride to the potent microbicide HOCl. Neutrophils from individuals with CGD are unable to provide the H_2O_2 substrate for MPO and are similarly unable to synthesize HOCl. Despite these metabolic deficiencies, both CGD and MPO-deficient neutrophils were able to kill *E. coli* as efficiently as normal neutrophils under the in vitro conditions employed in this study. The nonessential role for MPO was emphasized by the observation that reconstitution of CGD and MPO-deficient cells with H_2O_2 and MPO, respectively, failed to produce an incremental microbicidal effect (Fig. 1B and C). The observation of normal neutrophil killing of *E. coli* by CGD neutrophils is concordant with several (9, 37, 38) but not all (8, 13, 14, 16) other studies. The bases for the discrepancies among studies have not been explicitly characterized but may relate to incubation conditions that permit significant secretion of MPO, with attendant extracellular antimicrobial activity of the MPO-mediated antimicrobial system (25). In any case, it is clear that under some incubation conditions, such as those employed in this study, the nonoxidative microbicidal systems of neutrophils are sufficient to produce a normal microbicidal effect against *E. coli*.

Since there was no requirement for a functional MPO system in mediating bactericidal activity, one might ask whether this particular system is sufficiently active to engender an antimicrobial effect. An evaluation of neutrophil-mediated inhibition of bacterial DNA synthesis identified a major metabolic effect of the MPO system on *E. coli* cells. Normal neutrophils suppressed *E. coli* DNA synthesis nearly as extensively as they diminished survival (Fig. 2). In CGD and MPO-deficient cells, there was a defect in suppression of DNA synthesis that could be reversed by supplements expected to reconstitute their MPO systems (Fig. 3 and 4). Rapid inhibition of *E. coli* DNA synthesis is thus a clearly discernible biochemical effect of the neutrophil MPO system. The degree of suppression would be sufficient, in a cell-free system, to account for most of the microbicidal effect (32). Thus, while not detectable by virtue of its microbicidal effects, the MPO system appears to be active at "physiological" levels of opsonization, phagocytosis, and respiratory burst function.

In a cell-free MPO antimicrobial system, suppression of bacterial DNA synthesis was evaluated by comparison of the system with equally bactericidal oxidative (acetaldehyde–xan-

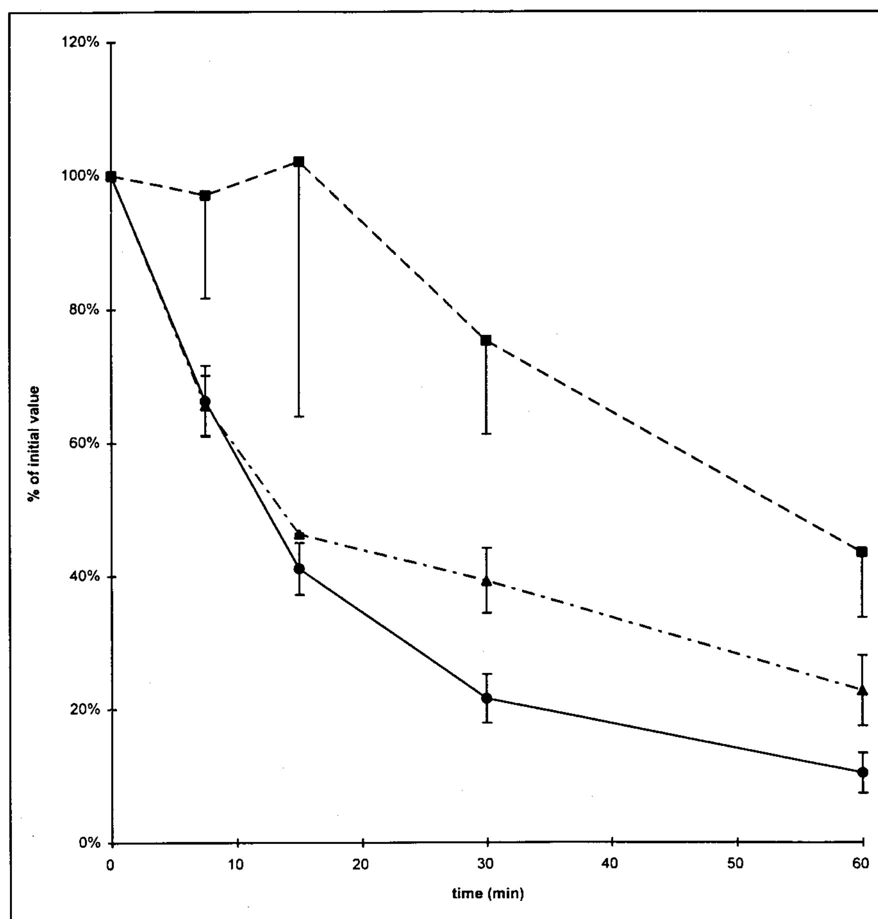


FIG. 4. Decline in *E. coli* DNA synthesis following incubation with MPO-deficient neutrophils. Conditions were as described in Fig. 1. Neutrophils from an individual with hereditary complete MPO deficiency (squares) were evaluated on five occasions. Where indicated (triangles), bacteria were preincubated with purified human MPO as described in Materials and Methods. Average values of initial thymidine incorporation into DNA ± standard deviations were 6.6 ± 4.0 (MPO-deficient neutrophils; $n = 5$) and 6.6 ± 1.2 (MPO-deficient neutrophils supplemented with MPO; $n = 2$) pmol of [^{3}H]thymidine per ml of suspension per 45 min. Results from Fig. 2 for normal neutrophils are included for comparison (circles).

thine oxidase–iron-EDTA) and nonoxidative (gentamicin) antibacterial systems (32). The MPO system was distinct from the other two in the rapidity and extent of suppression of bacterial DNA synthesis. Similar to inhibition by the cell-free MPO system, rapid neutrophil inhibition of *E. coli* DNA synthesis is relatively specific to the MPO component of the antimicrobial armamentarium. Limited inhibition of bacterial DNA synthesis by CGD cells indicated that the respiratory-burst-independent microbicidal systems were insufficient to produce the effect. In accord with these observations, lysates of rabbit neutrophils incubated under conditions where the MPO system should be inactive exhibited minimal effects on bacterial DNA and protein synthesis while producing substantial bactericidal effects (4). Impaired inhibition of bacterial DNA synthesis by MPO-deficient cells indicated that non-MPO oxidative microbicidal systems, such as those dependent on superoxide, hydrogen peroxide, and hydroxyl radicals, were also insufficient to produce major early suppression of *E. coli* DNA synthesis.

These findings should not be taken to suggest that the MPO system is broadly irrelevant to neutrophil-mediated antimicrobial effects. The importance of MPO and other oxidative systems has been demonstrated for neutrophil-mediated killing, in vitro, of *Staphylococcus aureus* (9, 10, 12, 13, 26, 27), *Burkholderia* (formerly *Pseudomonas*) *cepacia* (15, 35), and aspergillus (24, 26, 36) and candida (2, 6, 19, 20) species. The importance of oxidative antimicrobial mechanisms is further emphasized by the clinical importance of the above pathogens in afflicting CGD patients. While it should be noted that these patients are also susceptible to serious *E. coli* infections, there is no reason to presume a lesser-than-normal susceptibility of these patients to this moderately virulent organism.

The diverse elements of the antimicrobial armamentarium of neutrophils constitute an overlapping, broad-spectrum antimicrobial system in which the relative contribution of each component varies according to the species and growth phase (1, 18, 21, 29) of the pathogen encountered, as well as the metabolic microenvironment (e.g., aerobic or anaerobic) in which the phagocyte-microbe encounter takes place (5). We conclude that, for *E. coli*, there is a substantial redundancy of neutrophil bactericidal systems and that, in our system, participation of the MPO system was probably sufficient but not necessary for the observed microbicidal effects.

ACKNOWLEDGMENT

This work was supported by grant AI 25606 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service.

REFERENCES

1. **Bortolussi, R., C. M. Vandenbroucke-Grauls, B. S. van Asbeck, and J. Verhoef.** 1987. Relationship of bacterial growth phase to killing of *Listeria monocytogenes* by oxidative agents generated by neutrophils and enzyme systems. Infect. Immun. **55:**3197–3203.
2. **Diamond, R. D., R. A. Clark, and C. C. Haudenschild.** 1980. Damage to Candida albicans hyphae and pseudohyphae by the myeloperoxidase system and oxidative products of neutrophil metabolism in vitro. J. Clin. Invest. **66:**908–917.
3. **Dinauer, M. C., J. T. Curnutte, H. Rosen, and S. H. Orkin.** 1989. A missense mutation in the neutrophil cytochrome b heavy chain in cytochrome-positive X-linked chronic granulomatous disease. J. Clin. Invest. **84:**2012–2016.
4. **Elsbach, P., S. Beckerdite, P. Pettis, and P. Franson.** 1974. Persistence of regulation of macromolecular synthesis by *Escherichia coli* during killing by disrupted rabbit granulocytes. Infect. Immun. **9:**663–668.
5. **Elsbach, P., and J. Weiss.** 1983. A reevaluation of the roles of the O_2-dependent and O_2-independent microbicidal systems of phagocytes. Rev. Infect. Dis. **5:**843–853.
6. **Fleischmann, J., J. A. Church, and R. I. Lehrer.** 1986. Primary Candida meningitis and chronic granulomatous disease. Am. J. Med. Sci. **291:**334–341.
7. **Francke, U., H. D. Ochs, B. T. Darras, and A. Swaroop.** 1990. Origin of mutations in two families with X-linked chronic granulomatous disease. Blood **76:**602–606.
8. **Hamers, M. N., A. A. Bot, R. S. Weening, H. J. Sips, and D. Roos.** 1984. Kinetics and mechanism of the bactericidal action of human neutrophils against Escherichia coli. Blood **64:**635–641.
9. **Hampton, M. B., and C. C. Winterbourn.** 1995. Modification of neutrophil oxidant production with diphenyleneiodonium and its effect on bacterial killing. Free Radical Biol. Med. **18:**633–639.
10. **Humphreys, J. M., B. Davies, C. A. Hart, and S. W. Edwards.** 1989. Role of myeloperoxidase in the killing of *Staphylococcus aureus* by human neutrophils: studies with the myeloperoxidase inhibitor salicylhydroxamic acid. J. Gen. Microbiol. **135:**1187–1193.
11. **Hurst, J. K., J. M. Albrich, T. R. Green, H. Rosen, and S. J. Klebanoff.** 1984. Myeloperoxidase-dependent fluorescein chlorination by stimulated neutrophils. J. Biol. Chem. **259:**4812–4821.
12. **Jackson, S. H., J. I. Gallin, and S. M. Holland.** 1995. The p47phox mouse knock-out model of chronic granulomatous disease. J. Exp. Med. **182:**751–758.
13. **Johnston, R. B., III, R. J. Harbeck, and R. B. Johnston, Jr.** 1985. Recurrent severe infections in a girl with apparently variable expression of mosaicism for chronic granulomatous disease. J. Pediatr. **106:**50–55.
14. **Johnston, R. B., Jr., B. B. Keele, Jr., H. P. Misra, J. E. Lehmeyer, L. S. Webb, R. L. Baehner, and K. V. RaJagopalan.** 1975. The role of superoxide anion generation in phagocytic bactericidal activity. Studies with normal and chronic granulomatous disease leukocytes. J. Clin. Invest. **55:**1357–1372.
15. **Keusch, G. T., S. D. Douglas, and K. Ugurbil.** 1975. Intracellular bactericidal activity of leukocytes in whole blood for the diagnosis of chronic granulomatous disease of childhood. J. Infect. Dis. **131:**584–587.
16. **Klebanoff, S. J., and R. A. Clark.** 1978. The neutrophil. North-Holland Publishing Co. Amsterdam, The Netherlands.
17. **Klebanoff, S. J., A. M. Waltersdorph, and H. Rosen.** 1984. Antimicrobial activity of myeloperoxidase. Methods Enzymol. **105:**399–403.
18. **Lehrer, R. I., A. Barton, K. A. Daher, S. S. L. Harwig, T. Ganz, M. E. Selsted, and S. S. Harwig.** 1989. Interaction of human defensins with *Escherichia coli*. Mechanism of bactericidal activity. J. Clin. Invest. **84:**553–561.
19. **Lehrer, R. I., and M. J. Cline.** 1969. Leukocyte myeloperoxidase deficiency and disseminated candidiasis: the role of myeloperoxidase in resistance to *Candida* infection. J. Clin. Invest. **48:**1478–1488.
20. **Lehrer, R. I., and M. J. Cline.** 1969. Interaction of *Candida albicans* with human leukocytes and serum. J. Bacteriol. **98:**996–1004.
21. **Lehrer, R. I., T. Ganz, D. Szklarek, and M. E. Selsted.** 1988. Modulation of the in vitro candidacidal activity of human neutrophil defensins by target cell metabolism and divalent cations. J. Clin. Invest. **81:**1829–1835.
22. **Levy, O.** 1996. Antibiotic proteins of polymorphonuclear leukocytes. Eur. J. Haematol. **56:**263–277.
23. **Mannion, B. A., J. Weiss, and P. Elsbach.** 1990. Separation of sublethal and lethal effects of polymorphonuclear leukocytes on Escherichia coli. J. Clin. Invest. **86:**631–641.
24. **Odell, E. W., and A. W. Segal.** 1991. Killing of pathogens associated with chronic granulomatous disease by the non-oxidative microbicidal mechanisms of human neutrophils. J. Med. Microbiol. **34:**129–135.
25. **Okamura, N., S. Ishibashi, and T. Takano.** 1979. Evidence for bactericidal activity of polymorphonuclear leukocytes without phagocytosis. J. Biochem. (Tokyo) **86:**469–475.
26. **Pollock, J. D., D. A. Williams, M. A. Gifford, L. L. Li, X. Du, J. Fisherman, S. H. Orkin, C. M. Doerschuk, and M. C. Dinauer.** 1995. Mouse model of X-linked chronic granulomatous disease, an inherited defect in phagocyte superoxide production. Nat. Genet. **9:**202–209.
27. **Quie, P. G., J. G. White, B. Holmes, and R. A. Good.** 1967. In vitro bactericidal capacity of human polymorphonuclear leukocytes: diminished activity in chronic granulomatous disease of childhood. J. Clin. Invest. **46:**668–679.
28. **Rakita, R. M., B. R. Michel, and H. Rosen.** 1990. Differential inactivation of *Escherichia coli* membrane dehydrogenases by a myeloperoxidase-mediated antimicrobial system. Biochemistry **29:**1075–1080.
29. **Rest, R. F., and J. K. Spitznagel.** 1978. Myeloperoxidase-Cl^--H_2O_2 bactericidal system: effect of bacterial membrane structure and growth conditions. Infect. Immun. **19:**1110–1112.
30. **Rosen, H., and S. J. Klebanoff.** 1976. Chemiluminescence and superoxide production by myeloperoxidase-deficient leukocytes. J. Clin. Invest. **58:**50–60.
31. **Rosen, H., B. R. Michel, and A. Chait.** 1991. Phagocytosis of opsonized oil droplets by neutrophils: adaptation to a microtiter plate format. J. Immunol. Methods **144:**117–125.
32. **Rosen, H., J. Orman, R. M. Rakita, B. R. Michel, and D. R. VanDevanter.** 1990. Loss of DNA-membrane interactions and cessation of DNA synthesis in myeloperoxidase-treated *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**10048–10052.
33. **Selvaraj, R. J., J. M. Zgliczynski, B. B. Paul, and A. J. Sbarra.** 1978. Enhanced killing of myeloperoxidase-coated bacteria in the myeloperoxidase-H_2O_2-Cl^- system. J. Infect. Dis. **137:**481–485.
34. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35. **Speert, D. P., M. Bond, R. C. Woodman, and J. T. Curnutte.** 1994. Infection with Pseudomonas cepacia in chronic granulomatous disease: role of nonoxidative killing by neutrophils in host defense. J. Infect. Dis. **170:**1524–1531.
36. **Washburn, R. G., J. I. Gallin, and J. E. Bennett.** 1987. Oxidative killing of *Aspergillus fumigatus* proceeds by parallel myeloperoxidase-dependent and -independent pathways. Infect. Immun. **55:**2088–2092.
37. **Weiss, J., L. Kao, M. Victor, and P. Elsbach.** 1987. Respiratory burst facilitates the digestion of Escherichia coli killed by polymorphonuclear leukocytes. Infect. Immun. **55:**2142–2147.
38. **Weiss, J., M. Victor, O. Stendhal, and P. Elsbach.** 1982. Killing of gram-negative bacteria by polymorphonuclear leukocytes: role of an O_2-independent bactericidal system. J. Clin. Invest. **69:**959–970.

Editor: T. R. Kozel