Kinetics and Mechanism of the Bactericidal Action of Human Neutrophils Against Escherichia coli

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A mutant strain of Escherichia coli (E. coli ML-35) was used to follow the kinetics of phagocytosis, perforation of the bacterial cell envelope, and inactivation of bacterial proteins by human neutrophils. This particular E. coli mutant strain has no lactose permease, but constitutively forms the cytoplasmic enzyme $\beta$-galactosidase. This implies that the artificial substrate ortho-nitrophenyl-$\beta$-D-galactopyranoside cannot reach the $\beta$-galactosidase unless the bacterial cell envelope has been perforated. Thus, the integrity of the E. coli envelope can be measured simply by the activity of $\beta$-galactosidase with this substrate. Indeed, ingestion of E. coli ML-35 by human neutrophils was followed by perforation of the bacteria (increase in $\beta$-galactosidase activity). Subsequently, the $\beta$-galactosidase activity decreased due to inactivation of the enzyme. With a simple mathematical model and a curve-fitting computer program, we have determined the first-order rate constants for phagocytosis, perforation, and $\beta$-galactosidase inactivation. With 32 normal donors, we found an inter-donor variation in these rate constants of 20% to 30% (SD) and an assay variance of 5%. The perforation process closely correlated with the loss of colony-forming capacity of the bacteria. This new assay measures phagocytosis and killing in a fast, simple, and accurate way; it is not hindered by extracellular bacteria. Moreover, this method also measures the postkilling event of inactivation of a bacterial protein, which permits a better detection of neutrophils deficient in this function. The assay can also be used for screening neutrophil functions without the use of a computer program. A simple calculation suffices to detect neutrophil abnormalities. Neutrophils from patients with chronic granulomatous disease (CGD) showed an impaired rate of perforation and thus also of inactivation. Neutrophils from myeloperoxidase-deficient patients or from a patient with the Chediak-Higashi syndrome only showed a retarded inactivation of $\beta$-galactosidase, but normal ingestion and perforation. The role of myeloperoxidase in the killing process is discussed. Although myeloperoxidase does not seem to be a prerequisite for perforation, it probably plays a role in bacterial destruction by normal cells, because the inactivation of bacterial proteins seems strictly myeloperoxidase dependent.

With the use of a mutant strain of Escherichia coli, in which the lactose permease is absent and the cytoplasmic enzyme $\beta$-galactosidase is formed constitutively, we have shown previously that killing of E. coli by the myeloperoxidase (MPO)/hydrogen peroxide/chloride system proceeds via an increased permeability of the bacterial cell envelope. The properties of this particular E. coli mutant imply that the chromogenic substrate, ortho-nitrophenyl-$\beta$-D-galactopyranoside, cannot pass the bacterial cell envelope and thus cannot reach the $\beta$-galactosidase under normal conditions. Perforation of the E. coli cell envelope can thus be monitored simply by measuring the accessibility of the intracellular enzyme for its substrate.

The correlation between the perforation of the bacterial cell wall and the loss of colony-forming capacity has been demonstrated in this system and in preliminary experiments with human neutrophilic granulocytes. In the present article, the kinetics of the bactericidal action against E. coli by human blood neutrophils were studied with a simple mathematical model to evaluate the rate constants for phagocytosis, for perforation of the E. coli cell envelope, and for the inactivation of E. coli $\beta$-galactosidase.

Materials and Methods

Neutrophils

Neutrophilic granulocytes were isolated from fresh, defibrinated blood, according to Weening et al. The neutrophils were suspended in incubation medium (140 mmol/L NaCl, containing 10 mmol/L phosphate buffer, pH 7.4, 1.0 mmol/L CaCl$_2$, 0.6 mmol/L MgCl$_2$, 5.5 mmol/L glucose, and 10% (wt/vol) human albumin). The cell suspensions contained > 90% neutrophils; the remaining cells were mainly lymphocytes and some (< 3%) eosinophils.

Patients

Patients with chronic granulomatous disease (CGD) were diagnosed, clinically, by recurrent infections of skin, lungs, and lymph nodes with catalase-positive bacteria and yeasts, and biochemically, by failure of their blood granulocytes to mount a respiratory burst (O$_2$ consumption, NBT reduction on the single cell level, H$_2$O$_2$ formation) upon incubation with opsonized zymosan or phorbolmyristate acetate and the reduced capacity of these cells to kill Staphylococcus aureus in vitro. Patients with myeloperoxidase deficiency were detected by absence of peroxidase staining of their neutrophils and monocytes in a Hemalog-D system (Technicon Instruments Corp, Tarrytown, NY), and the myeloperoxidase was quantitated afterwards in isolated neutrophils, as described previously. The patient with the Chediak-Higashi syndrome was diagnosed and the neutrophil defects characterized as described by Weening et al.

E. coli

The E. coli mutant, ML-35 (lac $i^{-}$ z$^{-}$ y$^{-}$), was supplied by Dr A. Kepes, Institut Pasteur, Paris. The bacteria were grown aerobically...
at 37 °C overnight in nutrient broth (Difco Laboratories, Detroit), supplemented with 100 mmol/L NaCl. The next day, bacteria (6 × 10^8) were added to 10 mL of fresh medium and subsequently incubated at 37 °C for about three hours, until an optical density of 1.0 at 600 nm was reached (this equals 10^9 bacteria/mL). Bacteria were harvested by centrifugation (1,700 g, 15 minutes, 4 °C) and washed twice with phosphate-buffered saline, pH 7.4 (PBS, 140 mmol/L NaCl, 10 mmol/L sodium phosphate). The bacteria were suspended in incubation medium. These culture conditions of the bacteria were found to be essential for reproducible results in the killing assay: reculturing for more than about three hours (ie, when optical densities of > 1.0 are reached) produced bacteria that were more easily perforated by the neutrophils.

**Killing Assay (Standard Conditions)**

Bacteria (10^8/mL) were osazonized in incubation medium with 10% (vol/vol) pooled human AB serum for five minutes at 29 °C. At t = 5 minutes, 0.2 mL of the osazonized bacteria suspension was added to 1.8 mL of the neutrophil suspension (10^7 cells/mL) that had been prewarmed for ten minutes in a shaking water bath at 29 °C. The bacteria-to-neutrophil ratio was ten, and the final serum concentration in the killing assay was 1% (vol/vol).

At the indicated times, 0.1-mL samples were taken, and each sample was added to 0.9 mL of ice-cold phosphate-buffered-saline (PBS). The samples were stored on ice for maximally one hour before β-galactosidase was assayed. Furthermore, two samples of the osazonized bacteria were taken and diluted 100 times in PBS to measure the “background” β-galactosidase input of intact bacteria. One diluted sample of bacteria was sonicated (2 × 30 seconds at 0 °C, MSE sonicator, maximal power) to measure the total β-galactosidase input. The “background” β-galactosidase activity was always less than 2% of the total input and was subtracted from all other values. Sometimes, background values are higher than normal, probably due to reverted mutation: such cultures are to be discarded. This has happened to us only once in three years.

Two more samples from the incubations of neutrophils and bacteria were taken at t = 8 and 14 minutes, centrifuged (350 g, ten minutes, 4 °C), and resuspended in 1 mL of fresh medium and subsequently sonicated to expose the β-galactosidase activity of the ingested bacteria. Thereafter, β-galactosidase was measured (see below) to ascertain whether normal β-galactosidase had occurred (phagocytosis check).

When all samples had been taken, 1 mL of saponin (0.1% wt/vol; Merck, Darmstadt, W. Germany) was added to each sample to lyse the neutrophils (E. coli are resistant to this treatment). Thereafter, ortho-nitrophenyl-β-D-galactopyranoside (Sigma, St Louis) was added (final concentration 1 mmol/L), and the β-galactosidase assay was started by warming the tubes to 37 °C in a shaking water bath. After 30 minutes, the tubes were put on ice again, and the reaction was stopped by addition of 1 mL of 300 mmol/L glycine/NaOH buffer, pH 10.6. The amount of ortho-nitrophenol formed was calculated from the absorbance at 420 nm (absorbance coefficient 7.5 mmol/L cm^-1). The β-galactosidase activities of the samples were corrected for the “background” value and expressed as the fraction of the total input value. The resulting relative β-galactosidase activity was plotted against the sample time. Under these conditions, the intrinsic neutrophil β-galactosidase activity was negligible.

**Mathematical Model**

When human neutrophils (10^7 cells/mL) are incubated with 10 E. coli per neutrophil, phagocytosis behaves as a pseudo-first-order reaction with respect to the amount of bacteria. If we assume that the perforation of the E. coli cell envelope and the inactivation of the bacterial β-galactosidase are also first-order reactions, a model with three consecutive first-order reactions can be applied. This leads to the following reaction scheme:

\[
B + N \overset{k_1}{\rightarrow} P \overset{k_2}{\rightarrow} I
\]

where B is the concentration of bacteria and N is the concentration of neutrophils at the start of the killing assay; x, y, and z are the concentrations of free bacteria, ingested bacteria, and perforated bacteria, respectively, at time t after the start of the killing assay. The following differential equations have to be solved:

\[
\begin{align*}
\frac{dx}{dt} &= k_1Nx - k_2x + \frac{k_2}{k_3} (k_-k_1)(k-k_3) e^{-k_1t} \quad \text{with } x = B \text{ at } t = 0 \quad \text{and } z = 0 \\
\frac{dy}{dt} &= k_2y + k_2x - \frac{k_2}{k_3} (k_1-k_3)(k_1-k_3) e^{-k_1t} \\
\frac{dz}{dt} &= k_2y - k_2z + k_2z \quad \text{with } z = 0 \text{ at } t = 0.
\end{align*}
\]

The resulting expression for z as a function of time is:

\[
(4) \quad \left(\frac{z}{B}\right) = \frac{k_1k_2}{k_3-k_2} \left[ e^{-k_1t} - \frac{k_2}{k_3} \frac{(k_1-k_2)e^{-k_1t}}{(k_1-k_3)(k_1-k_3)} \right].
\]

The concentration of perforated bacteria, z, is measured as the β-galactosidase activity exposed by the neutrophils, whereas B (concentration of added E. coli) is taken as the input β-galactosidase activity measured in a sonicated E. coli sample. Thus, (z/B), in terms of β-galactosidase activity, represents the relative β-galactosidase activity as a function of time. The measured relative β-galactosidase activity at different times was fitted in expression (4), and the values of the three first-order rate constants were calculated by computer analysis. The computer program used was the Steepest Descent Method, described by Ruckdeschel with Marquarts algorithm. The described method was adapted to this three-dimensional problem. Because substitution of k2 = k1 and k3 = k1 in expression (4) leads to the same values for (z/B), it should be verified as to whether phagocytosis is normal. When phagocytosis is normal, k2 > k1 holds, and it is easy to find the right k values. Furthermore, the computer program should be started with the mean control k1, k2, and k3 as a first approximation, leading automatically to the right k values. In case phagocytosis is abnormal, no reliable values of k1, and k2 will be found without further experiments.

**RESULTS**

**Standard Conditions**

Figure 1 shows the β-galactosidase activity of E. coli ML-35 after phagocytosis by normal human neutrophils under standard assay conditions. Clearly, the β-galactosidase activity first increased because of phagocytosis and perforation of the bacteria. Thereafter, the β-galactosidase activity decreased again, due to inactivation of the bacterial protein. Computer analysis of this curve resulted in the following rate constants: for phagocytosis (k1) 0.216 min^-1; for perforation (k2) 0.057 min^-1; and for inactivation (k3) 0.090 min^-1. The computer-simulated curve (Fig 1, dotted line) closely fits the experimental points except at t = 60 minutes. In most controls, the computer-
BACTERICIDAL ACTION OF HUMAN NEUTROPHILS

Simulated curve shows lower relative β-galactosidase activities at t = 60 minutes, and sometimes also at t = 45 minutes, than the measured activities. This is probably due to a decrease in the metabolic activity of the cells at that time. The incubation temperature of 29°C was chosen because the process of phagocytosis and perforation is too fast at 37°C to measure the increase of β-galactosidase activity accurately.

Furthermore, it was tested whether the test samples had to be assayed for β-galactosidase activity immediately or could be kept on ice until the last sample had been drawn. No differences in the curves were observed, provided the saponin was added just before starting the β-galactosidase assay (not shown). So, during the period (one hour) in which the diluted samples were kept on ice, no further phagocytosis, perforation, or inactivation took place.

In each experiment, opsonized E. coli were also incubated for one hour without neutrophils (in the presence of 1% [vol/vol] human serum). In these samples, the β-galactosidase activity never exceeded the “background” value of the input activity. Thus, the opsonization and incubation procedures as such had no effects on the results.

To test the effect of the neutrophils on free bacteria during the incubation, the following experiment was performed (Fig 2). From each sample, the free E. coli were collected by centrifugation. These bacteria were assayed for β-galactosidase before and after sonication, while part of the bacteria were plated in triplicate to measure their colony-forming ability. Unsonicated, free bacteria showed input “background” β-galactosidase activity and thus were not perforated during the entire assay. The total amount of β-galactosidase in the sonicated, free bacteria corresponded with the amount of viable bacteria deduced from the plating assay (Fig 2). It is concluded from this experiment that free bacteria are neither perforated nor killed.

An optimal effect of opsonization was found under the standard conditions; longer opsonization times with 10% serum, or opsonization with higher serum concentrations, resulted in a slow perforation and killing of the E. coli in the control experiments without neutrophils (not shown).

To test the effect of the final serum concentration in the incubation medium (1% vol/vol), the following experiment was performed. After opsonization, part of the E. coli was washed twice and suspended again in the incubation medium. The standard procedure (with opsonized, unwashed E. coli and a final serum concentration of 1% [vol/vol]) was compared with the procedures with opsonized, washed E. coli, with or without the addition of 1% (vol/vol) serum. Although both procedures with 1% serum present during the assay gave slightly higher results, no significant differences were found (not shown).

The influence of the substrate concentration in the β-galactosidase assay was also checked. With ortho-nitrophenyl-β-D-galactopyranoside concentrations of 0.25, 0.50, and 1.00 mmol/L, almost identical curves were obtained, illustrating that the substrate concentration was not rate-limiting in the β-galactosidase assay. A concentration of 1.0 mmol/L was chosen as standard condition.

With this standard procedure, the neutrophils from 32 healthy donors were assayed, and the first-order rate constants were calculated. The mean bacteria-to-neutrophil ratio was 10.6 ± 1.1 (mean ± SD, N = 32). The following results were found (see Table 1): (1) the rate constant for phagocytosis (k1) is 0.187 ± 0.042 min⁻¹; (2) the rate constant for perforation (k2) is 0.054 ± 0.011 min⁻¹; and (3) the rate constant for inactivation (k3) is 0.118 ± 0.037 min⁻¹. The variance
of the individual data points, evaluated from 44 duplicate measurements, was 5%. The rate of phagocytosis ($k_1$) was also measured in a direct assay with radiolabeled E. coli. In two experiments, a $k_1$ of 0.14 min$^{-1}$ and 0.19 min$^{-1}$ was found, which is in reasonable agreement with the calculated mean value of 0.187 min$^{-1}$. This indicates that phagocytosis under these conditions indeed follows first-order kinetics.

We conclude from these experiments that this method can be used under standard conditions to screen neutrophils for killing disorders and that the mathematical model is a reasonable approximation for the kinetics of the complex intracellular processes of perforation and bacterial breakdown.

**Killing Disorders**

To demonstrate the correlation between the perforation of E. coli bacteria and the killing phenomenon, the following experiment was performed (Fig 3). E. coli were incubated with normal neutrophils under standard assay conditions. At the indicated times, parallel samples were taken. In one series of samples, the β-galactosidase activity was measured as described above. The samples from the other series were diluted appropriately and plated in triplicate on solidified nutrient broth. The plates were incubated overnight at 37 °C, and the number of colony-forming units were counted. Figure 3 shows that when the maximum β-galactosidase activity has been reached (at 20 minutes), about 75% of the added E. coli have been killed. After one hour, 97% of the added bacteria were killed, demonstrating that sample times beyond 60 minutes are not needed under these conditions to evaluate the bactericidal action of normal neutrophils.

The effect of neutrophils from a patient with CGD on the β-galactosidase activity of phagocytosed E. coli is shown in Fig 4A. A very slow increase in β-galactosidase was observed, and the calculated first-order rate constants showed a significant decrease in the rate constant for perforation ($k_2$) and in the rate constant for inactivation ($k_3$). The same was found for two other CGD patients (see Table 1). In parallel experiments, the E. coli were found to survive ingestion by these neutrophils, as judged by the criterion of colony-forming ability (Fig 4B).

In parallel to the experiment described above, samples were taken that were washed to remove free bacteria and subsequently sonicated to expose the β-galactosidase activity of the ingested bacteria (phagocytosis check; see Materials and Methods). Figure 4C shows the results. In normal neutrophils, an optimum in the β-galactosidase activity was reached 20 minutes after addition of the bacteria to the cells. At that time, only about 65% of the input β-galactosidase activity was recovered, although all bacteria had been ingested (as judged from microscopic examination and from phagocytosis studies with radiolabeled E. coli). Apparently, the normal neutrophils had inactivated already about 35% of the input β-galactosidase activity at that time. In contrast, the β-galactosidase activity of bacteria ingested by CGD neutrophils was fully recovered at 20 minutes, indicating that these cells indeed inactivated bacterial proteins to a minor extent.
Figure 5 shows the effect of neutrophils from a patient with MPO deficiency (<5% residual activity) on the β-galactosidase activity of ingested E. coli. Analysis of the curve revealed a significant decrease in the rate constant $k_3$ for inactivation (see Table 1). A comparable curve, revealing the same decrease in $k_3$, was found with neutrophils from a patient with the Chediak-Higashi syndrome (Fig 6 and Table 1).

Similar results were obtained with two other MPO-deficient patients, under slightly different assay conditions. Washed and sonicated samples again revealed a very high recovery (80% to 90%) of the β-galactosidase activity in the ingested bacteria, comparable with the results shown with CGD neutrophils in Fig 4B. These curves were mimicked by treatment of normal neutrophils with sodium azide (2 mmol/L) to inhibit myeloperoxidase (not shown).

DISCUSSION

Assay

This article describes a simple and rapid technique to measure the killing of E. coli by human neutrophils. This new assay avoids the disadvantages of the classic assay with triplicate plating and counting of colonies after overnight incubation. The counting of colonies is time-consuming, and the assay is not very accurate (variance 12%). Moreover, extracellular bacteria have to be killed or removed before plating, if intracellular killing has to be measured. This can be achieved either by the addition of antibiotics (or lysostaphin when staphylococci are used), which slowly penetrate the
neutrophils and lead to an overestimation of the killing
capacity, or by centrifugation and washing of the
samples, which may lead to loss of neutrophils with
ingested bacteria.

Our new method avoids the problem of extracellular
bacteria, because unphagocytosed bacteria are not
perforated and thus do not contribute to the \( \beta \)-galactosi-
dase assay. Furthermore, the use of radiolabels, as
used in alternative killing assays (eg, ref 13), is also
avoided. Another advantage of our assay is the possi-
bility of analyzing the relatively fast kinetics of dif-
cerent cellular processes related to the killing and
destruction of the bacteria. For instance, in our hands,
neutrophils from patients with a myeloperoxidase defi-
ciency only sometimes show a slight retardation in
killing when assayed with \( S. \) aureus in the standard
plating technique at 37 °C, whereas in the present
technique, the “postkilling” inactivation of \( \beta \)-galactosi-
dase activity was found to be significantly abnormal.
Thus, a better resolution between normal and abnor-
mal neutrophils has now been obtained.

Analysis of the curves obtained in our assay can also
be performed without a curve-fitting computer pro-
gram, in the following way. Because the three rate
constants govern the shape of the curves, and thus also
the maximal \( \beta \)-galactosidase activity exposed at a
certain time, this maximum is a characteristic of the
curves. The 32 controls show a maximum relative
\( \beta \)-galactosidase activity of 0.211 ± 0.042 (mean ± SD)
at time \( t = 17.6 ± 3.2 \) minutes. When this \( \beta \)-galactosi-
dase value is compared with the relative \( \beta \)-galactosi-
dase activity exposed by neutrophils of different
patients, a significant decrease is observed in CGD
patients, and a significant increase is observed in the
MPO-deficient patients and in the Chediak-Higashi
patient.

When the opsonized \( E. \) coli are attached to the
neutrophils, but not phagocytosed, a normal phagocy-
tosis check will be found, but an abnormal killing must
be expected. To discriminate between CGD and the
above defect, additional experiments are needed (such
as \( O_2 \) consumption or \( H_2O_2 \) production measure-
ments). However, phagocytosis deficiencies are rare
and have not yet been tested in our assay. When the
opsonized \( E. \) coli are not attached to the neutrophils,
the phagocytosis check will be abnormal, and neutro-
phils with this defect can thus be easily discriminated
from CGD neutrophils, which show a normal phagocy-
tosis.

Although, for convenience, \( 2 \times 10^7 \) neutrophils are
used under standard conditions, this amount can be
decreased to \( 4 \times 10^6 \) neutrophils per assay (ten sam-
ple) by a decrease in the incubation volume to 0.4 mL
and in the sample volume to 0.02 mL, together with a
corresponding increase in the time of the \( \beta \)-galactosi-
dase assay. This “small-scale” assay is particularly
useful in investigating the bactericidal activity of neu-
trophils from very young children. Another simplifica-
tion can be reached by using 3% (wt/vol) sodium
cholate (final concentration) instead of sonication of
the 100% sample and the phagocytosis check samples.
Identical results, as compared to the standard assay,
are obtained with the above modifications (unpub-
lished results).

The new technique (standard conditions) is used in
our laboratory as a screening assay for the diagnosis of
killing disorders. In the first two years, the new assay
was used together with the classical plating technique
with \( S. \) aureus. No false-positive or false-negative
results were observed with the new technique during
this period. Since then, the plating technique has been
abandoned.

The principle of our assay (use of a membrane-
impermeable substrate for a cytoplasmic enzyme) can
be applied to any other microorganism of choice, provided interference of neutrophilic enzymes is negli-
gible.

**Effect of Serum Factors**

Opsonization of \( E. \) coli in 10% (vol/vol) pooled
serum for longer than five minutes or opsonization for
five minutes in higher serum concentrations (> 10%)
led to perforation of the \( E. \) coli in the control experi-
ments without neutrophils. This effect is probably due
to serum complement action on the permeability prop-
erties of the inner membrane of the \( E. \) coli.

Opsonization of \( E. \) coli in low concentrations of
serum (≤ 2% vol/vol) or in heat-inactivated serum led
to impaired phagocytosis and impaired triggering of
the respiratory burst (not shown).

**Function of Myeloperoxidase**

From the experiments with the MPO-deficient (Fig
5) or azide-treated neutrophils, it follows that MPO
itself is not needed to perforate and kill \( E. \) coli, in
agreement with the lack of recurrent infections in most
patients with MPO deficiency. Furthermore, the
results obtained with the neutrophils from the patient
with the Chediak-Higashi syndrome (Fig 6) support
this conclusion. Because the release of MPO from
these cells upon stimulation has been found to be
grossly impaired, one could speculate that the fusion
of the MPO-containing azurophilic granules with the
phagosomes will also be impaired. Thus, these neutro-
phils might mimic an MPO deficiency.

The difference between these two types of neutro-
phil with CGD neutrophils is the ability to generate
hydrogen peroxide. MPO-deficient cells produce even
more hydrogen peroxide than do normal neutrophils.\textsuperscript{1,6} It is conceivable that the lack of MPO, and thus the lack of hypochlorous acid production, which is quite high in normal cells,\textsuperscript{18} is compensated by an increased hydrogen peroxide production.

In our previous study,\textsuperscript{1} we had shown that the \textit{E. coli} could only be perforated when the complete MPO-H\textsubscript{2}O\textsubscript{2}-Cl\textsuperscript{-} system or hypochlorous acid was applied. Hydrogen peroxide alone (0.5 mmol/L) was unable to expose the \(\beta\)-galactosidase. However, in the present report, we suggest that H\textsubscript{2}O\textsubscript{2} compensates for the lack of myeloperoxidase in MPO-deficient cells. The reason for this apparent discrepancy is the difference between the incubation conditions in both studies. In our previous study,\textsuperscript{1} unopsonized \textit{E. coli} were used, whereas in the present study, opsonized \textit{E. coli} were employed. Indeed, we found that opsonized \textit{E. coli} are susceptible to hydrogen peroxide: \(\beta\)-galactosidase is exposed and the bacteria are killed. The rate of perforation and killing is dependent on the amount of hydrogen peroxide as well as on the opsonization procedure (unpublished results).

Thus, it is conceivable that hydrogen peroxide can compensate for the lack of myeloperoxidase. Furthermore, it should be realized that other H\textsubscript{2}O\textsubscript{2}-dependent mechanisms may also play a role in the aerobic killing of bacteria.

The function of MPO in the inactivation of \(\beta\)-galactosidase is evident from the significant decrease in \(k_3\) in MPO-deficient neutrophils, in azide-treated neutrophils, and in Chediak-Higashi neutrophils. Furthermore, it has been shown that hypochlorous acid is very reactive with proteins containing an active sulfhydryl group. For instance, the iron-sulphur centers in the bacterial respiratory chain are inactivated very quickly by minute concentrations of hypochlorous acid.\textsuperscript{19,20} Thus, the function of MPO might be the fast inactivation of bacterial respiration and subsequent perforation of the bacterial membrane, followed by inactivation of cytoplasmic enzymes. In this way, myeloperoxidase might help in the destruction of microorganisms.

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