Myeloperoxidase targets oxidative host attacks to *Salmonella* and prevents collateral tissue damage

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Supplementary Information

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**Supplementary Note**

**Computational model parameters**

We build a diffusion-reaction model based on a previous neutrophil phagosome model\(^1\) combined with experimental data on *Salmonella* protective enzyme expression as obtained by *ex vivo* proteomics\(^2\), estimated bacterial ROS production\(^3,4\), adjusted vacuole volume based on electron microscopy\(^5\), and updated reaction kinetics and diffusion constants based on recent reports. Our combined model included four compartments (host cell cytosol, phagosome lumen, *Salmonella* periplasm, *Salmonella* cytosol), 8 small molecules, 10 host and bacterial enzymes, and 27 different reactions including generation and interconversion of various reactive oxygen species (ROS), their diffusion across the phagosomal membrane and the two *Salmonella* membranes, as well as ROS detoxification by *Salmonella* superoxide dismutases, catalases, and peroxidases with quantitative abundance data based on our *ex vivo* proteomics results\(^13\).

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*Salmonella* / phagosome geometry

*Salmonella* shape was approximated as a cylinder with a length of 2 µM and a diameter of 0.8 µM (ccdb.wishartlab.com/CCDB/intron_new.html). We assumed an outer membrane area of 5.8x10^{-12} m^2, an inner membrane area of 5.4x10^{-12} m^2, a periplasm volume of 5.7x10^{-17} l, and a cytoplasm volume of 8.3x10^{-16} l. The phagosomal membrane was assumed to enclose one single *Salmonella*\(^5\). The distance between phagosomal membrane and *Salmonella* outer membrane was set to 200 nm based on TEM images\(^5\). This yielded a phagosomal membrane area of 1.1x10^{-11} m^2, a total phagosome volume of 2.59x10^{-15} l and a phagosome lumen of 1.7x10^{-15} l (excluding the volume occupied by *Salmonella*).

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Generation of reactive oxygen species

*Salmonella* was assumed to endogenously generate O_2^- in periplasm and cytoplasm at rates of 3 µM s^{-1} and 5 µM s^{-1}, respectively\(^3\); and 10 µM s^{-1} H_2O_2 in the cytoplasm based on a total generation of 14 µM s^{-1} H_2O_2, which included 4 µM s^{-1} due to dismutation of endogenously generated O_2^-\(^4\). Neutrophil phagosomes were assumed to generate O_2^- at rates of 5.2x10^{-3} mol l^{-1} s^{-1} in the phagosome lumen\(^1\), and to contain 1 mM MPO (normal levels)\(^1\). Rates for the various reactions of MPO and chloride concentration (100 mM) were used as reported\(^1\).
Membrane permeability for reactive oxygen species

For superoxide in the protonated form $\text{HO}_2^\cdot$, membrane permeability was set to $9 \times 10^{-6}$ m s$^{-1}$ for all three membranes (phagosomal membrane, *Salmonella* outer and inner membranes)$^6$, while the deprotonated form was assumed to permeate poorly ($< 10^{-9}$ m s$^{-1}$) based on reported values for liposomes$^6$.

For $\text{H}_2\text{O}_2$, a membrane permeability of $3.2 \times 10^{-5}$ m s$^{-1}$ was assumed based on the reported permeability of intact *E. coli* ($1.6 \times 10^{-5}$ m s$^{-1}$; two membranes)$^7$. The phagosomal membrane was assumed to have the same permeability based on the range of reported values for mammalian membranes$^8$.

$\text{OCl}^-$ was predicted to have a short reach (33 nm) based on phagosomal protein concentrations$^1$, reaction rate constants$^9$, and its diffusion coefficient$^{10}$. This short reach prevented leakage of $\text{OCl}^-$ through membranes.

Spontaneous dismutation of superoxide

Superoxide dismutation depends on its protonation state. The total dismutation rate is the sum of rates of two different mechanisms, (i) $\text{HO}_2^\cdot + \text{O}_2^- + \text{H}_2\text{O} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 + \text{HO}^-$ with a second order rate constant $k_{AB} = 8.5 \times 10^7$ M$^{-2}$ s$^{-1}$; (ii) $2 \text{HO}_2^\cdot \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$, $k_{AA} = 7.6 \times 10^5$ M$^{-2}$ s$^{-1}$. The relative proportions of $\text{HO}_2^\cdot + \text{O}_2^-$ were calculated based on its pKa of 4.88$^{11,12}$. We assumed pH 7.4 in the neutrophil phagosome lumen and *Salmonella* periplasm in macrophages.

Detoxification of reactive oxygen species by *Salmonella* enzymes

Absolute *Salmonella* in vivo enzyme copy numbers were obtained from our recent study$^{13}$:
SodCI, 39’800 ± 8’300 copies per *Salmonella* cell; SodCII, 370 ± 200; SodA, 4’000 ± 1’000;
SodB, 10’200 ± 2’100; KatG, 2’600 ± 500; KatE and KatN, below detection limit; Tpx, 22’000 ± 4’000; AhpC, 15’200 ± 3’500; AhpF, 1’000 ± 400; TsaA, 21’700 ± 3’600.
We used the following kinetic parameters for these enzymes: SodCl, SodCII, $k_{\text{cat}}/K_M = 4 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ diffusion-limited$^{14}$; SodA, SodB, $k_{\text{cat}}/K_M = 7 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ diffusion-limited$^{15}$; KatG, $k_{\text{cat}} = 14'000 \text{ s}^{-1}$, $K_M = 5.9 \text{ mM}^{16}$; Tpx, $k_{\text{cat}} = 76 \text{ s}^{-1}$, $K_M = 1.7 \text{ mM}^{17}$; AhpC (and its paralog TsaA), $k_{\text{cat}} = 55.1 \text{ s}^{-1}$, $K_M = 1.4 \mu\text{M}^{18}$; AhpF, $k_{\text{cat}} = 25.5 \text{ s}^{-1}$, $K_M = 14.3 \mu\text{M}^{18}$).
a) Intact cells vs. lysed cells

b) Effects of ABAH on Annexin V and PI staining

- Positive cells
- Annexin Vhi PIlo
- Annexin Vhi PIhi

% Inhibition

ABAH (μM): 0.1, 1, 10, 100

3uM 200uM

C) MPO activity and H2O2 release

- ABAH (μM): 100
- DPI

D) Oxygen consumption vs. H2O2 release

- Normal
- Def.

E) Oxygen consumption vs. H2O2 release for Salmonella and Candida

F) MFI of CD66, CD63, and MPO activity

- ABAH
- DPI

G) Annexin V and PI fluorescence

- Annexin Vhi PIlo
- Annexin Vhi PIhi

H) Correlation between MPO activity and MPXI

r = 0.60
P = 0.0012

r = -0.67
P = 0.0003

I) MPO activity and H2O2 release
Supplementary Figure 1: Reactive oxygen species generation and leakage of human neutrophils in vitro.

a, Inhibition of myeloperoxidase activity by ABAH in intact or lysed neutrophils. Data (means and standard deviations of six technical triplicates) from one representative experiment out of two are shown. The dotted lines indicate 50% activity and the corresponding ABAH concentrations for lysed or intact cells, respectively. b, MPO activity measured as APF oxidation in neutrophils from seven different normal donors after 60 min stimulation in presence/absence of the MPO inhibitor ABAH (AB) or the NADPH oxidase inhibitor DPI (Wilcoxon test; *, P < 0.05). c, MPO activity and H$_2$O$_2$ release of neutrophils from eight different normal donors (black circles), two partially deficient donors (grey), and two severely deficient donors (empty circles) during stimulation with Candida in presence/absence of the MPO inhibitor ABAH (AB) or the NADPH oxidase inhibitor DPI (Kruskal-Wallis multiple comparisons test; **, P < 0.01). d, Relationship between oxygen consumption and H$_2$O$_2$ release in neutrophils stimulated with heat-killed Candida for 75 min. Mean values of triplicate wells containing 100’000 neutrophils are shown. e, Impact of inhibiting myeloperoxidase with ABAH, or NADPH oxidase with DPI, on oxygen consumption rate after stimulation with heat-killed Salmonella and Candida. f, Impact of inhibitors on neutrophil degranulation. Cells from three different donors were stimulated with PBS (Contr), heat-killed Salmonella (Salm), Candida (Cand), or PMA for 75 min in presence/absence of the MPO inhibitor ABAH (AB) or the NADPH oxidase inhibitor DPI. Surface expression of degranulation markers CD63 and CD66 was measured by flow cytometry. MPO release to the extracellular medium was quantified using ELISA. Different stimuli triggered the three degranulation processes to a various degree, but all three assays demonstrated no impact of inhibitors ABAH and DPI on neutrophil degranulation. g, Impact of 500 μM ABAH and 10 μM DPI on neutrophil viability. The fraction of cells undergoing apoptosis [annexinV$^{hi}$ propidium iodide (PI)$^{lo}$, left] and cells undergoing necrosis (annexinV$^{hi}$ PI$^{hi}$, right) at various times after inhibitor addition is shown. Means and standard deviations for four donors measured in one experiment are shown. In all other experiments of this study, cells were exposed to these inhibitors for a maximum of 75 min. h, Relationship between donor mean peroxidase index (MPXI) and HOCl generation (left) or H$_2$O$_2$ release (right) after stimulation with live or heat-killed Salmonella (r, Spearman correlation coefficient). Each dot represents data for an individual human donor. i, MPO activity and H$_2$O$_2$ release of neutrophils from seven different normal donors during stimulation with 1 nM PMA in presence/absence of the MPO inhibitor ABAH (AB) or the NADPH oxidase inhibitor DPI (Wilcoxon test; *, P < 0.05).
Supplementary Figure 2: Lipid peroxidation in uninfected spleen as detected by an antibody to 4-hydroxynonenal (4-HNE).

Representative micrograph of a spleen cross-section from one out of four uninfected MPO-deficient mice. CD11b<sup>hi</sup> cells populate the red pulp. 4-HNE signals are undetectable in these areas, whereas some background staining is visible in the white pulp areas (which are negative for CD11b). The scale bar represents 500 μm.
Supplemental References


