Mild Hyperthermia Downregulates Receptor-dependent Neutrophil Function

Dieter Fröhlich, M.D.*, Sigrid Wittmann, M.D.¶, Gregor Rothe, M.D.†, Daniel I. Sessler, M.D.¶, Peter Vogel, M.D.§, and Kai Taeger, M.D.@

* Associate Professor, Department of Anesthesia, University of Regensburg.
¶ Resident, Department of Anesthesia, University of Regensburg.
† Associate Professor, Department of Clinical Chemistry and Laboratory Medicine, University of Regensburg.
# Vice Dean for Research and Associate Vice President for Health Affairs, Director Outcomes Research™ Institute, Lolita & Samuel Weakley Distinguished University Research Chair, Professor of Anesthesiology and Pharmacology, University of Louisville.
§ Associate Professor, Department of Surgery, University of Regensburg.
@ Professor and Chair, Department of Anesthesia, University of Regensburg.

Abstract

Mild hypothermia impairs resistance to infection and, reportedly, impairs phagocytosis and oxidative killing of un-opsonized bacteria. We evaluated various functions at 33 to 41°C in neutrophils taken from volunteers. Adhesion on endothelial cells was determined using light microscopy. Adhesion molecules expression and receptors, phagocytosis, and release of reactive oxidants were assessed using flow cytometric assays. Adhesion protein CD11b expression on resting neutrophils was temperature independent. However, upregulation of CD11b with TNF-α was increased by hypothermia and decreased with hyperthermia. Neutrophil adhesion to either resting or activated endothelial cells was not temperature dependent. Bacterial uptake was inversely related to temperature, more so with *E. coli* than *S. aureus*. Temperature dependence of phagocytosis occurred only with opsonized bacteria. Hypothermia slightly increased N-Formyl-L-methionyl-L-leucyl-phenylalanine (FMLP) receptors on neutrophils: hyperthermia decreased expression, especially with TNF-α. FMLP-induced *H₂O₂* production was inversely related to temperature, especially in the presence of TNF-α. Conversely, phorbol-13-myristate-12-acetate, an activator of protein kinase C, induced an extreme and homogenous release of reactive oxidants that increased with temperature. In contrast to non-receptor dependent phagocytosis and oxidative killing, several crucial receptor-dependent neutrophil activities show temperature-dependent regulation, with hypothermia increasing function. The temperature dependence of neutrophil function is thus more complicated than previously appreciated.

Address correspondence to Dieter Fröhlich, M.D.: Department of Anesthesia, University of Regensburg, Franz-Josef-Strauß-Allee 11, Regensburg, 93053, Germany Tel: +49-941-944-7801, Fax: +49-941-944-7802. Email: dieter.froehlich@klinik.uni-regensburg.de. On the world wide web: www.or.org.

Received from the Departments of Anesthesia, Clinical Chemistry and Laboratory Medicine, and Surgery, University of Regensburg, Regensburg, Germany; the Outcomes Research™ Institute and Departments of Anesthesiology and Pharmacology, University of Louisville, Louisville, KY.

Supported by Deutsche Forschungsgemeinschaft #FR1165/1-2 (Bonn, Germany), NIH Grant GM 061655 (Bethesda, MD), the Joseph Drown Foundation (Los Angeles, CA), and the Commonwealth of Kentucky Research Challenge Trust Fund (Louisville, KY). Dr. Sessler has a personal financial interest in Radiant Medical, Inc. Presented in part at the annual meeting of the International Anesthesia Research Society in Los Angeles, March 12-16, 1999.
Keywords

Adhesion; Immunology; Infection; Leukocytes; Oxidative killing; Phagocytosis; Temperature; Thermoregulation

Implications Statement

In contrast to non receptor-dependent phagocytosis and oxidative killing, several crucial receptor-dependent functions of neutrophils have a temperature-dependent regulation, with increased function during hypothermia and reduced function during hyperthermia.

Introduction

Unwarmed surgical patients typically become 2–3°C hypothermic (core temperature of 34–35°C) because anesthetics impair thermoregulatory control (1). Mild core hypothermia impairs wound healing and resistance to infection in animals (2–4) and humans (5). There are at least two mechanisms by which hypothermia might impair resistance to surgical wound infection. The first is that thermoregulatory vasoconstriction decreases tissue perfusion and local oxygen partial pressure (6,7). Oxygen is required for scar formation (8) and is a substrate for oxidative killing by neutrophils (9). Consistent with this theory, tissue oxygen tension correlates well with the incidence of clinical infection (10) and supplemental oxygen reduces infection risk (11).

The second mechanism is that hypothermia may directly impair immune function (12). Neutrophils are the major defense against surgical pathogens. Neutrophils clear bacteria from infected and damaged tissue in stages (13). At the infection site, neutrophils roll along the vessel wall. This so-called rolling adhesion is regulated by L-selectin and its counter-part at the endothelial lining (14). After adhering, neutrophils migrate through the vessel wall, being guided to the area of infection by chemoattractants such as Interleukin-8 (IL-8) or N-Formyl-L-methionyl-L-leucyl-phenylalanine (FMLP). Subsequent degradation of bacteria involves phagocytosis first and then killing, which is mediated by release of oxygen radicals, proteases, and other bactericidal products (Fig. 1).

The effects of mild hypothermia on neutrophil function in vitro and in vivo have been evaluated by Wenisch, et al. (15). They reported that mild hypothermia profoundly impairs both phagocytosis and oxidative killing. A limitation of this study, though, is that neutrophils were stimulated by un-opsonized bacteria. This situation is non-physiological in that bacteria are probably always opsonized in vivo. More importantly, un-opsonized bacteria provoke activation of oxidative killing through a phagosomal pathway.

It is likely, though, that at least some measure of regulatory control is maintained even in contaminated and injured tissue. We therefore evaluated the temperature dependence of various neutrophil functions under conditions in which normal cellular control mechanisms remain intact. As a positive control, we also evaluated oxidative killing during PMA (phorbol-13-myristate-12-acetate) stimulation (16). PMA is a protein kinase C activator that bypasses receptor-mediated control over free radical production, thus mimicking the “oxidative potential” reported previously (15).
Methods

The local Ethics Board of the University of Regensburg Medical School approved this study. After informed consent, venous blood was drawn from six healthy donors with no history of infection in the two prior weeks.

Donors ranged in age from 27 to 38 years (mean 32). Average white blood cell count was 6,300 ± 1,200 cells/µl (mean ± SD), and hemoglobin concentration was 15.1 ± 1.3 g/dl. Differential leukocyte counts were as follows: neutrophils 53 ± 8%, lymphocytes 35 ± 7%, monocytes 8 ± 3%, eosinophils 3 ± 2%, and basophils 1 ± 1%. Complete blood count and the differential count were determined prior to each experiment using an Advia 120 counter (Bayer, Tarrytown, NY, USA).

Leukocyte preparation and quantification of H₂O₂ production

Leukocytes were isolated by sedimentation of erythrocytes on a Ficoll-Paque gradient (Pharmacia, Upsalla, Sweden). Heparinized (10 U/ml) whole blood (3 ml) was layered upon 3 ml lymphocyte separation medium (Ficoll, density 1.077 g/ml). Erythrocytes aggregated at the interface and settled at room temperature without centrifugation. After 40 minutes, the upper 800 µl of the supernatant leukocyte-rich plasma was withdrawn, avoiding contact with plasma near the separation medium interface. To avoid artifactual activation of cells, the isolation process did not include lysis, centrifugation, or washing.

The supernatant leukocyte-rich plasma was suspended 1:50 in Dulbecco’s PBS with Ca²⁺ to a total volume of 1 ml. The leukocytes were loaded with the fluorogenic substrates dihydrorhodamine 123 (DHR) and Carboxy-seminaphthorhodafluor-1-acetoxyethylster (SNARF1/AM) for 10 minutes (both dyes from Molecular Probes, Eugene, OR, USA). The final concentrations were 1 µM for DHR and 0.1 µM for SNARF1/AM. After addition of TNF-α (10 ng/ml; DPC Biermann, Bad Nauheim, Germany), samples were incubated for 30 minutes at 33, 35, 37, 39, or 41°C. Next, N-formyl-L-methionyl-L-leucyl-phenylalanine (FMLP) was added at a final concentration of 10⁻⁷ M to stimulate H₂O₂ production by neutrophils (Sigma Chemicals, Deisenhofen, Germany). After 15 minutes of incubation at the designated temperature, the reaction was stopped by putting the samples on ice. Dead cells were counterstained with propidium iodide (PI; Serva, Heidelberg, Germany) at a final concentration of 30 µM. The specimens were stored on ice in the dark and measured within one hour.

In all experiments a separate tube with an amount of fluid comparable to the experimental tubes was processed in parallel. The tube was equipped with a thermocouple probe to ensure the correct experimental temperature and temperature equilibrium during the experiments.

For analysis, we used a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) with argon ion laser excitation at 488 nm and measured 10,000 cells from each stained sample. Data were acquired and processed using Cell Quest software. Following calibration with standard dye-beads (Quantum 26; Flow Cytometry Standards Europe, Leiden, The Netherlands), the results of the cellular fluorescence were expressed in Molecule Equivalents of Soluble Fluorochrome (MESF). These MESF units were used for the absolute quantification of cellular fluorescence, allowing inter-assay and inter-laboratory comparison of data. Dead cells were identified and excluded by their lack of esterase activity and their propidium iodide fluorescence above 600 nm. Leukocyte esterase activity was determined based on SNARF1-related orange fluorescence. SNARF1/AM is cleaved in vital leukocytes by esterases to SNARF1. Neutrophils were identified by their typical side scatter light (SSC) and forward scatter light (FSC) patterns, and their esterase-activity. SSC depends on the granularity of cells, whereas FSC is related to cell size.

Anesth Analg. Author manuscript; available in PMC 2005 November 2.
The small amount of intracellular H$_2$O$_2$ generated was quantified by measuring intracellular oxidation of the indicator dye dihydrorhodamine 123 to rhodamine 123. The former is a non-fluorescent and membrane-permeable fluorogenic substrate, whereas the latter oxidation product emits a green light (510–530 nm) upon excitation (17). In fact, this method has several advantages over those previously used for quantifying neutrophil oxidative function. Purification of neutrophils, which often leads to artifactual activation of cells, is unnecessary. Flow cytometric results are also unaffected by variations in the concentration of neutrophils in the assay, since the oxidative response is analyzed on the single cell level. And finally, flow cytometry reveals a heterogeneous oxidative response following receptor-dependent stimulation with FMLP (100 nM) whereas unstimulated neutrophils demonstrate very low fluorescence or free oxygen radical generation. The ratio between reacting and non-reacting neutrophils upon stimulation with FMLP is modified in the presence TNF-$\alpha$ towards a higher number of neutrophils releasing H$_2$O$_2$. In contrast, PMA, which served as a positive control, induces a homogeneous, very high response of neutrophils (Fig. 2).

Expression of FMLP receptors

To quantify the expression of receptors for FMLP, we used the assay established by Allen and colleagues without modification (18). Leukocyte-rich plasma was suspended in Dulbecco's PBS (1:50) and, after addition of TNF-$\alpha$ (final concentration 10 ng/ml), samples were incubated for 30 minutes at 33, 35, 37, 39, or 41°C. Controls remained unstimulated and were processed in parallel. The samples were subsequently cooled to 4°C to avoid further activation of neutrophils. For staining of FMLP receptors, fluorescein labeled formyl-Nle-Leu-Phe-Nle-Tyr-Lys (FLPEP) was added in a final concentration of 100 nM, incubated for 20 minutes at 4°C, and washed twice. FLPEP, a highly specific binding analog of FMLP, fluoresces at 520 nm when excited at 488 nm (Molecular Probes, Eugene, OR, USA). Performing flow cytometric analysis, identification of polymorphonuclear leukocytes based on their side scatter (SSC) characteristic. Non-specific binding of FLPEP was determined by addition of excess FMLP (0.1 mM) in a parallel set of tubes.

Expression of adhesion molecules

Leukocytes were isolated by sedimentation of erythrocytes on a Ficoll-Paque gradient (Pharmacia, Upsalla, Sweden) as described above. Leukocyte-rich plasma was diluted 1:50 in Dulbecco's PBS to a total volume of one ml. We were able to exclude significant differences in expression of PMN surface markers by performing experiments with whole blood and leukocyte-rich plasma in parallel. After addition of TNF-$\alpha$, samples were incubated for 30 min at 33, 35, 37, 39, or 41°C. Samples were then gently centrifuged and the volume reduced to 100 µl. Fluorescence labeled monoclonal antibodies to CD11b (CD11b FITC; Medac, Hamburg, Germany), CD62L (CD62L FITC; Immunotech, Krefeld, Germany) were added and samples were stored at 4°C for 15 minutes. The cells were then fixed using 1% paraformaldehyde and samples were washed twice. Mac-1 and L-selectin were chosen since both molecules are relevant to transmigration and, therefore, to the immunological functions of neutrophils.

Mac-1 (CD11b) belongs to the integrin family of adhesion molecules and forms together with CD18 a dimer. This dimer on the one hand serves as an adhesion molecule promoting tight adhesion of neutrophils and on the other hand as a receptor (CR3) for the split product C3b of complement factor 3 (13). C3b is a major part of the alternative complement pathway and serves as an opsonin on bacterial surfaces. CR3 promotes several neutrophil functions including the oxidative response and phagocytosis.

L-selectin (CD62L) belongs to the selectin family of adhesion molecules. It promotes the so-called rolling adhesion of neutrophils on endothelial cells. Rolling adhesion leads to reduction...
of the neutrophil at the vessel wall and precedes tight adhesion and transmigration. L-selectin is cleaved by a membrane-bound endopeptidase and shed. The soluble L-selectin molecules interact with their bound counter-parts on endothelial cells and are therefore thought to regulate adhesion. In addition to its properties as an adhesion-molecule, L-selectin reacts receptor-like triggering a signal transduction chain.

Adhesion assay

Neutrophils were isolated from heparinized blood of healthy donors by dextran sedimentation and Ficoll-Paque (Pharmacia, Uppsala, Sweden) density centrifugation. The neutrophils were washed twice with Hanks balanced salt solution (HBSS) and resuspended in HBSS with 10% of human serum albumin in a concentration of 106 cells/ml. Cell purity was checked using flow cytometry and exceeded 96%. Cell viability exceeded 95%, as indicated by trypan blue staining.

Endothelial cells were isolated by rinsing human umbilical veins with collagenase 0.5%. The cells were washed twice with cell culture medium (VLE RPMI; Life Technologies, Karlsruhe, Germany), and resuspended in the same medium in T25 cell culture flasks. VLE RPMI contains 10% human serum, penicillin, streptomycin, glutamin and endothelial cell growth factor. Only endothelial cells from the second or third passage were used. Cells were characterized by their typical morphology and by factor VIII staining.

Endothelium was cultivated in 96-well micro titer plates until confluence to quantify the adhesion of PMN on endothelial cells. Endothelial cells were incubated with IL-1β (DPC Biermann, Bad Nauheim, Germany) at a final concentration of 30 ng/ml for 4 hours at 37°C. Thereafter, the cells were washed twice with VLE RPMI, and 100,000 neutrophils per well were added. After incubation at 35, 37, or 41°C in a water bath for 15 minutes, the endothelial cells were washed twice with medium, and the number of adherent neutrophils counted with a microscope. In each plate, a well with an equal amount of fluid was equipped with a thermocouple probe to ensure the correct experimental temperature and temperature equilibrium during the experiments. Four random high-power fields were counted per sample.

Phagocytosis of bacteria

*S. aureus* (DSM 1104) and *E. coli* (ATCC 25922) were grown over night. Bacteria were washed and then suspended in carbonate/bicarbonate buffer (pH 9.5) with 0.01 mg/ml fluorescein isothiocyanate (FITC) for 30 minutes at 37°C. Fluorescence-labeled bacteria were washed and stored at −70°C. For opsonization, 1 ml of bacterial solution was incubated with 1 ml of serum for 30 minutes at 37°C. The bacteria were then washed twice.

To assay phagocytosis, 1 ml leukocyte-rich plasma was added to 1 ml bacterial solution resulting in a 20:1 ratio of bacteria and cells. Bacteria and leukocytes were incubated for 30 minutes at 37°C. Phagocytosis was then stopped by abruptly cooling to 4°C. Before the fluorescence of the bacteria was assessed using flow cytometry, 1 ml of trypan blue (3 mg/ml) was added to exclude extracellularly attached bacteria from measurement.

Data analysis

Results at each temperature were compared with a one-ANOVA. Dunnett’s test was used for post hoc comparison to values obtained at 37°C. Data are expressed as means ± SDs; *P* < 0.05 was considered statistically significant. Pearson’s correlation coefficient (r) was calculated where appropriate and accepted as significant at *P* < 0.05.
Results

Expression of the adhesion protein CD11b on resting neutrophils remained stable throughout the whole range of tested temperatures. TNF-α induced an increase of CD11b on the cell surface. This process took less than five minutes (data not shown) suggesting a transport of transformed CD11b molecules from intracellular storage sites to the cell surface. The upregulation of CD11b was found to be temperature dependent (Table 1). Upregulation of CD11b with TNF-α was increased by hypothermia and significantly decreased with hyperthermia ($r = -0.808$ with $P < 0.01$).

Baseline expression L-selectin was not affected by temperature. Following stimulation with TNF-α, there was almost complete shedding of L-selectin from the cell surface that was almost independent of the assay temperature (Table 1).

FMLP is a constituent of bacterial proteins. Neutrophils bear a receptor for this chemoattractant. Neutrophil functions that can be induced by FMLP include chemotaxis, phagocytosis, and the release of neutrophils bactericidal products such as proteases and oxygen free radicals. Lower temperatures were associated with a slightly increased expression of receptors for FMLP on the surface of the neutrophils failing to reach the necessary levels of significance, whereas hyperthermia decreased expression - an effect that was most pronounced in the presence of TNF-α (Table 1).

Proinflammatory activation of endothelial cells led to a fivefold increase in the number of adhering neutrophils. This well-known increase in adhesion of neutrophils is caused by expression of adhesion molecules (e.g., E-selectin or ICAM-1) at the endothelial lining. But in the tested temperature range, 33 to 41°C, neutrophil adhesion to either resting or activated endothelial cells was not temperature dependent (Table 2).

Uptake of fluorescence-labeled bacteria by neutrophils was tested with a gram-positive and gram-negative species. The number of phagocytized bacteria was inversely related to temperature (Fig. 3). The effect was most prominent with gram-negative E. coli. Interestingly, temperature dependence of phagocytosis was only apparent using opsonized bacteria (i.e., pre-incubation with autologous serum which leads to deposition of opsonins, mainly complement factors, on the bacterial surface). Opsonin coating increases bacterial phagocytosis by changing phagocytosis into a receptor-triggered process. In contrast, phagocytosis of non-opsonized bacteria was not temperature dependent (Table 3).

Using FMLP as a stimulus, H$_2$O$_2$ production was inversely related to temperature. However, this effect that was only statistically significant in the presence of TNF-α. Reactive oxidant-release under these conditions from 33°C to 41°C decreased by a factor of approximately three (Fig. 4). In marked contrast, PMA (an activator of protein kinase C) induced an extreme and homogenous release of reactive oxidants (Fig 2, Fig 5). As might be expected from the thermodynamic effects of temperature on chemical reactions, the response increased with temperature.

Discussion

Hypothermia reduces resistance to test infections in animals (2,3) and augments the risk of surgical wound infections in humans (4,5). Non-receptor-dependent (i.e., PMA-stimulated) neutrophil function is also reduced in hypothermic humans (15). Furthermore, fever enhances immune function and resistance to infection (19). We therefore anticipated that receptor-dependent neutrophil function would also be impaired by hypothermia. Our results, though, demonstrate just the opposite: numerous aspects of neutrophil function were impaired by hyperthermia and facilitated by hypothermia.
The initial step in neutrophil defense is the adhesion to the vessel wall. This process depends on adhesion molecules at the endothelial lining and on the neutrophil surface. We found adhesion of neutrophils to be independent of temperature within the investigated range, whether or not endothelial cells were activated with IL-1β. This suggests the expression of adhesion molecules on endothelial cells is resistant to temperature, since adhesion of neutrophils to endothelial cells remained unchanged within the range of tested temperatures. This result is consistent with our finding that expression of adhesion molecules at the surface of neutrophils was unchanged by temperature. In contrast, expression of CD11b with TNF-α activation was temperature sensitive. Low temperatures increased expression, whereas expression declined with hyperthermia. TNF-α induced nearly complete shedding of L-selectin from neutrophils at all tested temperatures.

Following tight adhesion, neutrophils transmigrate through the vessel wall. Neutrophil migration to the inflammatory site is guided by chemo-attractants such as IL-8 or the bacterial peptide FMLP. In our experiments, we found the number of FMLP receptors declined with increasing temperature. However, our study did not evaluate neutrophil migration. Consequently, the biological relevance of the observed reduction in the number of FMLP receptors remains unclear.

Phagocytosis of opsonized *E. coli* by neutrophils was significantly reduced with increasing temperature. It is well established that phagocytosis of *E. coli* depend at least in part on FMLP receptors (20). The observed reduction of receptors for FMLP may well thus be clinically relevant. Additionally, the uptake of bacteria by neutrophils is facilitated by opsonins. Opsonins are proteins that coat bacteria and fit into receptors on the surface of neutrophils to induce and enhance uptake of pathogens. Major opsonins for neutrophils include immunoglobulins and the split product C3b of the complement factor C3 that is generated through the alternative pathway of complement activation and sticks to the bacterial surface. Major receptors involved include those for immunoglobulins and for C3b. The receptor for C3b is Mac-1, also known as CD11b, which also serves as an important adhesion molecule as described above (13). Interestingly, only the opsonin- or receptor dependent phagocytosis of bacteria was impaired with increasing temperature while the uptake of non-opsonized bacteria remained essentially temperature-independent. Wenisch et al. (15) described an increase of phagocytosis with temperature. This was visible in our data but only as a trend failing to reach the required levels of significance (Table 3). The protocol of Wenisch et al. (15) used to assess phagocytosis included an over all incubation time of 10 minutes for bacteria and blood and is therefore more comparable to a setting of non-opsonized bacteria. Opsonization via the alternative pathway of complement involves several proteolytic steps and takes some time. Opsonization becomes detectable after 7–8 minutes and is maximal after 20 minutes (data not shown).

Oxidative killing of bacteria by neutrophils is generally considered the single most important defense against surgical wound infections (13). In our study, we induced oxidative responses from neutrophils with either FMLP or PMA. Induction via FMLP involves a G-protein-coupled receptor and subsequent activation of an intracellular signaling pathway including the protein kinase C and release of Ca²⁺ from intracellular storage places. PMA, in contrast, bypasses this signaling pathway by directly activating protein kinase C (16). It is of considerable interest that receptor-dependent production of oxygen free radicals was inversely related to temperature whereas induction with PMA increased radical production as a function of temperature. This is consistent with data previously reported by Wenisch et al. (15) and might be expected from thermodynamic effects of temperature on chemical reactions. Our findings of an increased release of hydrogen peroxide following receptor-dependent stimulation are consistent with results reported by Salman et al. on peritoneal macrophages from hypothermic rats, although macrophages and neutrophils differ considerably (21). Nevertheless, the increased generation...
of reactive oxygen products of neutrophils in hypothermia may not be enough to fully compensate for the higher susceptibility to infection in hypothermia.

PMA stimulation is unlikely to represent a typical physiological situation in circulating neutrophils because it provokes oxidative responses two orders of magnitude greater than high-dose TNF-α combined with the bacterial agonist FMLP. Furthermore, no known physiological stimulus reproduces the exaggerated responses of PMA since PMA binds irreversibly to protein kinase C leading to an complete, unmodulated neutrophil response (16). The critical aspect of neutrophil function is defense against tissue infections. There is considerable evidence that neutrophil function differs substantially after transmigrating through the endothelial layer into tissues. An alternative theory is that in some infectious conditions, receptor-mediated control may no longer be rate limiting. Maximum oxidative potential, as represented by PMA stimulation, may therefore dominate. This theory is consistent with observations that substrate limitation frequently appears to dominate clinical responses. For example, hypovolemia and tissue hypoxia are highly correlated with surgical infections which suggests that cytokine mediation may not be the only element controlling oxidative responses in vivo. Under such circumstances, simple temperature-dependent kinetic factors may become critical determinants of response magnitude.

All our tests were performed at constant cytokine concentrations. However, in vivo concentrations of critical neutrophil activators such as TNF-α are highly temperature-dependent. Temperature dependence of cytokine release has been demonstrated for a variety of pro- and anti-inflammatory cytokines in vivo (22-24). In vitro experiments with monocytes and macrophages as major generators of cytokines show that cytokine production is clearly temperature dependent, with decreased production in hypothermia and hyperthermia (25,26). Interestingly, suppression of cytokine production in hyperthermia appears to be restricted to monocytes. Macrophages display the opposite response, namely an increased release of cytokines, especially TNF-α, during hyperthermia (26). In patients with accidental hypothermia, rewarming is also accompanied by a release of cytokines (24). These effects have been shown for TNF-α, IL-8, and IL-6 — all of which are major proinflammatory cytokines (25,26). The observed increase in neutrophil function during hypothermia might well compensate for reduced cytokine concentrations, whereas reduced production during hyperthermia is consistent with the concept that tissues require protection against damage resulting from excessive neutrophil activation during hyperthermia (27).

Neutrophils are hardly the only defense against bacterial infection and the temperature-dependence of other critical immune functions may differ substantially. Furthermore, in vivo neutrophil function in large measure depends on signals and activation by other immune-competent cells and tissues. For example, production of cytokines may be sufficiently reduced by hypothermia to produce an overall reduction in neutrophil function, despite hypothermia-induced activation at constant cytokine concentrations.

In summary, our results show that several crucial receptor-dependent neutrophil activities have a temperature-dependent regulation, with increased function during hypothermia and reduced function during hyperthermia. These mechanisms appear to offer increased protection of tissues against pathogens during hypothermia while simultaneously preventing tissue damage during hyperthermic (e.g., septic) conditions. However, control of neutrophil function is complex and probably controlled differently under various conditions and even in different regions of the body. While the clinical importance of these observations remains unclear, temperature dependence of neutrophil function is clearly far more complicated than previously appreciated.
References


Fig. 1.
Major steps in neutrophil host defense.
Fig. 2.
Flow cytometric analysis. The oxidative burst in 10,000 leukocytes of a single representative donor induced by phorbol-13-myristate-12-acetate (PMA) (B), N-Formyl-L-methionyl-L-leucyl-phenylalanine (FMLP) (C) and FMLP in the presence of TNF-α (D). Panel A shows unstimulated leukocytes. The X-axis indicates the side scatter of leukocytes, showing the cellular granularity: neutrophils (PMN), monocytes (MO) and lymphocytes (LY). The Y-axis shows the H₂O₂ generation (rhodamine 123 fluorescence) in arbitrary units. PMA induced a homogeneous very high release of H₂O₂ in almost all neutrophils. In contrast, the response to FMLP was heterogeneous, with only about 40% of the neutrophils generating oxidative products in this example. The percentage of non-responsive cells was lower in the presence of TNF-α compared to control.
Fig. 3.
Uptake of fluorescence labeled bacteria by neutrophils. Fluorescence reflects the number of ingested bacteria and is presented in arbitrary units ($r = -0.632$, $P < 0.01$ for *E. coli* and $r = -0.332$, $P = 0.07$). Data presented as means (SDs) of 6 independent experiments; *$P < 0.05$ compared to 37°C.*
Fig. 4.
Receptor-dependent Oxidative response of neutrophils following stimulation with N-Formyl-L-methionyl-L-leucyl-phenylalanine (FMLP) ($r = -0.563, P < 0.01$) and FMLP plus TNF-$
\alpha$ ($r = -0.694, P < 0.01$). Fluorescence is a measure of the $H_2O_2$ release and is presented in arbitrary units. Data presented as means (SDs) of 6 independent experiments; *$P < 0.05$ compared to 37°C.
Fig. 5.
Receptor-independent oxidative response of neutrophils following stimulation with phorbol-13-myristate-12-acetate (PMA) ($r = 0.754$, $P < 0.01$). Fluorescence is a measure of the H$_2$O$_2$ release and is presented in arbitrary units. Data presented as means (SDs) of 6 independent experiments; *$P < 0.05$ compared to 37°C.
Table 1
Temperature dependence of expression of adhesion molecules and receptors for FMLP.

<table>
<thead>
<tr>
<th></th>
<th>33°C</th>
<th>35°C</th>
<th>37°C</th>
<th>39°C</th>
<th>41°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11b</td>
<td>68 ± 21</td>
<td>66 ± 13</td>
<td>57 ± 13</td>
<td>61 ± 12</td>
<td>61 ± 16</td>
</tr>
<tr>
<td>CD11b + TNF-α</td>
<td>432 ± 25*</td>
<td>411 ± 57*</td>
<td>332 ± 56</td>
<td>297 ± 66</td>
<td>249 ± 56*</td>
</tr>
<tr>
<td>CD62L</td>
<td>93 ± 25</td>
<td>90 ± 23</td>
<td>96 ± 20</td>
<td>94 ± 24</td>
<td>91 ± 23</td>
</tr>
<tr>
<td>CD62L + TNF-α</td>
<td>8 ± 6</td>
<td>9 ± 5</td>
<td>13 ± 12</td>
<td>12 ± 11</td>
<td>24 ± 4*</td>
</tr>
<tr>
<td>FMLP-R</td>
<td>58 ± 4</td>
<td>55 ± 4</td>
<td>55 ± 6</td>
<td>53 ± 3</td>
<td>39 ± 8*</td>
</tr>
<tr>
<td>FMLP-R + TNF-α</td>
<td>121 ± 10</td>
<td>120 ± 11</td>
<td>110 ± 17</td>
<td>110 ± 9</td>
<td>74 ± 21*</td>
</tr>
</tbody>
</table>

Expression of CD11b, CD62L and receptors for N-Formyl-L-methionyl-L-leucyl-phenylalanine (FMLP) on the cell surface of neutrophils. Data presented as means ± SDs of 6 independent experiments; *P < 0.05 compared to 37°C.
Table 2
Temperature dependence of neutrophil adhesion on endothelial cells.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>HUVEC not activated</th>
<th>IL-1β activated HUVEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>33°C</td>
<td>28 ± 10</td>
<td>200 ± 56</td>
</tr>
<tr>
<td>37°C</td>
<td>26 ± 9</td>
<td>162 ± 15</td>
</tr>
<tr>
<td>41°C</td>
<td>34 ± 15</td>
<td>172 ± 34</td>
</tr>
</tbody>
</table>

Adhesion of neutrophils to human umbilical vein cells (HUVEC) is given in adherent neutrophils per high power field. Data presented as means ± SDs of 6 independent experiments.
Table 3
Temperature dependence of phagocytosis of opsonized vs. non-opsonized *E. coli*.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Opsonized</th>
<th>Non-opsonized</th>
</tr>
</thead>
<tbody>
<tr>
<td>33°C</td>
<td>1369 ± 244</td>
<td>1149 ± 81</td>
</tr>
<tr>
<td>37°C</td>
<td>1003 ± 141</td>
<td>1262 ± 83</td>
</tr>
<tr>
<td>41°C</td>
<td>530 ± 84*</td>
<td>1331 ± 85</td>
</tr>
</tbody>
</table>

Uptake of fluorescent labelled *E. coli* by neutrophils given in arbitrary fluorescence units. Fluorescence reflects the number of ingested bacteria. Data presented as means ± SDs of 6 independent experiments;

*P < 0.05 compared to 37°C.