

Neutrophil Migration In Vitro and In Vivo During Hypothermia

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The effect of hypothermia on pig leukocyte migration in vitro and in vivo was studied. Neutrophil chemotaxis in vitro under agarose was significantly impaired at 29°C (2.7 ± 0.6 [mean ± standard error]; 37°C, 7.1 ± 1.1). Leukocytes isolated from hypothermic pigs and tested at 37°C migrated normally (7.8 ± 0.6). Neutrophil and monocyte migration in vivo was markedly reduced at 29°C. Reduced inflammatory responses may contribute to increased infections during hypothermia.

Hypothermia, controlled or accidental, may significantly compromise host defenses. Children managed with controlled hypothermia of 29°C are frequently leukopenic and may have severe bacterial infections (D. J. Bohn, A. Conn, C. Smith, and W. D. Biggar, submitted for publication). Frewen et al. (3) reported an increased incidence of bacterial infections in children with Reye's syndrome managed with controlled hypothermia and barbiturates. Observations in experimental animals have varied and associate hypothermia with leukopenia, neutropenia, increased or decreased bacterial infections, and enhanced antibody production (2, 4, 5, 10, 12, 15, 17). We have established an experimental pig model of hypothermia, since the clinical circumstances surrounding controlled and accidental hypothermia in humans are so variable and the effects of hypothermia on the inflammatory response so difficult to study.

Under experimental conditions similar to those used for humans, the number of blood neutrophils ($\times 10^9$ /liter) fall from 6.5 ± 0.6 (mean \pm standard error of the mean) at 37°C to 2.3 ± 0.3 at 29°C and do not normalize over the subsequent 5 h of hypothermia (1). When the bone marrow is stimulated during hypothermia with either hydrocortisone sodium succinate or endotoxin, the release of mature and immature neutrophils is markedly reduced. Hypothermic (30 to 34°C) rabbits have a diminished inflammatory response to an intradermal challenge of bacteria (10). A reduced inflammatory response may occur in humans during accidental hypothermia, and serious infections may have few clinical signs (9). We report here our in vitro and in vivo studies of hypothermia and neutrophil migration.

Pigs (10 to 20 kg) were anesthetized and maintained in the supine position as previously described (1). Core temperatures and arterial pressures were recorded continuously (1). Small (10-Fr) plastic intercostal drains were inserted into the right and left pleural spaces. The pleural space was irrigated with 25 ml of normal saline, and if the irrigation fluid was bloody and did not clear with two to three lavages of normal saline, the experiment for that pleural space was terminated. For hypothermia, pigs were surface cooled and maintained at $29 \pm 0.5^\circ\text{C}$ on a cooling blanket.

When the pigs were stabilized at either 37 or 29°C, 5 ml of zymosan-activated serum (ZAS) was diluted to 10 ml with normal saline and infused into the pleural space. The ZAS was left in the pleural space for 60 min and then was removed by aspiration. Pleural washes (25 ml) were then

performed hourly with normal saline. The lavage fluid was examined for cell number and content. Smears were prepared for cell identification by cytocentrifugation and stained with Wright's stain.

Neutrophil migration in vitro was studied by the agarose method (11) with minor modifications (6). Neutrophils were obtained by dextran sedimentation, washed, and suspended to concentration of 2.5×10^9 neutrophils per liter.

ZAS from pigs was the chemotactic factor used. Cell migration as determined by the leading-front method (12) was measured in centimeters under magnification ($\times 45$) and expressed as the mean \pm the standard error of the mean. For each experiment, neutrophil migration was tested in triplicate for a minimum of seven pigs.

Pig neutrophil migration under agarose in vitro was similar to human neutrophil migration studied in parallel (6). When normal saline was placed in the chemotactic well, no enhanced directed or random migration of neutrophils was observed. Migration of neutrophils isolated from normothermic pigs and tested at 29°C toward ZAS was markedly reduced (2.7 ± 0.6) compared with migration at 37°C (7.1 ± 1.1). The number of cells migrating toward the chemotactic stimulus at 29°C, as estimated by the density of cells migrating, appeared to be fewer than the number migrating at 37°C. Random migration at 29°C was also significantly reduced (29°C, 0.5 ± 0.15 ; 37°C, 1.2 ± 0.8).

After 300 min at 29°C, neutrophil migration was greater (3.8 ± 1) than migration after 90 min (2.7 ± 0.6), but remained less than that observed at 37°C. Although neutrophil migration increased with longer periods of incubation, the number of cells migrating as judged by cell density did not increase significantly. When neutrophils were cooled in vitro to 29°C for 30, 60, and 90 min and then rewarmed, neutrophil migration was normal. Cell migration by neutrophils isolated from hypothermic pigs and tested at 37°C (7.7 ± 0.6) was normal (37°C, 7.1 ± 1.1). Migration by neutrophils isolated from hypothermic pigs and tested at 29°C was significantly reduced (2.9 ± 0.7). Migration by neutrophils obtained from pigs at either 37 or 29°C and tested at 29°C was similar (2.7 ± 0.6 and 2.9 ± 0.7 , respectively).

Leukocyte migration into the pleural space after a chemotactic stimulus of ZAS at 37°C is summarized in Fig. 1. One hour after the intrapleural challenge of ZAS, $33 \times 10^6 \pm 8 \times 10^6$ leukocytes were lavaged from the pleural space. The number of leukocytes migrating continued to increase and peaked at $300 \times 10^6 \pm 30 \times 10^6$ cells 3 h after challenge. Mature neutrophils were the predominant cell type over the 5 h of observation. One hour after ZAS challenge, greater

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than 85% of the migrating leukocytes were mature neutrophils. The number of neutrophils continued to increase and peaked after 3 h. By 5 h, mature neutrophils still predominated, although the percentage had decreased to ca. 60%. Monocytes, identified as peroxidase-containing cells, were isolated as early as 1 h after ZAS. The number of monocytes continued to rise and by 5 h, $75 \times 10^6 \pm 9 \times 10^6$ cells were isolated from the lavage fluid. Migration of immature neutrophils or bands was delayed by 1 to 2 h. Significant numbers (5×10^6) were seen over the subsequent 3 h. No inflammatory response was created by inserting the chest drains. Although the number of circulating blood neutrophils at the beginning of the study varied from 3×10^9 to 8×10^9 /liter, there was no significant change in the number of neutrophils isolated from the pleural space. No accumulation of erythrocytes, lymphocytes, or platelets into the pleural space was observed. When the animals were sacrificed and their lungs and pleural cavities examined, no gross or histological abnormalities were apparent. There were no pleural adhesions.

The effect of hypothermia on leukocyte migration *in vivo* is summarized in Fig. 2. When pigs were challenged with ZAS at 29°C, migration of all cell types of markedly diminished over the 5 h of observation.

Immunological defenses are influenced by changes in temperature. Mild hyperthermia may be beneficial (16), whereas hypothermia appears to compromise inflammatory responses and increase susceptibility to infection. Our studies utilized *in vitro* and *in vivo* techniques to assess the effects of lowered temperature on leukocyte migration. Each technique has its advantages and limitations. *In vitro* techniques allow one to observe a given cell population in isolation and under different *in vitro* conditions. Neutrophil migration is markedly reduced at 29°C compared with that at 37°C. With longer periods of incubation, migration continued but never equalled the distances that neutrophils achieved at 37°C. Further, the number of cells migrating at 29°C did not appear to increase significantly with longer periods of incubation as observed at 37°C.

In contrast to *in vitro* studies, our *in vivo* model monitored

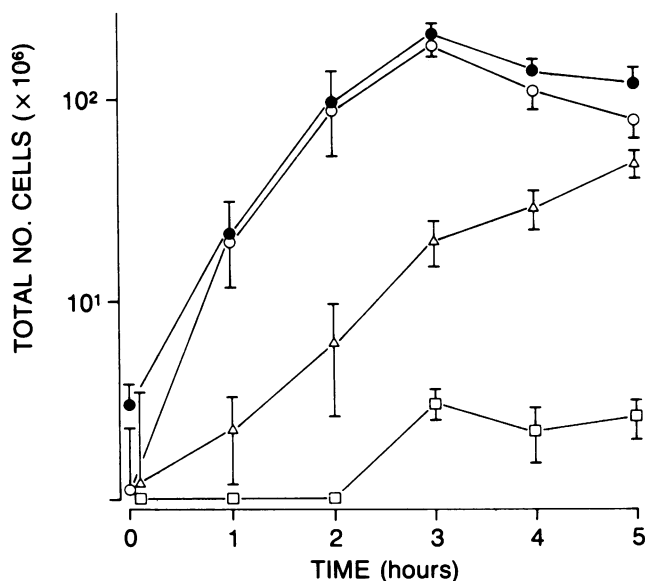


FIG. 1. Pattern of leukocyte migration during inflammation. Symbols: ●, total leukocytes; ○, neutrophils; △, monocytes; □, immature neutrophils. Mean \pm standard error of the mean.

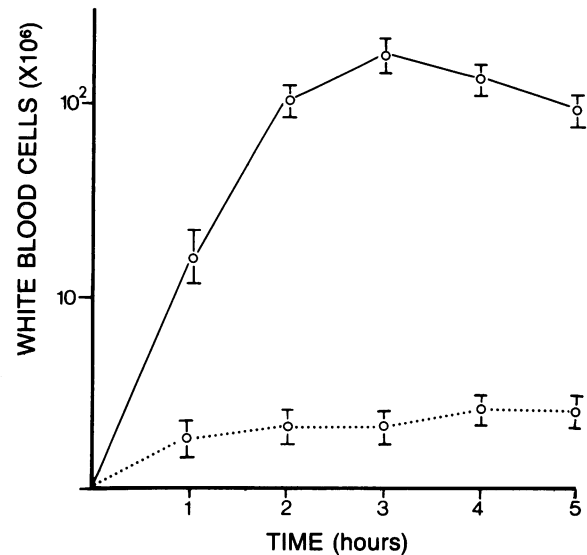


FIG. 2. Effect of hypothermia on leukocyte migration during inflammation. Symbols: —, total leukocytes at 37°C; ····, total leukocytes at 29°C. Mean \pm standard error of the mean.

the neutrophil in concert with all the other components of the inflammatory cycle. At 37°C, the neutrophils migrated first and were always the predominant cell. These findings are similar to those with chromium-labeled rabbit neutrophils (7). Monocyte migration in these studies and others reported previously (8, 13) occurred soon after challenge, in contrast to other sites, e.g., skin, where it may be delayed by several hours (14). It will be important to study inflammatory response over 12 to 18 h to determine if monocytes become the predominant cell type as they may in skin (14). Immature neutrophils or bands have not been observed frequently in other models of inflammation. Immature neutrophils do migrate although migration was delayed compared with that of neutrophils and monocytes. This delay may be due, in part, to diminished responsiveness to inflammatory stimuli and to the time required for recruiting immature cells from the bone marrow.

Our findings also illustrate that large numbers of neutrophils can leave the circulation without any change in the number of circulating neutrophils being detected. For example, ca. 5×10^9 to 10×10^9 neutrophils were recovered from two pleural cavities during a 5-h period. For a 10-kg pig the total blood neutrophil pool would be ca. 7×10^9 (blood volume calculated at 70% of body weight; circulating neutrophil pool at 5×10^9 /liter; marginating neutrophil pool at 5×10^9 /liter) (18). Thus, with this rough approximation, the total blood neutrophil pool can be mobilized into the pleural spaces without significant changes being detected in the number of circulating neutrophils.

Leukocyte migration was negligible at 29°C. The decreased number of circulating neutrophils during hypothermia may contribute in a small way to the decreased migration. However, other factors, such as vasoconstriction, decreased blood flow, decreased vascular permeability, and delayed cell migration likely had an important influence on reducing leukocyte migration. These findings may explain, in part, the increased incidence and the occult and malignant behavior of infections during hypothermia in man.

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