INFECTION AND IMMUNITY, May 1983, p. 708–712
0019-9567/83/050708-05$02.00/0

Vol. 40, No. 2

Neutrophil Circulation and Release From Bone Marrow During Hypothermia

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Received 29 November 1982/Accepted 24 February 1983

The effect of hypothermia on neutrophil circulation and release from bone marrow has been studied. Pigs were anesthetized and maintained at 37°C or surface cooled to 29°C over 60 min. As the core temperature was reduced to 29°C, the number of circulating neutrophils ($\times 10^9$ per liter) fell from 6.0 ± 0.6 to 2.3 ± 0.3 by 60 min. No significant change in the number of circulating mature or immature neutrophils was observed over the 4 h of observation at 29°C. Neutrophil demargination after administration of intravenous catecholamines was similar at 37 and 29°C. Steroid stimulation of bone marrow to release neutrophils was markedly impaired at 29°C. Circulating mature neutrophils in normothermic pigs increased from 5.6 ± 1.2 to 10.4 ± 1.2 by 120 min after administration of intravenous hydrocortisone sodium succinate. Circulating immature neutrophils increased from 1.7 ± 0.3 to 5.3 ± 0.4. At 29°C, no significant changes in the number of circulating mature or immature neutrophils occurred. Endotoxin also failed to stimulate neutrophil release from the bone marrow. Furthermore, a marked neutropenia occurred in hypothermic pigs after intravenous endotoxin, which persisted for the 3 h of observation. Neutrophil circulation and release from bone marrow are compromised by hypothermia, which may increase the risk for bacterial infection.

Controlled hypothermia has been used in the management of several clinical problems. The early experimental work and clinical observations of Bigelow et al. clearly established an important role for hypothermia during cardiac surgery (2). Others have advocated its use in the management of septicemia, intracranial hemorrhages, and cerebral edema, for example.

The effects of hypothermia on bacterial defenses in experimental animals and humans are not clearly understood. Leukopenia has been observed in experimental animals (6, 11, 19) and may be profound when the core temperature reaches 17°C. This leukopenia may persist during the period of hypothermia or it may be transient (6). Fedor et al. (6) found that circulating neutrophils in hypothermic (23°C) dogs fell by 80% soon after hypothermia induction. Of importance was their observation that the leukocyte count in most dogs returned to normal, or greater than normal, while the dogs remained hypothermic. Furthermore, a significant number of circulating immature neutrophils, indicating recent release from the bone marrow, was observed in some studies (6) but not others (19). The degree of leukopenia and its associated compromise of bacterial defenses likely varies with the experimental model tested and the degree of hypothermia. Little information is available concerning the effect of hypothermia on circulating neutrophils in humans. An increase in circulating neutrophils was reported in septic patients who were cooled to 34°C (4).

Recently, concern has been raised that patients cooled to 29°C may have an increased incidence of bacterial infection (8). Despite its use in clinical medicine, virtually no information is available concerning the effect of lowered temperatures on circulating leukocytes in humans or in carefully monitored, biochemically balanced, and well-hydrated experimental animals. Since abnormalities of these parameters are likely to have an important effect on leukocyte circulation, function, and release from bone marrow, we established an experimental pig model to begin to address the question of hypothermia and bacterial defenses. We report here the effects of hypothermia on leukocyte circulation and release from bone marrow in pigs at 29°C.

MATERIALS AND METHODS

Pig model. Pigs weighing 10 to 15 kg were used. The pigs were permitted water but were otherwise fasted for 12 h before anesthesia. The pigs were sedated with acepromazine (2 mg/kg intramuscularly) 45 min before

transfer to the operating room. Anesthesia was then induced, without resistance, with a nasal facepiece and a mixture of 4% halothane and 100% oxygen and then maintained on 1.5% halothane after intubation. Under aseptic conditions, arterial and venous cannulae were inserted. Arterial pressure was monitored with a Statham arterial transducer (model 1280) and Hewlett-Packard recorder (model 7700). Arterial blood gases were monitored hourly. Core temperatures were monitored continuously with YSI model 43TK Thermisters (Yellow Springs Instrument Co., Yellow Springs, Ohio) placed in the rectum and esophagus. For hypothermia, pigs were surface cooled and maintained at 29°C by a cooling blanket. This maintained their core temperature at 29 ± 0.5°C. Normothermic pigs were maintained at 37°C. An intravenous glucose-saline solution was administered at a rate of 15 ml/kg per h. After 6 to 8 h, the pigs were rewarmed if hypothermic and the halothane was discontinued. The pigs were extubated when spontaneous respirations were established.

Leukocyte studies. Blood samples were taken into heparinized plastic syringes and processed immediately. Hemoglobin, hematocrit, leukocyte count and differential, and blood smears were determined by standard laboratory techniques. These were obtained immediately after anesthetic induction and subsequently as the experimental protocol dictated.

Neutrophil demargination. Once anesthetized and metabolically balanced, pigs were given a bolus of epinephrine or isoproterenol (9) intravenously over 2 to 3 min. The dose was titrated for each pig to ensure that the heart rate increased by approximately twofold over preinjection values. Leukocyte counts were measured at 0, 5, 10, 15, 30, and 60 min postinfusion and then hourly for the duration of the experiment.

Bone marrow stimulation of neutrophil release. Two stimuli for neutrophil release were used. Anesthetized and metabolically balanced pigs were given either hydrocortisone sodium succinate (HC; 100 or 200 mg intravenously) (3) or *Escherichia coli* endotoxin (0111-B4; Difco Laboratories) (0.25 to 1.5 μg/kg intravenously) over 5 min (1). Leukocyte counts and differentials were done at 0, 30, 60, 90, and 120 min postinfusion and then hourly for the duration of the experiment.

The volume of blood taken for these studies was recorded, and the total volume did not exceed 1.0% of the total body weight of the pig.

RESULTS

In early experiments and before the use of acepromazine, wide fluctuations in peripheral leukocyte counts were observed. Peripheral leukocyte counts in nonanesthetized pigs were elevated and ranged from 20×10^9 to 40×10^9 per liter. After anesthesia was induced, the cell counts usually fell to 10×10^9 to 30×10^9 per liter by 45 to 60 min. With acepromazine premedication, the pigs remained quiet and tolerated the anesthetic induction without resistance. Under those conditions, the leukocyte counts stabilized at $14.1 \times 10^9 \pm 0.9 \times 10^9$ per liter (n = 28). The blood neutrophil count was 6.0 ± 0.6. If the initial, postanesthetic leukocyte count was greater than 20×10^9 per liter, the experimental data were excluded.

Arterial blood gases remained stable and required little adjustment of ventilatory rates or pressures. The partial pressure of O_2 in arterial blood varied between 300 and 400 mmHg (39.990 and 53.320 kPa), and the CO_2 pressure varied between 35 and 40 mmHg (4.665 to 5.332 kPa), which are normal for an animal on 100% oxygen. Serum electrolytes (Na, Cl, and K), blood urea nitrogen, and blood glucose remained normal throughout the 6 to 8 h of observation.

Neutrophil changes with hypothermia. The effects of normothermia and hypothermia on the number of circulating neutrophils in six normothermic and six hypothermic pigs are summarized in Fig. 1. When the core temperature of the anesthetized pigs was maintained at 37 ± 0.5°C, there was no significant change in the number of circulating neutrophils over the 5 h of observation. Similarly, no changes in hemoglobin concentration, hematocrit, and lymphocyte counts were observed (unpublished observations). By contrast, as the core temperature was reduced to 29°C, there was an immediate fall in the number of circulating neutrophils from 6.0 ± 0.6 ($\times 10^9$ per liter) to 2.3 ± 0.3. Once the core temperature reached 29°C and was maintained, no significant change in the number of circulating neutrophils was observed over the next 4 h of observation. Furthermore, no immature neutrophils or band forms were seen in the circulation. Clinical examination of the pigs during hypothermia did not reveal any apparent increase in spleen size or altered pulmonary function suggestive of massive leukocyte sequestration. Hemoglobin concentration and hematocrit were similar at 37 and 29°C.

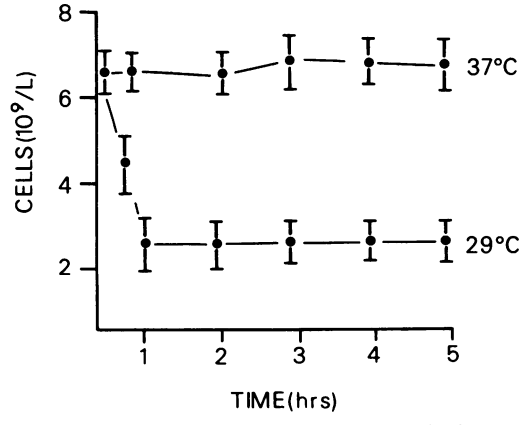

FIG. 1. Number of circulating neutrophils in normothermic (37°C) and hypothermic pigs (29°C). Pigs were anesthetized with 1.5% halothane and oxygen and maintained within ±0.5°C of the desired temperature.

Neutrophil demargination. The effect of hypothermia on neutrophil demargination at 37 and 29°C was studied in four pigs by epinephrine or isoproterenol administration. At 37°C, when the pulse rate had increased by approximately twofold, the number of circulating neutrophils increased by 77% (range, 50 to 100%) after 15 min. Similarly, at 29°C, after a catecholamine-induced tachycardia, the neutrophil count increased by 77% (range, 60 to 100%) after 15 min. Neutrophil counts returned to preinjection values within 30 min. The number of circulating immature neutrophils was not affected by catecholamine administration at 37 or 29°C.

Bone marrow stimulation of neutrophil release. Two stimuli for neutrophil release from the bone marrow were examined: HC (Fig. 2) and *E. coli* 0111-B4 endotoxin (Fig. 3). When normothermic pigs were given 100 mg of HC intravenously, a brisk increase in the number of circulating neutrophils was observed. The number of circulating neutrophils ($\times 10^9$ per liter) increased from 5.6 ± 1.2 to 10.4 ± 1.2 by 120 min after HC administration in the six pigs studied. Immature neutrophils increased from 1.7 ± 0.3 to 5.3 ± 0.4 over the same time period. Thus, the number of circulating immature cells was approximately 50% of the number of circulating mature neutrophils.

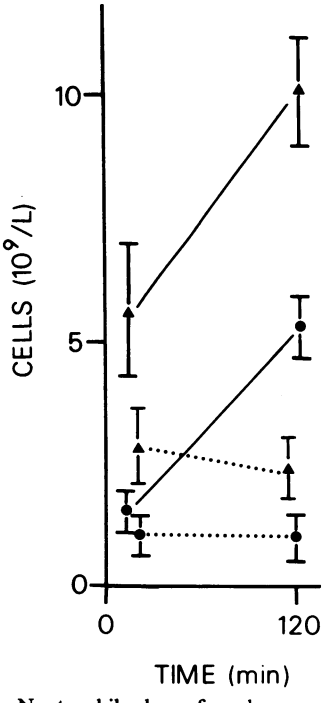

FIG. 2. Neutrophil release from bone marrow after HC (100 and 200 mg intravenously) stimulation at 37 (solid lines) and 29°C (dotted lines). Pigs were anesthetized with 1.5% halothane and oxygen and maintained within ±0.5°C of the desired temperature. Symbols: ▲, polymorphonuclear leukocytes; ●, bands.

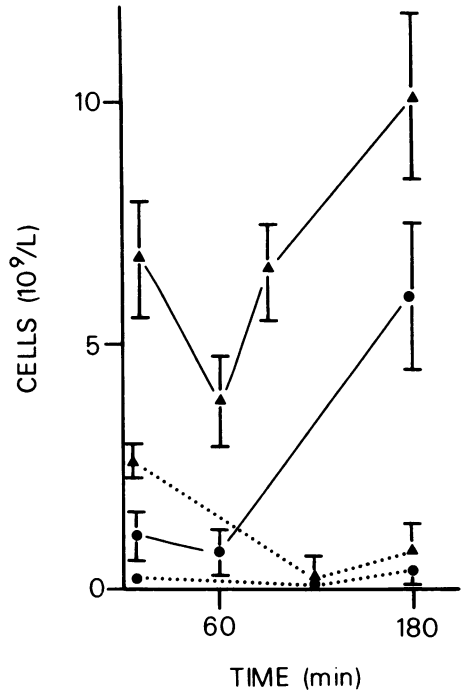

FIG. 3. Neutrophil release from bone marrow after *E. coli* 0111-B4 endotoxin (0.5 mg/kg intravenously) stimulation at 37 (solid lines) and 29°C (dotted lines). Pigs were anesthetized with 1.5% halothane and oxygen and maintained within ±0.5°C of the desired temperature. Symbols: ▲, polymorphonuclear leukocytes; ●, bands.

In six pigs cooled to a core temperature of 29°C, the number of circulating neutrophils ($\times 10^9$ per liter) fell to 2.5 ± 0.5. When hypothermic pigs were given 100 mg of HC intravenously, there was no significant increase in the number of circulating mature or immature neutrophils (Fig. 2). Similar results were obtained when the intravenous dose of HC was increased to 200 mg.

The ability of endotoxin to stimulate neutrophil release from the bone marrow in normothermic pigs is summarized in Fig. 3. Six pigs were given 0.5 μg of endotoxin per kg intravenously. Sixty minutes after endotoxin administration, the number of circulating neutrophils ($\times 10^9$ per liter) fell from 6.1 ± 0.5 to 3.2 ± 0.4. After this, the number of circulating neutrophils increased to 10 ± 2 by 180 min. The number of immature neutrophils (1.0 ± 0.3) did not fall after endotoxin (0.9 ± 0.11), and by 180 min they had increased to 4.5 ± 1.2. Thus, approximately half of the circulating neutrophils were immature. When pigs were cooled to a core temperature of

29°C, the number of circulating neutrophils fell to 2.3 ± 0.3. After endotoxin administration, the number of circulating neutrophils fell even further (0.3 ± 0.1) by 120 min and did not increase over the subsequent 60 min. Similarly, there was no increase in immature cells. The findings were similar when the dose of endotoxin was increased to 1.5 μg/kg or decreased to 0.25 μg/kg.

DISCUSSION

Lowered temperatures, environmental, body, or both, may alter host immunity and increase susceptibility to infection. The effects on immunity are variable and depend to a considerable extent on the magnitude of the temperature change and the animal model examined. For example, antibody synthesis may be increased in rabbits during lowered environmental temperatures (15). On the other hand, delayed hypersensitivity, passive cutaneous anaphylaxis (17), and the Arthus reaction (16) may be inhibited by lowered body temperatures.

Phagocytic cells are the primary defense mechanism against bacteria. Hypothermia may slow bacterial replication and has been reported to be beneficial in experimental peritonitis (5). Most reports, however, conclude that host defenses are compromised more than bacterial growth is. Therefore, the net effect of hypothermia is not protection but an increased susceptibility to bacterial infection. Neutrophils have several important steps to take from the time their production is complete to their final role in bacterial killing, including their release from bone marrow, circulation, and, finally, migration into extracellular spaces to participate in the inflammatory response. Any delay or interruption in this sequence of events may render the host susceptible to bacterial infection. We have examined the first two steps in this reaction and found that hypothermia is detrimental to both.

Hypothermia was associated with a marked reduction in the release of mature and immature neutrophils from the bone marrow into the circulation. HC was found to be a potent stimulator of neutrophil release from the bone marrow at 37°C. The large number of immature neutrophils circulating after HC stimulation suggests that this was a potent stimulus for neutrophil release. The failure of HC to stimulate the bone marrow to release neutrophils during hypothermia may have been secondary to endorgan hyporesponsiveness (12). Endotoxin, a second, potent stimulator of neutrophil release from the bone marrow, was also ineffective at 29°C. Our findings during hypothermia would suggest that endorgan marrow unresponsiveness is not restricted to steroids alone (12) since the release of neutrophils was reduced after steroid and endotoxin stimulation.

Neutrophil circulation, the second step of the inflammatory cycle studied, was also affected by hypothermia. At 29°C the number of circulating neutrophils fell by approximately 50%. This decreased number of neutrophils persisted for the 4 h of hypothermia. The number of circulating neutrophils has been reported to fall during hypothermia in some experimental animals. The magnitude of this fall appears to depend on the degree of hypothermia. Circulating neutrophils in the hypothermic pigs described in this report fell by approximately 50%, whereas in dogs cooled to 17°C, circulating neutrophils fell by 80% (19).

Our findings in pigs differed from those reported in dogs where the number of circulating neutrophils returned to normal during hypothermia (6). In our previous work with dogs, we found that a significant splenomegaly developed soon after anesthesia induction (unpublished observations). This resulted in a 25 to 30% decrease in the circulating blood volume. The blood neutrophil count fell rapidly by 25 to 30%. The number of circulating neutrophils would gradually return to baseline values over the subsequent 2 to 4 h. Similar observations have not been made in pigs and humans.

Hypothermia may also compromise the third step of the inflammatory response, namely neutrophil migration. The degree of impairment may vary with different sites of inflammation. Rabbits cooled to 30 to 34°C, while awake, had a diminished inflammatory response to an intradermal challenge of viable *Streptococcus pneumonia* (14). This is due, in part, to the severe degree of peripheral vasoconstriction. Of importance in those studies was the finding that a strain of bacteria which was usually avirulent for normothermic rabbits caused an overwhelming bacteremia and death during hypothermia in these same animals. The intestinal hemorrhage observed in over 50% of dogs after hypothermia may have been attributed, in part, to intestinal necrosis secondary to ischemia and impaired inflammatory responses necessary for maintaining the integrity of the intestinal wall (11). A compromised inflammatory response has also been reported in humans who, when hypothermic, may have serious infections which are clinically occult (13).

Finally, the reticuloendothelial network, a central site for bacterial clearance and modulated by mediators of the inflammatory response, is impaired during hypothermia. This impaired function is evidenced by delayed intravascular clearance of an experimental bacteremia (7, 10, 18). These findings are also likely due in part to impaired circulation and delivery of microorganisms to the reticuloendothelial network.

We conclude that acute hypothermia of 29°C

in pigs for 4 h is associated with a reduced number of circulating neutrophils and an inability to release new neutrophils from the bone marrow. Finally, endotoxin, a potent stimulator for neutrophil release from the marrow at 37°C, was associated with a profound neutropenia in hypothermic pigs. Our findings, and those reported previously, suggest that an increased susceptibility to bacterial infection, perhaps by organisms not usually considered as virulent and life-threatening, may occur during hypothermia.

ACKNOWLEDGMENTS

We acknowledge the skilled assistance of C. Barker.

This work was supported by the Medical Research Council of Canada, MA 6897.

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