In Vivo pH of Induced Soft-Tissue Abscesses in Diabetic and Nondiabetic Mice

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Infections in the diabetic host have been shown to persist longer than those in the nondiabetic host. To investigate whether intra-abscess milieu might be a contributing factor to this persistence, the in vivo intra-abscess pH was measured in induced soft-tissue abscesses in diabetic and nondiabetic mice. Two models (female genetically obese insulin-resistant and male streptozocin-induced diabetic mice) were used with appropriate controls. The bacteria injected to produce the soft-tissue abscesses were *Bacteroides fragilis* and *Enterococcus* (B+E), *Staphylococcus epidermidis* and *Enterococcus* (S+E), and *S. aureus* (SA). Intra-abscess pH measured on day 3 was consistently and significantly lower in all diabetic mice compared with their controls. In the diabetic mice, the pH of an abscess induced with B+E, S+E, and SA was 6.28 (n = 17), 6.79 (n = 10), and 6.52 (n = 10), respectively; the pH in the controls was 7.21 (n = 20), 7.30 (n = 10), and 7.17 (n = 10), respectively. Differences in all groups between diabetic and nondiabetic mice were significant. The blood glucose values of the diabetic mice averaged 722 mg/dl, and in the nondiabetic mice were 210 mg/dl. No animals were ketotic. There were no significant differences in total colony counts between any groups. In conclusion, there is a significantly lower pH in the abscess of the diabetic host compared with the nondiabetic host that is not related to the numbers or types of causative bacteria. *Diabetes* 38:659–62, 1989

Increased susceptibility of the diabetic patient to various infections has been postulated for many years. Clinical impressions have supported the view that diabetic patients suffer more severe and prolonged infections than do nondiabetic patients. Alterations and deficiencies of various aspects of the immune system, e.g., granulocyte adherence, granulocyte phagocytosis, microbicidal activity, and monocyte activity, have been found in the uncontrolled diabetic patient (1–5). In vivo granuloma formation was found to be less effective in poorly controlled diabetic animals than in their better-controlled diabetic and nondiabetic counterparts (6). In vivo soft-tissue infections in the diabetic mouse model have been shown to persist for longer periods than equivalent infections in nondiabetic controls (7). The reason for this persistence of soft-tissue infections is not entirely clear. Because pH of the immediate environment may influence bacterial growth and local cell function and may affect antimicrobial activity of antibiotics (8,9), we measured the in vivo pH of soft-tissue abscesses in diabetic and nondiabetic mice to investigate whether a difference in abscess pH was present. An altered local pH might be one of the host factors affecting persistence of microbial growth in diabetic soft-tissue infections.

MATERIALS AND METHODS

Mouse models. In some studies, female *db/db* mice and their nondiabetic female littermates, aged 10–11 wk, were used. These mice (C57BL/KsJ *db/m*) were obtained from Jackson, (Bar Harbor, ME). The obese diabetic mice, aged 10–11 wk, weighed 45.3 ± 4.62 g, and their nondiabetic littermates weighed 20.3 ± 1.83 g. In other studies, diabetes was induced in 9- to 13-wk-old male Swiss-Webster mice (obtained from a local vendor) by streptozocin (STZ, 4 mg · kg^{-1} · 5 $days^{-1}$) injected intraperitoneally. The diabetic mice weighed 32.35 g (5.09SD), and the nondiabetic mice weighed 33.59 g (4.95SD) when injected with STZ. In both sets of animals, mice were considered diabetic when

Glucose 1 mM = 18 mg/dl

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glucosuria appeared. Injections of bacteria were given 3–4 days after the onset of glucosuria.

Animals were housed in groups of 3 and were checked daily for glucosuria and ketonuria. Urine was collected from each mouse individually by stroking and/or compressing the bladder and obtaining fresh urine onto a Keto-Diastix reagent strip.

Induction of soft-tissue abscesses. Soft-tissue abscesses were induced by intradermal injection in the groin, according to the method of Brook et al. (10). The organism or combinations of organisms used in this study were *1*) 0.1 ml · 10^{-8} · cm^{-3} *Bacteroides fragilis*/0.1 ml · 10^{-8} · cm^{-3} *Enterococcus* (B+E), *2*) 0.1 ml · 10^{-6} · cm^{-2} *Staphylococcus aureus* (SA) diluted to 0.2 ml, and *3*) 0.1 ml · 10^{-8} · cm^{-3} *S. epidermidis*/0.1 ml · 10^{-8} · cm^{-3} *B. enterococcus* (S+E).

A total volume of 0.2 ml was injected intradermally into the groin of each mouse. The *B. fragilis* was obtained from a foot (plantar) abscess of a diabetic patient and was capsule positive. *Enterococcus* was obtained from the same abscess and was capsule negative. The SA and *S. epidermidis* were obtained from two patients with infected diabetic gangrene. The 0.1 ml of the SA was injected in dilution of $10^6/cm^3$ because 0.1 ml of $10^8/cm^3$ or $10^7/cm^3$ caused either a high mortality (10^8) or a high incidence of local tissue necrosis and spontaneous drainage (10^7).

The abscesses appeared within 48 h and started to decrease in size 3–4 days after injection of bacteria. The measurement of pH of the abscesses and removal of the abscesses for quantitative bacterial counts were done on days 3–4 after injection of inocula.

In vivo measurement of abscess pH. After anesthetizing the mice with Nembutal given intraperitoneally, the subcutaneous abscess and adjacent peritoneum and soft tissue were exposed. The pH was measured with a needle microelectrode, microreference electrode (Micro-electrodes, Londonderry, NH), and a Corning model 12 pH meter (American Scientific Products, Irvine, CA). The microelectrode was guided under magnification into at least two separate sites of the abscess. The external peritoneal tissue pH was measured before and after measurement of the abscess pH.

Colony counts, blood glucose determinations, and arterial pH studies. After pH measurements, the abscesses were excised for total colony count by a sterile technique and placed in an appropriate transport medium. During measurement of pH, the cardiorespiratory system of the mice remained intact and functional. Quantitative colony counts were done by grinding the removed tissue in a Beck's tissue grinder, according to methods previously described (11). The media used were Columbia CNA agar and sheep blood agar for *Enterococcus, Brucella* menadione in blood agar and kanamycin-vancomycin for *B. fragilis*, and Columbia CNA agar and sheep blood agar for SA and *S. epidermidis*. Standard aerobic and anaerobic dilution and incubation techniques were used. The grinding and other procedures were performed in an anaerobic chamber where *B. fragilis* was one of the organisms. After completion of these measurements, blood was drawn by cardiac puncture for blood glucose and pH determinations. Blood samples for pH determinations were collected in heparinized, capped iced syringes. Only samples of free-flowing arterial (bright red) blood without air bubbles were measured for arterial pH.

RESULTS

For intra-abscess pH, peritoneal pH, and blood glucose levels of female *db/db* mice and their controls and the STZ-induced diabetic (STZ-D) mice in the male Swiss-Webster mice and their controls, see Tables 1 and 2.

Table 1 details the results for abscesses induced with B+E. The intra-abscess pH in the diabetic *db/db* mouse model was 6.29 (±0.37), and in the STZ-D model the pH was 6.27 (±0.42). In the nondiabetic controls, the pH was 7.23 (±0.13) and 7.19 (±0.21), respectively.

Table 2 gives the intra-abscess pH values for abscesses induced by the SA. The intra-abscess pH was 6.52 (±0.22) in the STZ-D host and 7.17 (±0.14) in the nondiabetic control. The pH of S+E–induced abscesses in the STZ-D host was 6.79 (±0.29), and in the nondiabetic control the intra-abscess pH was 7.30 (±0.12; Table 3).

The peritoneal pH measured 7.2–7.3 in all animals. The arterial pH measured 7.26 ± 0.12 in all samples. There were no differences in the average of the blood pH levels in diabetic and nondiabetic animals. The intra-abscess pH of the abscesses in the diabetic mice was uniformly and significantly lower than the intra-abscess pH in nondiabetic mice. This finding was consistent in the genetically obese, insulin-resistant female mouse model, and in the STZ-D insulin-deficient male-mouse model. The finding of decreased intra-abscess pH in the diabetic compared with the nondiabetic mice also occurred consistently with varied bacteria, both in combination (B+E, *B. fragilis*, and *S. epidermis*) and with a single agent (SA). There were no significant differences in pH values that related to the bacteria type or the mouse model.

In all abscesses from diabetic and control mice, the colony-forming units (CFU) were too numerous to count at 10^3 and were absent at 10^7. At 10^5, the counts ranged from 15 to 190 CFU. There were no significant differences in the colony counts between the diabetic and the nondiabetic mice. There was no significant difference between the blood glucose levels of the *db/db* and the STZ-D models. Daily urine ketones remained negative in all animals throughout the study.

DISCUSSION

Many in vitro studies have demonstrated altered phagocytic function in diabetic patients (1–6). These alterations in function included decreased chemotaxis, phagocytosis, and microbicidal activity. Recent studies have confirmed the long-

TABLE 1
Intra-abscess pH of abscesses induced with *Bacteroides fragilis* and *Enterococcus*

	n	Abscess pH	Peritoneal pH	Glucose (mg/dl)
db/db				
Diabetic	11	6.29 ± 0.37 *	7.32 ± 0.11 *	704 ± 120
Nondiabetic	15	7.23 ± 0.13	7.32 ± 0.14	200 ± 53
Streptozocin				
Diabetic	6	6.27 ± 0.42 *	7.30 ± 0.12	694 ± 84
Nondiabetic	5	7.19 ± 0.21	7.31 ± 0.13	235 ± 42

Values are means ± SD.
*$P < .001$ by Student's 1-tailed test. All other comparisons not significant.

held clinical impression that infections in the hyperglycemic diabetic host persist longer than similar infections in the nondiabetic host (7). In these studies, identical polymicrobial inocula were injected to produce soft-tissue abscesses in diabetic and nondiabetic mice. Various combinations of bacteria were used. Colony counts of the abscesses 1 wk after induction showed no significant differences between nondiabetic and diabetic animals with the exception of B+E. At 2 wk, there were higher colony counts with all combinations of bacteria in the diabetic compared with the nondiabetic animals. In this study, there were no differences in colony counts between diabetic and nondiabetic animals, even with B + E. However, in this study, the colony counts were done 3 days after abscess induction compared to 7 days in the previous study.

There have been few in vivo studies of the local milieu of the abscess itself. Because various bacteria have optimum growth at various pH levels, and because many cellular functions are affected by pH levels, the goal of this study was to examine the in vivo intra-abscess pH in the hyperglycemic diabetic host compared with the nondiabetic host.

One of the factors contributing to the local intra-abscess pH might be the bacterial growth itself. For example, it is known that members of the *Bacteroides* genus produce organic acids (12,13), including succinic acid, that might be responsible for producing a locally acidic milieu. However, as can be seen in the results, there were no significant differences in the quantitative colony counts of any of the organisms injected between the diabetic and nondiabetic hosts. Although there was a slight trend for the pH of the abscesses induced by B+E (Table 1) to be lower (6.29 and 6.27) than the pH of the abscesses induced by SA (6.52) and S+E (6.79), these differences are not statistically significant. Furthermore, comparing the relative pH of the abscesses induced by various combinations of bacteria in two different models of diabetes, and by use of male or female mice, the pH in the diabetic host is consistently lower than the pH of the abscess in the control nondiabetic host. Therefore, bacterial products are probably not the primary agents responsible for the demonstrated abscess pH differences in diabetic and nondiabetic mice. There were no significant differences in peritoneal or arterial pH levels in the various groups of diabetic and nondiabetic mice, an indication that systemic acidosis did not play a role in the local pH differences.

Metchnikoff, as reviewed by Dubos (14), noted the extracellular environment in an inflammatory area to be markedly acidic whether the inflammation was induced by chemical or bacterial agents. If the host was rendered leukopenic, the environment was less acidic. If glucose was injected, the environment was more acidic. That increased glucose might cause increased glycogen in the white cell and that the white cell used the extra glycogen as the energy source to produce lactic acid (from pyruvate) from glycolysis were postulated. Potts et al. (15) measured the pH of low-glucose pleural effusions and found there was an inverse correlation of pH levels and the number of white blood cells. They postulated that the pleural fluid acidosis was related to increased lactic acid production by leukocytes, which were metabolizing glucose via glycolysis and the hexose monophosphate shunt.

TABLE 2
pH in *Staphylcoccus aureus*–induced abscesses in mice

	n	Abscess pH	Peritoneal pH	Glucose (mg/dl)
Diabetic	10	6.52 ± 0.22	7.21 ± 0.06	747 ± 133
Nondiabetic	10	7.17 ± 0.14	7.2 ± 0.12	181 ± 23
P		<.001	NS	<.001

Values are means ± SD.

TABLE 3
pH in *Staphylcoccus epidermis* plus *Enterococcus*–induced abscesses in mice

	n	Abscess pH	Peritoneal pH	Glucose (mg/dl)
Diabetic	9	6.79 ± 0.29	7.26 ± 0.08	742 ± 277
Nondiabetic	10	7.30 ± 0.12	7.28 ± 0.09	223 ± 51
P		<.001	NS	<.001

Values are means ± SD.

In phagocytosis, granulocytes demonstrate increased activity of the hexose monophosphate shunt, a respiratory burst that affects the monovalent reduction of oxygen by use of the NADPH generated by the accelerated shunt. In turn, activated oxygen radicals and related products are produced that assist in the bactericidal function of the phagocyte (16). Increased activity of the hexose monophosphate shunt and glycolysis also results in increased lactic acid production. This pathway is not affected by inhibitors of oxidative phosphorylation (17). The enzymes for gluconeogenesis (e.g., pyruvate carboxylase) are absent in leukocytes (18). Therefore, any lactate that is formed must be processed through the tricarboxylic acid cycle (oxidative phosphorylation). This pathway is carried out on the mitochondria (19).

In his review of the diabetic leukocyte, Essman (20) states that administration of insulin increases glucose utilization after 24 h of treatment in the diabetic patient, but insulin administration has no effect on leukocyte glycolysis. The presumed locus for this effect of insulin (increased glucose utilization) was the mitochondrial tricarboxylic acid cycle.

Few mitochondria are found in phagocytes. However, a functional citric acid cycle has been demonstrated in the neutrophil. Under normal circumstances, only ~5–10% of glucose carbon is processed through this cycle (16,20). McCall et al. (21) have observed that stimulated phagocytes increase the metabolism of C1 of the glucose molecule ~8 times that of control. The metabolism of the C6 of the glucose molecule, which represents tricarboxylic acid cycle metabolism, was increased only 1.5 times, despite the lesser percentage contribution to overall metabolism. Citric acid cycle metabolism is felt to contribute substantially to the overall energy source for phagocytes (22).

Repine et al. (23) have shown that neutrophils from diabetic subjects fail to increase their bactericidal activity in response to acute bacterial infection and are unable to eradicate bacteria in the face of higher bacteria to white cell ratios. Although this phenomenon is more pronounced in subjects with poorly controlled diabetes, the defect persisted in subjects with well-controlled diabetes. The difference in the in vivo intra-abscess milieu (a lowering of pH in the hy-

perglycemic diabetic host compared with the nondiabetic control) may be an additional reflection of altered white blood cell function in diabetes. Most (90–95%) glucose metabolism in the phagocyte is related to the non-insulin-dependent glycolysis and hexose monophosphate shunt pathways, which terminate in the production of lactic acid. As described, these pathways are accelerated by phagocytosis and hyperglycemia. The lactic acid must then be processed through the Krebs cycle by the mitochondria, which are insulin dependent. As mentioned, only 5–10% of glucose carbon goes through the Krebs cycle. Thus, if increased lactate is produced via the glycolytic pathways and this lactate is not processed via the Krebs cycle, the result would be a more acidic milieu. Increased activity of the glycolytic pathway because of an elevated local glucose level would contribute further to the accumulation of lactate.

Our studies show that the in vivo pH of abscesses caused by various bacteria are consistently lower in the diabetic mouse model (whether STZ-D or the genetically obese model) compared with the corresponding nondiabetic control. For some antibiotics, the most important chemical factor creating differences between in vitro and in vivo activities is the pH of the medium (8,9). In general, antibiotics are more active at an electroneutral pH. It is theorized that uncharged molecules can reach their sites of action more freely if the barrier (e.g., lipophobic cell membrane) is more easily crossed. For example, the activities of gentamicin and clindamycin are greater at an alkaline than acidic pH. The mean inhibitory concentration (MIC) of gentamicin against *Klebsiella*, *Proteus*, *Enterobacter*, and *Serratia* has been shown to be markedly affected by pH changes. The MIC required increases markedly with decreasing pH. The pH levels in these studies ranged from 5 to 8.

We postulate that the decreased pH of the intra-abscess milieu in the diabetic host compared with the nondiabetic control may be due to the imbalance of glycolytic (non-insulin-dependent) and citric acid (insulin-dependent) pathways in the hyperglycemic diabetic host. The altered pH of the milieu may affect host defense mechanisms, facilitate bacterial growth, and influence antibiotic kinetics and activity.

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