The Effect of Lactic Acid on Mononuclear Cell Secretion of Proinflammatory Cytokines in Response to Group B Streptococci

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This study found that lactate alone had a stimulatory effect (207.1 ± 16.3%; $P = .001$) on tumor necrosis factor (TNF)-α production by human mononuclear cells with the most profound secretion being at pathologic concentrations of 4–8 m*M* lactate. Furthermore, exposure of these mononuclear cells to group B streptococci (GBS, 10^5 cfu) resulted in TNF-α production of up to 621.1 ± 42% of control; the combination of lactic acid and GBS increased TNF-α production up to 1019.3 ± 16.1% ($P = .001$). The combination of GBS and lactate also enhanced the secretion of interleukin (IL)-1β and IL-6. Lactate in pathologic concentrations, therefore, likely enhances the secretion of these inflammatory mediators and contributes to septic shock and meningitis caused by GBS.

In previous studies, we demonstrated that group B streptococci (GBS) induce the production of tumor necrosis factor (TNF)-α by neonatal monocytes [1, 2]. TNF-α may contribute to the overwhelming septic shock that often accompanies early-onset GBS infections. We have previously shown that recombinant TNF-α depresses neutrophil chemotaxis, an effect that can be partially reversed by the methylanthine derivative, pentoxifylline, which antagonizes the action of TNF-α. [3] We have also found that TNF-α decreases neutrophil membrane fluidity and markedly up-regulates cell membrane expression of the adhesive glycoprotein CD11b/18. [3] Up-regulation of this membrane receptor may cause capillary plugging with neutrophils, which may contribute to capillary leak, alveolar and interstitial pulmonary edema, and increased lung lymph flow, contributing to the severe morbidity and mortality observed in GBS infection. During meningeal infections, neutrophil adhesion and emigration through intercellular junctions also contribute to the increased permeability of the blood-brain barrier, allowing leakage of serum proteins into the cerebrospinal fluid.

In addition to the elevated protein concentration found in the cerebrospinal fluid in bacterial meningitis, there is a concurrent rise in lactic acid and TNF-α concentrations. Because lactic acid is commonly found in association with elevated concentrations of TNF-α in the cerebrospinal fluid during meningeal infections and lactic acidosis accompanies septic shock, we hypothesized that elevated concentrations of lactic acid might modulate TNF-α production induced by GBS. We therefore examined the production of the proinflammatory cytokines, including TNF-α, interleukin (IL)-1β, and IL-6, by human mononuclear cell preparations after exposure to various concentrations of lactate in the presence or absence of GBS.

Materials and Methods

Preparation of organisms. A human blood isolate of encapsulated type III GBS (COH1; courtesy of C. Rubens, University of Washington, Seattle) was cultured in Todd-Hewitt broth (Difco, Detroit) at 37°C for 16–18 h. The organisms were then washed three times in Hanks' balanced salt solution (HBSS) and resuspended in HBSS at a standard concentration of 5×10^8 cfu/mL, as determined by an optical density of 0.9 at 620 nm (Spectronic 20; Bausch & Lomb, Rochester, NY). Bacterial suspensions were then aliquoted, heat-killed at 56°C for 1 h, and stored at −70°C for later use.

Mixed mononuclear cell cultures. Whole heparinized blood was diluted 1:2 with HBSS (Whittaker Bioproducts, Walkersville, MD), and mononuclear cells were separated by density-gradient centrifugation (Ficoll-Paque; Pharmacia, Piscataway, NJ). The mononuclear cell layer was washed twice with HBSS; the cells were counted and adjusted to 10^6/mL. To minimize pH change in our culture medium, sodium lactate (MW 112.1; Sigma, St. Louis) was mixed with HBSS to a concentration of 200 m*M* and then diluted with RPMI 1640 (Whittaker Bioproducts) to concentrations of 0.5–20 m*M*.

Cytokine assays. TNF-α, IL-1β, and IL-6 were assayed by EIA (Immunotech, Westbrook ME) in supernatants of mononuclear cell cultures. Samples were frozen at −70°C until time of assay.

Endotoxin. All buffers, reagents, and culture media were assayed for the presence of endotoxin with the limulus amebocyte lysate reaction (Endotect; ICN Biomedicals, Costa Mesa, CA), which is sensitive to 0.1 ng/mL endotoxin, a level lower than that required to induce TNF-α production by human monocytes.

Results

As reported previously [1, 2], TNF-α secretion by mononuclear cells is induced by GBS in a dose-dependent fashion. We

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Informed consent was obtained from all blood donors. The work was conducted in accordance with the guidelines of the Institutional Review Board of the University of Utah.

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found that concentrations of GBS of $\geq 10^5$/mL resulted in a pronounced increase in TNF-α production, with the maximum response at 10^6/mL GBS. In previous studies, [1] we also used live organisms, with similar results. Because of cellular toxicity associated with using live bacteria and the inability to regulate the concentrations of organisms in these cultures, we elected to use heat-killed organisms in this and other reported studies [1, 2]. For these studies, we chose a concentration of 10^5/mL GBS and a 48-h incubation period, which provided a submaximal stimulus and optimal timing for TNF-α production. Additional experiments indicated that 48 h was also the maximal response time for IL-1β and IL-6.

Figure 1 shows the cumulative results from the mononuclear cells of 5 subjects exposed to increasing concentrations of lactate in the presence and absence of GBS. The average baseline TNF-α secretion in the presence of media alone was 80 pg/mL. Physiologic concentrations of lactate <2 m*M* showed a slight stimulatory effect; concentrations >2 m*M* resulted in a significant increase in TNF-α production up to 180 pg/mL. Expression of TNF-α production as percent change from baseline for concentrations of 4–16 m*M* lactate showed an average enhancement of 207.1% ± 16.3% ($P = .001$). Mononuclear cells stimulated with GBS (5×10^5/mL) alone produced ~550 pg/mL TNF-α. When mononuclear cells were exposed to the combination of lactate and GBS, TNF-α production was further enhanced by an additional 400–500 pg/mL. This increase in production when cells were exposed to the combination of GBS and lactate in concentrations >2 m*M* is greater than an additive effect. When TNF-α production is calculated as percent change from baseline unstimulated cell secretion, GBS enhanced TNF-α production an average of 621.1 ± 42% of unstimulated controls ($P = .001$); the combination of GBS and lactate increased production by an average of 1019.3 ± 16.1% of unstimulated controls ($P = .001$).

When mixed mononuclear cells from 3 persons were examined for IL-1β production after exposure for 48 h to lactate in various concentrations, no statistically significant response was found (data not shown). The combination of GBS and lactate, however, enhanced IL-1β production above that of mononuclear cells stimulated by GBS alone (figure 2). Mixed mononuclear cells exposed to GBS alone secreted an average of 138.73 ± 11.6 pg/mL ($P = .001$); the combination of GBS plus pathophysiologic concentrations of lactate (≥ 4 m*M*) induced an average of 295.0 ± 23.95 pg/mL ($P = .0001$).

Finally, secretion of IL-6 was not significantly enhanced when mononuclear cells were exposed to lactate alone (data not shown). When mixed mononuclear cells were exposed to the combination of GBS (10^5 cfu) and various concentrations of lactate, IL-6 production was significantly enhanced, with concentrations of ≥ 3 m*M* (figure 2). If pathophysiologic con-

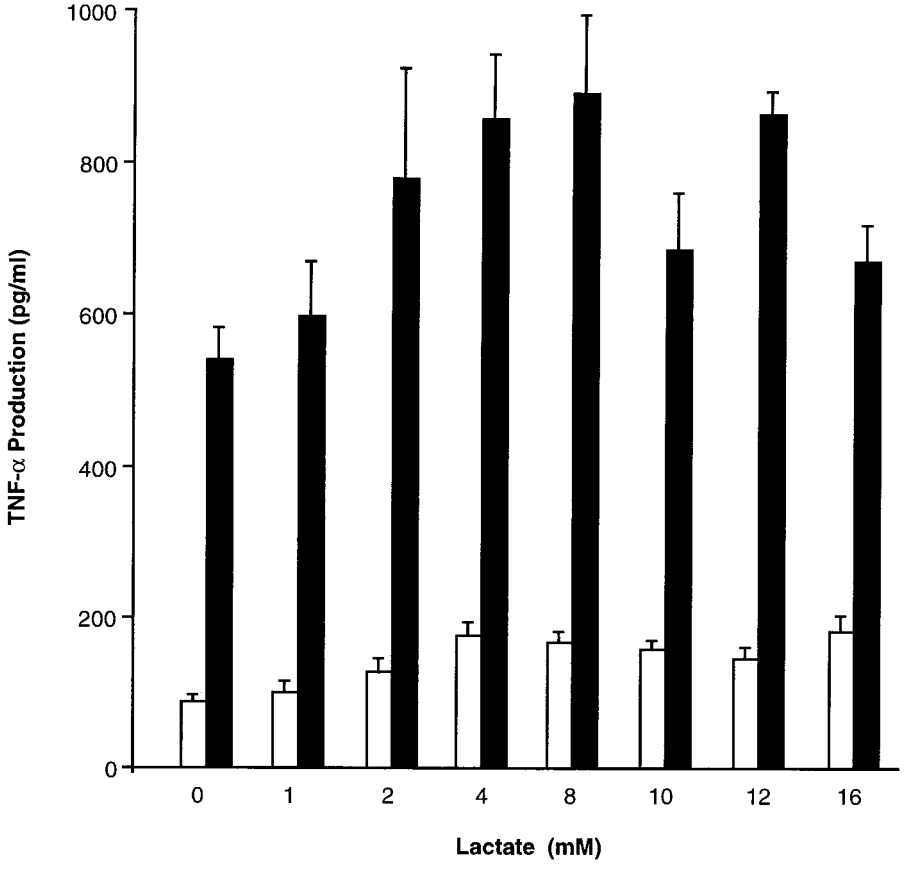

Figure 1. Effect of lactate on mononuclear cell secretion of tumor necrosis factor (TNF)-α in presence (■) or absence (□) of group B streptococci (10^5 cfu/mL; $n = 5$). Bars indicate SE.

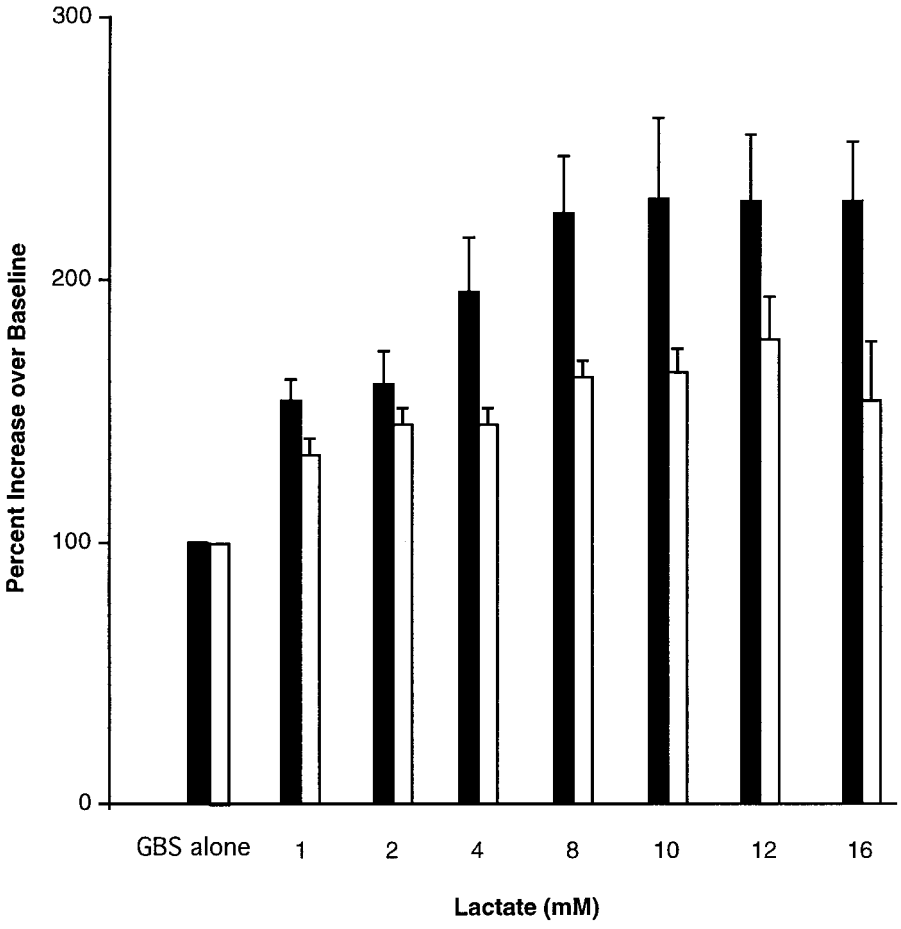

Figure 2. Effect of lactate on mononuclear cell secretion of interleukin (IL)-β (■) and IL-6 (□) in response to group B streptococci (10^5; n =3). Bars indicate SE.

centrations of lactate plus GBS were averaged, concentrations of 3–16 m*M* enhanced IL-6 production by an average of 59.6 ± 10.9% (P = .0002) over baseline GBS-stimulated mixed mononuclear cells.

Discussion

Lactate alone or in combination with GBS significantly enhanced mononuclear cell secretion of proinflammatory cytokines. The environment in which proinflammatory cells (e.g., macrophages and monocytes) function is often glucose-poor and acidic, and contains an abundance of lactic acid or lactate [4, 5]. Despite this hostile environment, macrophages are able to carry out their varied functions. Many cells are required to function in lactate-rich or anaerobic conditions. Because proinflammatory cells must be able to rapidly produce large quantities of energy for their metabolic needs, a lactate-rich environment provides substrate for energy production. Loike et al. [4] and others [5, 6] have shown that murine macrophages take up lactate preferentially in an acidic environment, thus appearing to utilize a stereospecific proton cotransporter that is inhibited by the anion transport blocker probenecid [4–6].

When we examined mixed mononuclear cells for TNF-α production after exposure to various levels of lactate, we found that lactate alone had a stimulatory effect on TNF-α production by human mononuclear cells. Although neutrophils may also produce TNF-α, IL-1, and IL-6, we chose to concentrate these studies on mononuclear cells. Exposure of these mononuclear cells to a combination of lactate and GBS increased TNF-α production, suggesting a synergistic effect of lactate with bacteria on TNF-α production.

Along with TNF-α, IL-1β is thought to be one of the principal cytokines responsible for the induction of fever, changes in leukocyte numbers, and synthesis of acute-phase reactants following acute bacterial infection [7, 8]. Monocyte/macrophage secretion of IL-1β was also affected by increasing lactate concentrations. Although no effect was found when mononuclear cells were exposed to lactate alone, there was a significant increase in the production of IL-1β after exposure to lactate plus GBS. Similarly, IL-6 production was not enhanced after exposure to lactate alone, but a significant response was observed with the combination of GBS and lactate. Failure to detect an effect of lactate alone on IL-1 and IL-6 lactate occurred at earlier time points as well, suggesting that the kinetics of the cytokine response were not responsible.

In summary, lactate significantly up-regulates TNF-α production by itself and profoundly enhances that induced by GBS. Lactate also enhances the production of IL-1β and IL-6 induced by GBS. There is considerable evidence for a pathogenic role for TNF-α and other cytokines in the septic shock syndrome and in bacterial meningitis. Lactic acid, which is elevated in the blood during septicemia and in the cerebrospinal fluid in meningitis, most likely acts in a synergistic fashion with bacterial products to cause enhanced release of proinflammatory cytokines. Furthermore, the combination of lactate and GBS likely contributes significantly to the severe morbidity observed in early and late onset forms of neonatal GBS infection.

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