Review

Science review: Extracellular acidosis and the immune response: clinical and physiologic implications

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Abstract

Metabolic acidosis is among the most common abnormalities seen in patients suffering from critical illness. Its etiologies are multiple and treatment of the underlying condition is the mainstay of therapy. However, growing evidence suggests that acidosis itself has profound effects on the host, particularly in the area of immune function. Given the central importance of immune function to the outcome of critical illness, there is renewed interest in elucidating the effects of this all too common condition on the immune response. In this review we concentrate on the effects of extracellular acids on production and release of inflammatory mediators, and we demonstrate that different acids produce different effects despite similar extracellular pH. Finally, we discuss potential clinical implications.

Keywords acidosis, cytokines, immune response, pH, sepsis

Introduction

Critical illness is exemplified by a state of profound disruption in normal homeostatic mechanisms. Patients who remain critically ill may progress to a poorly understood condition known as multiple organ failure, which is characterized by widespread alterations in both individual organ function and integrative function across organs. Although our understanding of this condition is extremely limited, numerous observations suggest that alterations in the immune response are not only caused by but may also be the cause of ongoing organ injury, and these alterations may adversely affect patients' ability to recover. Both increased inflammation and immune suppression have been implicated in the pathogenesis of multiple organ failure. Little is known about the influences that therapies have on the immune response. Emerging evidence suggests that ventilator-associated lung injury results in increased systemic inflammation [1] and that systemic inflammation resulting from local tissue injury appears to have effects on remote organs [2]. Drugs that appear to modify the course of organ injury such as activated protein C and corticosteroids appear to have a broad range of effects on the immune system [3,4]. Abnormalities in systemic acid–base balance may also induce significant alterations in the immune response. The clinical significance of these alterations is not yet known, but their magnitude suggests that they may play an important role in the development or maintenance of immune dysfunction. If this is the case, then they represent attractive targets (or even tools) for therapy. Extracellular pH (pH_o) for circulating leukocytes (i.e. blood pH) is easily altered and thus, for good or bad, changes in pH may rapidly alter the immune response in these cells.

bHS = 6% hetastarch in a balanced electrolyte solution; IL = interleukin; iNOS = inducible nitric oxide synthase; LPS = lipopolysaccharide; LR = lactated Ringer's; MAP = mean arterial pressure; NF-κB = nuclear factor-κB; NO = nitric oxide; NS = normal (0.9%) saline; pH_i = intracellular pH; pH_o = extracellular pH; SBE = standard base excess; TNF = tumor necrosis factor.

Table 1

Effects of acids on inflammatory mediators in macrophages

Acid	pH_o	Cells	LPS	Effect	Reference
HCl	6.5	Alveolar macrophages	(+)	↑TNF mRNA	5
HCl	5.5	Alveolar macrophages	(+)	↑TNF mRNA/↓TNF secretion	5
HCl	5.5	RAW	(+)	No ΔTNF mRNA/↓TNF secretion	7
HCl	7.0	Alveolar macrophages	(+)	↓TNF secretion	8
HCl	7.0	Peritoneal macrophages	(–)	↑NO, ↑TNF*, ↑NF-κB	6
HCl	7.2	RAW	(+)	↑NO	9
LA	6.7	Peritoneal macrophages	(+)	↑TNF mRNA/↑TNF secretion	10
DS	6.0	Peritoneal macrophages	(+)	↓TNF mRNA/↓TNF secretion	14
DS	6.5	Human blood-borne macrophages	(+)	↓TNF mRNA, ↓NF-κB	15

*Tumor necrosis factor (TNF) was not measured directly. DS, lactate-based dialysis solution; LA, lactic acid; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; NO, nitric oxide; NR, not recorded; pH_o, extracellular pH.

Effects of extracellular acidosis on inflammatory mediator release

There are now several studies documenting the effects of decreased pH_o on the synthesis and release of inflammatory mediators, especially tumor necrosis factor (TNF) and nitric oxide (NO). Most of these studies were conducted in resident macrophages or macrophage-like cell lines and yielded conflicting results (Table 1). However, studies using HCl have consistently shown proinflammatory effects at the level of nuclear factor-κB (NF-κB) DNA binding or TNF synthesis provided pH_o was not less than 6.0 [5–7], although TNF secretion was reduced even at pH_o as high as 7.0 [5,7,8]. Studies of nonstimulated resident peritoneal macrophages [6] and lipopolysaccharide (LPS)-stimulated RAW 264.7 cells [9] have shown increased NO formation at moderately reduced pH_o (7.0–7.2). However, more severely acidic pH_o reduces NO formation [6,9], and there is an apparent dissociation between the pH_o effects on inducible nitric oxide synthase (iNOS) mRNA, protein, and final NO release [9]. Thus, HCl appears to affect inflammatory mediators differently at different stages in their synthesis and release. Little is known about the effects of HCl on other cytokines or on the kinetics of pH_o mediated effects.

Lactic acid has been studied in an even more limited way than HCl. Lactic acid (pH_o 6.75) was shown in one study [10] to result in increased TNF release in LPS-stimulated peritoneal macrophages. This finding is surprising in light of the growing evidence of a protective effect of lactic acid in neuronal injury [11–13]. Several studies have sought to explore the effect of dialysis solutions on the immune response [14,15]. These acidic, lactate-based solutions have been shown to decrease various aspects of the immune response, including TNF synthesis and release [14,15]. Douvdevani and coworkers [15] also demonstrated a decrease in LPS-induced NF-κB DNA binding in human blood-derived macrophages when incubated with dialysis solution. Although these solutions are also hyperosmolar and have excessive glucose concentrations – variables that are known to influence immune function [14,16] – they provide additional evidence of a potential anti-inflammatory role of lactate and highlight potential differences between various acids and their effects on the immune response.

We conducted a series of experiments in LPS-stimulated RAW 264.7 murine macrophage-like cells in which we decreased the pH_o of the medium using different acids. Remarkably, dramatically different patterns of inflammatory mediator expression occurred with different acids, despite normalization to the same pH_o. In our first set of experiments [17] we acidified the cell culture medium using HCl and stimulated the cells with 10 ng/ml LPS (*Escherichia coli* 0111:B4) for 24 hours. Acidic medium itself barely affected the release of inflammatory mediators, including NO, IL-6, and IL-10. However, compared with pH_o 7.4, acidosis (pH_o 7.0) was associated with significantly increased NO release in response to LPS stimulation. Interestingly, under more extreme acidic conditions (pH_o 6.5), NO release decreased in response to LPS and was again similar to pH_o 7.4 (Table 2). At pH_o 6.5, release of both IL-6 and IL-10 was significantly less than at pH_o 7.0 or 7.4. However, IL-10 release was reduced to a far greater extent than was IL-6, and thus the ratio of IL-6 to IL-10 increased significantly from 5:1 at pH_o 7.4 to 55:1 at pH_o 6.5.

These findings suggest a proinflammatory effect of HCl, which is consistent with the existing literature on the effects of HCl on TNF synthesis [5–7]. Furthermore, the paradox in which mild and severe acidosis induced by HCl results in opposite effects on NO has now been explained. Pedoto and colleagues [18] first suggested that the optimal intracellular pH (pH_i) for iNOS was near 7.0 and that the addition of acid

Table 2

Summary of effects of lactic acid versus HCl on lipopolysaccharide-stimulated RAW 264.7 cells

	Lactic acid (pH 7.0)	Lactic acid (pH 6.5)	HCl (pH 7.0)	HCl (pH 6.5)
NO	↓	↓↓	↑	–
iNOS mRNA	↓	↓↓	↑	↑↑
IL-6	↓	↓↓	–	↓
IL-6 mRNA	↓	↓↓	–	↓
IL-10	↓	↓↓	↓	↓↓↓
IL-10 mRNA	↓↓	↓↓	–	–
IL-6 : IL-10 ratio	–	–	–	↑↑
NF-κB	↓	↓↓	↑	↓

IL, interleukin; iNOS, inducible nitric oxide synthase; NO, nitric oxide. Adapted from Kellum and coworkers [19].

would lower the pH_i toward the optimal value, thus increasing iNOS activity and NO production. Further addition of acid would cause pH_i to fall below the optimal value, leading to decreased NO production [18]. This hypothesis was recently tested by Huang and coworkers [9], who demonstrated that the optimal pH_o for NO formation by iNOS was 7.2 in RAW 264.7 cells. However, they also noted that alkaline pH_o favored expression of iNOS protein but that post-transcriptional mechanisms predominated, resulting in increased NO release at slightly acidotic pH_o.

To clarify the mechanism by which HCl influenced the release of cytokines from LPS-stimulated cells, we measured NF-κB DNA binding using electrophoretic mobility shift assay after exposure to different concentrations of HCl [17]. Again, acidosis (pH_o 7.0) significantly increased LPS-induced NF-κB activation, as compared with pH_o 7.4, whereas more extreme acidosis (pH_o 6.5) actually attenuated NF-κB activation. Thus, different degrees of hyperchloremic acidosis have differing effects on inflammatory mediator release as well as on NF-κB activation. Overall, the effects of HCl appear to be proinflammatory. These findings are in accordance with those of a study conducted in resident peritoneal macrophages by Bellocq and colleagues [6]. Those investigators found that these cells produced more NO when incubated in medium at pH_o 7.0 than at pH 7.4, and that this effect was associated with upregulation of iNOS mRNA as well as with activation of NF-κB.

By contrast, our data using lactic acid demonstrates that this acid is anti-inflammatory to RAW 264.7 cells, as indicated by decreased cytokine expression and NF-κB activation [17]. In these experiments, increasing concentrations of lactic acid (0–30 mmol/l) caused increasing acidification of the media, and trypan blue exclusion and lactate dehydrogenase release demonstrated that lactic acid did not reduce cell viability. However, lactic acid inhibited LPS-induced NF-κB DNA binding (Table 2). Lactic acid also significantly decreased LPS-induced expression of NO, IL-6, and IL-10, both RNA and protein, in a dose-dependent manner.

The mechanisms by which these acids exert their effects on innate immunity are presently unknown. The effects are not limited to LPS-stimulated cells, however, because the results have been (preliminarily) reproduced in interferon-γ stimulated RAW 264.7 cells [19], suggesting that the effects are not mediated through pH-induced changes in the LPS molecule or LPS-binding protein, or at the receptor. The effects may be partly mediated through NF-κB because DNA binding of this transcription factor is generally consistent with effects on NO and IL-6 (Table 2). However, extracellular acids also have effects on IL-10, which is outside the NF-κB pathway. What is apparent is that the effects of extracellular acids are not limited to the effects on pH_o because different acids produce different effects despite similar pH_o. Whether different effects can be explained by differences in pH_i are as yet unknown, although the patterns of response (Table 2) suggest that this is likely.

Effects of extracellular acidosis on other aspects of immune cell function

While this review focuses on the effects of extracellular acids on inflammatory mediator release, there is evidence that acidosis influences other aspects of the immune response. As detailed in the excellent review by Lardner [20], extracellular acidosis has far reaching effects on the immune response. For example, leukocyte chemotaxis is impaired at extreme acidic pH_o, generally beginning between pH 6.0 and 5.5 [21–23] with an additive effect of hypoxia [22,24]. Activation of oxygen burst in neutrophils [25], production of reactive oxygen species [26–28], neutrophil phagocytosis [25,29], and intracellular killing [30] all appear to be influenced by pH_o, as does neutrophil apoptosis [31,32]. Finally, there is evidence that complement activation by C-reactive protein may be the result of a pH_o-dependent conformational change in the protein [33].

Thus, pH_o, or the effects of the separate ions involved, appears to influence multiple aspects of the inflammatory response. In addition, extracellular acidification may exert its effects by altering pH_i. Indeed, several studies have identified a relationship between pH_i and pH_o, regardless of which milieu is altered experimentally [34,35]. For example, when pH_o was increased a subsequent increase in pH_i, mediated by the N^+/H^+ exchanger (NHE-1), was observed, along with augmented leukotriene release by neutrophils [34]. These events were followed by extracellular acidification. Of note, studies conducted in bicarbonate-buffered medium [32] have shown effects on neutrophil function that are at odds with other literature. Those investigators hypothesized that acid titration of bicarbonate with generation of CO_2 leads to a rapid decrease in pH_i. Alternatively, the CO_2 effect may be independent from the effect on pH_i.

In vivo effects of hyperchloremic acidosis

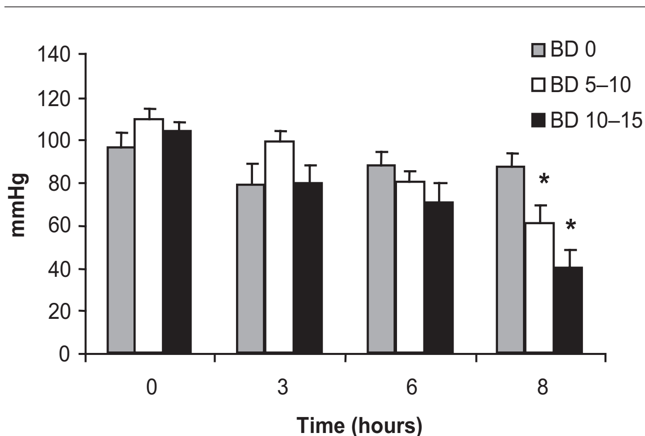
Experiments using cells in culture exposed HCl or lactic acid provide a highly reproducible but less clinically relevant model for study. By contrast, saline resuscitation is an extremely common cause of hyperchloremic acidosis. By using a mathematical model based on a physicochemical acid–base analysis, we accurately predicted the serum Cl^- concentration and resulting arterial blood pH changes in healthy dogs given large volumes of intravenous 0.9% saline [36]. By applying this model to dogs given an intravenous bolus of LPS (1 mg/kg) and subsequent large volume saline resuscitation (100 ml/kg over 3 hours), we quantified the effects on acid–base balance [36]. The total acid load was calculated from the change in standard base excess (SBE) attributable to each source. In LPS-treated animals mean arterial pH decreased from 7.32 to 7.11 ($P < 0.01$); partial CO_2 tension and lactate were unchanged. Saline accounted for 38% of the total acid load. Although serum Na^+ did not change, serum Cl^- increased (128 to 137 mmol/l; $P = 0.016$). From these experiments we concluded that saline resuscitation alone accounts for more than a third of the acidosis seen in this canine model of acute endotoxemia, whereas lactate accounts for less than 10%. Furthermore, a large amount of the unexplained acid load in this model appears to be attributable to differential Na^+ and Cl^- shifts, presumably from extravascular to vascular or intracellular to extracellular spaces.

In a recent study [37], we found that normal (0.9%) saline (NS) resuscitation resulted in a decreased survival time and reduced the SBE by 5–10 mEq/l as compared with a balanced colloid solution. In this experiment, we studied 60 rats for 12 hours after intravenous infusion of LPS (20 mg/kg). We resuscitated to maintain a mean arterial pressure (MAP) above 60 mmHg using NS, 6% hetastarch in a balanced electrolyte solution (bHS), or lactated Ringer's (LR). We showed that mean survival time among animals treated with NS or LR was 45% less than in bHS-treated animals ($P < 0.0001$) and that overall survival (at 12 hours) was 0% with NS or LR versus 20% with bHS ($P = 0.05$). After resuscitation with NS, arterial SBE and plasma apparent strong ion difference were both significantly lower and plasma Cl^- was significantly higher than with bHS. Resuscitation with LR resulted in a SBE and plasma Cl^- between those with NS and bHS. Importantly, we observed an inverse relationship between the change in serum Cl^- and survival time in these animals ($R^2 = 0.37$; $P < 0.001$). From these data we concluded that, as compared with bHS, volume resuscitation with NS was associated with more metabolic acidosis and shorter survival in this experimental animal model of septic shock. Furthermore, we hypothesized that hyperchloremia may play a role in reducing short-term survival, but that other factors must also be involved because LR-treated rats fared no better than did those treated with NS, even if they had less hyperchloremia.

Metabolic acidosis might reduce survival from sepsis through a variety of mechanisms. First, acidosis has been associated with hemodynamic instability [38], although the association is not always consistent [39] and the underlying mechanisms are uncertain. Pedoto and colleagues [18] recently showed that metabolic acidosis may increase iNOS expression in animals and that this could exacerbate vasodilation and shock. Second, acidosis, even in the absence of sepsis or endotoxemia, is associated with gut barrier dysfunction [40,41]. Finally, acidosis can lead to oxidative stress by promoting delocalization of protein-bound iron stores in cells leading to Fenton-type biochemistry and redox stress [42], and by causing protonation of the peroxynitrite anion ($ONOO^-$) and thereby increasing the tendency of this moiety to behave like the potent free radical hydroxyl ($OH^\bullet$) [43,44]. Pedoto and colleagues demonstrated that hyperchloremic acidosis increases lung [18] and intestinal injury [45] in healthy rats.

In order to control for other effects of large-volume resuscitation (e.g. cell swelling), we next increased serum Cl^- concentration by infusing a dilute HCl solution into rats with sepsis induced by cecal ligation and puncture [46]. Eighteen hours after cecal ligation and puncture, we randomly assigned 24 rats to three groups. In groups 2 and 3 we began an 8-hour intravenous infusion of 0.1 N HCl to reduce the SBE by 5–10 and 10–15 mEq/l, respectively. We measured MAP, arterial blood gases, electrolytes, and plasma nitrate/nitrite levels at 0, 3, 6 and 8 hours. MAP remained stable in group 1 but decreased in groups 2 and 3 ($P < 0.001$), such that at 8 hours MAP was much higher in group 1 than in either group 2 or group 3 (Fig. 1). This change in MAP correlated with the increase in plasma Cl^- ($R^2 = 0.50$; $P < 0.0001$) and less well with the decrease in pH ($R^2 = 0.24$; $P < 0.001$). After 6 hours of acidosis plasma nitrite levels were significantly higher in group 2 animals than in group 1 or group 3 animals ($P < 0.05$). We concluded that moderate acidosis, induced by HCl infusion, worsened blood pressure and increased plasma nitrate/nitrite levels in septic rats. Some other mechanism is needed to account for the further reduction in MAP in group 3

Figure 1



Mean arterial pressure for septic animals (induced by cecal ligation and puncture) after infusion of 0.1 N HCl acid to reduce the base deficit (BD) by 5–10 mEq/l (white bars) or 10–15 mEq/l (black bars). A control group was given a similar volume of lactated Ringer's (gray bars). Shown are group means ($n = 8$) ± SEM. *$P < 0.05$. Adapted from Kellum and coworkers [46].

animals, however, because NO release was not increased in that group. Our results are in general agreement with reports by Pedoto and coworkers [18,45] that demonstrated that metabolic acidosis increased iNOS, leading to vasodilation and shock in healthy rats. Our study extends these findings by examining the effects of acidosis in nonshocked, septic animals. These data are also consistent with our data from RAW 264.7 cells (presented above), in which a decreased pH_o (7.0) resulted in increased NO release but more severe acidosis ($pH_o = 6.5$) did not [17].

Clinical implications

Understanding the effects of acid–base balance on the inflammatory response is highly relevant to clinical medicine for a variety of reasons. First, current deficiencies in our understanding of the effects of acidosis on a wide range of cellular processes have led to controversy in the way in which patients are managed in a variety of clinical settings. Most clinicians tend to ignore the effects of exogenous Cl^- on pH_o, but many will treat even mild forms of acidemia. In addition, all forms of metabolic acidosis appear to be associated with prolonged hospital and intensive care unit length of stay [47]. Because metabolic acidosis is both commonly caused and treated by clinicians, an understanding of the physiologic consequences of altered pH_o is imperative.

Second, our ability to alter acid–base balance as a tool with which to manipulate cellular processes will be dependent on an improved understanding of the relationship between pH_o and the synthesis and release of inflammatory molecules. Investigators continue to seek means to modulate the inflammatory response as primary therapy for sepsis and related conditions. These efforts have focused not only on reducing proinflammatory mediators in an effort to reduce tissue injury, but also on the converse – augmenting the inflammatory response to infection. This interest also extends into other fields, including autoimmune disease and cancer therapy. For example, decreased lymphocyte function has been documented with decreased pH_o in human lymphokine-activated killer cells [48], human IL-2 stimulated lymphocytes [49], as well as murine natural killer cells [50]. The mechanisms responsible for these effects are unknown but probably do not include energy substrate depletion [50].

Third, even when it is not practical or desirable to manipulate pH_o as a primary means of altering the inflammatory response, an understanding of how pH_o affects this response is necessary to interpret data from studies of immunomodulation; to avoid unintended immunomodulation in clinical and laboratory settings; and to explore the capacity of pH_o to improve the effectiveness of existing treatments. Finally, an understanding of how pH_o is involved in the regulation of inflammation by intracellular signaling pathways or other mechanism might ultimately lead to other strategies for immunomodulation.

Conclusion

Little is currently known about the effects of acid–base abnormalities on innate immunity. Acidosis produces significant effects on immune effector cell function *in vitro*. The regulation of NO release and synthesis has been found to be significantly effected by pH_o both *in vitro* and *in vivo*, and may be partially responsible for acidosis-associated hemodynamic instability. Production of inflammatory cytokines, as well as DNA-binding of transcription factors in their control pathways, appears to be sensitive to pH_o as well. However, emerging evidence suggests that different forms of acidosis (respiratory versus metabolic) and even different types of metabolic acidosis (lactic versus hyperchloremic) produce different effects. Overall, lactic acid appears to be anti-inflammatory whereas HCl is proinflammatory. The extent to which these effects apply to the clinical situation has yet to be determined, but given that acidosis is an extremely common problem in the intensive care unit, and immune function is of critical importance, efforts to elucidate these relationships are quite justified.

Competing interests

JAK has received research grants and consulting fees from Abbott Laboratories.

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