

The effects of extracellular pH on immune function

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Abstract: The effect of alterations in extracellular pH on cellular and humoral immune function is reviewed. Because acidic pH predominates at inflammatory loci and other sites of immune activity, most studies to date focus on the effect of acidic rather than alkaline pH. Investigations on polymorphonuclear leukocytes demonstrate mainly inhibition of chemotaxis, respiratory activity, and bactericidal capacity at reduced pH. Evidence of impaired lymphocyte cytotoxicity and proliferation at acidic pH is also beginning to emerge. Many of the clinical acidoses are accompanied similarly by immunodeficiency. Studies on macrophages and eosinophils are few and inconclusive. A small number of studies demonstrate acid-induced activation of complement proteins and the alternative complement pathway, plus increased antibody-binding to leukocytes at lowered pH. A differential effect of acidic pH on humoral and cellular immunity may, therefore, exist. Increasing recognition of the significance of extracellular pH in relation to immune function warrants further studies in this presently incomplete but rewarding field. *J. Leukoc. Biol.* 69: 522–530; 2001.

Key Words: *immunity · acidosis · leukocytes · immunodeficiency · humoral · cellular*

INTRODUCTION

Review of the effects of extracellular pH on immune function

The importance of acid-base homeostasis in the maintenance of normal cellular responses and physiological integrity has long been recognized. Many cellular responses are diminished at lowered extracellular pH, including cytosolic- and membrane-associated enzyme activities, ion transport activity, protein and DNA synthesis, and cAMP and calcium levels. The activities of hydrolytic enzymes, which are released to the extracellular fluids or medium during cell activation, have also been found to be pH-sensitive [1, 2]. Apropos the immune system, alterations in the microenvironment at the sites of infection and inflammation have been studied since the 1940s (for example, see [3–7]). For instance, a characteristic feature of the inflammatory locus is local acidosis, which is attributed to the local increase of lactic-acid production by the anaerobic, glycolytic activity of infiltrated neutrophils and to the presence of short-chain, fatty acid by-products of bacterial metabolism [3, 4]. The interstitial fluid of tumors and abscesses also has shown

pH values of less than 6.0, averaging 0.2–0.6 units lower than mean extracellular pH of normal tissues (for review, see [8]). Recently, it has been suggested that acidic microenvironments may play a role in inhibiting immune function in certain respiratory conditions such as cystic fibrosis [9], and during neoplastic growth and invasion [8, 10]. Therefore, perhaps surprisingly, there are relatively few studies on the effect of altered extracellular pH on immune cells and their function. Also considering the clinical frequency of acid-base disturbances and how these might affect host immunity, an understanding of how direct and indirect immune function might be altered by ambient variations in pH is increasingly warranted.

In contrast, the role of intracellular pH in the regulation of various cellular activities has been the focus of a considerable body of work over the last 20 years. For example, an increase in cytosolic pH is known to be responsible for the increase in DNA and protein synthesis, enhanced metabolic rate accompanying oocyte fertilization, and cell proliferation and mitosis in general (for review, see [11]). In addition, the stimulus response coupling certain activities of neutrophils and lymphocytes has been linked unequivocally to cytoplasmic alkalization [12–15]. The maintenance of a resting intracellular pH of 6.8–7.3 and alterations in intracellular pH accompanying the cellular events outlined are considered primarily to be the result of an energy-requiring, active proton-extrusion system [11]. A major mechanism for active H⁺ extrusion that has been documented in the plasma membrane of a large variety of cells is the carrier-mediated Na⁺-H⁺ exchanger NHE-1 (anti-port, counter-transport system) that catalyzes the exchange of sodium for hydrogen [11, 16]. This Na⁺-H⁺ exchanger constitutes a form of secondary, active transport, relying on the steep, extracellular Na⁺ gradient provided by the primary Na⁺-K⁺ ATPase pump to extrude one hydrogen ion from the cytosol in exchange for the entry of one Na⁺. It is also capable of mediating the exchange of a narrow selection of other monovalent ions, for example external H⁺ for internal Na⁺, external Li for internal H⁺, internal Li for external H⁺, and Na⁺ for Na⁺ [11]. The primary physiological role of this exchanger is to maintain intracellular pH homeostasis by extruding metabolically generated H⁺ ions [11, 16]. It does so by allosterically activating a region of the inner cytoplasmic surface up to a critical set-point of pHi, beyond which further increases in the cytosolic H⁺ concentration results in diminished activity of the

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transporter [11]. The latter mechanism serves to protect against the generation of an intracellular alkalosis.

A variety of extraneous stimuli such as extracellular acidification, hormones, and growth factors are also capable of modulating the activity of the NHE-1 exchanger [16]. Acute and chronic acidosis, for example, causes an increase in the mRNA and the activity of the exchanger in many cells, including lymphocytes [17, 18]. The exchanger also acts as a signal transducer for various stimuli that modulate cell function by altering intracellular pH. For example, activation of mature lymphocytes is accompanied by an abrupt rise in intracellular pH associated with increases in RNA and protein synthesis and with increased activity of the Na^+/H^+ exchanger [12, 13]. In addition, macrophages, neutrophils, and lymphocytes possess two further Cl/HCO_3^- exchangers, one sodium-dependent and the other sodium-independent [4, 19]. The former is thought to play a role in the cell defence against intracellular pH under physiological conditions, and the latter may assist in the restoration of pH_i after an alkali load [19]. Macrophages also possess an additional ATP-dependent, proton-extrusion mechanism, which assists in the recovery from an intracellular acid load [19].

This article reviews the studies available to date on immune function and extracellular pH, with due consideration, where appropriate, to the simultaneous involvement of intracellular pH effects and their consequences for modulation by extracellular acid-base changes.

STUDIES ON POLYMORPHONUCLEAR LEUKOCYTES

Most of the studies reviewed focus on the effect of extracellular acidification more than on alkaline pH. One of the earliest investigations of pH and leukocyte activity was carried out by Nahas *et al.* [20]. They measured the rate of random leukocyte motility as a function of pH *in vitro* and demonstrated no adverse effect when the pH was decreased from 7.4 to 6.5. Locomotion was impaired significantly below pH 6.5. Given that the lowest pH studied at the site of an inflammatory locus is 5.5 [3], a similar pH-related retardation of leukocyte migration *in vivo* is likely. Increasing the ambient pH beyond 7.6 also produced a significant decrease in movement, with complete and irreversible inhibition of motility occurring at pH 7.9. Because increased pH has not been found in the microenvironment of immune reactions, this finding may not be clinically relevant. Leblebicioglu *et al.* [21] showed an impairment of chemotaxis at pH 7.7 and 8.2 but found no change at pH 6.7. In contrast, several groups have shown a reduction in specific chemotaxis at acidic pH. Rabinovitch *et al.* [22] showed an inhibition of chemotaxis at acidic pH, with a paradoxical stimulation of random neutrophil movement at pH 6.0. They speculate that the inhibition of chemotaxis could be related in part to the enhanced spontaneous migration, which may blunt migrational movement. Rotstein *et al.* [6] also demonstrated progressive impairment of chemotactic migration to formyl-Met-Leu-Phe (fMLP) as the medium pH was lowered from 7.0 to 5.5, decreasing to half of control values at pH 5.5. However, in contrast to the findings of Rabinovitch *et al.* [22], random

migration in their study was also decreased at pH 6.0 and 5.5. This is in general accord with findings of Nahas *et al.* [20]. Two groups showed an additive effect of hypoxia combined with lowered pH on inhibition of chemotaxis [6, 23]. In summary, the available data suggest impairment of chemotaxis and random migration of polymorphs at acidic, extracellular pH. A significant reduction in the migration of eosinophils when acidic extracellular pH was combined with increased, extracellular NaCl concentrations has also been found *in vitro* [24].

STUDIES ON NEUTROPHILS

There are a number of studies, mostly *in vitro*, investigating extracellular pH effects on isolated neutrophils. Gabig *et al.* [25] showed an 80–90% decrease in oxygen consumption and O_2^- production with acidic pH. Leblebicioglu *et al.* [21] examined selected functions of human polymorphs *in vivo* following exposure to a range of altered extracellular pHs. Activation of the respiratory burst was optimal at pH 7.2 but was depressed significantly at pH 6.7 and 8.2. Lactoferrin release and phagocytosis of opsonized bacteria were inhibited at pH 7.2. The group concluded that the pH of the periodontal environment could influence neutrophil activation selectively, thus altering the balance between bacteria and the host response. Rotstein *et al.* [7] examined the effect of succinic acid, a metabolite of the anaerobic bacterial species *Bacteroides*, on the respiratory burst of neutrophils. An inhibitory effect was observed at pH 5.5 but not at 7.4. The inhibition correlated with a decrease in intracellular pH, suggesting that succinic acid exerted its inhibitory effect by decreasing intracellular pH. In an investigation of the effect of intracellular pH changes on superoxide production by neutrophils, Simchowicz [26] noted a direct correlation between activation of the cells by fMLP and an internal alkalinization mediated by the Na^+/H^+ exchanger. He demonstrated a relationship between the quantity of O_2^- produced and cellular alkalinization. He also found that the intracellular pH (pH_i) of stimulated neutrophils was dramatically dependent on extracellular pH (pH_o) and that lowering the pH_o caused a reduction in the amount of superoxide generation increasing pH_o , resulting in enhanced O_2^- release. Likewise, Araki *et al.* [27] showed increased tumor cytotoxicity and H_2O_2 production by neutrophils with increasing pH_o . Inhibition of the Na^+/H^+ exchanger resulted in inhibition of the pH-mediated activity. This is consistent with the correlation of superoxide inhibition with pH_i by Rotstein's group [7]. No mechanism has been forwarded to explain how pH_o alters pH_i so dramatically, which is somewhat surprising in view of the relatively large volume of literature investigating the role of the Na^+/H^+ exchanger on pH_i regulation and immune cell activation. Given the ability of the cation exchanger to operate in alternative modes under experimental conditions, it is possible that the convergence of pH_i and pH_o values may result from alternative activation of the cation exchanger by initial extracellular hydrogen in exchange for intracellular sodium. However, this needs to be demonstrated under suitable experimental conditions. Stimulation of mammalian neutrophils by complement component C5a and fMLP is also accompanied by a sharp rise in pH_i [14]. The latter has

also been correlated with the subsequent migration, degranulation, and superoxide production by neutrophils [15]. It is interesting that two studies showed the extracellular release of protons by activated neutrophils following the respiratory burst [28, 29]. On the strength of the available evidence to date and the absence of any evidence of intracellular hydroxyl anion generation during polymorph (or indeed monocyte and lymphocyte) activation, it seems likely that this efflux of protons is the primary source of intracellular alkalization accompanying many of the functional changes in neutrophils and other immune cells. Others have discovered a direct relationship between intracellular and extracellular pH, regardless of which milieu is altered experimentally [30, 31]. For example, Osaki *et al.* [30] found that initial, extracellular alkalization facilitated cytoplasmic alkalization and subsequent leukotriene B₄ production of neutrophils, which was intimately related to Na⁺/H⁺ anti-port activity. However, the shift in pH_i subsequent to neutrophil activation was accompanied in both cases by proton efflux and extracellular acidification [30]. Weisman *et al.* [32] have also correlated intracellular alkalization of activated neutrophils with increased activity of the Na⁺/H⁺ exchanger. Initial stimulation of neutrophils by fMLP results in an initial, rapid acidification of the cell, lasting for about 50 s, followed by a return to baseline and a plateau of alkalization lasting several minutes. Grinstein and Furuwaya [31] have correlated the source of intracellular H⁺ with generation of superoxide and the hexose monophosphate shunt. Irrespective of the source of intracellular protons, it is likely that the efflux of hydrogen ions during the respiratory burst of neutrophils contributes to acidification of the extracellular milieu in addition to that resulting from lactate production by leukocytes and bacterial fatty acid production. This may well serve to paradoxically confound the acid-pH-related inhibition of other cellular functions, such as chemotaxis and phagocytosis.

The sequence of cellular events linking activation of the cation exchanger with the observed experimental changes in polymorph and neutrophil function has yet to be elucidated. The Na⁺/H⁺ exchanger plays a role in the regulation of cell volume as a result of sodium influx [11]. Alterations in cell volume of immune cells consequent to activation of the cation exchanger could theoretically play a role in triggering certain indirect functions such as chemotaxis and phagocytosis. One early study, for example, has found an inverse relationship between cell shrinkage and phagocytosis [33].

In contrast to the decreases shown in cytotoxicity at acidic extracellular pH, a marked increase in cytotoxicity induced by zymosan, fMLP, and concanavalin A (Con A) in human neutrophils and monocytes at pH 6.2 compared with 7.4 has been demonstrated by two groups [34, 35]. In one study, cytotoxicity, as measured by the luminescence response of stimulated neutrophils and by percentage lysis of target cells, was increased significantly at pH 6.2 [34]. These cellular assays have not been used by others, and it is possible that acidic pH values enhance these two functions selectively. In a detailed study, Trevani and co-workers [35] studied the effect of ambient pH on a range of nonspecific, neutrophil activities. Lowering the extracellular pH from 7.0 to pH 6.5 resulted in a transient increase in the intracellular concentration of calcium, a delay in the rate of apoptosis, up-regulation of the surface expression

of the β2-integrin CD-18 (which plays a role in the binding of neutrophils to endothelial cells during inflammation), and an increase in H₂O₂ production by neutrophils. They [35] have suggested that the production of reactive oxygen species other than O₂⁻ could be increased in acidic conditions, resulting in the observed increase in cytotoxicity. Commensurate measurements of intracellular pH changes were obtained, and a direct correlation between the observed changes and intracellular pH alterations was observed. In addition, all experiments were carried out in bicarbonate-based medium to reproduce as closely as possible the environmental conditions predominating *in vivo*. They found that all of the changes shown were dependent on the presence of extracellular bicarbonate, and only a slight change in the intracellular pH and neutrophil function was observed when HCl was added to bicarbonate-free medium. They postulate that the presence of HCO₃⁻ is necessary for the titration of extracellular H⁺, thus allowing inward diffusion of CO₂ and subsequent intracellular acidification. In this way, they propose a central role for external HCO₃⁻ in the development of the functional changes observed. Although the parameters investigated differ somewhat from those of others, their findings of an overall increase in neutrophil activity disagree with the observed inhibition of neutrophil function of most other groups. Further investigations of phagocytosis, intracellular killing, and chemotaxis by neutrophils in bicarbonate-based medium may help to clarify these apparent discrepancies. Craven *et al.* [36] tested the effect of moderate extracellular acidification on bovine neutrophil function and in contrast to the findings of Leblebicioglu *et al.* [21], found that phagocytosis of *Staphylococcus aureus* was hardly affected by ambient pH changes except at pH 5.0 [37]. However, intracellular killing was inhibited markedly, and optimal killing of bacteria was observed above neutral pH. This points further to a role for intracellular alkalization on nonspecific, neutrophil function. However, given the highly acidic pH optima of the lysosomal hydrolytic enzymes involved in bactericidal killing, the positive effect of alkaline pH is somewhat unexpected. In an *in vivo* study, Leblebicioglu and Walters [38] investigated the effect of asphyxia and accompanying acidosis on neutrophil number and function in rats. They found an increase in the neutrophil number but impaired phagocytosis and bactericidal activity for 24 h after exposure to the asphyxia. They speculated that asphyxia might predispose patients to sepsis as a result of impaired neutrophil function but failed to forward any possible explanation for this effect. Their finding of impaired phagocytosis *in vivo* is in agreement with the data of Leblebicioglu *et al.* [21], and the combined effects of hypoxia and pH to diminish neutrophil function are also consistent with the findings of Rabonovitch *et al.* [22] and Simchowitz [26]. An additive effect of asphyxia or hypoxia and pH is to be expected, given the adverse effects of compromised cellular oxygen levels on cell metabolism and energy charge. Finally, another study investigated the effect of external acidity and alkalinity on the rate of apoptosis of polymorphs *in vitro* and found an increased rate of apoptosis with increasing external pH [39]. This agrees with the recent findings of Trevani *et al.* [35], who showed delayed apoptosis at acidic pH.

STUDIES ON MACROPHAGES

Because the microenvironment of inflammatory lesions and abscesses is recognized as a locus of decreased pH [3–5], a small number of studies of macrophage function and pH effects are beginning to emerge. Bidani *et al.* [9] have investigated the role of the vacuolar-type H^+ -ATPase in the regulation of intracellular pH of alveolar macrophages and found that extracellular acidification had a similar effect on superoxide production and Fc-mediated phagocytosis as inhibition of the H^+ -ATPase activity with bafilomycin. Both strategies resulted in inhibition of the functions examined. This membrane-bound, H^+ -ATPase proton pump does not appear to exist in other populations of immune cells. The same group also measured the release of tumor necrosis factor- α (TNF- α) by stimulated macrophages and showed a reduction in the amount released and overall activity with diminished extracellular pH [9]. Alveolar macrophages function in highly acidic microenvironments, prompting these workers to suggest that the diminished extracellular pH may play a role in suppressing cytokine production and cytotoxic activities by pulmonary macrophages, thus impairing host response to infection. Nakagawara *et al.* [39] showed a direct correlation between H_2O_2 release by monocytes and pH_o , increasing pH_o and resulting in augmented hydrogen peroxide production. However, there is one study that shows an increase in nitric oxide synthase (NOS) activity in rat peritoneal macrophages following incubation with medium at pH 7.0 [40]. The authors conclude that exposure of macrophages to an acidic microenvironment in inflammatory lesions leads to up-regulation of NOS. The significance of this finding remains to be established as a result of the inhibitory effects of acidic pH_o on superoxide and TNF production in macrophages.

STUDIES ON LYMPHOCYTE FUNCTION

There are relatively few studies on the effect of extracellular pH on lymphocyte function. Those that have been carried out tend to be focused on the role of ambient pH alterations within tumor microenvironments or on bovine lymphocytes following ketosis.

The average pH in many tumors is about 0.5 unit lower than normal surrounding tissues [9, 41, 42]. Therefore, there is a growing awareness among immunologists and oncologists of the potential modulatory role of the prevailing tumor microenvironment on immune cell function. Necrotic foci are a common feature of solid tumors, probably as a result of, in part, insufficient vascularization and subsequent hypoxia [41]. Hypoxic cells are dependent on glycolysis for their energy needs, and the production of large amounts of lactic acid is an inevitable consequence of such anaerobic metabolism. Lactate accumulation results in a decrease in extracellular pH, which, when combined with hypoxia, results in diminished viability of healthy and cancerous cells. In addition, a drop in pH_i from 7.0 to 6.0 results in inhibition of glycolysis with concomitant inhibition of glucose consumption and lactate production (see [41]). Therefore, a further consequence of acidic pH is energy

deprivation, and this effect is as applicable to healthy immune cells as it is to cancer cells. Extracellular pH effects are, therefore, becoming increasingly germane to studies of tumor immunology. In an attempt to simulate the three-dimensional milieu of solid tumors, Ratner [43, 44] investigated lymphocyte motility in neutral and acidified extracellular matrix following stimulation with interleukin (IL)-2 in three-dimensional gels. He found increased motility at pH 6.7 compared with pH 7.1, an effect that was abolished in one-dimensional gels. He concluded that the pH effect represents a modification of lymphocyte-matrix interactions and forwarded ambient pH as a microenvironmental factor that can influence lymphocyte motility within tumors. He further speculated on a stimulatory role for weakly acidic tumor microenvironments, and extremely low pH was toxic to lymphocyte activity. Conversely, a role for extracellular pH in stimulating tumor development through inhibition of immune function is emerging from other *in vitro* studies. Severin *et al.* [45] showed diminished cytotoxic activity of human lymphokine-activated killer (LAK) cell activity in acidic pH; Loeffler *et al.* [46] found a similar depression of murine natural killer (NK) cell activity with lowered pH_o . The conditions replicated *in vitro* were designed to simulate as closely as possible the microenvironment predominating in areas of necrotic or poorly vascularized tumor foci. In attempting to explain the mechanism by which cytotoxicity was impaired, they ruled out depletion of energy substrates as the sole cause of inhibition [46]. These workers also demonstrated inhibition of IL-2-stimulated lymphocyte proliferation by acid pH_o when tumor-like physico-chemical conditions of anoxia, low pH, and low glucose were simulated experimentally [47]. They suggest that inhibition of IL-2-stimulated lymphocyte proliferation by such conditions may be a factor in the relatively poor success rate of IL-2-LAK cell immunotherapy. They further argue for the temporary improvement of physical conditions such as pO_2 within tumors during IL-2 administration to determine if the response of tumor-infiltrated lymphocytes to IL-2 could be improved. Similarly, decreased lysis of various tumor cell lines by cytotoxic T-lymphocytes at acidic extracellular pH has been demonstrated by Redegeld *et al.* [48]. On the basis of theoretical computer modeling based on experimental data available to date, Kraus and Wolf [8] propose that acidification of the microenvironment by malignant cell “enslaves” processes normally counteracting neoplastic growth and invasion. The lowest pH values are found in those regions where tumor cells are growing adjacent to a basement membrane, causing necrotic foci and promoting tumor invasion. Thus, they suggest that the success of immunotherapies may critically depend on the number of tumor cells and the microenvironment at the beginning of the therapy. They further propose a reassessment of the benefit of therapeutic approaches that aim to decrease tumor pH selectively. According to this theory, artificial tumor acidification may lead to a reduction in the primary tumor size in the first phase, whereas the second phase may generate malignant, acid-insensitive subclones. In contrast, Ratner [43] proposes that manipulation of intra-tumor pH via systemic acidosis, glucose infusion, or hyperthermia might constitute a useful adjuvant to immunotherapy if lymphocytic infiltration were increased as a result. However, it must be considered that tumor pH may not necessarily decrease in a

homogenous manner; low pH may be present in large tumors, and elevated pH may be found in some necrotic areas because of the depletion of glycogen stores in these areas [41, 46]. Also, hypoxia in combination with low-glucose concentration and acidic pH_o is capable of killing tumor cells themselves [42], rendering obsolete the targeted immunotherapy of such cells. In view of the likelihood of variations in pH existing within and around solid tumors, there are likely to be equally heterogeneous effects of pH on immune-cell function at the locus of activity, and much research remains to be done in this area. It should be also considered that cancer cells use glucose at far higher rates than normal cells; thus, it is possible that insufficient glucose may be available for lymphocytes and other immune cells operating within a tumor region, further compromising the overall effectiveness of the immune response. Similarly, the inadequate perfusion of diseased tissues, which are involved in a variety of other pathological conditions such as infarction and resulting necrosis, will result in similar metabolic effects on the surrounding milieu. The effectiveness of the resultant inflammatory processes is just as likely to be compromised by low extracellular pH as are populations of immune cells congregating at the site of tumors.

Because of the clinical frequency of bovine ketosis, there are a number of studies about the effect of ketoacids on bovine lymphocyte function, and all but one paper shows inhibition of lymphocyte proliferation in the presence of varying concentrations of ketones. Two groups investigated the mitogenic response of peripheral bovine lymphocytes from ketotic cows or calves and found a significantly lower glucose consumption index value for phytohaemagglutinin in ketotic cows compared with healthy cows [49–51]. However, one study showed that high concentrations of butyrate and physiological acetate concentrations inhibited lymphocyte proliferation, and only supraphysiological levels of β -hydroxybutyrate affected increased proliferation [52]. The authors concluded that the ketone bodies tested had minimal effects on bovine lymphocyte proliferation *in vitro* and in comparing their results with others, suggest that species differences or variations in assay conditions may account for their contrasting results. One study has investigated the influence of spontaneous ketosis in cows on interferon α and γ production [53]. A high negative correlation was found between blood ketone concentration and interferon release in response to known inducers. Further studies on the effect of acidosis and alkalosis on interferon production are required to confirm this finding. Consistent with previous studies, the same group showed reduced mitogenic response by bovine lymphocytes from ketotic cows [53].

SPECIFIC IMMUNE FUNCTION: COMPLEMENT ACTIVATION AND ANTIBODY PRODUCTION

Several studies have been published about the effect of extracellular pH on complement activation. Hammer *et al.* [54] showed acid-induced activation of C5 when combined with C6, resulting in a lytic complex called C5b,6^a. They proposed that during acid activation of C5 and C6, the high local H⁺ concentration alters the tertiary structure of either or both of these components, resulting in formation of the C5,6^a complex for-

mation and its subsequent cleavage to generate lytic capacity. Fishelson *et al.* [55] investigated the effect of pH on the alternative complement pathway and demonstrated superior lysis of sheep erythrocytes at pH 6.4 compared with 7.4, in addition to an increase in the generation of the two C3 convertases and increased binding of complement proteins to human erythrocytes. They concluded that the optimal pH for the initiation and amplification of the alternative pathway and for the formation of the membrane attack complex is 6.4. Sonntag *et al.* [56] investigated the effect of lactate and of hydrochloric acid [57] on selected complement proteins in blood from healthy volunteers and found significantly increased levels of activated products C3a and C5a in blood and plasma compared with untreated controls. They concluded that neither cellular interaction nor contact with destroyed cells is necessary to initiate the complement system in acidosis [56] and that acidosis *per se* rather than lactate is the trigger for activation of complement *in vitro* [57]. In a clinical study of neonatal hypoxic-ischaemic acidosis by the same group, similar increases in C3a and C5a, along with increased factor X11a, were shown, commensurate with complement activation [58]. However, they also demonstrated a reduction in median-complement function as measured by lysis of sensitized sheep erythrocytes by activated plasma-complement factors and a reduction in levels of the C1 inhibitor, Clq, and factor B compared with healthy controls. They concluded that the activation of complement was probably a result of cellular disintegration because of ischaemia, causing the release of subcellular constituents such as mitochondrial proteins, which activate the complement cascade *in vitro* and *in vivo*. No explanation was offered for the decrease in functional activity. Miyazawa and Inoue [59] demonstrated activation of the complement system by C-reactive protein in mildly acidic conditions via a pH-dependent, conformational change of the protein.

One study examined the effect of chronic, compensated acidosis and alkalosis on antibody synthesis in rats and showed decreased synthesis of antibody with acidosis [60]. A pH dependence of immunoglobulin G (IgG) binding by the neonatal Fc receptor has also been shown, and high-affinity binding was observed at pH 6–6.5 and weak or no binding at pH 7.5 [61]. The differential binding reflects the physiological variations in pH between the gut and bloodstream of the neonate, and the lower pH predominates in the gut where binding occurs. In another study, acid-pH-treated TB sera resulted in significantly greater titres of antibodies to *Mycobacterium tuberculosis* and higher antigen-binding ability of the former [62]. The changes were shown to be irreversible. Recently, Lopez *et al.* [63] have shown that acidic pH increases the avidity of human IgG binding to human neutrophils, monocytes, and NK cells. There are several structural and molecular studies on the pH dependence of antibody/antigen association; however, their functional relevance remains to be evaluated, and, therefore, they will not be reviewed here. In conclusion, there is a growing body of evidence suggesting a positive effect of acidic pH on complement activation, and more research is required to clarify the effects of ambient pH on antibody synthesis and binding.

CLINICAL STUDIES

By their nature, clinical studies are largely empirical in their findings and offer little in the way of mechanistic explanations. However, they constitute a valuable repository of general data and provide very useful pointers to further experimental research. Numerous studies of immunodeficiency associated with organic acidurias have been shown. Propionic and methylmalonic acidemia are associated frequently with immunological defects. In three cases of propionic acidemia, low levels of γ globulin and frequent infections were demonstrated [64–67]. A temporary B-cell lymphopaenia has also been demonstrated in an infant with propionic acidemia plus parathyroid hormone resistance, and numbers of circulating B cells returned to normal within 2 months of treatment of the acidosis [68]. However, this does not prove that acidosis *per se* was the primary culprit. Current opinion favors accumulated organic acids as the cause of the neutropenia and haematological abnormalities found frequently in propionic acidemia. Likewise, immunodeficiency has been shown to accompany methylmalonic acidemia frequently, characterized by severe neutropenia, lymphopaenia, and infection [69–71]. In an *in vitro* study, the growth of bone marrow stem cells from a patient with pancytopenia was found to be inhibited by concentrations of methylmalonic acid found *in vivo* [72], and impaired neutrophil and monocyte chemotaxis have been found in some patients with methylmalonic aciduria [73]. Not surprisingly, there are several studies of diabetic ketoacidosis and impaired immunity. One of the earliest clinical studies investigated the local inflammatory response in patients with controlled and uncontrolled diabetes [74]. A markedly impaired inflammatory response was shown in diabetic patients with accompanying ketoacidosis, relative to well-controlled diabetic patients' diabetes. The inflammatory response of uncontrolled patients returned to normal with correction of the acidosis, prompting speculation on a role for acidosis in the overall inflammatory response [74]. Menkin [75] also demonstrated that granulocytes in local exudates decreased in number as the local pH decreased and showed higher-than-normal concentrations of lactic acid and hydrogen ion in exudates from diabetic hosts. A subsequent study showed decreased chemotactic indices in diabetic patients compared with controls, which was corrected by the addition of insulin to the culture medium. The mechanism by which restoration of insulin resulted in normalization of the pH was not investigated and warrants further investigation. Blasetti *et al.* [76] demonstrated decreased percentages of several subclasses of T lymphocytes, in addition to impaired neutrophil chemotaxis in young children with diabetic ketoacidosis. One clinical study of invasive aspergillosis and diabetic ketoacidosis has been published, although no deficiencies in lymphocyte numbers or mitogenic responses to known mitogens were found [77]. On the basis of previously demonstrated findings, the investigators postulated a delay in the phagocytic response as the most likely predisposing factor to infection [77]. However, no experimental evidence exists to date to substantiate this theory.

In summary, the overwhelming evidence from the studies outlined points quite unequivocally to an impairment of immune function consequent to organic acidosis. The data

indirectly point to a reduction in the proliferation of lymphocytes and polymorphs, impaired chemotaxis, and inhibition of antibody production. The reduction in antibody production is also in agreement with the findings of Zhuravskii *et al.* [60].

SUMMARY AND CONCLUSIONS

It is evident from the above review of the literature that the pH of the extracellular milieu has a direct influence on a broad range of immunological functions. The majority of the work to date has focused primarily on cell-mediated immunity, with very few studies on humoral immunity. Apropos the latter, the small number of studies to date on complement activation and antibody synthesis suggests an enhancement of both at acidic pH. On the contrary, an overall trend toward inhibition of cell-mediated immunity is emerging. How these two disparate trends integrate with each other at the physiological level remains to be established. With respect to the studies of cell-mediated immunity, caution must be exercised in the extrapolation of experimental data, most of which is carried out *in vitro* using nonphysiological buffers. The suggestion from earlier studies, for example that acidic pH results in diminished random migration and chemotaxis of neutrophils, plus a reduction in phagocytosis and bactericidal capacity are complicated by the more recent findings of increased respiratory and cellular activity of neutrophils cultured in bicarbonate-based medium of acidic pH. Therefore, it is important that further studies in this area emulate as closely as possible the physiological milieu existing *in vivo*, including the use of bicarbonate-based buffers. Although many studies indicate that acidic pH has a negative effect on polymorph activation and function, the data from Trevani's group [35] mean that it is still too early to say for sure whether neutrophil function is impaired at acidic pH. Experimental evidence is emerging gradually for an inhibition of lymphocyte activity when the surrounding pH of tumors is reduced. This may constitute a very significant finding in the context of tumor immunotherapy, and further consideration by clinicians of the effects of the acidic microenvironment of tumors on immune function would appear to be warranted. However, there are too few studies on the response of lymphocytes to infection when the ambient pH is lowered, and given the ubiquity of lymphocyte activity in the immune response, much work remains to be done in this area.

Evidently, the inter-relationship between extracellular and intracellular pH on immune function cannot be ignored, especially in light of the myriad findings implicating a role for the Na^+/H^+ exchanger prior to activation of certain immune activities. The available data strongly suggest that the Na^+/H^+ exchanger is a *sine qua non* in generating a rapid intracellular alkalization prior to differential activation of certain immune activities. It seems reasonable to speculate on a similarly central role for the exchanger in altering pH_i in the same direction as pH_o . However, the mechanism by which pH_i alters in response to changes in pH_o warrants investigation. If the cation exchanger in neutrophils or lymphocytes, for example, were experimentally inhibited *in vitro* with amiloride or related compounds dur-

ing extracellular acidification, measurement of the pH; would help to ascertain whether the exchanger contributes to intracellular acidification following an increase in extracellular H⁺ levels. Although extracellular acidification may be triggered by a multiplicity of humoral or pathological factors originating in lesions remote from the site of immune activity, it is also evident from the available data that the extracellular burden of hydrogen ion concentration existing at an inflammatory locus or within the extracellular fluids may be augmented by the activity of the Na⁺/H⁺ pump extruding protons to the outside medium. Thus, a “catch-twenty-two” situation may potentially arise, further confounding any modulatory effects arising from the original acidotic insult. It is also possible that a variety of other cellular factors and molecular effectors, e.g., pH-induced alterations in membrane permeability, receptor binding at the plasma membrane, and intracellular trafficking, are involved in the differential effects of extracellular pH. Appropos of peripheral molecular effectors, primary candidates for such an effector role are the glucocorticoids. It is well-established that transcription and translation of glucocorticoids are increased during metabolic acidosis. Given the well-documented, inhibitory effects of the glucocorticoids on immune function, an additive effect of acidosis and glucocorticoid action should be considered. There is little or no experimental data on such an effect, and, thus, it is an area ripe for exploration. Likewise, a variety of endocrine effectors are known to alter the activity of the Na⁺/H⁺ exchanger, including thyroid hormone, insulin, glucocorticoids, and parathyroid hormone [78–80]. An additive or interactive effect of these hormones with altered interstitial pH may also exist and is worth investigating.

Perhaps the most unequivocal data providing evidence for an impairment of the immune response emerge from the clinical studies of the organic acidoses and ketoacidosis. In general, the clinical acidaemias are accompanied by immunodeficiency, including a decrease in white cell numbers, γ globulins, and mitogenic responses, a diminution of the inflammatory response and delayed phagocytosis. In many cases, the immunodeficiency is reversed on correction of the acidosis. Despite the valuable research carried out to date, a chasm exists in our knowledge of extracellular, acid-base effects on a wide range of other immune activities. There is a dearth of experimental data about the effect of ambient pH on antibody production, antigen processing and presentation, opsonization of bacteria, antibody synthesis activation and effectiveness of NK and cytotoxic T cells, hypersensitivity, and pathogen resistance and activities of the myriad cytokines and lymphokines. These include the interferons and interleukins. Hopefully, an increasing awareness of the relevance of the environmental pH surrounding immune cells and organs will encourage more research in what is undoubtedly a field ripe with research possibilities.

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