II: A Perspective on Endotoxemia

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Introduction
One of the most commonly encountered life-threatening conditions in horses with gastrointestinal disease is endotoxemia. The results of studies of horses presented with abdominal pain (colic) to university clinics in various parts of the world indicate that gram-negative bacterial endotoxins commonly are detected in circulation.1–4 Although much of the focus of research on endotoxemia in horses has centered on diseases that produce intestinal strangulation obstruction, these are not the only disease conditions characterized by endotoxemia. Similar clinical findings and problems often occur in adult horses with colitis, pleuropneumonia, retained placenta, and peritonitis.5 The most common clinical findings in horses presumed to be endotoxemic include abnormalities in mucous membrane color, with one of the initial findings being a ‘toxic line’; prolongation of capillary refill time; increased heart and respiratory rates; reduced borborygmi; fever; and hemoconcentration. Some horses may exhibit clinical signs of abdominal pain whereas others will appear to be depressed.

The situation is equally serious in neonatal foals that develop a nidus of bacterial infection. Approximately 50% of foals with clinical signs of septicemia have endotoxins in their circulation.6 Septicemia is the leading cause of death in foals less than a week old, primarily because the foal’s immune system lacks the ability to prevent dissemination of the bacteria from the initial nidus. The most common organisms responsible for endotoxemia and septicemia in neonatal foals are gram-negative bacteria such as E. coli, Klebsiella, Enterobacter, Actinobacillus, and Pseudomonas, although gram-positive or mixed gram-negative and gram-positive bacterial infections also occur.7

Consequently, endotoxemia is associated with life-threatening diseases affecting horses of all ages. In the past decade, there has been a virtual explosion of new information about the mechanisms responsible for the deleterious effects of endotoxins, with some of the more exciting findings occurring within the past 2–3 years. Because so much has been learned in such a short period of time, it is often difficult for people intimately involved in this field of research to keep up with what is known. The four basic goals of this article are to provide the equine practitioner with an appreciation of the historical background associated with endotoxemia, a better understanding of this serious and life-threatening complication of many diseases, an appreciation of the impact of new findings regarding the ways endotoxins exert their deleterious effects, and some thoughts regarding potential new methods of treatment.

Historical Perspective
Approximately 100 years ago, Richard Pfeiffer, a student in Professor Robert Koch’s laboratory in
Germany, made the observation that *Vibrio cholerae* organisms released two different types of toxins. A heat-labile exotoxin was released into the growth medium when the organisms were alive and a potent heat-stable toxin became evident after the organisms were killed. Based on his inability to detect the latter toxin when the organisms were alive, Pfeiffer concluded that the heat-stable toxins had been sequestered within the organisms when they were alive. He coined the term *endotoxins* for these toxins. In independent studies in other parts of Europe, Centanni made similar observations regarding extracts from gram-negative bacilli, which he called *pyrotoxina* because of their ability to induce a febrile response; Buchner reported that the bacterial extracts also caused characteristic changes in circulating white blood cell counts and leukocytosis. At approximately the same time, extracts of heat-killed bacteria were used in the United States as a vaccine to induce “fever therapy” in the treatment of tumors in humans; the active principle of this vaccine was proven to be endotoxin. Therefore, not long after Koch and his colleagues began documenting cause-and-effect relationships between bacteria and diseases, scientists in various laboratories became intrigued by the obviously deleterious yet potentially beneficial effects of bacterial endotoxins.

In the 1920s and 1930s, chemists in various parts of Europe began the initial chemical characterization of these bacterial extracts and reported that they were complex structures composed of polysaccharide, phospholipid, and protein. Subsequent studies indicated that the endotoxins consisted of phospholipopolysaccharide, hence the interchangeable use of the terms lipopolysaccharide, phospholipopolysaccharide, endotoxin phospholipopolysaccharide, and endotoxin in the literature.

Interest in the effects of endotoxemia intensified after World War II because of the devastating effects of sepsis and the development of septic shock that were apparent on the battlefield. As a result, numerous research studies were performed in the 1950s and early 1960s in an effort to understand the mechanisms responsible for trauma-induced shock and the effects of bacterial infections. Many of these early studies, which were performed at the University of Minnesota and Harvard University, involved the administration of large amounts of endotoxin to anesthetized dogs and the measurement of cardiopulmonary parameters that might be assessed in mobile army hospitals. It became evident very quickly that intravenous administration of endotoxin caused an abrupt reduction in systemic arterial pressure and cardiac output. These effects were caused by decreased venous return of blood to the heart. Early investigators also reported that a compensatory release of catecholamine-like substances caused an increase in total vascular resistance and an early restoration of blood pressure. If, however, the dose of endotoxin was increased sufficiently, blood pressure deteriorated because of reductions both in cardiac output and in vascular resistance. These responses to endotoxin resulted in poor tissue perfusion of vital organs and eventual death. In part because of the concurrent association between systemic hypotension and poor clinical outcome in human patients with circulatory shock, a considerable amount of effort was spent in identifying ways to restore blood pressure to normal values. As a result of its effects in experimental studies, a new synthetic norepinephrine anlogue gained widespread use in human hospitals despite the fact that the increase in blood pressure was not accompanied by an improvement in survival rate.

A review of the veterinary literature indicates that endotoxins were first incriminated in a new clinical syndrome designated ‘colitis X’ by Rooney and colleagues in 1963. The potential involvement of endotoxins in this syndrome was based on clinical findings of intractable diarrhea, fever, dehydration, and colic. In an experimental study reported two years later, Carroll and colleagues administered 0.025 mg/kg endotoxin intraperitoneally to a horse and reported pronounced clinical signs, development of diarrhea, hemoconcentration, severe leukopenia, and death. An additional important finding in this initial study was the comparative sensitivity of different animal species to the same source of endotoxin. In contradistinction to the effects on the horse, mice, a cat, and a cow recovered rather quickly after receiving 20 mg/kg, 0.48 mg/kg, and 0.12 mg/kg endotoxin, respectively.

The next decade of study brought to light more of the body’s responses to endotoxin, including the synthesis and release of histamine, bradykinin, catecholamines, various coagulation factors, a myocardial depressant factor, and the first of the lipid-derived mediators, the arachidonic acid metabolites. These findings caused Lewis Thomas to note in his 1974 book *The lives of a cell: Notes of a biology watcher,* “Our arsenals for fighting off bacteria are so powerful and involve so many different defense mechanisms, that we are in more danger from them than from the invaders.... The (endotoxin) macromolecules are read by our tissues as the very worst of bad news. When we sense lipopolysaccharide, we are likely to turn on every defense at our disposal.”

At the same time, several studies were performed to more fully characterize the responses of horses and ponies to administration of endotoxins in various dosages via various routes. Some of the studies published in the early 1970s described the effects of endotoxin administration on anesthetized ponies, with the primary focus again being effects on the cardiopulmonary system. The most commonly reported effects included hypotension, decreased central venous pressure, leukopenia, hemoconcentration, and alterations in blood glucose concentrations. Many of the studies performed during this
decade involved the administration of approximately 150–200 μg of *E. coli* endotoxin per kg of body weight, given as a bolus or delivered via rapid infusion or slow infusion, either to anesthetized or conscious ponies.\(^{29–31}\) These studies documented the development of arterial hypoxemia, pulmonary hypertension, increased plasma protein concentration, lactic acidosis, and biphasic reductions in systemic arterial pressure and cardiac output.

Toward the end of the 1970s, a series of studies was performed on conscious ponies and horses, who were given a bolus injection of what we considered to be a very small amount of endotoxin (10 μg/kg).\(^{32–34}\) Although the responses of the animals were quantitatively less pronounced, there were many similarities to the results of studies using 10–20 times as much endotoxin. For instance, the development of arterial hypoxemia, pulmonary hypertension, tachycardia, tachypnea, leukopenia followed by leukocytosis, and lactic acidosis was consistently documented. Clearly, a lot was learned in these initial studies about the physiologic responses to a sublethal challenge with endotoxin. However, the abrupt onset and severity of the responses did not accurately mimic some of the clinical signs commonly associated with endotoxia as recognized in the clinic. These latter findings alone indicated that the experimental model needed to be improved and that the mechanisms responsible for the effects of endotoxin could be identified only by performing studies under more controlled circumstances.

During the last twenty years, a large number of elegant studies were performed that dealt with various aspects of endotoxemia in horses. These studies included in-vivo experiments in which the effects of more physiologic amounts of endotoxin (20–30 ng/kg given via slow infusion) were quantified, measurements of plasma concentrations of endotoxin in horses with clinical diseases, in-vitro studies on the effects of endotoxin on isolated equine cells, measurements of circulating concentrations of specific inflammatory mediators in endotoxemic horses, and assessments of the efficacy of individual treatment regimens.\(^{3,5,6,35–61}\) As a result of the findings of these studies, there is now a far more complete appreciation of the association between endotoxia and specific diseases of horses, how endotoxin exerts many of its deleterious effects, and treatment options that need to be studied in more detail.

### Important Concepts Concerning the Source, Structure, and Detection of Endotoxin

Endotoxin, the lipopolysaccharide component of the outer cell envelope of enteric gram-negative bacteria, is comprised of three portions: an outer polysaccharide (O-antigenic) region, a unique core region consisting primarily of monosaccharides, and an inner fatty acid–rich region termed ‘lipid A.’ Bacteria containing all three portions are *smooth* strains whereas strains lacking the O-antigenic portion are *rough.* Based on numerous studies, the majority of the deleterious effects of endotoxin have been linked to the lipid A moiety.\(^{33,62,63}\)

Under normal circumstances, the horse’s intestinal tract contains large amounts of gram-negative bacteria. Because endotoxins are released from these bacteria when they die or multiply rapidly, the intestinal lumen normally contains a large amount of endotoxin. Based on one estimate, the cecum and ventral colons of healthy horses contain more than 2 g of free endotoxin.\(^{64}\) These endotoxins are restricted to the intestinal lumen by an efficient intestinal mucosal barrier,\(^{62}\) which is comprised of mucosal epithelial cells, antibodies and enzymes secreted by those cells, and the adjacent resident bacteria. Endotoxins that cross this barrier either are bound to specific plasma proteins, neutralized by circulating antibodies, or cleared from the circulation by Kupffer cells in the liver.\(^{62,64}\)

Diseases that impair the intestinal mucosal barrier allow endotoxins to enter either the peritoneal cavity or the portal circulation.\(^{62,64}\) This can occur if the local mucosal blood supply is reduced sufficiently, which causes mucosal epithelial cells to detach from the underlying lamina propria, or if the intestinal wall is inflamed. As a result, the clinical conditions most commonly associated with clinical evidence of endotoxia are those that involve severe intestinal displacements with obstruction of the blood supply and intestinal lumen (e.g., volvulus, incarcerations) or severe inflammation (e.g., colitis and proximal enteritis).\(^{1,4,5,63–66}\)

Endotoxin can be detected in body fluids by using an assay based on the response of lysates from the circulating hemolymph cells (amoebocytes) of the horseshoe crab, *Limulus polyphemus.*\(^{67}\) This Limulus Amoebocyte Lysate assay, though highly sensitive for the detection of endotoxin, requires collection of blood cleanly into pyrogen-free syringes and transfer to tubes containing endotoxin-free heparin, followed by dilution and heating of the plasma to reduce the effects of interfering substances. By paying strict attention to these details of sample collection and preparation, the reliability of the Limulus Amoeocyte Lysate Assay has improved markedly and has strengthened the effect of data obtained from horses with disease conditions causing clinical signs of colic. Although differences in the detection limits of the assay as performed in different laboratories make it difficult to compare exact plasma concentrations of endotoxin among the studies, the overall results suggest that 35–45% of horses presented to veterinary colleges with gastrointestinal disease are endotoxemic.\(^{1,2,4,5,63,65,66}\) Clinical conditions commonly associated with the presence of endotoxin in circulation include intestinal strangulation obstruction, colitis, proximal enteritis, and carbohydrate overload. Because there is evidence that the mucosa of normal intestine is damaged by the presence of intestinal ischemia elsewhere in the gastrointestinal tract or by hypovolemia, horses with ischemic

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**AAEP PROCEEDINGS** / Vol. 47 / 2001 63

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intestine may exhibit clinical signs of endotoxemia for several days after ischemic intestine has been removed. Similarly, transmural movement of endotoxin across an inflamed intestinal wall may persist for days in horses with enteritis or colitis.

When the Limulus Amoebocyte Lysate Assay was initially being tested in clinical studies, the gold standard against which the results were compared was the simultaneous isolation of Gram-negative bacteria from the patient. In fact, a considerable amount of controversy arose when investigators failed to culture Gram-negative organisms from many patients who had endotoxin in their blood samples. Over the years, it has become apparent that lipid-rich endotoxins are able to traverse the intestinal mucosal barrier and enter the circulation far more readily than can intact bacteria. This situation appears to hold true for horses as well, making it common for laboratories to detect endotoxins in the absence of bacteria. Finally, it is important to realize that the Limulus Amoebocyte Lysate Assay is not routinely performed in most veterinary pathology laboratories.

Clinical Effects of Endotoxemia

Although most veterinarians are acutely aware of many of the clinical signs associated with endotoxemia (e.g., hyperemic or discolored mucous membranes, alterations in capillary refill time, decreased gastrointestinal sounds, increased heart and respiratory rates, and evidence of dehydration), there are other effects that may not come to mind quite as quickly. Based on the responses of horses to whom small amounts of endotoxin were administered in recent experimental studies, endotoxemia by itself may cause the horse to exhibit clinical signs indicative of mild or moderate abdominal pain (i.e., pawing, looking at the flank region, stretching out, lying down) or the horse may appear to be depressed. Presumably, these effects are caused by the synthesis and release of proinflammatory substances that inhibit normal gastrointestinal muscular activity, reduce the threshold for painful stimuli, and/or cause alterations in intestinal blood flow. As an extension of these effects of endotoxin being administered to otherwise healthy horses, it may be surmised that endotoxemia may increase the likelihood that a horse with gastrointestinal disease will experience acute pain, as often occurs when horses have intestinal strangulation obstructions or acute colitis.

The Fate of Endotoxin in Circulation: Interaction with Plasma Proteins and Inflammatory Cells

After endotoxin gains access to the circulation, it may interact with circulating proteins and blood cells or it may be removed by tissue-fixed macrophages in the liver, spleen, and pulmonary vasculature. Because of the hydrophobic nature of its lipid A region, endotoxin tends to form aggregates in plasma. As the aggregates interact with high density lipoproteins and a unique plasma protein named lipopolysaccharide binding protein, they are dispersed into endotoxin monomers (Figs. 1 and 2). The binding of endotoxin with high density lipoproteins prolongs the half-life of endotoxin in circulation but reduces its ability to interact with proinflammatory cells.

In direct contrast, lipopolysaccharide binding protein removes individual endotoxin molecules from aggregates and markedly facilitates the ability of these endotoxin monomers to interact with the horse’s mononuclear phagocytes (peripheral blood monocytes, macrophages). This special lipopolysaccharide binding protein, which in effect serves to shuttle endotoxins from the aggregates to the inflammatory cells, is synthesized by the liver and normally exists in trace amounts in plasma. As part of the acute phase response to inflammatory stimuli such as surgery, infection, or endotoxemia itself, circulating concentrations of this protein may increase 100-fold. Lipopolysaccharide binding protein interacts strongly with the lipid A portion of endotoxin, an effect that is heat sensitive. Thus, serum samples heated to 56°C lose more than 70% of their ability to bind endotoxin, which suggests that lipopolysaccharide binding protein may be one of the interfering substances encountered if the Limulus Amoebocyte Lysate Assay is performed on undiluted, unheated plasma.

Because of its function in shuttling endotoxin to the host’s inflammatory cells, lipopolysaccharide binding protein substantially increases the sensitivity of the host to endotoxin. It achieves this effect by transferring endotoxin monomers to a cell surface receptor called CD14 that exists primarily on mononuclear phagocytes (Fig. 3). The interaction among endotoxin, lipopolysaccharide binding protein, and CD14 increases the sensitivity of these cells to endotoxin and results in the synthesis of a potpourri of proinflammatory mediators. In invitro studies, this latter effect of endotoxin can be prevented with monoclonal antibodies directed against CD14 or by enzymatically removing CD14 from the surface of the cells. These results suggest a central and important role of CD14 in the cellular response to endotoxin. Furthermore, there is evidence that endotoxin and lipopolysaccharide binding protein may interact with a soluble form of CD14 that is released from the surface of mononuclear phagocytes. Through these interactions, the endotoxin-soluble CD14 complex interacts with cells lacking CD14, such as endothelial cells, thereby initiating inflammatory effects involving these cells. Until the year 2001, it was not possible to determine the role of lipopolysaccharide binding protein or either form of CD14 in horses because the purified equine proteins and the antibodies that crossreact with these proteins did not exist. We recently cloned and have begun expressing recombinant equine CD14 and are in the process of cloning the gene for equine lipopolysaccharide binding pro-
tein. Having these new tools will allow the importance of these proteins in the response of horse cells to endotoxin to be characterized and their importance in clinical cases of colic to be elucidated.

Although the CD14 receptor plays a central role in the response of host cells to endotoxins, this receptor lacks the ability to transmit the endotoxin signal to the interior of the cell. In essence, CD14 is a cell surface receptor that is attached to the exterior of the cell by a carbohydrate linkage; it does not include portions that cross the cell membrane and therefore cannot initiate the release of second messenger systems or signal transduction pathways that lead to the synthesis of proinflammatory mediators. For approximately a decade, the link between CD14 and the response of the cell was missing. Three years ago, however, investigators working with Drosophila identified a family of receptors named the Toll receptors that are necessary for fruitflies to respond to bacterial and fungal toxins. The Toll receptors have transmembrane and intracellular components that allow them to communicate between the exterior and interior aspects of a cell (i.e., initiate the inflammatory response to toxins).90–92 Shortly thereafter, scientists in other laboratories discovered an analogous family of receptors on mammalian cells, which they named Toll-like receptors. As of this writing, 10 mammalian Toll-like receptors have been identified and Toll-like receptor 4 has been discovered to be responsible for delivery of the endotoxin signal from CD14 to the interior of a cell93–97 (Figs. 4 and 5). Interestingly, CD14 is not selective for endotoxins because it also recognizes and binds to cellular components of gram-positive bacteria, such as peptidoglycan. Toll-like receptor 2 then delivers that signal to the interior of a cell, thereby initiating its own specific series of cellular responses.98–100 We recently cloned equine Toll-like receptor 4 and are hopeful that future studies will allow us to identify new methods for controlling the effects of endotoxins at the molecular level.

Proinflammatory Mediators in Endotoxemia

Based largely on the results of studies of monocytes and macrophages from a variety of species, including the horse, binding of endotoxin to CD14 results in transmission of this endotoxin signal to the interior of the cell, mobilization of intracellular messengers, and interactions within the nucleus that induce genes to encode proinflammatory substances. Although there is a considerable amount of debate about which cellular signaling systems are involved, there is agreement that many of the deleterious effects of endotoxin are mediated by cytokines and proinflammatory substances synthesized.
and released by mononuclear phagocytes. These cells generate many of the inflammatory mediators that have been detected in the circulation of horses to whom endotoxin was administered under experimental conditions and in horses with naturally occurring gastrointestinal diseases.\textsuperscript{46,55,57,64,66,101–109} The most widely studied of these mediators include tumor necrosis factor, the interleukins, the eicosanoids (arachidonic acid metabolites), and tissue factor.

Tumor necrosis factor is a small polypeptide cytokine that is synthesized by mononuclear phagocytes exposed to endotoxin and other proinflammatory stimuli. Serum concentrations of tumor necrosis factor increase shortly after in vivo administration of endotoxin and initiate a cascade of responses that include hypotension, hemoconcentration, metabolic acidosis, and disseminated intravascular coagulation.\textsuperscript{5,56,109,110} Tumor necrosis factor exerts these effects by stimulating the synthesis of other inflammatory mediators (including the interleukins, eicosanoids, and tissue factor), the acute phase response, and fever.\textsuperscript{111} In laboratory animal species, administration of antibodies directed against tumor necrosis factor has increased the survival rate of animals subjected either to endotoxic or septic shock, thereby exemplifying the pivotal role of tumor necrosis factor in these complex syndromes. There have been several experimental and clinical studies documenting increases in serum concentrations of tumor necrosis factor in horses and neonatal foals and showing the associations of these values with the prognosis for survival.\textsuperscript{5,6,35,56,105,109,110,112,113}

Arachidonic acid, a well-described 20-carbon fatty acid in cell membrane phospholipids, is released by phospholipase A\textsubscript{2} and metabolized by cyclooxygenase or lipoxygenase. The most widely studied cyclooxygenase-derived metabolites include thromboxane A\textsubscript{2}, prostaglandin I\textsubscript{2}, prostaglandin E\textsubscript{2}, and prostaglandin F\textsubscript{2\alpha}. Because of difficulties related to their stability in plasma, relatively little is known about the lipoxygenase-derived metabolites of arachidonic acid in horses. In contrast, there is considerable information regarding the cyclooxygenase-derived metabolites of arachidonic acid. Although these metabolites are associated with a reduction in the threshold for certain painful stimuli, their effects on vascular smooth muscle activity and, therefore, tissue perfusion are more commonly discussed. For instance, thromboxane A\textsubscript{2} and prostaglandin F\textsubscript{2\alpha} cause vasoconstriction by decreasing cAMP concentration in vascular smooth muscle cells whereas prostaglandins I\textsubscript{2} and E\textsubscript{2} cause vasodilation by increasing cAMP. Many of the early hemodynamic effects of endotoxemia are mediated by these cyclooxygenase-derived metabolites of arachidonic acid.
Specifically, increases in plasma concentrations of the stable metabolite of thromboxane A2 are associated with the early development of hypoxemia, dyspnea, and pulmonary hypertension that occur within minutes of endotoxin administration. These findings corroborate the vasoconstrictive and bronchoconstrictive effects of thromboxane A2 reported in other species. Plasma concentrations of the stable metabolite of prostaglandin I2 increase later, reaching peak values approximately 90 min after endotoxin administration. They are associated with discoloration of the horse’s mucous membranes, prolongation of capillary refill time, and the onset of hypotension. Increased plasma concentrations of prostaglandin F2α also are associated with endotoxin-induced abortion in mares early in gestation.49,116

Tissue factor (‘procoagulant activity’ or thromboplastin) is a glycoprotein synthesized by monocytes, macrophages, and, to a lesser degree, by endothelial cells in response to endotoxin. The majority of the tissue factor synthesized by these cells remains on the surface and may be exposed to coagulation factor VII in plasma. As a result, the extrinsic arm of the coagulation cascade can be stimulated, causing the stimulated cells to be a focus for the formation of microthrombi. Tissue factor activity has been shown to increase significantly in horses to whom endotoxin was administered as well as in horses with naturally occurring gastrointestinal diseases.3,5,117,118 Importantly, in a clinical study of horses with colic, the magnitude of the increase in tissue factor activity correlated inversely with the animal’s prognosis for survival.118

Anti-Inflammatory Mediators in Endotoxemia

Although the focus of most endotoxemia studies, particularly those performed on horses or associated with clinical diseases, has been on the specific effects of proinflammatory mediators, there is increasing evidence that endotoxin also causes the synthesis and release of anti-inflammatory mediators. The most well-studied of these is interleukin-10, which is synthesized by monocytes, macrophages, and lymphocytes.119,120 Synthesis of interleukin-10 also appears to be regulated by tumor necrosis factor in some species, interleukin-1, transforming growth factor-β, and interferon γ. The primary anti-inflammatory effect of interleukin-10 appears to involve deactivation of mononuclear phagocytes and inhibition of proinflammatory cytokine synthesis.119 Studies performed in other species indicate that neutralization of interleukin-10 causes enhanced production of tumor necrosis factor and increased lethality.121 In the only study performed to date involving equine cells, human recombinant
interleukin-10 significantly reduced endotoxin-induced synthesis of tumor necrosis factor, prostaglandin E₂, and interleukin-6.⁵³ Other potentially anti-inflammatory mediators that deserve study in horses include interleukins 4, 6, 11, and 13 and transforming growth factor-β.

Treatment of Endotoxemic Horses

There are four primary therapeutic targets that should be considered in the treatment of endotoxemia: 1) prevention of movement of endotoxin into the circulation, 2) neutralization of endotoxin before it interacts with inflammatory cells, 3) prevention of the synthesis, release, or action of inflammatory mediators, and 4) prevention of endotoxin-induced cellular activation.

The first therapeutic target may be the most difficult to achieve, although efforts should be expended to reduce the time available for translocation of endotoxin into the circulation by identifying the underlying disease process and initiating appropriate therapy as rapidly as possible. For horses with intestinal ischemia, this means making the decision for surgery early and removing the affected intestine. Similarly, infected umbilical remnants or other sources of bacterial infection should be removed to prevent the continued movement of endotoxin and bacteria into the circulation.

The second target in treating endotoxemic horses is to neutralize endotoxin before it activates the horse’s inflammatory cells. This can be accomplished with antibodies directed against the core and lipid A regions of endotoxin or by administering polymyxin B, which binds avidly to lipid A. The rationale for using antibodies is to enhance opsonization of intact bacteria, remove endotoxin from the blood, and interfere with the interaction of endotoxin with inflammatory cells.¹²² Because the core and lipid A regions are well conserved among gram-negative bacteria, antibodies against these regions usually are utilized in clinical situations.⁶⁴,¹²³

Commercially available antiendotoxin antibodies are harvested from horses vaccinated against the core regions of rough strains of J5 Escherichia coli or the Re Salmonella mutant. These antibodies have been used in several clinical and experimental trials with conflicting results. In one clinical study, this form of treatment resulted in reduced mortality and fewer days of hospitalization when compared with horses that did not receive antibodies or that received plasma from nonvaccinated horses.¹²⁴ These results were similar to those reported for humans who received a monoclonal antibody that binds specifically to lipid A.¹²² In contrast, the effects of antiendotoxin antibodies in other studies of humans and horses have been equivocal.¹²⁵ There are sev-
eral potential reasons for these discrepancies. Because the interaction between endotoxin and the horse's inflammatory cells occurs rapidly, the antibodies may have been administered too late in some of the studies. It is also feasible that anti-core antibodies are unable to penetrate the O-antigenic chains of intact smooth endotoxin to neutralize the core region. Finally, the failure of antiendotoxin antibodies to produce beneficial effects may reflect the inability of the host's compromised inflammatory system to opsonize or phagocytize bacteria. Currently, a common treatment technique involves administering 1.5 ml/kg J5 hyperimmune serum IV diluted at least two-fold in balanced intravenous fluids. Clearly, additional controlled clinical trials need to be performed using strict entrance criteria, during which antiendotoxin antibodies or nonspecific immunoglobulins would be administered early in the course of the disease, when this form of treatment might have its best opportunity to be successful.

As an alternative approach, polymyxin B, an antibiotic that forms a stable complex with lipid A, has been used in an attempt to prevent endotoxin from interacting with the horse's inflammatory cells. Although this approach is being used in clinical cases, concerns have arisen because of polymyxin B's inherent toxicity. Although pretreatment with polymyxin B (2.5 mg/kg) did not alter the development of shock, acidosis, or lameness in horses administered a carbohydrate overload to produce acute laminitis, results in a less severe model of endotoxemia in foals have been more positive. In that study, pretreatment with polymyxin B (6,000 u/kg) ameliorated clinical signs, prevented leukopenia, and resulted in significantly lower serum concentrations of tumor necrosis factor and interleukin-6 activity, when compared with foals not receiving polymyxin B and foals receiving J5 hyperimmune serum (1.5 ml/kg). Additional well-controlled clinical and experimental studies are needed to determine the relative usefulness of this approach in the treatment of endotoxemia.

The third target in treating endotoxemic horses is to prevent or reduce the synthesis, release, or effect of inflammatory mediators. A variety of studies have been performed to evaluate specific treatments, most of which have focused on one or a few specific mediators. The primary treatments that have been evaluated include nonsteroidal anti-inflammatory drugs, corticosteroids, monoclonal antibodies directed against cytokines, platelet activating factor receptor antagonists, pentoxifylline, and omega-3 fatty acids.
Nonsteroidal anti-inflammatory drugs have been a mainstay in the treatment of endotoxemic horses for two decades; the results of comparative studies involving different nonsteroidal anti-inflammatory drugs (phenylbutazone, dipyramine, ibuprofen, flunixin meglumine, and a thromboxane synthetase inhibitor) clearly indicate that flunixin meglumine is most effective in preventing endotoxin-induced synthesis of prostaglandins and thromboxane and clinical signs of endotoxemia. Additionally, a reduced dosage of flunixin meglumine (0.25 mg/kg) more effectively prevented synthesis of prostaglandins and thromboxane than did phenylbutazone at nearly ten times the dosage (2 mg/kg). With concern about toxic side effects of nonsteroidal anti-inflammatory drugs (gastrointestinal ulceration and renal papillary necrosis) prominent in the minds of equine practitioners, many began treating endotoxemic horses with a reduced dosage of flunixin meglumine (0.25 mg/kg intravenously every 8 h). Although this has become standard practice in many clinical settings, especially in the treatment of horses after colic surgery, to my knowledge no clinical studies have been performed to test whether or not this form of treatment is efficacious or even how long it should be continued after surgery. Clearly, there are many hypotheses that remain to be tested regarding the use of nonsteroidal anti-inflammatory drugs.

Another effective way of reducing synthesis of proinflammatory cyclooxygenase-derived metabolites of arachidonic acid is by replacing it with other fatty acids. This is most effectively achieved with omega-3 fatty acids (e.g., alpha-linolenic acid and eicosapentanoic acid), whose metabolites lack the proinflammatory effects of the metabolites of arachidonic acid. This approach has been evaluated in three studies in horses. In one study, monocytes from horses consuming a ration containing 8% linseed oil as a source of alpha-linolenic acid synthesized less thromboxane and expressed less tissue factor in response to endotoxin than did monocytes from horses consuming a control ration. Similarly, peritoneal macrophages isolated from horses consuming the omega-3 fatty acid–enriched ration produced less tumor necrosis factor. Unfortunately, the clinical signs and laboratory findings caused by intravenous administration of endotoxin were indistinguishable between the two dietary groups.

To circumvent the time required for dietary omega-3 fatty acids to be incorporated into membrane phospholipids, a recent study compared the effects of intravenous administration of 20% lipid emulsions enriched either with omega-3 or omega-6 fatty acids to horses. In that study, the fatty acid composition of monocytes isolated from the horses were altered within 8 h after lipid infusion and persisted for 1 week. Furthermore, production of thromboxane and tumor necrosis factor by cells from horses administered the omega-3 fatty acid enriched emulsion were reduced, which suggested that short-term infusion of omega-3 fatty acids rapidly reduces the inflammatory responses to stimuli such as endotoxin.

An alternative approach to modulating the effects of inflammatory mediators is to prevent the interaction between the mediator and its own receptors. This approach was utilized with monoclonal antibodies directed against tumor necrosis factor, as well as platelet activating factor receptor antagonists and alpha-2 adrenergic receptor antagonists. Pretreating miniature ponies with monoclonal antibodies against equine tumor necrosis factor reduced the severity of clinical signs, the synthesis of tumor necrosis factor, interleukin-6, and thromboxane B2, and the development of leukopenia and lactic acidosis induced by endotoxin administration. Based on the fact that the tumor necrosis factor gene must be upregulated very soon after administration of endotoxin, so that the rapid changes in serum concentration of this cytokine occur, this method of therapy may have limited clinical use in horses.

Using a similar approach, platelet activating factor receptor antagonists have been evaluated during experimentally induced endotoxemia and reduced endotoxin-induced ileus, fever, leukopenia, tachycardia, and lactic acidemia. A similar protective effect of the alpha-2 antagonist was seen in a study of endotoxin-induced ileus. Although administering a platelet activating factor antagonist reduced the degree of leukopenia and thrombocytopenia caused by experimentally induced colonic ischemia–reperfusion, it did not have positive effects on the cardiovascular system and exacerbated the degree of metabolic acidosis.

Another drug that has been used both experimentally and clinically on endotoxemic horses is pentoxifylline, a methylxanthine derivative used as a rheologic agent in humans. In in vitro and ex vivo experimental studies, pentoxifylline reduced endotoxin-induced production of cytokines, thromboxane, and expression of tissue factor while simultaneously increasing plasma concentrations of prostaglandin I2. In vivo studies, the beneficial effects associated with administering only pentoxifylline to endotoxemic horses were limited. When a combination of pentoxifylline and flunixin meglumine was compared to the effects of either drug alone in endotoxemic horses, the combination of the two drugs appeared to offset some of the hemodynamic responses to endotoxin more effectively than either drug alone. Pentoxifylline has been used rather widely in clinical situations. However, as is the case for many current treatment techniques, there are no clinical studies documenting its effects.

The final therapeutic approach is to prevent or interfere with endotoxin-induced cellular activation. At present, this approach to therapy is in its formative stage. Additional information is required regarding the intracellular signaling mechanisms through which endotoxin causes the synthesis...
and release of inflammatory mediators. To date, most studies have involved evaluation of the effects of one or a select few of the large number of mediators synthesized in response to endotoxin. As a direct result of genomic-based investigations and the development of microarray technology, it should be feasible in the near future to follow temporal changes in the activities (i.e., up-regulation, down-regulation, or no change) of literally thousands of equine genes in response to endotoxin. Data gleaned from such studies should provide the foundation for the development of new treatments designed to modulate the proinflammatory and anti-inflammatory mediators released in response to challenges such as endotoxin.

References


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