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Evidence of Species Specificity in the Cytocidal Effects of *Pasteurella haemolytica*

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Pasteurella haemolytica was found to have a cytotoxic effect on the peripheral blood mononuclear leukocytes of cattle, sheep, and goats, but no effect on the mononuclear leukocytes of swine, horses, or humans. In contrast, *Escherichia coli* caused marked cell death among mononuclear leukocytes of all species tested.

Pasteurella haemolytica is a major cause of illness and death in young feedlot cattle and sheep (6–8). A more severe, septicemic form is a common cause of death among very young lambs (7). However, reports of other species contracting severe pneumonia caused by this bacterium are rare.

It has been reported previously that *P. haemolytica* has a cytocidal effect on bovine alveolar macrophages (1, 9). Recently, we have found that *P. haemolytica* and *Escherichia coli* have a cytocidal effect on bovine mononuclear leukocytes. As many as 90% of mononuclear leukocytes may be killed with high concentrations of bacteria (K. L. Kaehler, R. J. F. Markham, C. C. Muscoplat, and D. W. Johnson, Am. J. Vet. Res., in press). The present study was undertaken to determine whether the mononuclear leukocytes of other species are similarly affected.

Blood was obtained from sheep, goats, pigs, and calves kept as research animals, from horses brought into the University of Minnesota's large animal clinic, and from the laboratory staff. Venous blood was drawn into sterile, heparinized vacuum tubes. The mononuclear leukocytes were separated from whole blood on Ficoll-Hypaque by the method of Boyum (3). Cells aspirated from the interface were washed twice with Hanks balanced salt solution and suspended in RPMI 1640 (IX, with 25 mM HEPES buffer [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid], Biolabs, Northbrook, Ill.) to a concentration of 4×10^6 cells per ml.

P. haemolytica, type A, obtained from clinical isolates, and *E. coli* ATCC 25922 were maintained on blood agar. A single colony was inoculated into brain heart infusion broth and incubated overnight at 37°C. Bacteria were washed twice with Hanks balanced salt solution and suspended to approximately 10^{12} cells per ml, a concentration previously determined to be cytoxic to 40% of bovine mononuclear leukocytes (Kaehler et al., in press). An equal volume of bacterial suspension or RPMI 1640 alone was added to 0.5 ml of mononuclear leukocyte suspension (ratio of bacteria to cells, approximately 10^6:1), and the mixture was incubated in a 37°C water bath for 30 min. Cell viability was determined by trypan blue dye exclusion. Statistical significance was determined at $P < 0.01$.

Figure 1 shows the results of exposure of mononuclear leukocytes of various species to *P. haemolytica*. Mean percent cell death (MPD) in bovine cultures (MPD = 41.7%) was as expected for the concentration of bacteria used and was significantly greater than cell death in control cultures (MPD = 1.5%). *P. haemolytica* also caused significant cell death in sheep (MPD = 29.3%) and goat (MPD = 33.2%) mononuclear cell cultures, when compared with controls. Cell death in cultures of these three species was significantly greater than death among mononuclear cells of horses (MPD = 3.6%), swine (MPD = 4.0%), and humans (MPD = 1.4%). Cell death in swine and horse cultures was somewhat higher than that in the controls, but human cells showed no greater death than did the controls.

Figure 2 shows the results of exposure of the mononuclear leukocytes of the same animals to *E. coli*. Pronounced cell death was observed among cells of all species; in all cases, cell death was significantly greater than that in the controls.

In contrast to the cytotoxic capabilities of *E. coli*, which extend to the cells of all species tested, the cytotoxicity of *P. haemolytica* appears to be limited to ruminants. These and earlier results, which indicate that neither heat- nor X-ray-killed *P. haemolytica* organisms have the cytotoxic potential of live cells, lead to the conclusion that the cytotoxic factor in *P. haemolytica* is not the lipopolysaccharide endotoxin usually associated with gram-negative bacteria.

There are several possible explanations for the observed differential sensitivity of the mononuclear leukocytes of various species to the cyto-

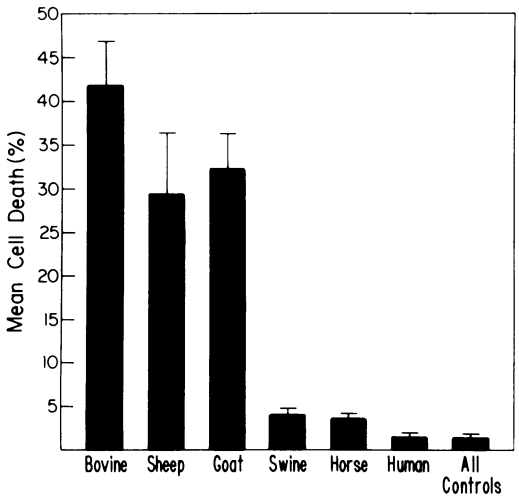

FIG. 1. *Cytocidal effect of P. haemolytica on the mononuclear leukocytes of various species (mean + standard error).*

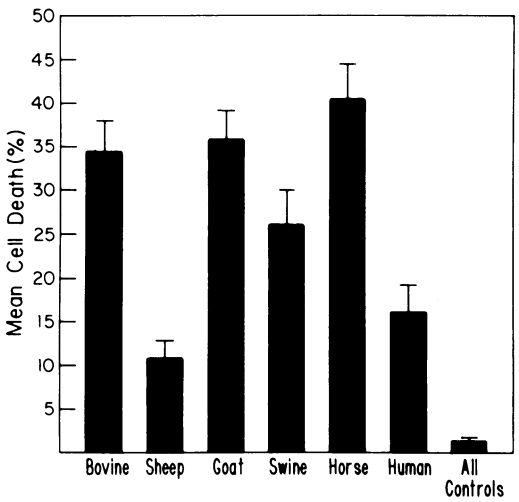

FIG. 2. *Cytocidal effect of E. coli on the mononuclear leukocytes of various species (mean + standard error).*

toxic effects of *P. haemolytica*. First, the mononuclear leukocytes of ruminants may not have the cellular mechanism necessary to detoxify a factor released by *P. haemolytica* that the cells of other species are capable of neutralizing, or this cytotoxic factor may act in ruminants on a metabolic pathway that nonruminants lack or for which they can compensate. Second, if cell death is caused by damage to the plasma membrane or lysosomal membrane, ruminant mononuclear leukocytes may possess membrane receptor sites for the cytotoxic factor which nonruminant cells lack. We previously reported that lymphocytes and monocytes were killed, decreasing the possibility that differences in leukocyte death are due to a differential rate of phagocytosis by monocytes (Kaehler et al., in press).

Although *Pasteurella* pneumonia has been reported in many species, including mice (4), fowl (4), swine (4, 5, 8), and humans (2), the causal agent is generally certain serotypes of *Pasturella multocida*, not *P. haemolytica*. If the results of our studies with *P. haemolytica* and mononuclear leukocytes reflect the interaction of the bacterium with alveolar macrophages, the differential sensitivity to the cytotoxic effects of *P. haemolytica* may be an explanation for the species-associated occurrence of *Pasteurella* pneumonia. The cytotoxic effects of *P. haemolytica* on alveolar macrophages may contribute to the severity of lung damage: dying macrophages release factors which cause irritation and fibrin deposition. In addition, the ability of the bacteria to debilitate macrophages and lymphocytes may be important in the initiation of the disease.

Identification of the toxic factor in *P. haemolytica* and the reasons for the species-associated difference in mononuclear leukocyte sensitivity to this toxic factor may permit the devising of more suitable prophylactic measures.

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