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THE ABILITY OF NEUTROPHILS FROM CALVES WITH BOVINE  
GRANULOCYTOPATHY SYNDROME TO PRODUCE  
REACTIVE OXYGEN SPECIES

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The bactericidal properties of phagocytes are dependent on the production of reactive oxygen species by the respiratory burst. Using electron spin resonance (ESR) and chemiluminescence spectrometries, the ability of neutrophils from Holstein-Friesian calves with bovine granulocytopeny syndrome (GPS) to produce reactive oxygen species was examined. ESR spectrometry combined with spin trapping and luminol chemiluminescence spectrometry were employed to measure qualitatively and quantitatively the reactive oxygen species from stimulated neutrophils. 5, 5-Dimethyl-1-pyrroline N-oxide (DMPO) was used as a spin trapping reagent. Phorbol 12-myristate 13-acetate (PMA) and opsonized zymosans were used to stimulate neutrophils.

ESR examination showed that when the neutrophils were stimulated by PMA, the ESR spectrum, which was regarded as originating from the spin adduct between DMPO and superoxide anions ( $O_2^-$ ), was clearly observed in both the GPS calves and healthy calves, and no difference was obtained in the spectral intensities between the two. However when the neutrophils were stimulated by opsonized zymosans, the appearance of the ESR spectrum was recognized in the case of healthy calves but not in the GPS calves. Similar results were obtained from chemiluminescence experiments.

PMA is known to stimulate neutrophils by acting on protein kinase C which is located near the cell membrane. Stimulation of neutrophils by opsonized zymosans is achieved by interaction with C3b receptors on the cell membrane, followed by the activation of protein kinase C through several enzymatic pathways. Therefore, it was concluded from both ESR and chemiluminescence experiments that such a low activity of  $O_2^-$  generation of neutrophils in the GPS calves could be attributed to a functional deficiency in the enzymatic pathways from C3b receptor to protein kinase C.

When the phagocytic activities of both neutrophils were measured by small chemiluminescent particles which were specially designed for phagocytic measurements, the activity of GPS neutrophils was about two-thirds that of normal neutrophils. The dysfunction of phagocytic activity of GPS neutrophils seems to be one of the causes for the low activity of  $O_2^-$  generation.