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ANALYSIS OF UNUSUAL SUPEROXIDE GENERATION IN
NEUTROPHILS FROM A CALF WITH BOVINE
LEUKOCYTE ADHESION DEFICIENCY

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The ability of neutrophils from a Holstein-Friesian calf with bovine leukocyte adhesion deficiency (BLAD) (the proband with a genetic deficiency of the CR3 (Mac-1, CD11b/CD18) glycoprotein corresponding to the receptor of complement iC3b) to generate oxygen radicals was examined using electron spin resonance spectrometry (ESR) combined with a spin-trapping technique. When neutrophils from BLAD were stimulated by serum-opsonized zymosan (s-OPZ), monomeric-, heat aggregated-IgG and IgG-opsonized zymosan, the generation of superoxide anions was low, whereas when they were stimulated with a protein kinase C stimulator, phorbol 12-myristate 13-acetate, and calcium ionophore A23187, the generation was normal, indicating that signal transduction pathways for superoxide generation via IgG-FcR activation might be affected by the presence of CR3. To clarify the roles of CR3, neutrophils from BLAD as well as normal calves were stimulated with those stimuli in the presence or absence of several inhibitors of signal transduction pathways (H-7 and staurosporine as protein kinase C (PKC) inhibitors, genistein as a tyrosine kinase inhibitor, and wortmannin as a phosphatidyl inositol 3-kinase (PI 3-kinase) inhibitor). It was found that PKC inhibitors largely affected superoxide generation when stimulated by s-OPZ in normal neutrophils, but not do so in BLAD neutrophils. Genistein depressed the generation of superoxides in both types of neutrophils regardless of the difference of stimulators. Wortmannin inhibited the superoxide generation in normal neutrophils but did not in BLAD neutrophils when stimulated by s-OPZ, while this did not inhibit the superoxide generation in both neutrophils when stimulated by IgG. These results indicated that the signal transduction pathways for the superoxide generation in BLAD neutrophils were insensitive to PKC inhibitors and the PI-3 kinase inhibitor but were sensitive to the tyrosine kinase inhibitor, which are different from normal neutrophils.