THE SERUM NEUTRALIZING ANTIBODY RESPONSE IN CATTLE TO *Fusobacterium necrophorum* LEUKOTOXOID AND POSSIBLE PROTECTION AGAINST EXPERIMENTALLY INDUCED HEPATIC ABSCESSES

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ABSTRACT


The serum antileukotoxin antibody response and protection against subsequent experimental challenge with *Fusobacterium necrophorum* were investigated in 30 steers vaccinated with crude *F. necrophorum* strain 25 leukotoxoid. Culture supernatant of *F. necrophorum*, strain 25, containing leukotoxoid was concentrated. The steers were assigned randomly to six groups (n = 5): PBS control with Stimulon adjuvant; vaccinated with concentrated supernatant diluted to provide 2.5, 5.0, 10.0, or 20.0 ml with the water-soluble Stimulon adjuvant; and 5.0 ml with the Ribi oil-emulsion adjuvant. The steers were injected subcutaneously on days 0 and 21. Blood samples were collected at weekly intervals to monitor serum antileukotoxin antibody titres. On day 42, all the steers were challenged intraportally with *F. necrophorum* culture. Three weeks later (day 63), the steers were killed and necropsied for examination of their livers and assessment of protection. Steers vaccinated with crude leukotoxoid tended to have higher antileukotoxin titres than the controls, but the difference was not significant. Also, the antibody titre did not appear to be dose-dependent. In the control group, 3 out of 5 steers developed liver abscesses. The incidence of liver abscesses in steers vaccinated with Stimulon adjuvant was not dose related; however, only 8 of the 25 vaccinated steers developed abscesses. None of the steers vaccinated with the 5.0 ml dose with Ribi had any abscesses. Evidence for a relationship between antileukotoxin antibody and protection was shown by the lower titre in those steers that developed abscesses compared to those that did not. It was concluded that antileukotoxin antibody titres probably provided some degree of protection against experimentally induced liver abscesses, but further dose-titration studies using Ribi or possibly another more effective adjuvant will be needed to confirm this.

Keywords: abscesses, adjuvant, antibody, antileukotoxin, cattle, *Fusobacterium necrophorum*, immunity, leukotoxoid, liver, protection

Abbreviations: BHI, brain-heart infusion; CFU, colony-forming units; ELISA, enzyme-linked immunosorbent assay; MTT, 3-[4,5-(dimethylthiazol-2-yl)]-2,5-diphenyltetrazolium] bromide; PBS, phosphate-buffered saline; PMN, polymorphonuclear neutrophils
INTRODUCTION

Bovine hepatic abscesses are of economic concern to the beef cattle industry because they cause condemnation of livers, reduced feed efficiency, and reduced weight gain (Brink et al., 1990). *Fusobacterium necrophorum*, a Gram-negative anaerobic rod-shaped bacterium and an inhabitant of the ruminal flora, is the primary aetiological agent of bovine hepatic abscesses (Langworth, 1977). Hepatic abscesses in cattle are related to feeding of high-grain diets. Rapid fermentation of grain in the rumen and the resultant accumulation of organic acids, particularly lactate, results in ruminal acidosis and rumenitis. Ruminal lesions appear to be predisposing factors for liver abscesses (Smith, 1944; Jensen et al., 1954a). *F. necrophorum* penetrates the rumen epithelial wall, reaches the liver via portal circulation and causes abscesses (Scanlan and Hathcock, 1983).

The pathogenicity of *F. necrophorum* is attributed mainly to a potent leukotoxin, an extracellular, high-molecular-weight (> 300,000) protein that is cytotoxic to ruminant leukocytes and possibly to hepatocytes (Roberts, 1967, 1970; Emery et al., 1984; Tan et al., 1992). There is evidence of a correlation between leukotoxin production and the infectivity of the organism (Coyle-Dennis and Lauerman, 1979; Emery et al., 1985). Therefore, immunity against the leukotoxin may provide protection against *F. necrophorum* infection. Studies have indicated that antileukotoxin immunity reduces the incidence of hepatic abscesses and interdigital necrobacillosis (Garcia et al., 1974). Clark and colleagues (1986) reported that cattle injected with the supernatant of *F. necrophorum* culture containing leukotoxin had a low incidence of interdigital necrobacillosis. Evaluation of the efficacy of a vaccine for liver abscesses is difficult because of the unpredictability of their occurrence in grain-fed cattle. Ultrasound-guided, intraportal inoculation of *F. necrophorum* offers a simple, non-invasive technique for experimentally inducing liver abscesses in cattle (Lechtenberg and Nagaraja, 1991). The objective of this study was to determine the protective immune response against experimentally induced liver abscesses elicited by a crude supernatant from a culture of *F. necrophorum* containing leukotoxoid.

MATERIALS AND METHODS

Preparation of antigen

*Fusobacterium necrophorum* subsp. *necrophorum*, strain 25, previously shown to be a high leukotoxin producer (Tan et al., 1992), was grown in pre-reduced, anaerobically sterilized, brain–heart infusion broth (BHI, Difco Laboratories, Detroit, MI, USA; Tan et al., 1992). A late log-phase culture (7 h) was centrifuged at 13,500 g for 30 min at 4°C. The culture supernatant was filter-sterilized through a 0.45 μm membrane filter (Micron Separation Inc., Westborough, MA, USA) and concentrated 58-fold at 4°C using a hollow fibre concentration/desalting system with a 100,000 molecular weight exclusion filter (Amicon Co., Danvers, MA, USA).