LEUKOTOXIN PRODUCTION BY *FUSOBACTERIUM NECROPHORUM* BIOTYPES

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Summary

Fusobacterium necrophorum biotypes A and B were grown anaerobically to detect their leukotoxin production. Both biotypes exerted the highest leukotoxic effect on bovine leukocytes in the late logarithmic and early stationary growth phases. Biotype A produced more leukotoxin than biotype B throughout all phases of bacterial growth. Results are consistent with the findings that biotype A is more virulent than biotype B.

(Key Words: Leukotoxin, *F. necrophorum*, Biotype, Growth Phase, Liver Abscesses.)

Introduction

Liver abscesses in feedlot cattle result in condemnation of over three million livers annually. The principal causative agent is *F. necrophorum*, a normal inhabitant of the rumen. This bacterium secretes leukotoxin, a substance that kills leukocytes and suppresses the body defense functions, allowing the establishment of the bacterium in the liver. Therefore, leukotoxin may be an important factor contributing to the pathogenesis of liver abscesses in cattle.

Two biotypes of *F. necrophorum* have been described. Biotype A is found most frequently in liver abscesses. Biotype B, which predominates in ruminal contents, is also isolated from liver abscesses. However, it is usually found in mixed infections and is considered less pathogenic than biotype A. We determined leukotoxin production of both biotypes in different growth phases.

Experimental Procedures

F. necrophorum biotypes A 25 and B 35 were isolated previously from liver abscesses collected at a packing plant. Bacteria were grown aerobically in brain heart infusion (BHI) broth. Samples were collected at 0, 2, 4, 6, 8, 10, 12, 30, and 45 hr for measurements of growth (colony counts) and for assay of leukotoxin production.

Culture supernatants prepared by centrifugation were used for leukotoxin assay. Polymorphonuclear neutrophil (PMN) leukocytes were isolated from cattle blood and labeled with ^{51}Cr-sodium chromate. Labeled cell suspensions were mixed with culture supernatants,

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incubated for 2 hr, and centrifuged. Supernatants were harvested by an automatic supernatant collection system. The release of radioactivity into the supernatant, indicating leukotoxicity (percentage of cell lysis), was measured in a gamma counter.

Results and Discussion

The leukotoxicities of biotypes A and B at different stages of growth are shown in Figure 12.1. Both biotypes A and B showed the same trend of toxin production in relation to bacterial growth. The percentage of toxicity increased with increasing bacterial growth, was highest at the late logarithmic and early stationary growth phases, and then began to decline. Leukotoxicity completely disappeared after 30 hr incubation, implying that bacteria possibly secreted proteolytic enzyme(s) that destroyed the toxin.

Biotype A produced more leukotoxin than biotype B at both the logarithmic and stationary growth phases. This may be one of the reasons why biotype A is more virulent and more frequently encountered in liver abscesses.

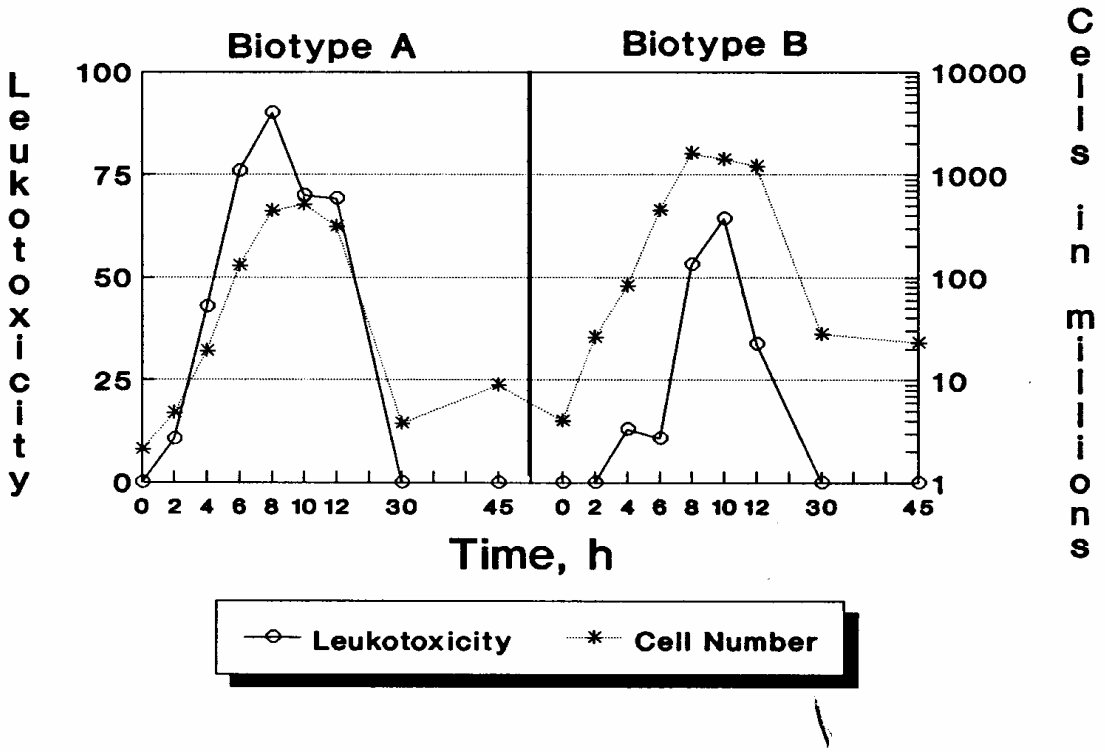

Figure 12.1. Leukotoxin Production in *Fusobacterium necrophorum* Biotypes A and B