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Commentary Response

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The authors are pleased to respond to the excellent observations and commentary provided by Fluit et al. During the current studies we were unable to obtain a neutralizing monoclonal antibody to cytotoxin B or the enzyme enolase (EC 4.2.1.11). Results of efforts to separate cytotoxic and enolase activities suggested that cytotoxin B may represent an enzyme. Based on the data available at the time of investigation the authors proposed that cytotoxin B may be an enolase.

Fluit et al. have presented evidence that cytotoxin B and enolase are separate entities which are complexed or associated during attempts at fractionation on DEAE-Sepharose CL-6B and Sephacryl S300. Further evidence presented by Fluit et al. related to the production of enolase activity by nontoxigenic isolates of *Clostridium difficile* and the neutralization of cytotoxic, but not enzymatic, activity associated with cytotoxin B by antiserum to *Clostridium sordellii* are compelling facts which indicate a lack of homology. These results also indicate that cytotoxin B remains associated or complexed with enolase activity after Biogel A5m chromatography, phenyl boronate hydrophobic interaction chromatography, density gradient ultracentrifugation, and SDS-PAGE used in our investigations.

The cytotoxin B preparation in our studies had a molecular weight of 163 kDa on SDS-PAGE. As Fluit et al. indicate this molecular weight does not agree with expected literature values of 250 kDa [1], 270 kDa [2], or 240 kDa or greater [3]. In further studies we have repeatedly substantiated a molecular weight of 163 kDa when estimated by SDS-PAGE or sucrose density gradient ultracentrifugation. The enzyme enolase has been described as a dimer with a molecular weight of 82 kDa [4]. Assuming the protein is conserved, an enolase–cytotoxin B complex would have an estimated molecular weight of 352 kDa, yielding molecular weights of 41 and 270 kDa when subjected to SDS-PAGE. We have not observed a 41 or 270 kDa protein band on SDS-PAGE. Although these discrepancies cannot be reconciled at present the available data suggests that the varied molecular weights may be the result of differences in innate cytotoxin structure produced by different isolates, the presence of protoxins that are cleaved during release from the bacterial cell or during sporulation [5], or the production of multiple toxic forms [6]. Johnson et al. [3] have demonstrated a number of bands when the recombinant toxin is subjected to immunoblot analysis. It is also possible that separate and distinct toxic entities are produced by different isolates of *C. difficile* [6,7].

In view of the information provided by Fluit et al. the authors conclude that cytotoxin B may be complexed or associated to the enzyme enolase. However, further studies will be required to separate the complex, resolve the ambiguities and discrepancies in reported molecular weights, and determine what role enolase plays in the stability or biological expression of cytotoxin B.

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