

ABSTRACT

Stx1 and Stx2 were purified by the procedures consisting of ammonium sulfate fractionation, DEAE-Sepharose column chromatography, chromatofocusing column chromatography and FPLC. Both toxins showed toxicity to the Vero cells. About 0.87 µg of purified Stx1 caused 50% cytopathic effect (CD₅₀) to the cells. The CD₅₀ of partially purified Stx1 and Stx2 obtained after DEAE-Sepharose column chromatographies (Stx1-IEC and Stx2-IEC) were 6.8 µg and 1.38 ng, respectively. It was shown that Stx2-IEC was less toxic to the Vero cells than the Stx1-IEC. Mouse MAbs against A- and B-subunits of both Stx1 and Stx2 were produced through hybridoma technology. Four hybridomas, namely clones 11E3G3 and 3C9D4 which produced MAbs specific to A- and B-subunits of Stx1 and 26E9F9 and 13D4D2 which produced MAbs specific to the A- and B-subunits of Stx2, respectively were selected for use in the subsequent toxin neutralization assays. MAbs produced by the first three clones were IgG₁ while those MAbs from clone 13D4D2 was IgG_{2b}. MAbs secreted by each clone were tested for their neutralizing capability and ability to inhibit 100 CD₅₀ of these respective toxins. The results indicated that MAbs to B-subunits of both toxins conferred higher neutralizing capability to the respective holotoxins than the MAbs to the A-subunits and could completely inhibit the 100 CD₅₀ of respective toxin activities. The MAbs to A-subunit could only partially inhibited the activities of the toxins; however, they conferred synergistic toxin inhibitory effect when mixed with the MAbs to B-subunit.