CHARACTERIZATION OF NEUTRALIZING HUMAN ANTI-TETANUS MONOCLONAL ANTIBODIES PRODUCED BY STABLE CELL LINES

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Tetanus is an infectious and non-contagious, neuromuscular disease caused by the action of anaerobic *Clostridium tetani* exotoxin (TeNT). One of the most potent known toxins, it can cause death when appropriate treatment is not provided. The bacteria are everywhere spread in the soil and feces of animals and infect people through cuts, nails, needles, surgery, tattoo, piercing, etc. TeNT affects the nervous system by blocking the release of inhibitory neurotransmitters glycine and gamma-aminobutyric in the synapses. TeNT is synthesized as a single-chain polypeptide, then cleaved into a two-chain polypeptide linked by a disulfide bridge. The heavy chain with 100kDa contains two domains (fragment C, responsible for the toxin binding to the neuron surface, and domain B (promotes the translocation in the neural membrane). The light chain with 50kDa contains a zinc-binding motif responsible for the proteolytic toxin action. Despite a safe and low-cost vaccine, tetanus cases persist, mainly in low- and middle-income countries, due to unimmunized or poorly immunized populations, the decline of protection in elderly people, and drug abuse, among others. Only polyclonal antibodies, of equine or human origin, are available for use in cases of infection or potential risk of tetanus. In this context, the development of human-neutralizing mAbs could represent a more specific treatment.

Previously, we have obtained a trio of human mAbs that, in combination, neutralizes TeNT in animal trials compared to hyperimmune equine serum. Besides, we identified two other mAbs that inhibit the binding of TeNT to GT1b (ganglioside receptor of tetanus toxin in neurons) (1). In the current work, stable cell lines were established for those five mAbs. The expression in CHO-S cells was accomplished by transfection of pCHO 1.0 vector containing the heavy and light variable regions of each mAb. The variable regions were derived from the sequencing of PCR products of RNA extract from single human B cells sorted by flow cytometry. To express a large number of variable regions into the vector, we ordered the separate synthesis of IgG1 and kappa constant sequences, which, through a series of sequential steps, were inserted into pCHO 1.0 vector, that after were cloned with each individual paired heavy and light variable region of each mAb. The CHO-S cells underwent four-step selection with methotrexate and puromycin, generating stable pools for the five mAbs. A 14-day fed-batch was run for the 28 pools, sampled along the time for cell counting and viability, and mAb production. The purified mAbs were tested by ELISA against TeNT immobilized in the plates. Some differences were observed for each of the five mAbs. The pools generated in the most stringent selection were in affinity assays by surface plasmon resonance). Only two mAbs could have the kinetic affinity calculated, the other three could not dissociate even at the extended time of 36,000 s. The steady-state affinity assay was used instead. An analysis of inhibition of binding of TeNT to GT1b was performed with individual and mixed mAbs, confirming the results obtained in the transient transfection for two mAbs, that reached 100% inhibition. The mixed assay with the five mAbs showed that only these mAbs contributed to the inhibition indexes. Molecular docking and binding prediction were also evaluated for one mAb (2).

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