Construction of a Single-Chain Variable-Fragment Antibody against the Superantigen Staphylococcal Enterotoxin B

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Staphylococcal food poisoning (SFP) is one of the most prevalent causes of food-borne illness throughout the world. SFP is caused by 21 different types of staphylococcal enterotoxins produced by Staphylococcus aureus. Among these, staphylococcal enterotoxin B (SEB) is the most potent toxin and is a listed biological warfare (BW) agent. Therefore, development of immunological reagents for detection of SEB is of the utmost importance. High-affinity and specific monoclonal antibodies are being used for detection of SEB, but hybridoma clones tend to lose their antibody-secreting ability over time. This problem can be overcome by the use of recombinant antibodies produced in a bacterial system. In the present investigation, genes from a hybridoma clone encoding monoclonal antibody against SEB were immortalized using antibody phage display technology. A murine phage display library containing single-chain variable-fragment (ScFv) antibody genes was constructed in a pCANTAB 5E phagemid vector. Phage particles displaying ScFvs were rescued by reinfection of helper phage followed by four rounds of biopanning for selection of SEB binding ScFv antibody fragments by using phage enzyme-linked immunosorbent assay (ELISA). Soluble SEB-ScFv antibodies were characterized from one of the clones showing high affinity for SEB. The anti-SEB ScFv antibody was highly specific, and its affinity constant was 3.16 nM as determined by surface plasmon resonance (SPR). These results demonstrate that the recombinant antibody constructed by immortalizing the antibody genes from a hybridoma clone is useful for immunodetection of SEB.

Staphylococcus aureus is one of the most prevalent causative agents of food-borne illness throughout the world. The illness occurs following ingestion of staphylococcal enterotoxins (SEs) produced by S. aureus in the contaminated food. The symptoms of food poisoning include nausea, vomiting, abdominal cramps, and diarrhea. Twenty-one different types of SEs, i.e., staphylococcal enterotoxin A (SEA) to staphylococcal enterotoxin V (SEV), have already been discovered (33, 40). The immunomodulatory effects of SEs, such as immunosuppression, enhancement of endotox shock, and induction of cytokine release, can be attributed to the fact that SEs are potent T-cell mitogens. The ability of SEs to induce proliferation of T cells is somewhat similar to conventional antigen presentation by major histocompatibility complex (MHC) class II molecules to T-cell receptors (TCRs). However, unlike conventional antigens, the T-cell mitogenicity of SEs does not require antigen processing and lacks the normal specificity to the TCR for specific epitopes in response to conventional antigens. This bypass of normal TCR specificity for conventional T-cell epitopes results in the stimulation of a substantial proportion of the total T-cell population, and therefore, staphylococcal enterotoxins are referred to as superantigens. The stimulation of T cells leads to overproduction of cytokines, causing clinical symptoms that include fever, hypotension, and even death in severe cases (3, 30, 39).

Among SEs, SEB is the most potent toxin secreted by S. aureus (3, 35). This is a single polypeptide containing 239 amino acids with a molecular mass of 28 kDa. SEB is highly resistant to proteases, boiling temperature, and extremes of pH because of its compact tertiary structure (5, 27). In humans, 3.5 µg of SEB ingested by the oral route causes emesis (5). SEB is extremely toxic by inhalation, and as little as 30 ng is sufficient to cause fever, respiratory complaints (cough, dyspnea, and retrosternal discomfort or chest pain), and gastrointestinal symptoms. Severe intoxication results in pulmonary edema, adult respiratory distress syndrome (ARDS), shock, and death (28, 37, 42). Although exposure to SEB by the inhalation route is not a common feature of S. aureus infection, the possibility of it makes SEB a candidate weapon for biological terrorism and, hence, it is a listed biological warfare agent (21, 26). Therefore, its quick and unambiguous detection is of paramount importance.

The availability of specific and high-affinity antibodies is the major bottleneck in the development of an immunodetection system for SEB. Any immunological detection system for SEB requires specific and high-affinity antibodies, but SEB being a superantigen leads to the generation of low-titered polyclonal antibodies. The problem is further aggravated if SEB is contaminated even slightly with other, undesired proteins, leading to nonspecific antiserum. Polyclonal antibodies are generated using SEB purified by conventional protein purification meth-