Production of functional ScFv inhibiting streptococcus mutans glucosyltransferase activity from a hybridoma P126

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Abstract

Streptococcus mutans has been considered the principal etiologic agent of dental caries in humans. The glucosyltransferase-I (GTF-I), which synthesized adhesive water-insoluble glucans from sucrose, has been demonstrated to be an important cariogenic property. Water-insoluble glucans (WIG) synthesized by S. mutans GTF-I can mediate sucrose-enhanced colonization on tooth surfaces and form dental plaque. It has been suggested that inhibition of WIG synthesis decreases bacterial colonization and cariogenicity. Indeed, the use of GTF enzymes as a vaccine antigen resulted in protection from experimental dental caries in rodents. However, it is preferable to eliminate unwanted immune response during active immunization of humans. To prevent this incidence, we attempted to produce the single-chain variable fragment (ScFv) antibody against GTF-I to develop passive immunization for dental caries. Hybridomas producing monoclonal antibody (MAb) that inhibited WIG synthesis by GTF-I have been constructed. Using mRNA from an IgG1 hybridoma P126, cDNAs encoding the variable fragments of the L and H chains of IgG1 from the hybridoma P126 were cloned by RT-PCR-based techniques and then transformed into an Escherichia coli expression system. The purified ScFv antibody recognized the recombinant (r) GTF-I proteins and was capable of inhibiting the WIG synthesis of rGTF-I.