Characterization and molecular modeling of a highly stable anti-Hepatitis B surface antigen scFv

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Date: 2003

Abstract

We raised a mouse monoclonal antibody (5S) against the 'a' epitope of the Hepatitis B surface antigen (HBsAg) by selecting for binding of the hybridoma supernatant in conditions that usually destabilize protein-protein interactions. This antibody, which was protective in an in vitro assay, had a high affinity with a relative dissociation constant in the nanomolar range. It also displayed stable binding to antigen in conditions that usually destabilize antigen-antibody interactions, like 30% DMSO, 8 M urea, 4 M NaCl, 1 M guanidium HCl and extremes of pH. The variable regions of the antibody were cloned and expressed as an single chain variable fragment (scFv) (A5). A5 had a relative affinity comparable to the mouse monoclonal and showed antigen binding in presence of 20% DMSO, 8 M urea and 3 M NaCl. It bound the antigen in the pH range of 6-8, though its tolerance for guanidium HCl was reduced. Sequence analysis demonstrated a significant increase in the frequency of somatic replacement mutations in CDRs over framework regions in the light but not in the heavy chain. A comparison of the molecular models of the variable regions of the 5S antibody and its germ-line precursor revealed that critical mutations in the heavy and light chains interface resulted in better inter-chain packing and in the movement of CDR H3 and CDR L1 from their germline positions, which may be important for better antigen binding. In addition to providing a reagent for neutralizing for the virus, such an antibody provides a model for the evolution of stable high affinity interaction during antibody maturation.