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ANTIGENICALLY REACTIVE REGIONS WITHIN THE HEPATITIS A VIRUS (HAV) POLYPROTEIN

Objectives. The main objectives of this study were, (1) to map antigenic epitopes across the entire hepatitis A virus (HAV) polyprotein and (2) to identify the most diagnostically relevant antigenic regions.
Methods. The PCR walking technique was utilized to localize antigenically reactive regions within the HAV polyprotein. Fifty-three overlapping PCR fragments of ~300-400bp spanning the entire coding region of the HAV genome were cloned with pGEX-4T-2 and expressed in Escherichia coli as hybrid proteins with Glutathione S-transferase. The recombinant proteins were purified using ligand affinity chromotography and tested by enzyme immunoassay against a panel of acute (*n*=57) and convalescent (*n*=48) phase anti-HAV-positive human serum specimens and against serconversion panels obtained from experimentally HAV-infected chimpanzees.
Results. Recombinant polypeptides comprising regions from the HAV proteins VP1, P2A, P2B, P2C and P3A were found strongly and broadly immunoreactive. The most immunoreactive region was localized at position 722-830 a.a. A single recombinant protein containing this region detected IgM and IgG anti-HAV activity in 94.7% of acute-phase and IgG anti-HAV activity in 75% of convalescent-phase sera. Ten recombinant proteins strongly immunoreacted with IgG and IgM antibodies during the acute phase of experimentally infected chimpanzees. One protein comprising a region of the protein P2C at position 1121-1234 a.a. detected IgG anti-HAV activity in experimentally infected chimpanzees for more than 2 years following HAV inoculation.
Conclusion. The HAV polyprotein contains several diagnostically relevant regions, the antigenic properes or which can be efficiently modelled with recombinant proteins expressed in *E. coli.* These recombinant proteins can be used as diagnostic targets for the development of sensitive and specific assays for the detection of anti-HAV activity in serum specimens obtained from either the acute or convalescent phase.

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