A. OBRIADINA, J. MENG, E.N. LOPAREVA, J. SPELBRING, A. BURKOV, K. KRAWCZYNSKI

Centers for Disease Control and Prevention, Atlanta, Ga., USA;
NPO Diagnostic Systems, Nizhniy Novgorod, Russia;
Nanjing Railway medical College, Nanjing, China

ANTIGENIC PROPERTIES OF RECOMBINANT PROTEINS OF HEPATITIS E VIRUS (HEV)

Objectives. The purpose of this study was to evaluate the diagnostic relevance of a nested set of five recombinant proteins that efficiently model the neutralizing antigenic epitope(s) of the HEV open reading frame 2 (ORF2) encoded protein.
Methods. Five fragments of the ORF2 encoded protein of HEV Burma strain; namely, L4 (393-660 aa), L5 (421-660 aa), L6 (452-660 aa), B2 (421-617 aa) and Bl (452-617 aa), were expressed in *E. coli* as hybrid proteins with glutathione S-transferase. These proteins were purified using ligand affinity chromatography and tested by enzyme immunoassay against a panel of serum specimens obtained from patients acutely infected with HEV (n = 81) and from normal blood donors (n = 288). Additionally, serial specimens obtained from four experimentally HEV-infected chimpanzees were also tested.
Results. All five proteins detected seroconversion in experimentally infected chimpanzees and they strongly immunoreacted with IgG antibodies during the entire period of observation for more than 2.5 years after HEV inoculation. The sensitivity of the EIA for the detection of IgG anti-HEV activity in acute human serum specimens varied from 97.6% to 99.6% for each protein. The smallest protein, Bl, was the most broadly immunoreactive. However, 20 serum specimens (6.9%) from normal blood donors were also found to be immunoreactive with recombinant proteins L4, L5, L6 and B2. Protein Bl also immunoreacted with 44 (15.3%) of the normal serum samples. Additionally, these specimens were tested against a set of 71 overlapping synthetic 30-mer peptides spanning the entire ORF2 encoded protein. The data indicated that 14 out of the 20 serum specimens from normal blood donors that immunoreacted with L4, L5, L6 and B2 proteins contained antibodies that specifically bound to a large number of synthetic peptides. Each specimen immunoreacted with ~ 30 HEV peptides. Out of the 44 specimens that immunoreacted only with Bl protein, four of which also immunoreacted with synthetic peptides. Each specimen immunoreacted with ~ 40 peptides.
Conclusion. The recombinant proteins used in this study demonstrated significant potential as diagnostic reagents for the development of assays for the detection of anti-HEV activity. However, the immunoreactivity of some serum specimens, especially those obtained from normal blood donors requires further characterization before these recombinant proteins and synthetic peptides can be used in the development of specific and sensitive diagnostic tests.

10th International Symposium on Viral Hepatitis and Liver Disease, Atlanta, USA April 9-13, 2000
Antiviral Therapy 2000; 5 (Suppl. 1): P-17