T.I. ULANOVA, A.P. OBRIADINA, A.N. BURKOV, H.A. FIELDS, Y.E. KHUDYAKOV

Hepatitis Branch, CDC, Atlanta, Ga, USA;
National Institute of Hygiene and Epidemiology, Hanoi, Vietnam
'Diagnostic Systems', Nizhniy Novgorod, Russia

ARTIFICIAL MOSAIC ANTIGEN OF THE HEPATITIS E VIRUS

A second generation artificial multiple-epitope antigen (MA-II) containing all major immunoreactive regions of proteins encoded by open reading frame (ORF)2 and ORF3 of the Hepatitis E virus (HEV) genome was designed and constructed. Each region was preliminarily selected using a large number of synthetic peptides of different sizes tested against a panel of anti-HEV positive sera. The major design feature of MA-II is that each antigenic region was duplicated within the artificial protein using slightly different sequence variants of each region, which were evenly scattered along the MA-II polypeptide chain. Ten antigenic regions were selected from the ORF2 protein (31-66 aa, 39-64 aa, 85-114 aa, 95-119 aa, 398-427 aa, 403-432 aa, 614-638 aa, 626-655 aa, 631-660 aa, 635-660 aa), while two sequence variants were selected from the ORF3 protein (91-123 aa), one from the Burmese strain and the other from the Mexican strain. The MA-II gene was assembled from synthetic oligonucleotides by a new method termed Restriction Enzyme-Assisted Ligation (REAL). A full-length synthetic gene as well as various intermediate products of the gene assembly were cloned and expressed in *E.coli* as fusion proteins with glutation S-transferase (GST). Each protein was purified and tested against a panel of 132 anti-HEV positive and negative specimens. This comparative study showed that the full-length MA-II detected more anti-HEV positive specimens than fragments thereof. Furthermore, full length MA-II detected anti-HEV activity in 98.5% of serum specimens obtained from HEV infected patients. These findings strongly suggest that MA-II has significant diagnostic potential and should be considered as a new diagnostic target for the development of assays for the detection of anti-HEV activity in serum specimens.

American Society for Virology, 20th Annual Meeting, Madison. - USA, 2001. - P.197 (P 26-9).