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Monoclonal antibody against non-dominant epitopes of HBV e Ag was raised by antigen–antibody co-immunization

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Abstract

Detection of hepatitis B e antigen (HBeAg) in the sera of individuals infected with hepatitis B virus (HBV) can indicate both a high infectivity of the disease and a poor prognosis of disease treatment. Most of monoclonal antibodies raised against HBV e proteins interact with immuno-dominant epitopes, such as HBeAg-β. In order to raise antibodies against non-dominant epitopes of HBV e protein, in this study, mice were immunized with both recombinant HBeAg (rHBeAg) and an anti-HBeAg antibody (EWB) recognizing a dominant antigenic epitope of HBeAg (HBeAg-β epitope). With this strategy, we successfully selected two monoclonal antibodies, S-29-3 and S-72-3. Both S-29-3 and S-72-3 bind to recombinant HBeAg with a high affinity. The epitope mapping assay determined that the S-73-2 recognizes the N-terminal of HBeAg (1–118 aa) and the S-29-3 recognizes the C-terminal of HBeAg (91–149 aa). Further experiment showed that these two antibodies could be formed a pair-Abs that is used in detecting native HBeAg from the sera of HBV patients. The conclusion is that the developed method is useful to raise mAb against non-dominant epitopes in given Ag.

Keywords: Hepatitis B virus (HBV); HBeAg; Co-immunization; Epitope; Anti-HBeAg immunoassay; ELISA

1. Introduction

Hepatitis B virus (HBV) infects more than 300 million people worldwide (Beasley et al., 1981). It is a small DNA virus and consists of a lipid envelope embedded with surface antigen (sAg), HBeAg and the core antigen (cAg) as well as a DNA polymerase (Brunello et al., 1979).

Although it is not clear whether HBeAg plays a role in the pathogenesis of HBV, it has a great value in clinical diagnosis (Klein et al., 1991; Niermeijer et al., 1980). HBeAg is considered as a good marker for indicating HBV infection (Chen et al., 2006; Pal et al., 2005). In nature, HBeAg is always present in a non-particulate state in the serum of HBV-infected individuals (Pal et al., 2005; Takahashi et al., 1983; Magnius and Espmark, 1972). It can be used as a marker of infectivity and diagnosis of patients who have a high risk in transmission of the disease (Okada et al., 1976; Alter et al., 1976). During convalescence, antibodies to HBeAg and HBcAg are detected along with antibodies against the sAg. These antibodies are important serological markers to assess the immunity and the reduction of viral replication in the infected individual (Okada et al., 1976; Alter et al., 1976).

Based on literature, three major epitopes of HBV e protein have been described. They are HBeAg-β, HBeAg-1 and HBeAg-2 (Sallberg et al., 1991, 1993; Salfeld et al., 1989; Baumeister et al., 2000). HBeAg-β and HBeAg-1 are linear epitopes, located on the 128–133 aa and 76–89 aa of the HBeAg, respectively. HBeAg-2 is a conformational epitope, covering the total length

Abbreviations: HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; aa, amino acid; HRP, horseradish peroxidase; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay

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of the HBeAg (2–140 aa). Further studies show that HBeAg-β is a predominant epitope (Sallberg et al., 1991; Salfeld et al., 1989; Baumeister et al., 2000; Ferns and Tedder, 1986). In most case, following the immunization with recombinant HBeAg (rHBeAg), the antibodies are mainly against HBeAg-β epitope. To select mAbs recognizing different epitopes that could form a pair-Abs in detection of HBeAg has been proven to be difficult.

Since it is essential to have a pair-Abs recognizing a different epitopes and then to be formed a ELISA-kit in detecting a given Ag, in this study, HBV e protein was selected as an model system based on its available reagents and epitopes' information. To address our question, an antigen–antibody complex was used for co-immunization. Specifically, mice were immunized with an antigen–antibody complex containing the rHBeAg and the monoclonal antibody (EWB). The latter recognizes the HBeAg-β epitope, 128–133 amino acids. We hypothesized that adding of EWB may decrease the response to HBeAg-β epitope and but selectively enhance the immune response to other epitopes within HBeAg. Here we report that with this strategy, we successfully obtained two anti-HBeAg monoclonal antibodies, S-29-3 and S-72-3. Both of them showed a good specificity and affinity to rHBeAg. The S-73-2 recognizes the fragment of HBeAg (1–118 aa) and the S-29-3 recognizes the fragment of HBeAg (91–149 aa). Using these two mAbs, a sandwich ELISA-kit was developed and it has a potential in clinical diagnosis.

2. Materials and methods

2.1. Antigen and reagents

Recombinant HBeAg, cDNA of HBV e Ag and two commercialized monoclonal antibodies (EWB and LPA), were kindly provided by Kehua Bioengineering (Shanghai) Co. Ltd. LN 18 peptide (119–136 aa, LVSFGVWIRTPPAYRPPN of HBeAg-β epitope) and NN16 peptide (75–90 aa, NLEDPASRDLVVSYVN of HBeAg-1 epitope) were synthesized using a conventional solid-phase chemical method and purified with HPLC in GL Bio-Chem (Shanghai) Ltd. Bovine serum albumin (BSA) served as a carrier protein. Peroxidase labeled goat anti-mouse IgG were purchased form Sigma. Mouse sub-isotype panel was purchased from Bio-Rad. Glutathione sepharose™ 4B and ECL detection reagents were purchased from Amersham Pharmacia Biotech. *E. coli* DH5α and BL21 (DE3) strains were purchased from Novagen. Restriction enzyme was purchased from Takara. T4 DNA ligase was purchased from Sino-American Biotechnology Company. IPTG, PMSF, and Taq DNA polymerase were from Promega Company.

2.2. Animals

BALB/c mice were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences. Animals were kept in conventional conditions and were handled in compliance with Chinese Academy of Sciences guidelines for Animal Care and Use.

2.3. Expression and purification of rHBeAg fragments

Four fragments of rHBeAg (E1, E2, E3, E4) gene were inserted into vector pGEX-4T-1 and transformed into *E. coli* strain, BL21 (DE3). The bacterial cells were induced by IPTG (0.1 mM) at 30 °C for 4 h in tryptone-phosphate medium (Ying Lin et al., 2004). The cells were pelleted by centrifugation and resuspended. The cell lysates were prepared by sonication. Extracted proteins were purified by column purification with GST sepharose 4B beads (Smith and Johnson, 1988).

2.4. Immunization and monoclonal antibodies raising

Female BALB/c mice were immunized subcutaneously with 0.1 mg rHBeAg and excessive EWB mAb in emulsion 1:1 (v/v) complete Freund's adjuvant and boosted two times at 3-week interval in incomplete Freund's adjuvant. The mouse with the highest antibody titer was intraperitoneally boosted with 0.1 mg of the rHBeAg in 0.5 ml PBS. On the third day after the last boosting, the mouse was sacrificed and the spleen cells were harvested (Shang et al., 2005). The spleen cells were fused with murine myeloma cells (SP2/0) by 50% PEG. The hybridomas were generated through the selection of HAT medium. The supernatants of hybridoma cultures from each well was screened by detecting its binding activity to rHBeAg in ELISA. The positive hybridomas cultures were cloned by a limiting dilution. After three cycles of cloning, the stable hybridoma clones were obtained. Antibodies' isotype was tested by mouse sub-isotype panel (Bio-Rad).

2.5. HRP enzyme labeling purified monoclonal antibodies

The pristine-primed BALB/c mice were injected intraperitoneally with 1×10^6 hybridoma cells per mouse. The ascites fluids were collected and purified by protein G affinity column. The purified antibodies were then labeled with horseradish peroxidase (HRP) by two-step method with glutaraldehyde (Shang et al., 2005).

2.6. Measurement of antibody affinity constant

The antibody affinity constant was measured by BIAcore 3000. In brief, HBeAg was first immobilized to CM5 sensory chip. Various concentration (4, 2, 1, 0.5, 0.25, 0.125, 0 μM) of anti-HBeAg monoclonal antibodies was then added to the chip to measure the kinetic constant according to the binding model. The equilibrium dissociation constant K_D was estimated using the formula: $K_D = k_d/k_a$ (Canziani et al., 2004; Yao et al., 2006). The affinity constant K was calculated using the formula: $K = 1/K_D$. The system buffer used in this assay contains 20 mM HEPEs, 150 mM NaCl, 5 mM EDTA and 0.1% P20.

2.7. Western blotting

rHBeAg, E1, E2, E3, E4, LN18-BSA and NN16-BSA were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were then transferred onto a