

Construction of eukaryotic expression plasmids of hepatitis B surface antigen and helper T lymphocyte epitope

Yun-Feng Piao, Tong-Yu Tang, Jun-Qi Niu and Feng Wang

Changchun, China

BACKGROUND: DNA immunization provides a promising approach to elicit protective humoral and cellular immune responses against HBV. This study was to construct an eukaryotic expression plasmid containing helper T lymphocyte epitope, which will enhance the immunogenicity of a novel hepatitis B virus (HBV) fusion protein DNA vaccine.

METHODS: The target gene containing pan-DR helper T cell epitopes (PADRE) and HBsAg was amplified by polymerase chain reaction (PCR). The PCR products were linked with PMD-18T vector. Plasmid DNA was purified from transformed *E. coli* competent cell JM109 and digested with Hind III and EcoR I. Then, the target gene was cloned in pcDNA3.1(+) digested by Hind III and EcoR I. Finally, the identity of DNA was verified by digestion and DNA sequencing.

RESULTS: The recombinant expression vectors of pcDNA 3.1(+)-PADRE/HBs were identified by restriction enzyme digestion and DNA sequencing. The insert DNA fragment was consistent with the expected sequence.

CONCLUSIONS: The constructed eukaryotic expression plasmid of pcDNA3.1(+)-PADRE/HBs is convenient for further study of eukaryotic transfection and response for cellular and humoral immunity against HBV.

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KEY WORDS: hepatitis B virus;
helper T lymphocyte epitope;
eukaryotic expression plasmid

Introduction

Despite the existence of safe and efficacious vaccines, infection with hepatitis B virus (HBV) is one of the most common infectious diseases with estimated 350 million chronic HBV carriers worldwide.^[1] This infection leads in some carriers to cirrhosis and ultimately to hepatocellular carcinoma or noncarcinoma. About 170 million Chinese are infected chronically with HBV and around half a million Chinese die of hepatitis B caused hepatocellular carcinoma and end-stage cirrhosis each year.^[2] Since no efficient cure is available presently for HBV chronic infection,^[3-5] an alternative treatment is necessary. Animal studies have shown that DNA vaccine would be promising for immunotherapy of chronic hepatitis B infection.^[6-13] Because DNA vaccines can induce weak and short-lived immune response in large out-bred animal,^[14] it is more important to select an effective way to promote the immune response of DNA immunization. In the present study, we constructed a new expression plasmid, which contains a potent universal helper T lymphocyte (HTL) epitope pan-DR helper T cell epitopes (PADRE) and the HBsAg gene. The optimized construction would be more efficient to enhance the immunogenicity of HBV DNA vaccines.

Methods

Plasmids, competent cells and restriction enzyme

Plasmid expressing hepatitis B virus surface antigen (pcDNA3.1(+)-HBs) containing the full length HBsAg genomic DNA of subtype adr obtained from the Department of Infectious Diseases, First Hospital of Jilin University, Changchun, China. pcDNA3.1(+) was purchased from Introvigen, USA. Cloning plasmid vector PMD-18T, JM109 competent cells and restriction enzyme were bought from Takala, Dalian, China.

Target gene amplified by PCR

The PADRE and large envelope gene of HBV were amplified by polymerase chain reaction (PCR)

Author Affiliations: Department of Gastroenterology (Piao YF and Tang TY), and Department of Infectious Diseases (Niu JQ and Wang F), First Hospital of Jilin University, Changchun 130021, China

Corresponding Author: Tong-Yu Tang, MD, Department of Gastroenterology, First Hospital of Jilin University, Changchun 130021, China (Tel: 86-431-5612242; Email: tangtongyu@163.com)

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from plasmid pcDNA3.1(+)-HBs, which contains the full-length HBsAg genome. The upstream primer was P1: 5'-AAGCTTACCATGGCCAAGTTCGTGGCTGCCTGGACCCCTGAAGGCTGCCGCTATGGGAGGTTGGTCTTCC-3', containing a Hind III site and PADRE, preS1 sequences. The downstream primer was P2: 5'-GAATTCATGTCAAATGTATACCCAAAGACA-3', containing a EcoR I site, S sequences.

The reaction at 94 °C for 5 minutes was further subjected to 30 automated cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 1 minute. Finally, it was extended at 72 °C for 5 minutes. PCR products were analyzed and purified by 1% (w/v) agarose gel electrophoresis.

T/A cloning and construction of expression vector

After the PCR products were recovered and purified, fragments were directly ligated into the pMD18-T cloning vector. The resulting products were transformed into competent *E. coli* JM109 cells. Four ampicillin resistant clones were picked out and purified. The plasmids with proper inserts were recut with Hind III and EcoR I enzyme and cloned into the expression vector pcDNA 3.1(+) digested with Hind III and EcoR I. After transformation of *E. coli* JM109 cells, recombinant plasmid was selected on the ampicillin plate. Three positive colonies were selected for minipreparation and the insert evaluation was done by enzyme digestion and DNA sequencing.

Results

Construction of expression plasmid

Sequence analysis suggested that the target gene was correctly placed the downstream of CMV promoter of the pcDNA 3.1(+) vector and the insert gene belonged to HBV adr subtype, which is the epidemic type in China.

Target gene amplified by PCR

The PCR product was analyzed and purified by 1% (w/v) agarose gel electrophoresis. The PCR product results of electrophoresis showed that about 1240bp belt was seen as expected. It is consistent with HBsAg large surface protein (1200bp) and PADRE (39bp).

Identification with enzyme digestion

The cloning vector pMD18T-PADRE/HBs was digested by Hind III and EcoR I. The results of electrophoresis showed that the belts of the target gene and vector were about 1240bp and 2700bp, respectively. The expression vector pcDNA 3.1(+)-PADRE/HBs was digested by Hind III and EcoR I. The results of electrophoresis showed that the belts of the target gene and vector were about 1240bp and 5400bp, respectively (Fig.). The insert target DNA fragment was consistent with the

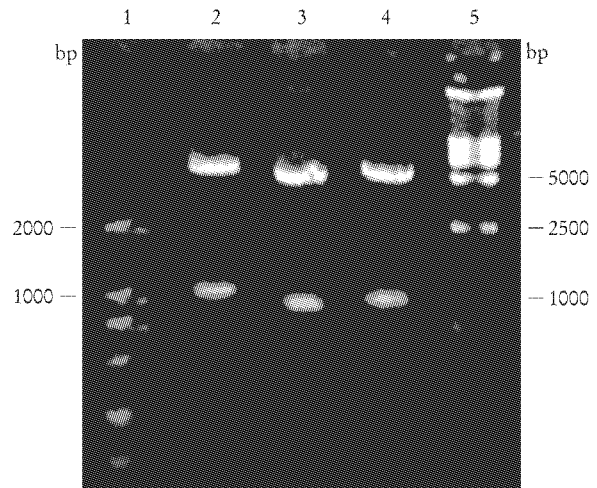


Fig. pcDNA3.1(+)-PADRE/HBs digestion. Lane 1: DL2000 marker; Lanes 2-4: pcDNA 3.1(+)-PADRE/HBs digested by Hind III and EcoR I; and Lane 5: DL15000 marker.

expected sequence.

Discussion

DNA-based immunization is a vaccine approach in which plasmid DNA encoding an antigen is introduced into the host and immune responses are generated after in vivo expression of antigenic gene product. This novel approach to immunization may overcome deficits of the traditional antigen-based approach and provide safe and effective prophylactic and therapeutic vaccines.^[15-17] Direct injection of DNA into skeletal muscles results in the synthesis of viral proteins in the host, which can enter both major histocompatibility complex (MHC) class I and class II antigen processing pathways to activate specific immunization. Cytotoxic T lymphocytes (CTLs) play an important role in combating HBV infection. Recovery from acute HBV infection requires strong humoral and cellular immune responses. The CTL response is multispecific and polyclonal during acute infection, but weak in chronic HBV carriers.^[18,19] In contrast to acute hepatitis B, however, TH1 cytokines (INF- γ) production after antigen stimulation of T cells in the periphery is weak or absent in chronic hepatitis B.^[20,21] This T cell response is believed to be responsible for the elimination or control of hepatitis B virus infection. Three HBsAg protein species are present in the envelope of HBV. Small S antigen contains conformational 'a' determinant in the S₁₂₄₋₁₄₇ region that is recognized by most human and murine anti-HBsAg antibodies. The small S antigen also contains murine CTL-defined epitopes, and the preS2 antigen contains many T cell and B cells epitopes, which can enhance the response to the S protein.^[22] AA₂₁₋₄₇ in the region of preS1 are thought to

be the site at which the virus attaches to hepatocytes.^[23] Hence, HBsAg is a well characterized candidate to HBV DNA vaccine-induced responses to B and T cells.

Helper T lymphocyte response plays an important role in the induction of both humoral and cellular immune responses. Therefore, HTL epitopes are likely to be a crucial component of prophylactic and immunotherapeutic vaccines. PADRE can bind most HLA-DR molecules with high affinity and act as a powerful immunogene. PADRE can obviously promote CTL response and humoral response.^[24-26] In a HBV transgenic murine model, CTL tolerance is broken by PADRE-CTL epitope lipopeptide, not by a similar structure containing a conventional HTL epitope.^[27] Therefore the use of a potent PADRE may play an important role in overcoming the CTL tolerance to HBV antigens. PADRE might be an important candidate epitope for new efficient HBV DNA vaccine.^[28-30]

Based on the knowledge available, we constructed successfully a recombination vector expressing HBsAg large surface protein, pcDNA3.1(+)-PADRE/HBs, which is convenient for further study of eukaryotic transfection and response for cellular and humoral immunity against HBV. The novel expression plasmids may overcome the tolerance to HBV and be possible to treat chronic HBV infection.

Competing interest

The author or authors do not choose to respond to the statements listed in Instructions for Authors.

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The reason that dripping wears away a hole in the stone does not lie in its force
but in its constancy.

— (Ancient Rome) Marcus Aurelius