

## Immunization of Mice with Plasmid DNA Encoding Hepatitis B Virus Core Antigen (HBcAg)

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**Abstract:** The aim of this study was to investigate the antibody response following immunization with plasmid vector DNA containing the hepatitis B virus core antigen (HBcAg) gene of a hepatitis B virus (HBV) isolated from a patient in Turkey. A HBcAg gene fragment was cloned into a pcDNA 3 eukaryotic expression vector. After confirmation of the cloning of the HBcAg gene, the expressing vector based HBcAg was used in DNA immunization experiments. Four- to 6-week-old Balb/c mice were immunized

with HBcAg expressing plasmids and the anti-HBcAg antibody responses of the mice were evaluated by enzyme immunoassay (EIA). The results of this study indicated that HBcAg expressed from pcDNA 3 based eukaryotic expression vector induced an anti-HBcAg response when introduced intramuscularly into Balb/c mice.

**Key Words:** Hepatitis B virus, core antigen, DNA immunization.

<sup>1</sup>Department of Microbiology, Faculty of Medicine, <sup>2</sup>Department of Virology, Faculty of Veterinary Medicine, Firat University, Elazığ, Turkey

### Introduction

Recent studies have shown that the injection of foreign DNA into muscle induces an immune response to the DNA-encoded protein (1,2). DNA immunization has the ability to induce both cell-mediated and humoral immunity. In addition, DNA-based immunization also lends itself to the design of multivalent immunogens targeted at various pathogens. This may facilitate economical immunization in developing countries. Thus, DNA-based immunization is a newly developed method of vaccination that has already been applied to prevent a wide range of infectious diseases (1,2).

Chronic hepatitis B virus (HBV) infection may lead to hepatocellular carcinoma (3,4). Individuals who are chronic carriers of the hepatitis B virus (HBV) have a greater than 100-fold increased relative risk of developing this tumor. The incidence of this cancer is rising globally and it is thus essential that means be found to prevent this lethal disease by prophylactic and therapeutic immunization (3-5). It has been reported by several researchers that DNA vaccines specific for hepatitis B virus (HBV) antigens induce a strong humoral and cell-mediated immunity in mice and other experimental animal models (6-8).

The hepatitis B virus core antigen is one of the major viral proteins present on the virion. It is about 183 aa long and has a molecular weight of 21 kDa (9). There are conflicting reports on the role of the immune responses to this protein (10,11). While some argue that the immune response to HBcAg facilitates viral clearance from the host, others favor the idea that anti-HBcAg T cell response is the major cause of immunopathology observed in the liver during HBV infection (5,12).

In the present study, we sought to investigate the antibody response following immunization with plasmid vector DNA containing a HBcAg gene segment.

### Materials and Methods

In the present study, the HBcAg gene segment amplified by a polymerase chain reaction (PCR) from HBV positive human serum was cloned into pUC19 plasmid, as described earlier (13). Later, HBcAg was excised from pUC-HBc with *Eco RI* and *Hind III* and inserted into a pcDNA3 eukaryotic expression vector (13,14). The recombinant plasmid (pcD-HBc) containing HBcAg gene inserts was identified with *Eco RI* and *Hind III* restriction endonucleases. Further confirmation of the presence of the HBcAg gene into pcD-HBc was carried out with PCR (13,14).

A 100 µg sample of the recombinant plasmid DNA (pcD-HBc) in 100 µl 0.01 M of phosphate buffered saline (PBS) was injected into the right tibial muscle of each mouse (1). The control animals received PBS only. After 2 weeks of immunizations, the animals were bled retro-orbitally for serum extraction. The resulting sera were tested for the presence of anti-HBcAg antibodies by a commercially available enzyme immunoassay (EIA) kit (Abbott Murex, Wiesbaden, Germany) (15). Booster immunizations were carried out with pcD-HBc 1 month after the first immunization; 2 weeks after the boost, blood samples were obtained and the sera were tested as mentioned earlier.

**Results**

For confirmation of the cloned HBcAg gene in pcDNA3 restriction endonuclease analysis on the recombinant plasmid pcD-HBc was carried out. The results of the analysis showed that the fragment excised into pcD-HBc with *Hind III* and *Eco RI* was about 550 base pairs (bp) long (Figure 1, lane 6). In further confirmation with the

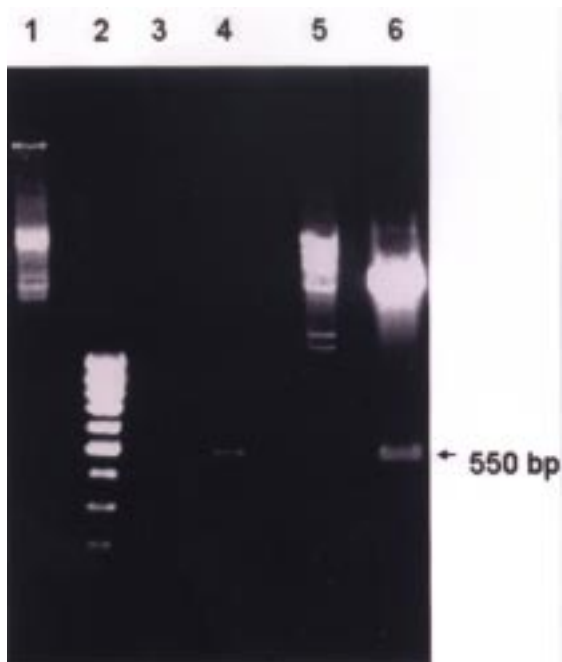


Figure 1. Confirmation with restriction endonuclease analysis and PCR of the cloning of the HBcAg gene into a eukaryotic expression vector. Lane 1: Uncut pcD-HBc, Lane 2: 100 bp DNA ladder; Lane 3: PCR products amplified from pcDNA3; Lane 4: About 560 bp PCR products amplified from pcD-HBc; Lane 5: *Hind III* cut Lambda DNA marker; and Lane 6: *Hind III* and *Eco RI* cut pcD-HBc.

PCR of pcD-HBc, an amplification product 550 bp long was visualized on a 1.5% agarose gel (Figure 1, Lane 4).

The results of anti-HBcAg antibody responses following the first and second immunizations as determined by sandwich EIA are shown in Figure 2. After the first immunization, four out of the 10 animals had antibody titers whereas all the animals had increased anti-HBcAg antibodies after the second immunization.

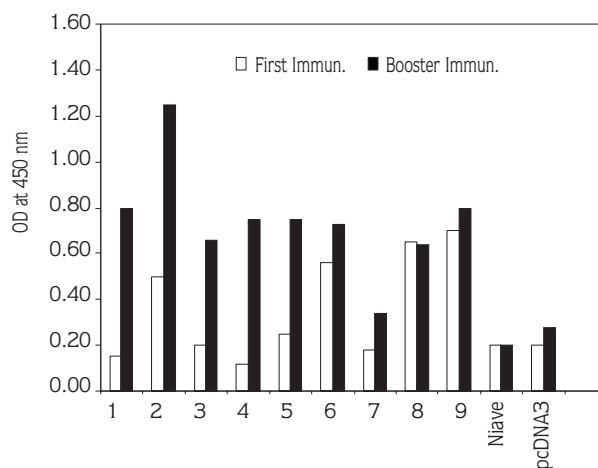


Figure 2. The results of anti-HBcAg antibody responses were determined by sandwich enzyme immunoassay.

**Discussion**

In the present study, we demonstrated that HBcAg expressed from a plasmid vector induced a detectable antibody response in Balb/C mice. Antibody response was shown to be induced in four out of 10 immunized mice following the first injection of plasmid DNA. However, after the second immunization, an antibody response was noted in all the animals. Similar findings have been reported by other researchers (1,2).

HBcAg specific cytotoxic T and CD4<sup>+</sup> T lymphocyte responses are thought to play a role in the clearance of acute and chronic HBV infections (12,16). In the present study, only the antibody response was investigated. It would be of interest to determine CTL and CD4<sup>+</sup> helper T cell activity in these mice.

There are a number of reports in the literature indicating that vectors carrying HBV core antigen sequences are effective in inducing anti-HBcAg immune reactions in chimpanzees and mice (6,17). In our model, we aimed to test the immunogenicity of HBcAg. After the

establishment of the model, other more effective vectors or other forms of the antigen would be investigated. It has been demonstrated with nucleic acid based vaccines that the plasmid DNA introduced into the muscle tissue would be taken up primarily by myocytes (2,18). It has also been postulated that some DNA would also enter dendritic cells and/or leak into the lymphatic drainage and reach local lymph nodes (2). Antigen carried into the tissues by the vector was shown to be processed and presented by both major histocompatibility complex molecules (1,2,18). In our study, we did not investigate the fate of the injected DNA. However, from the presence of antibody responses it would be inferred that at least

some HBcAg encoding plasmid DNA reached the appropriate antigen presenting cells, and HBcAg was processed and presented to the pathway leading to recognition by CD4<sup>+</sup> T lymphocytes. In future studies, the level of expression of HBcAg and the other arms of immunity stimulated by the expressed protein deserve to be researched.

*Correspondence author:*

*Yasemin BULUT*

*Department of Microbiology,*

*Faculty of Medicine,*

*Firat University, Elazığ - TURKEY*

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