Short Communication

CpG motif as an adjuvant in immunization of a recombinant plasmid encoding hepatitis C virus core protein

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Abstract
The immunogenicity and protective efficacy of DNA vaccines have been demonstrated in numerous animal models of infectious diseases. In order to increase the potency of DNA vaccines, in this study, conventional adjuvants such as aluminium phosphates, dendrosome, CpG motif and mixture of aluminium phosphate and CpG motif have been tested. Female BALB/c mice were immunized with mixture of 10, 25 and 50 μg HCV core pcDNA3. Each dose of recombinant pcDNA3 together with different adjuvants used as an immunogen were injected three times on day; 0, 30 and 50 days. Blood samples were collected at four different times intervals and antibody response against HCV core antigen was determined by HCV core ELISA kit. The results indicate that the best antibody response was with mixture of aluminium phosphate and CpG motif as an adjuvant. This data suggest that the antibody response induced following DNA immunization can be modified by formulation strategies.
Keywords*: CpG Motif; Dendrosome, Hepatitis C Virus; HCV Core pcDNA3.*

The hepatitis C virus (HCV) is the major causal agent of non A-non B viral hepatitis (Houghton *et al.*, 1991). More than 50% of those infected develop chronic hepatitis and some progress to cirrhosis and perhaps hepatocellular carcinoma (Saito *et al.*, 1990; Hem and White, 1995).

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Vaccines made from inactivated organisms or products derived from them are often formulated with adjuvants to enhance their immunogenicity. Other type of vaccines in development include peptides, recombinant viral or bacterial vectors expressing heterologous antigens, and plasmid. The potential use of plasmid DNA as a vaccine was first suggested by the observation that administration of DNA encoding hormones or reporter genes could result in expression *in vivo* after inoculation (Beneveinsty and Reshef, 1986; Wolff e*t al*., 1990). It was found that vaccination of mice with plasmid DNA resulted in the induction of specific antibodies (Tang *et al*., 1992 and Ulmer *et al*., 1993). Various means of enhancing immune responses induced by vaccines have been reported, including coadministration of DNA cytokines (Kim *et al*.,1997), sonicated calf thymus- DNA (Alvarez *et al*., 2002), dendrosome (Sarboloki *et al*., 2000), CpG motif (Tokanaga *et al*.,1988), cationic lipids (Ishi *et al*., 1997), aluminium and calcium salts (Warren *and* vogel, 1986), etc. In the present study we demonstrated that some, but not all, of these conventional adjuvants are compatible with DNA vaccines and strongly enhance immune response in animals.

For this purpose HCV cDNA was isolated from an Iranian individuals suffering with chronic hepatitis C (Montgomery *et al*., 1993). This cDNA was amplified in *Escherichia coli* (DH5α). Cells were grown under selective pressure with 50 microgram/milliliter ampicillin. Plasmid DNA was subsequently purified in free endotoxin method (Levy *et al*., 1997) by using diethyl amino ethyl sephadex (DEAE-sephadex) anion exchange chromatography column to increase the

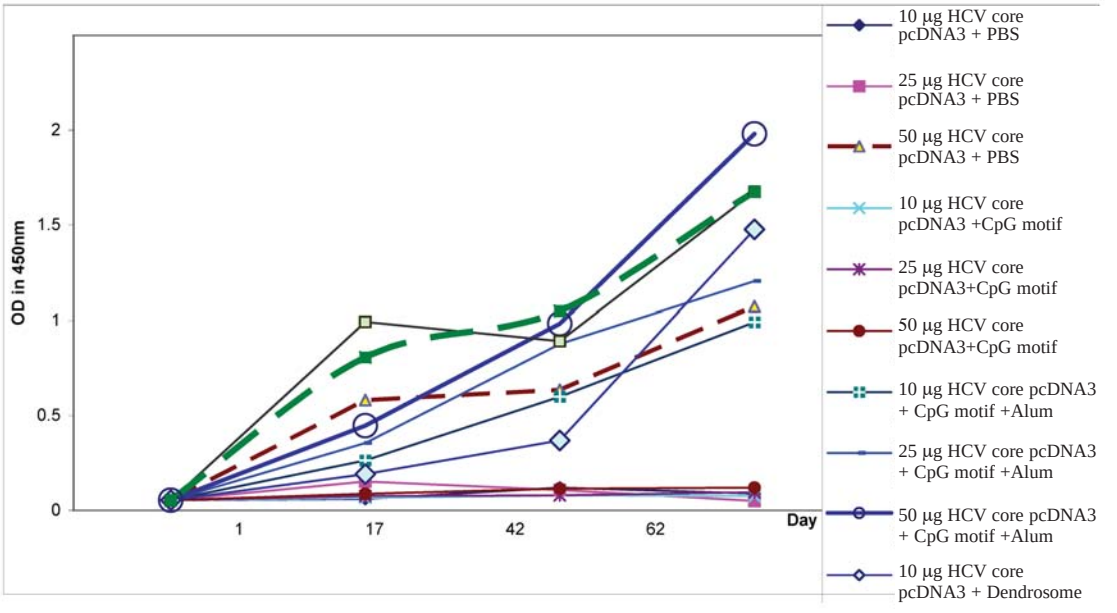

Figure 1. Combination of CpG motif and aluminium phosphate with 50 µg of plasmid encoding Hepatitis C core gene induces highest antibody titer. Comparative chart of core-specific antibody measurement by ELISA elicited in BALB/c mice by injecting 10, 25 and 50 µg HCV core pcDNA3 along with PBS, CpG motif, CpG motif + aluminium phosphate and dendrosome as an adjuvant. Explanation of bars is shown at the right box.

supercoiled plasmid and delete the open circular and linear plasmid.

Four types of adjuvants were used to study the potency of HCV *viz.*, (1) aluminium phosphate (450 µg/ml) with the negative charge that does not physically bind to DNA and greatly enhance antibody response to the construct (Ulmer *et al*., 1996), (2) CpG motif (5 µg/ml) as immunostimulatory sequence which was used a phosphorothioate (5´-TGACTGTGAACGTTCGAGATGA-3´) (Krieg, 2002 and Yi *et al*., 1999), (3) Dendrosome polymer (Den 123) synthesized in Iran (Sarboloki MN *et al*, 2000) (1/150 = Dendrosome/DNA) and finally (4) a mixture (100 µl) of CpG motif (5 µg/ml) and aluminium phosphate (450 µg/ml). The adjuvants have been compound to HCV core pcDNA3 and blended and stirred 1h.

Five groups of BALB/c female mice (n = 5-7) of 6 to 7 weeks at age (18-20 gram weight) were immunized with 100 µl of different immunogen. Mice were injected three times at 0, 30 and 50 days by insulin siring in the quadriceps muscle. Blood samples were collected from retro-orbital sinus at 1st, 17th, 42th and 72th days after injection. To determine anti-core antibodies 96-well plates (HCV Core kit, made in Spain by BIOKIT, S.A.) coated with core antigen were used. The cut-off value to consider a positive mouse anti-core antibody response was established (Sambrook *et al*., 1989).

The results revealed that the free endotoxin super coiled plasmids of HCV core pcDNA3 was obtained by using DEAE-Sephadex chromatography column after free-endotoxin extraction.

To investigate the effect of adjuvants on immunological properties of DNA vaccine, 10, 25 and 50 µg HCV core pcDNA3 with PBS, aluminium phosphate (450 µg/ml), Dendrosome (1/150 = dendrosome /DNA), CpG motif (5 µg) and CpG motif + aluminium phosphate (450 µg/ml) in total volume of 100 µl was injected into the female BALB/c muscle. The antibody response at 17, 42 and 72 days was determined by using HCV core ELISA kit (Fig.1).

It was found that PBS and aluminum phosphate do not improve the humoral immune response and increase in immune response was coordinated to quantity and number of doses of HCV core pcDNA3. Hence no valuable increase was seen. Antibody titer was found to increase a little after third injection of DNA (50 µg).

Dendrosome was found to improve the antibody response especially after third injection. CpG motif after third injection of 50 µg HCV core pcDNA3 improved the antibody response too. But the mixture of CpG motif and aluminum phosphate as an adjuvant was the best adjuvant and improves the antibody response as compared to other tested. Mixture of CpG motif and aluminum phosphate alongwith 50 µg HCV core pcDNA3 had highest antibody titer because of synergistic effect of CpG motif and phosphate aluminum.

However, nucleic acid vaccines do not seem to

induce a response as strong as conventional (lived attenuated) vaccines and consequently different approaches have been used to modulate the plasmid DNA vaccine response. These efforts have been directed mainly to recruit cells and facilitate the entry of plasmid DNA to cells. Previous studies have shown that the intramuscular injection of plasmid expressing the HCV core protein was capable of inducing detectable core specific antibody response (Gupta *et al*., 1995).

Aluminum salts have been used to increase antibody response in a formulation of DNA vaccine (Gupta *et al*., 1995). CpG motif is an immuno stimulatory sequence, which used as an adjuvant in DNA vaccine (Halperin *et al*., 2003). In the present study, mixture of aluminium phosphate and CpG motif when given with HCV core pcDNA3 stimulated the anti-core antibody response. Among 4 types of adjuvants used, *viz*. aluminium phosphate, CpG motif-dendrosome and mixture of aluminium phosphate and CpG motif, it was found that mixture of aluminium phosphate-CPG motif increases the efficiency of HCV core pcDNA3 immunization.

Acknowledgments

We would like to thank Minoo Alasti and Maryam Ebrahimi for their technical assistance. This work was supported by grants from ministry of Science, Research and Technology Islamic Republic of Iran.

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