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Plasmid DNA loaded chitosan nanoparticles for nasal mucosal immunization against hepatitis B

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Abstract

This work investigates the preparation and in vivo efficacy of plasmid DNA loaded chitosan nanoparticles for nasal mucosal immunization against hepatitis B. Chitosan pDNA nanoparticles were prepared using a complex coacervation process. Prepared nanoparticles were characterized for size, shape, surface charge, plasmid loading and ability of nanoparticles to protect DNA against nuclease digestion and for their transfection efficacy. Nasal administration of nanoparticles resulted in serum anti-HBsAg titre that was less compared to that elicited by naked DNA and alum adsorbed HBsAg, but the mice were seroprotective within 2 weeks and the immunoglobulin level was above the clinically protective level. However, intramuscular administration of naked DNA and alum adsorbed HBsAg did not elicit sIgA titre in mucosal secretions that was induced by nasal immunization with chitosan nanoparticles. Similarly, cellular responses (cytokine levels) were poor in case of alum adsorbed HBsAg. Chitosan nanoparticles thus produced humoral (both systemic and mucosal) and cellular immune responses upon nasal administration. The study signifies the potential of chitosan nanoparticles as DNA vaccine carrier and adjuvant for effective immunization through non-invasive nasal route.

Keywords: Chitosan nanoparticles; pDNA; Hepatitis B; Nasal immunization; Vaccine delivery

1. Introduction

Hepatitis B virus infection is a major global health concern and is the most common cause of chronic liver disease and mortality from chronic sequelae, especially primary hepatocellular carcinoma. There are approximately 350 million HBV carriers all over the world. Therefore, new generation of HBV vaccines are urgently needed in order to overcome problems encountered with the immunization of immunocompromised people and more importantly with the potential of using active immunotherapy in treating chronic patients.

The next generation DNA vaccines, however, has the potential to eliminate many of the limitations of current vaccine technologies. DNA vaccine technology is a simple concept based on relatively simple design and production technologies (Whalen, 1996). Another advantage of DNA vaccines over conventional protein vaccines is the low cost of producing a highly purified product. Furthermore, DNA vaccine distribution is not dependent upon maintaining a cold chain so the vaccines can be easily distributed, especially in developing countries. DNA vaccination works by using host cells as protein factories to produce the plasmid encoded antigen. The translated protein is then processed and presented by the immune system in a manner similar to that, which occurs following a natural infection. This elegant concept has been demonstrated successfully for a wide variety of vaccine antigens and has been effective in preventing both infectious diseases and cancer in mouse models (Donnelly et al., 1997).

The recombinant protein based vaccines presently available in market produce mainly humoral immune response. The antibodies generated in this manner, do not always reach the mucosal surfaces, which is the predominant entry site for most of the infectious pathogens including HBV. Mucosal immunization thus provides the first line of immunological defense, i.e., induction of secretory IgA (sIgA) that prevents the attachment of bacteria and viruses to the mucosa and, thereby, prevents any possible damage to host. Further, vaccination at mucosal surfaces may result in humoral and cellular responses, both systemic and local; the latter not at the site of vaccination, but also at distant mucosal epithelia. Furthermore, traditional vaccination

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strategies that use needles or require multiple dosages applied through an invasive route suffer from the problems of administration, needle-born cross contamination, expenses and patient compliance. Nasal route holds great promise for the perspective of vaccination due to particular organization of the nasal mucosa (Davis, 2001; Partidos, 2000). The nasal mucosae is the first site of contact with inhaled macromolecules, and the nasal-associated lymphoid tissue (NALT) at the base of the nasal cavity (Waldeyer's ring in humans) is important in the defense of mucosal surfaces. Additionally, the nasal epithelium is leaky and there are underlying blood vessels, cervical lymph nodes and lymphoid cells to which the macromolecule may have direct access if it can be adequately transported across the epithelium.

Major challenges in the development of new generation of prophylactic or therapeutic vaccines against chronic hepatitis B virus infection are the induction of potent cellular immune responses and the efficient targeting of the specifically induced immune effector specificities to the liver and spleen (Schirmbeck et al., 2000). Therefore, the use of cationic polymers particularly chitosan and gelatin in combination with DNA vaccination appears to be a very attractive alternative. Chitosan and gelatin form polycation–DNA complexes that are generally more stable when compared with other non-viral gene delivery systems especially liposomal or cationic lipid systems (Leong et al., 1998; Mao et al., 2001). Factors hindering the success of the liposomal approach appear to be instability of the complex and toxicity of the cationic lipid. Chitosan is a cheap, biocompatible, biodegradable and non-toxic cationic polymer that forms polyelectrolyte complexes with DNA. Therefore, chitosan and chitosan derivatives may represent potentially safe and efficient cationic carriers in gene delivery (Borchard, 2001). Previous studies carried out with protein and DNA vaccines clearly demonstrated potential of chitosan nanoparticles as a mucosal adjuvant. Oral immunization with chitosan nanoparticles loaded with plasmid DNA encoding a peanut allergen gene, protected AKR mice from food allergen induced hypersensitivity (Roy et al., 1999). Also, it has been reported that intragastric priming with GRA 1 protein vaccine loaded chitosan microparticles and boosting with GRA1pDNA vaccine resulted in high anti-GRA 1 antibodies, as characterized by a mixed IgG2a/IgG1 ratio (Bivas-Benita et al., 2003). Chitosan has been successfully used as a nasal delivery system for subunit influenza vaccine (Read et al., 2005), tetanus toxoid (Vila et al., 2004) and diptheria (McNeela et al., 2000). Surface modification of PLGA microspheres with chitosan containing HBsAg induced strong systemic and mucosal immune responses following intranasal immunization (Jaganathan and Vyas, 2006). Also, chitosan nanoparticles and chitosan coated emulsions adsorbed with ovalalbumin and cholera toxin induced systemic and mucosal immune responses in rats following intranasal immunization (Nagamoto et al., 2004). Chitosan nanoparticles loaded with pDNA encoding respiratory syncytial (SV) proteins reportedly induced protective Th1 type immune responses (Kumar et al., 2002) and CTL responses (Iqbal et al., 2003) in BALB/c mice following intranasal immunization. Also, prophylactic immunization using chitosan DNA intranasal vaccine induced Coxsackievirus B3 (CVB3) specific immune responses (Xu et al., 2004).

In the present study we prepared chitosan nanoparticles loaded with plasmid DNA encoding surface protein of Hepatitis B virus. The prepared nanoparticles were characterized for size, shape, zeta potential, loading efficiency and ability of nanoparticles to protect DNA from DNase I and for their transfection efficacy. The developed systems were also evaluated for their ability to induce humoral mucosal and cellular immune responses following intranasal immunization.

2. Materials and methods

2.1. Materials

Chitosan, 400 kD MW and 85% deacetylated, was purchased from Fluka Co. Ltd., Switzerland. DNase I was obtained from Sigma–Aldrich Corp. (USA). Hoechst 33258 was from Invitrogen. Plasmid pRc/CMV-HBs(S) was purchased from Aldeveron, Fargo, USA. λ Hind III DNA was purchased from Genei (India). Enzyme linked immunoassay kit (AUSAB and AUZYME) and cytokines (IL-2 and IFN-γ) estimation kit was purchased from Abbott Laboratories, USA and e-Bioscience, respectively. HBsAg (MW 24 kDa) was a kind gift from Shantha Biotech Ltd. (Hyderabad, India) and Metafectene pro was kindly provided by Biontex Laboratories (Germany). All other chemicals and reagents were of analytical grade.

2.2. Preparation of plasmid DNA/chitosan nanoparticles

Plasmid DNA/chitosan nanoparticles were prepared as described previously (Mao et al., 2001). In brief, 100 μl of chitosan (0.02%, w/v in 5 mM NaAc-Hac buffer pH 5.5) was added during high-speed vortexing to plasmid DNA (100 μl) (100 μg/ml in 50 mM Na_2SO_4) for 1 min. Both the solutions were preheated to 50–55 °C separately. The final volume of the preparation was limited to below 500 μl in order to yield uniform nanoparticles. The formulations were then lyophilized with mannitol for 24 h and kept in a dessicator at 2–8 °C until required. Before administration to mice, the lyophilized DNA formulation was reconstituted with ultrapure water.

2.3. Quantitation of plasmid DNA loading

The concentration of plasmid DNA in solution was quantified using the Hoechst 33258 dye. The pDNA-loaded nanoparticles were spun down at 14,000 rpm for 20 min. The supernatants were diluted (if necessary), stained with Hoechst 33258 dye and analyzed using spectrofluorimeter (Molecular Devices) at excitation and emission wavelengths of 350 and 455 nm, respectively.

2.4. Physical characterization of the nanoparticle size, shape and surface charge

The nanoparticle size was measured by dynamic light scattering (DLS) measurements using Zetasizer, Nano ZS (Malvern Instruments, UK). All the data analysis was performed in auto-

matic mode. Zeta potential measurements were also carried out using the same instrument in an automatic mode. Prepared nanoparticles were characterized for their shape using transmission electron microscopy (Philips Morgagni 268, Netherlands). Uranyl acetate was used as a negative stain for the transmission electron microscopy.

2.5. Protection against nuclease degradation

Chitosan–DNA nanoparticles were incubated with DNase I (0.1 μg/μg DNA) for 15 min at 37 °C. The DNase activity was stopped by adding iodoacetic acid solution to a final concentration of 5 mM. Samples were analyzed in a 0.8% agarose gel containing 1 μg/ml ethidium bromide, in TAE buffer. DNA was visualized under UV light using gel documentation system (Biorad, USA). The naked plasmid was used as control and a λ Hind III DNA ladder as a reference.

2.6. In vitro cell transfection

In vitro transfection studies were performed in HeLa cells (kindly provided by NCCS (Pune, India). Cells were seeded at 1×10^5 cells per well in 12–well plate and incubated for 24 h at 37 °C under a 5% CO_2 atmosphere. Immediately before transfection, cells were rinsed and supplemented with 900 μl of fresh culture medium DMEM supplemented with 10% v/v FCS, 2 mM L-glutamine, penicillin (50 U/ml) and streptomycin (50 μg/ml). Then, 100 μl of nanoparticles equivalent to 1 μg of DNA were added in each well and plates were incubated at 37 °C under a 5% CO_2 atmosphere. After 48 h incubation, cells were harvested and lysed by cold heat treatment (incubation at −20 °C for 30 min in a deep freezer and then 37 °C for 30 min). Cell debris was removed by centrifugation at 12,000 × *g* and the supernatant was analyzed for expression of HBsAg by ELISA. Transfection with Metafectene pro complexed with plasmid DNA was carried out as per manufacturers protocol and served as positive control.

2.7. Immunization studies

The immunogenecity of the pDNA loaded chitosan formulations were assessed in female BALB/c mice (6–8 weeks age). Animals were housed in groups of five ($n = 5$) with free access to food and water. They were withdrawn of any food intake 3 h before immunization. The study protocol as approved by Institutional Animals Ethical Committee of Dr. Harisingh Gour University was followed. The studies were carried out as per the guidelines of Council for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. To evoke an immune response, 50 μg of plasmid DNA loaded chitosan nanoparticles and naked plasmid DNA per animal was inoculated intranasally (IN) in small drops. Nasal dosing was performed by inserting a small piece of polyethylene tubing (sterile), attached to a Hamilton syringe, 0.2 cm into the nostril (10 μl nanoparticle formulation/nostril) of the non-anesthetized animal (supine position) and ejecting into the nasal cavity. Care was taken that a new drop was only given when the former had been entirely inspired. Secondary immunization was done after 2 weeks with the same dose of formulations. Mice were also immunized with intramuscular (IM) injection of naked plasmid DNA (50 μg equivalent dose in 100 μl normal saline) as well as alum adsorbed HBsAg (10 μg per animal in quadriceps muscles on day 0 and 14th with same formulations for comparison purposes.

2.8. Sample collection

Blood was collected by retro-orbital puncture (under mild ether anesthesia) after 2, 4, 6 and 8 weeks of primary immunization and sera were stored at −40 °C until tested by ELISA for antibody. The nasal, vaginal and salivary secretions were collected after 6 weeks of booster immunization. A vaginal wash was obtained according to the method reported by Debin et al. (2002). Briefly, 50 μl of PBS containing 1% (w/v) bovine serum albumin (1% BSA–PBS) was introduced into the vaginal tract of non-anesthetized mice using a Gilson pipette. These 50 μl aliquots were withdrawn and reintroduced nine times. A second vaginal wash was collected the following day and pooled with the first one. The nasal wash was similarly collected by cannulation of the trachea of sacrificed mice. The nasal cavity was then flushed three times with 0.5 ml of 1% BSA/PBS (pH 7.4). Salivation was induced as described in our previous studies (Jain et al., 2005). Mice were injected with 0.2 ml sterile pilocarpine solution (10 mg/ml) intraperitoneally. The saliva from mice after 20 min was collected using capillary tube. These fluids were stored with 100 mM phenylmethyl sulfonyl fluoride (PMSF) as a protease inhibitor at −40 °C until tested by ELISA for secretory antibody (sIgA) levels.

2.9. Measurement of specific IgG and IgA response

The concentration of anti-HBsAg antibody in the collected blood sample was determined by using commercially available solid-phase enzyme-linked immunoassay kit AUSAB®, Abbott Laboratories, USA). IgG antibodies present in sera were detected using 1/100 dilution as the first dilution of the serum. To signify actual antibody concentration (antibody titre) in mIU/ml, a standard curve was prepared using the calibrated anti-hepatitis B panel provided by Abbott Laboratories. Antibody response was plotted as total serum anti-HBsAg antibody titres (mIU/ml) versus time in weeks. Secretory IgA level in mucosal fluids (nasal, salivary and vaginal) was determined by ELISA using slightly modified method of Debin et al. (2002). Briefly, microtiter plates (Nunc-Immune Plate® Fb96 Maxisorb, Nunc, India) were coated with a solution of HBsAg at 2 μg/ml in carbonate buffer pH 9.6 for overnight at 4 °C. Wells were blocked with PBS–BSA (3% (w/v)) for 1 h. The plates were washed three times with 300 μl of PBS containing 0.05% Tween 20. Serial dilutions of mucosal fluids in PBS–BSA (0.1%, w/v) were added and the plates were held at room temperature for 2 h followed by washing and addition of horseradish peroxidase-conjugated goat anti-mouse IgA (Sigma, USA). IgA antibodies present in mucosal samples were analyzed using 1/10 dilution as the first dilution of the sample. After 1 h incubation and washing,

100 μl of *o*-phenylenediamine dichloride (OPD; Sigma, USA) in phosphate-citrate buffer pH 5.5 and H_2O_2 was added as a substrate. Colour development was stopped after 30 min via the addition of 50 μl of 1N H_2SO_4 and the absorbance was measured at 490 nm. The end point titre was expressed as the reciprocal of the last dilution, which gave an optical density at 490 nm above the optical density of negative control.

2.10. Estimation of cytokines levels

Endogenous levels of IL-2 and IFN-γ in mouse spleen homogenates were measured after 6 weeks of secondary immunization by using separate ELISA kits for these cytokines. Spleen homogenates were prepared by the method reported by Nakane et al. (1992) with slight modifications. Briefly, spleens were weighed and homogenized in ice-cold PBS containing 1% CHAPS (Sigma, USA) and 10% (w/v) homogenates were obtained with the help of tissue homogenizer (York, New Delhi, India). Homogenates were incubated in an ice-bath for 1–2 h at temperature below 0 °C and the insoluble matters were settled down. Supernatant were centrifuged at 2000 × *g* for 20 min and the clear supernatants were used for cytokines estimation by selected ELISA method.

2.11. Statistical analysis

Statistical analysis was performed on the data obtained by one-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparisons post test using GraphPad InStat™ software. Throughout, the level of significance was chosen as less than 0.05 (i.e., $p < 0.05$).

3. Results and discussion

Chitosan nanoparticles were obtained by coacervation between chitosan and DNA. Size of the prepared nanoparticles as determined by zetasizer was found to be 337 ± 27 nm. Transmission electron microscopy also confirmed that chitosan–DNA nanoparticles were approximately 300–400 nm in size and nearly spherical in shape (Fig. 1). The loading procedure of the nanoparticles was very efficient, yielding encapsulation efficiencies of the order of 96.2 ± 1.8%. Zeta potential of the prepared nanoparticles was found to be 13.8 ± 1.5 mV at complexation pH (pH ∼5.5) and 4.2 ± 0.6 mV at pH 7. The cationic character of chitosan is a crucial parameter for the formation of complexes between the polysaccharide and DNA. The pK_a of the amino group in the repeating units is ∼6.5 rendering the majority of amino groups (over 90%) protonated at pH ∼5.5, while at physiological pH most of the positive charge would be neutralized. This unique property ensured that nanoparticles formed at low pH could remain physically stable at physiological pH.

The nanoparticles were also evaluated for their ability to protect the DNA against degradation by DNase I. It has been found that the DNA associated to nanoparticles was more slowly degraded by DNase I: whereas naked DNA was completely degraded after 15 min, DNA was still protected by the nanoparticles during an incubation time of up to 15 min (Fig. 2).

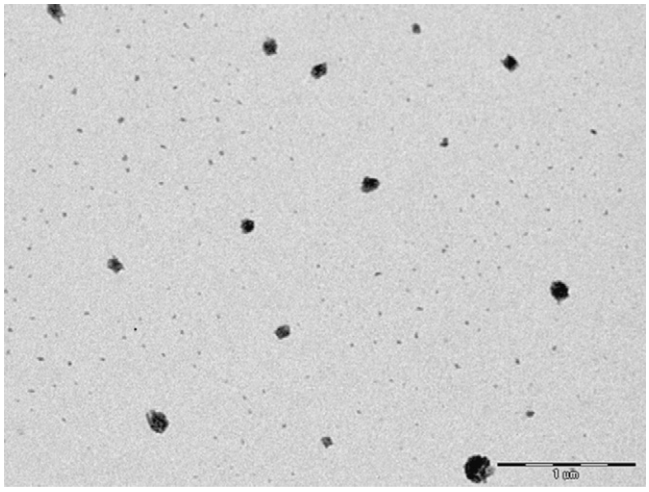

Fig. 1. Transmission electron microscopy image of chitosan pDNA nanoparticles.

This result suggests that chitosan could partially protect DNA from markedly high concentration of DNAse I digestion. At the physiological condition such as mucosal membrane surfaces with large quantities but lower concentration of nucleases, this protection to DNA by chitosan would be meaningful for the maintenance of integration and function of DNA vaccine. Transfection studies carried out in HeLa cells revealed that nanoparticles complexed with DNA could express HBsAg in vitro however maximum transfection was achieved with DNA complexed with Metafectene pro (Fig. 3).

To asses the potential utility of intranasal chitosan–DNA (pRC/CMV-HBs(S)) immunization in inducing HBsAg-specific systemic and mucosal humoral responses, female BALB/c mice were vaccinated intranasally with chitosan–DNA nanoparticles (CH-NP), with total 100 μg of pDNA. There were substantial differences in the levels of serum and mucosal secreted antibodies among 'naked' DNA-immunized IM, alum adsorbed HBsAg immunized IM, naked DNA and chitosan–DNA administered IN in mice. Nasal administration of nanoparticles resulted in serum anti-HBsAg titre that was less compared to that elicited by naked

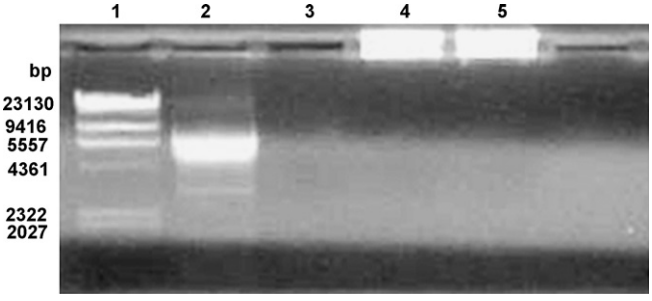

Fig. 2. Protection of pDNA from DNAse I digestion by chitosan. Naked pDNA and chitosan pDNA nanoparticles containing 1 μg pDNA were incubated with DNAse I for 15 min at 37 °C. Then, the reaction was stopped by adding iodoacetic acid solution to a final concentration of 5 mM. Finally, the samples were run on 0.8% agarose gel containing 1 μg/ml ethidium bromide, in TAE buffer. Lane 1: λ Hind III DNA; lane 2: pDNA ((pRC/CMV-HBs(S)); lane 3: pDNA + DNAse I; lane 4: chitosan pDNA nanoparticles; lane 5: chitosan pDNA nanoparticles + DNAse I.

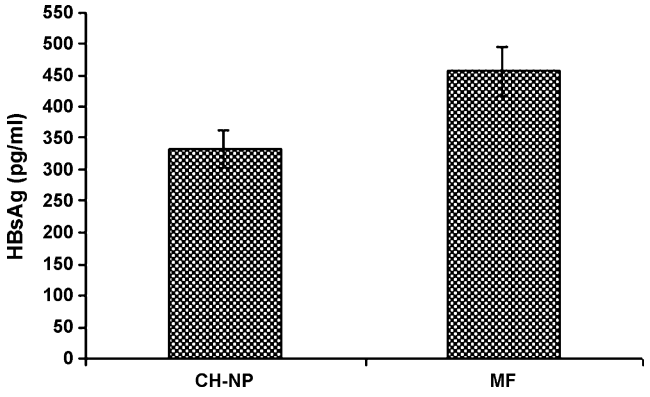

Fig. 3. HBsAg expression in HeLa cells transfected with chitosan pDNA nanoparticles (CH-NP) and metafectene pro complexed pDNA (MF). Values are expressed as mean ± S.D. ($n = 3$).

DNA and alum adsorbed HBsAg, but the mice were seroprotective within 2 weeks and the immunoglobulin level was above the clinically protective level (>10 mIU/ml) suggesting successful generation of systemic immunity (Fig. 4). Levels of 1 and 10 mIU/ml are well-established standards for anti-HBs antibody levels in mice and humans, respectively and are considered sufficient to confer protection against the disease (Davis et al., 1993; Szmuness et al., 1980).

Chitosan and positively charged chitosan complexes can bind strongly to negatively charged sites in the nasal cavity such as the sialic acid residues in the mucin. Furthermore, chitosan has in Caco-2 cell studies been shown to transiently open the tight junctions between cells thereby allowing the increased membrane transport of large molecular weight drugs (Illum, 1998) and to a lesser degree particulate systems coated with chitosan (Brooking et al., 2001). Hence, although the exact mechanism of action of nasally administered plasmid DNA–chitosan vaccine system is not known, it is envisaged that the small plasmid DNA–chitosan nanoparticles to some extent are able to pass the membrane and reach the underlying lymphoid tissue where they are transfected within antigen presenting cells. There might also be a possibility that the nanoparticles are taken up by the M-cell like cells in the NALT and presented to the underlying lymphoid tissue (Illum et al., 2001). Ishii et al. (2001) investigated the likely transfection mechanism of the plasmid DNA–chitosan complexes in cell lines and also the factors affecting transfectivity. They suggested that the complexes most likely condensed to form loose aggregates, which adsorbed to the cell surface before being endocytosed and transferred to the nucleus for transfection. Chitosan was also reported to have an immune stimulating activity such as increasing the accumulation and activation of macrophages and polymorphonuclear cells, promoting resistance to infections by cytokines, and enhancing CTL response (Seferian and Martinez, 2000; Kumar et al., 2002). Previous investigations also demonstrates that chitosan could not only slow down mucociliary clearance of encapsulated drugs but could also transiently increase paracellular absorption so as to improve immune stimulation (El-Shafy et al., 2000). As both mucosal absorption enhancer and immune stimulator, chitosan represents a good mucosal delivering vehicle for DNA or protein vaccines.

It is generally known that mucosal IgA plays an important role in protection against enteropathogens and viruses both in human and animal models (McNabb and Tomasi, 1981). Since most organisms enter the host through the respiratory, gastrointestinal and urinogenital routes, vaccine delivery at these sites could provide a first line of defence against infectious diseases. The mucosal surfaces are rich in B-cells, T-cells and plasma cells and such antigen-reactive cells are essential for the induction and maintenance of specific immune responses. Uptake of antigen by mucosal tissues such as the nasal-associated lymphoid tissue (NALT) is very important in the induction of immune responses. A particular advantage of the intranasal delivery of vaccine is that less antigen is required via this route as compared to that needed for oral immunisation. Moreover, production of HBsAg-specific mucosal IgA antibodies must be important for production from mucosally transferred HBV (Isaka et al., 2001). Fig. 5 depicts secretory IgA levels in nasal, salivary and vaginal secretions. It can be inferred from the data that chitosan nanoparticles efficiently elicited mucosal immune response (sIgA level). Negligible IgA response was observed on intramuscular administration of naked pDNA or alum adsorbed recombinant protein vaccine. It may be attributed to the fact that parenterally administered antigen lacks the ability to stimulate mucosal immune

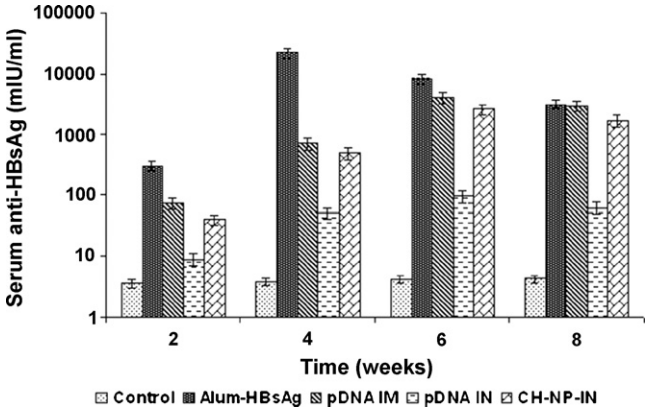

Fig. 4. Serum anti-HBsAg profile of mice immunized with different formulations. Values are expressed as mean ± S.D. ($n = 5$).

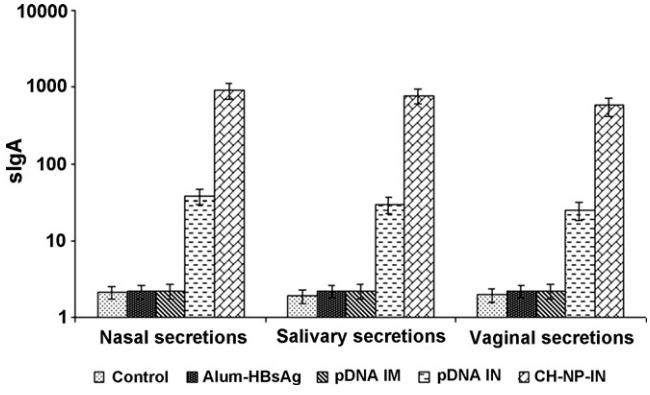

Fig. 5. Secretory IgA levels in nasal, salivary and vaginal secretions of mice immunized with different formulations. Values are expressed as mean ± S.D. ($n = 5$).

system. Thus, nasal administered mucoadhesive system can provide additional advantage of mucosal immunity, i.e. neutralizing the antigen at first exposure. The sIgA response of pDNA administered IM was found to be significantly less when compared with pDNA administered IN or loaded in chitosan nanoparticles ($p < 0.05$). In addition, only chitosan–DNA-treated mice had high levels of secretory IgA in nasal, salivary and vaginal secretions indicating successful induction of mucosal immunity as well. These results indicate chitosan–DNA intranasal immunization induced both systemic and mucosal humoral immune responses. Chitosan–DNA nanoparticles might adhere to the nasal or gastrointestinal epithelia, and are easily transported to the nasal-associated lymphoid tissue (NALT) and Peyer's patches of the gut associated lymphoid tissue (GALT) both as IgA inductive site (van der Lubben et al., 2001), in which chitosan–DNA might be taken in by M cell, and transported across the mucosal boundary, thereby transfect immune cells within NALT or GALT (Janes et al., 2001). And because of common mucosal immune system (CMIS), immunization at one mucosal site (IgA inductive site) always lead to specific immunity induction at distant mucosal effector sites. At such sites, antigen-specific IgA-committed B and Th cells interact with each other through cytokine network and function to produce secretory IgA, finally building up a first line of protection at mucosal surfaces (Yuki and Kiyono, 2003).

Endogenous cytokine levels (IL-2 and IFN-γ) were estimated in spleen homogenate after 6 weeks of booster immunization of different formulations. The significant levels of both IL-2 and IFN-γ (Fig. 6) were measured in mice immunized with pDNA administered IM and with pDNA loaded chitosan nanoparticles administered IN as compared to those recorded for control and alum adsorbed HBsAg ($p < 0.05$). Both Th1 dependent cytokines and their high levels are evidenced for the strong cell-mediated immune response elicited by pDNA loaded chitosan formulations administered intranasally. It is clear from the results that although conventional recombinant protein based vaccine elicited higher humoral response (serum antibody titer) but it fails to elicit cell-mediated immune response. Therefore, it can be used only for prophylactic immunization and is ineffective for treatment of chronic hepatitis B infection (therapeutic immunization). The results are in accordance with the previous findings (Leclere et al., 1997; Geissler et al., 1997), which clearly demonstrated the inefficiency of conventional protein based vaccine to elicit cellular response. When mice are immunized with recombinant HBsAg, the main type of immune response generated is the antibody response, since the exogenous antigen is mainly presented by B cells through the MHC class II pathway to Th2 cells. In the case of DNA vaccines, part of the HBsAg generated in vivo are secreted and presented by B cells through MHC class II pathway leading to a good antibody response, while the part of antigen is cleaved within the antigen presenting cells and presented through MHC class I pathway, leading to a Th1/CTL response. The generation of a dominant Th1 cytokine profile is important to facilitate eradication of HBV infection and thus it can be utilized for therapeutic immunization of HBV chronic carriers.

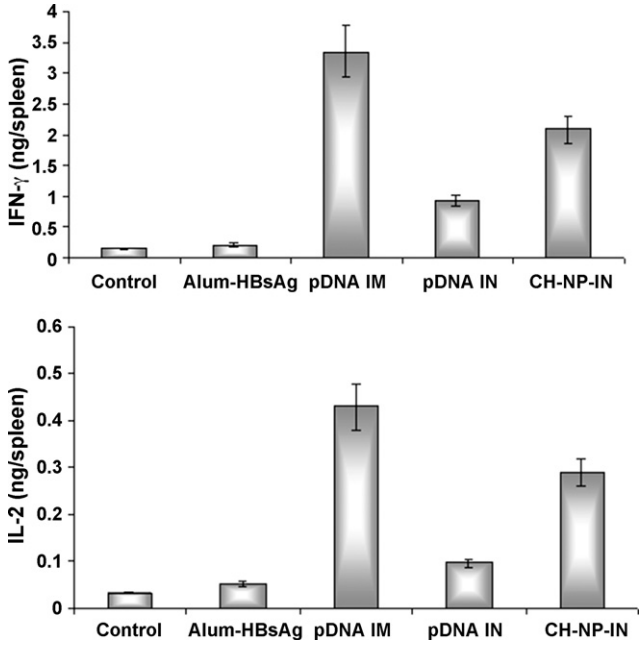

Fig. 6. Interferon-γ and interleukin-2 levels in the spleen of mice immunized with different formulations. Values are expressed as mean ± S.D. ($n = 5$).

4. Conclusion

Chitosan being natural biocompatible polysaccharide has been widely used in controlled drug delivery and it may provide non toxic carrier for successful nasal mucosal delivery of pDNA. Chitosan nanoparticles seems to be a clinically acceptable carrier system for pDNA delivery to NALT and capable of eliciting systemic, mucosal and cellular immune responses. The proposed mucoadhesive system being simple, economical, biocompatible and safe may have the potential to boost vaccine coverage worldwide, however, the practical utility could only be realized following elaborative studies elucidating the mechanisms of enhanced immune responses using this carrier system and clinical and preclinical trials taken up with large populations.

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