Med Microbiol Immunol (2002) 191: 187–190
DOI 10.1007/s00430-002-0144-z

REVIEW

A. Henke

DNA immunization – a new chance in vaccine research?

Received: 12 July 2002 / Published online: 14 September 2002

Abstract A novel class of vaccines, based on the immunization with "naked" DNA, may hold the promise of protecting against human disease without the disadvantages associated with vaccines presently used, and may help to prevent infections which are not curable today. Direct intramuscular or intradermal inoculation of plasmid DNA encoding sequences of viral proteins results in the synthesis of these proteins, causing humoral and/or cellular immune responses in the recipient. Several advantages are associated with DNA immunization, e.g., cheap to produce, heat stability, amenable to genetic manipulation, mimic viral infection, and no risk of reversion to pathogenicity. Nevertheless, some concerns remain regarding their safety, e.g., the possible integration of plasmid DNA into host chromosomes. In summary, the results concerning the efficiency of DNA vaccination demonstrate clearly that these new vaccines may have a promising future in human immunization.

Keywords DNA immunization · Influenza virus · Vaccination

On May 14, 1796, Edward Jenner administered cow-pox material to James Phipps and started thereby a new area in modern medicine – the concept of vaccination. Although vaccination has been used to combat diseases for two centuries, smallpox is the only disease that has been eradicated by this treatment so far. Current vaccines may be divided into two forms: "live" and "dead". Live vaccines comprise traditional attenuated microbes, selected for reduced pathogenicity with maintained immunogenicity as well as recombinant vaccines in which a foreign antigen is expressed, e.g., via a replicating viral vector. "Dead" vaccines consist of killed whole or subunit pathogens. The nature of the vaccine determines the type of the induced immune response. Nonviable vaccines cannot efficiently enter the MHC class I pathway, which means non- or very low cellular immune activation. Live vaccines can induce both humoral and cellular immune responses, but may be dangerous to pregnant women and immunocompromised hosts or may revert to pathogenicity within the immunized individual.

A new approach to protect susceptible individuals against a lethal disease is the administration of plasmid DNA by direct inoculations with the intent of inducing an immune response to the protein(s) encoded therein. The begin of this new area of vaccine development started with the fundamental experiment by Wolff et al. [19], who was able to demonstrate that simply a single intramuscular (i.m.) inoculation of plasmid DNA encoding the reporter protein β-galactosidase induces the expression of this protein without the use of a delivery vehicle under in vivo condition. A plasmid vector used for DNA vaccination usually consists of a cytomegalovirus promoter for efficient gene expression in mammalian cells. This promoter is linked to a region encoding the desired protein preceded by a cap site necessary for correct mRNA initiation in mammalian cells. The coding sequence of interest is completed by a polyadenylation (polyA) signal important for allowing correct termination of the mRNA. This polyA sequence is derived either from the bovine growth hormone or from the simian virus SV40 and allows very little readthrough transcription. A drawing of the general structure of such a plasmid vector (pCMV-β, Clontech Lab. Palo Alto, Calif.) is demonstrated in Fig. 1A, together with our own observations about the in vivo expression of β-galactosidase and the induction of a humoral immune response after DNA administration. Using histochemical staining procedures for β-galactosidase activity, blue-colored muscle cells were easily detectable at the site of inoculation 7 days after injection (Fig. 1B). Moreover, transfected muscle cells (blue) were surrounded by infiltrating leukocytes, indicating the activation of a specific immune response against the foreign

A. Henke
Institute of Virology, Medical Center,
Friedrich-Schiller University,
Winzerlaer Str. 10, 07745 Jena, Germany
E-mail: i6hean@rz.uni-jena.de
Fax: +49-3641-657202

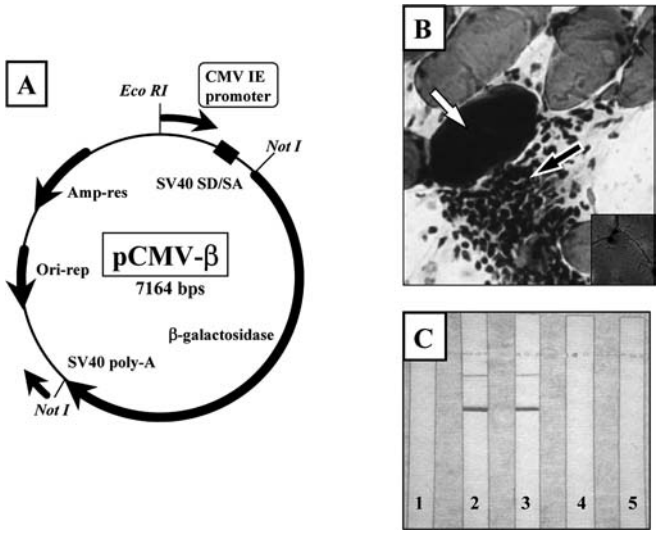

Fig. 1A–C Characterization of the eukaryotic expression vector. **A** pCMV-β (Clontech) is shown. The simian virus 40 control sequences flank the β-galactosidase gene are demonstrated (*SD/SA* splice donor-splice acceptor, *poly-A* transcription and processing signals), as is the cytomegalovirus immediate-early (*CMV IE*) promoter. **B** pCMV-β and pCMV was injected into the quadriceps muscle of male BALB/c mice. At 7 days post inoculation, muscle tissue was taken from the injection site and stained for β-galactosidase activity (*white arrow*). Infiltrating immune cells are indicated by a *black arrow*. The *inset* in the *right lower corner* demonstrates a section of muscle tissue from a pCMV-injected mouse serving as a negative control. Sections counterstained with hematoxylin-eosin, ×400. **C** At 28 day post inoculation, antibodies against β-galactosidase are only present in sera of pCMV-β-injected mice (*lane 2*) and in the positive control (*lane 3*). Sera of mice before immunization (*lanes 1* and *4*) and sera of pCMV-inoculated mice (*lane 5*) are negative

antigen, which was accompanied by the presence of β-galactosidase-specific antibodies as it is shown by Western blotting in Fig. 1C. For the delivery of plasmid DNA, not only i.m. injections but also intradermal (i.d.), mucosal, and biojector injections as well as direct skin delivery has been reported [2, 9, 11, 14, 16]. However, the majority of DNA vaccine studies have utilized skin or muscle as immunization targets. After i.m. injection, DNA is taken up by myocytes and/or professional antigen-presenting cells (APC) with subsequent expression of the foreign antigen via MHC class I molecules. Secreted antigens may be ingested by phagocytes and then presented via the MHC class II pathway. These exogenous antigens may prime the induction of antibody responses as well as $CD4^+$ T cell activation. For antigen-MHC class I presentation to activate naive $CD8^+$ T cells to become effector cells, only bone marrow-derived APC and not myocytes are able to present antigen to T cells. Using the gene-gun delivery system, which shoots plasmid DNA on gold particles into skin via helium gas acceleration, it was demonstrated, that DNA is delivered directly into Langerhans cells. Thereafter, these transfected cells migrate to draining lymph nodes presenting the foreign antigen to the immune system [5]. In general, the nature of the plasmid DNA-induced immune response is influenced by delivery routes and methods of delivery. Intramuscular inoculation drives immune responses mainly towards the Th1 response [17]. A possible explanation for this Th1 immune response is the initially strong induction of interferon-γ (IFN-γ) production, probably due to unmethylated CpG nucleotide sequences in the plasmid vector itself. Such CpG motifs consist of DNA sequences such as AACGTT. In fact, an efficient immune stimulation has been associated with the presence of such CpG motifs in the inoculated plasmid DNA and may contribute to the efficacy of DNA immunization, similar to the effect of adjuvants in other vaccines. However, the gene-gun delivery method requires much less DNA as compared to the i.m. method, and drives immune responses to both the Th1- [7] and the Th2-type [15] responses. The mechanism of this selective Th1- and Th2-specific immune activation by different DNA inoculation methods is presently not known.

During the last 10 years, DNA vaccine research has progressed from laboratory tests to initial clinical trials. The inoculation of plasmid DNA encoding sequences of viral proteins instead of reporter genes results in the synthesis of these proteins, causing a specific immune response in laboratory and preclinical models, and has suggested that immunization with DNA might be clinically useful for vaccination of humans. Both antibody and cell-mediated cytotoxic T lymphocyte (CTL) responses have been demonstrated, indicating that DNA vaccination can serve as an alternative to immunization with live attenuated viruses or killed virus vaccines. Advantages of this type of immunization are that (1) DNA can be produced inexpensively, at a high level of purity, and in large quantities; (2) heat-stability; (3) immunization against different pathogens with a single administration of a "vaccine cocktail"; (4) the plasmid vector itself is unlikely to be pathogenic, and (5) there is no or only little immune reaction to the vector. In addition, DNA vaccines mimic a natural viral infection, inducing a humoral as well as a cellular immune response, even in the presence of pre-existing host antibodies, which would interfere with an equivalent live-virus vaccine. DNA immunization can raise long-lived immune responses, including persistent and protective antibody levels, which has important implications for vaccination strategies. In view of the characteristics listed above, the development of DNA vaccines against HIV, malaria, and tuberculosis is probably most desirable.

To establish a DNA vaccine several requirements should be fulfilled: (1) protein sequences of the pathogen which induces immune responses need to be known, (2) an expression vector containing, for example, the CMV promoter is required, (3) an animal model to study the disease is needed, as are (4) assays to characterize the vaccine-caused immune reactions. This genetic immunization has been studied in many infectious disease models against several pathogens. Among them, fun-

damental investigations about the protective role of distinctive influenza virus proteins expressed by plasmid DNA were published soon after the first description of this new method [18]. Since than, many new and important results have been obtained, especially using murine influenza virus models. Several recent observations are:

- A DNA vaccine expressing a fusion protein, which consists of the influenza virus hemagglutinin (HA) and the cytotoxic T lymphocyte antigen 4 (CTLA-4), increases the antibody response and reduces the virus titer by targeting the DNA to APC [6].
- Coadministration of DNA coding for HA and cytokine DNA, such as interleukin-12 (IL-12) and granulocyte macrophage-colony-stimulating factor (GM-CSF), increases the protective immunity against influenza A virus challenge in mice [13].
- A mixture of plasmids encoding HA as well as neuraminidase (NA) induces high levels of specific antibodies in mice and improves protection against influenza virus challenge in comparison to inoculation of HA- or NA-expressing plasmid DNA alone [3].
- Electroporation with HA DNA induces strong antibody responses, weak CTL activities and protection in mice [10].
- Influenza virus H3N2 NA-DNA administration confers cross-protection against lethal challenge with antigenic variants within the same subtype, but failed to provide protection against infection by the different subtype virus H1N1 [4].
- Immunization with NP DNA is as effective as natural influenza virus infection in generating CTL precursors [8].
- Immunization of newborn mice with NP DNA primes specific cellular protective immune responses [1].

Despite these promising results in different murine models, the success in large animals and especially in humans will be highly important for future application of DNA vaccines. Therefore, increased efficacy is the major issue of DNA immunization research today. Different approaches to optimize DNA vaccines are under investigation, e.g., directing inoculated plasmid DNA to dendritic cells via technical devices, or using DNA together with chemical or biological components expressing fusion proteins which can be released from cells and can target specific APC. Increased efficiency can also be achieved by codon exchange, messenger stabilization, fusion to long-lived immunoglobulins, nuclear targeting or secretory signals for tissue-specific expression. A new effort to optimize DNA vaccination efficacy is focused on so-called prime-boost strategies. After the initial DNA inoculation the boosting is being performed by virus-like structures, liposomes, cross-linked peptides or peptides with adjuvants. Attenuated or killed viruses as well as bacteria or parasites have also been studied, together with RNA viral replicons.

Nevertheless, some concerns remain regarding the safety of DNA vaccines, e.g., the possible integration of plasmid DNA into host chromosomes. A variety of different factors may affect the frequency of integration of plasmid DNA into the host cell, including plasmid DNA sequences, the expressed gene product, the delivery method, the route of DNA administration, the formulation as well as the cell type exposed to the DNA vaccine. To analyze the putative plasmid integration into the host cell genome, an integration assay based on purification of high-molecular-weight genomic DNA away from free plasmid using gel electrophoresis was established [12]. The assay sensitivity was approximately 1 plasmid/μg DNA, which represents the genomic DNA of $\sim$ 150,000 diploid cells. Three different DNA vaccines – encoding either the influenza virus HA or matrix gene as well as the HIV gag gene – were inoculated i.m. After 6 weeks free plasmid DNA was detected in murine muscle tissue at levels ranging from 1,000 to 4,000 copies/μg DNA, and after 6 months the plasmid level ranged between 200 and 800 copies/μg DNA. Gel purification assays revealed that essentially all detectable plasmid DNA in treated muscle tissue was extrachromosomal. If integration had occurred, the frequency was below 1–8 integration per 150,000 diploid cells. This would be at least three orders of magnitude below the spontaneous mutation rate, indicating that the risk of mutation due to plasmid integration following i.m. inoculation is negligible. However, integration has been described using other routes and methods of DNA inoculation. Therefore, preclinical integration assays are necessary for each new plasmid DNA considered to be used as a clinical vaccine. Other theoretical risks include the induction of tolerance due to long-term presentation of antigen, adverse reactions due to the coadministration of cytokine DNA, or autoimmune reactions due to the induction of anti-DNA antibodies. Anti-DNA antibodies have been found to be increased by approximately 20–30% in human individuals, which at this level are not assumed to induce any disease. In contrast, 100- to 1,000-fold increases were detected in some patients suffering from the autoimmune disease systemic lupus erythematosus, indicating that before the application of DNA vaccines the individual health condition of each person should be considered. In general, DNA for clinical use should also be manufactured according to Good Manufacturing Practice (GMP).

In summary, animal and clinical DNA immunization studies have been focused on designing prophylactic and therapeutic vaccines to prevent or control specific infections. Although this new area of vaccine development started only 10 years ago, enormous progress has been made and several clinical trials to test various types of DNA vaccines are in progress. Despite the fact that several questions about specific safety issues remain, the results on the efficiency of DNA vaccination demonstrate clearly that these new vaccines may have a promising future in human immunization against viral disease.

References

1. Bot A, Bot S, Garcia-Sastre A, Bona C (1998) Protective cellular immunity against influenza virus induced by plasmid inoculation of newborn mice. Dev Immunol 5:197–210
2. Chen SC, Jones DH, Fynan EF, Farrar GH, Clegg JC, Greenberg HB, Herrmann JE (1998) Protective immunity induced by oral immunization with a rotavirus DNA vaccine encapsulated in microparticles. J Virol 72:5757–5761
3. Chen Z, Matsuo K, Asanuma H, Takahashi H, Iwasaki T, Suzuki Y, Aizawa C, Kurata T, Tamura S (1999) Enhanced protection against a lethal influenza virus challenge by immunization with both hemagglutinin- and neuraminidase-expressing DNAs. Vaccine 17:653–659
4. Chen Z, Kadowaki S, Hagiwara Y, Yoshikawa T, Matsuo K, Kurata T, Tamura S (2000) Cross-protection against a lethal influenza virus infection by DNA vaccine to neuraminidase. Vaccine 18:3214–3222
5. Condon C, Watkins SC, Celluzzi CM, Thompson K, Falo LD (1996) DNA-based immunization by in vivo transfection of dendritic cells. Nat Med 2:1122–1128
6. Deliyannis G, Boyle JS, Brady JL, Brown LE, Lew AM (2000) A fusion DNA vaccine that targets antigen-presenting cells increases protection from viral challenge. Proc Natl Acad Sci USA 97:6676–6680
7. Feltquate DM, Heaney S, Webster RG, Robinson HL (1997) Different T helper cell types and antibody isotypes generated by saline and gene gun DNA immunization. J Immunol 158:2278–2284
8. Fu TM, Guan L, Friedman A, Schofield TL, Ulmer JB, Liu MA, Donnelly JJ (1999) Dose dependence of CTL precursor frequency induced by a DNA vaccine and correlation with protective immunity against influenza virus challenge. J Immunol 162:4163–4170
9. Fynan EF, Webster RG, Fuller DH, Haynes JR, Santoro JC, Robinson HL (1993) DNA vaccines: protective immunizations by parenteral, mucosal, and gene- gun inoculations. Proc Natl Acad Sci USA 90:11478–11482
10. Kadowaki S, Chen Z, Asanuma H, Aizawa C, Kurata T, Tamura S (2000) Protection against influenza virus infection in mice immunized by administration of hemagglutinin-expressing DNAs with electroporation. Vaccine 18:2779–2788
11. Kuklin N, Daheshia M, Karem K, Manickan E, Rouse BT (1997) Induction of mucosal immunity against herpes simplex virus by plasmid DNA immunization. J Virol 71:3138–3145
12. Ledwith BJ, Manam S, Troilo PJ, Barnum AB, Pauley CJ, Griffiths TG, Harper LB, Beare CM, Bagdon WJ, Nichols WW (2000) Plasmid DNA vaccines: investigation of integration into host cellular DNA following intramuscular injection in mice. Intervirology 43:258–272
13. Operschall E, Schuh T, Heinzerling L, Pavlovic J, Moelling K (1999) Enhanced protection against viral infection by co-administration of plasmid DNA coding for viral antigen and cytokines in mice. J Clin Virol 13:17–27
14. Pertmer TM, Eisenbraun MD, McCabe D, Prayaga SK, Fuller DH, Haynes JR (1995) Gene gun-based nucleic acid immunization: elicitation of humoral and cytotoxic T lymphocyte responses following epidermal delivery of nanogram quantities of DNA. Vaccine 13:1427–1430
15. Prayaga SK, Ford MJ, Haynes JR (1997) Manipulation of HIV-1 gp120-specific immune responses elicited via gene gun-based DNA immunization. Vaccine 15:1349–1352
16. Ray NB, Ewalt LC, Lodmell DL (1997) Nanogram quantities of plasmid DNA encoding the rabies virus glycoprotein protect mice against lethal rabies virus infection. Vaccine 15:892–895
17. Raz E, Tighe H, Sato Y, Corr M, Dudler JA, Roman M, Swain SL, Spiegelberg HL, Carson DA (1996) Preferential induction of a Th1 immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization. Proc Natl Acad Sci USA 93:5141–5145
18. Ulmer JB, Donnelly JJ, Parker SE, Rhodes GH, Felgner L, Dwarki VJ, Gromkowski SH, Deck RR, DeWitt CM, Friedman A, Hawe LA, Leander KR, Martinez D, Perry HC, Shiver JW, Montgomery DL, Liu MA (1993) Heterologous protection against influenza by injection of DNA encoding a viral protein. Science 259:1745–1749
19. Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A, Felgner PL (1990) Direct gene transfer into mouse muscle in vivo. Science 247:1465–1468