DNA vaccines for prophylaxis and therapy

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DNA vaccines are tantalizingly giving hope of a third vaccine revolution. Ever since the serendipitous discovery of Wolff *et al.*[1] that naked DNA injection into the muscle of mice led to expression of the encoded marker protein, there has been a surge to use this approach to generate DNA vaccines against a variety of infectious diseases. While many aspects of the basis of genetic immunization need to be worked out, some unique features of this phenomenon have become evident. The injected plasmid DNA with appropriate promoter and regulatory sequences remains as an episome, but is transcribed and translated to give rise to the vector-encoded protein. This protein is processed similar to an antigen presented by an infectious virus, resulting in presentation of antigenic fragments in association with MHC-class I determinants, leading to activation of cytolytic cells. The generation of CD8(+) CTLs is proving to be crucial for defense against many viral, bacterial and parasitic diseases. Genetic immunization also results in stimulation of T helper cells and B cells leading to antibody production, which is generally weak, but long lasting. Intramuscular and intradermal routes for introducing DNA have been extensively used by investigators. Since muscle cells express only low levels of MHC-class I antigens and lack expression of MHC-class II antigens, it has been suggested that dendritic cells in muscle tissue could get activated and up-regulate MHC and co-stimulatory molecules. These start to secrete cytokines, migrate to lymphatic tissues and initiate immune response. Since the dendritic cells have a finite life span, the muscle cells being poor target for CTLs, might serve as a reservoir of antigen, providing a constant reminder to the immune system[2].

While more research is needed to reconcile a wide variety of immune responses to DNA vaccines described in literature, the dominant CTL response that is proving to be crucial for protection against major diseases such as malaria, AIDS and tuberculosis has led to high hopes on this approach for protection. Besides, DNA vaccine does not need an external adjuvant, sequences such as the CpG motif in the DNA providing this effect. Finding an appropriate adjuvant for the human, besides alum, has been a major effort.

In the case of malaria, studies with a whole gamut of antigens defining the pre-erythrocytic/liver, erythrocytic and mosquito stages, have indicated that induction of CD8(+) T cells is crucial and this would have to include multiple epitopes from single and many proteins, because of parasite polymorphism and genetic restriction of T cell response. One approach has been to stitch different epitopes and make a synthetic protein by recombinant route. Spf66 was the first recognized malaria vaccine developed by Patarroyo by joining three merozoite derived proteins with repetitive sequences derived from the circumsporozoite protein of *P. falciparum*. Despite the initial claims, this vaccine has given equivocal results on human trials in more than one location[3]. The latest in this effort is the development of a recombinant multistage *P. falciparum* candidate vaccine through an Indo-US collaboration[4]. A 41 kDa protein has been expressed in the Baculovirus system from a synthetic gene consisting of 12 B cell, 6 T cell proliferative and 3 CTL epitopes derived from 9 stage specific *P. falciparum* antigens, corresponding to sporozoite, liver, erythrocytic asexual and sexual stages. The candidate vaccine remains to be tested in animal models. One problem with such vaccines could be that the synthetic protein would fold and only expose certain epitopes but not others that are necessary. A vaccine with a mixture of several recombinant antigens would prove to be expensive, besides the effect of protein–protein interaction *in vitro* being anybody's guess. In this context, DNA vaccines have distinct advantage, where plasmid DNAs encoding different antigens and prepared by the same generic procedure can be mixed and administered. A mixture of 4 plasmid DNAs (pfCSP, pfSSP2, pfEXP-1 and pfLSA-1) has been injected into Rhesus monkeys and found to elicit multiple antigen-specific CTLs[5]. There is information that mixtures of 15–20 plasmid DNAs are being tested. In fact, Hoffman *et al.*[6] have suggested a 'vaccinome' approach to make a pre-erythrocytic vaccine. The scheme envisages construction of DNA vaccine plasmids from each of thousands of identified open reading frames in the parasite genome and used to immunize mice. Antisera from each immunized group is then used to identify the proteins expressed in irradiated sporozoite-infected hepatocytes. (Despite all the advances, the best result on protection against malaria has only been achieved by injecting irradiated sporozoites, which of course is not a practical proposition.) From the protein sequences of hundreds of expressed proteins, the full complement of degenerate HLA superfamily binding motifs are selected and validated experimentally. The DNA sequences of selected T-cell epitopes are linked on numerous plasmids giving rise to a vaccine comprised of tens to hundreds of DNA vaccine plasmids, each containing dozens of individual T-cell epitopes. A perfect example of mega science!

The importance of CTL response in HIV patients has also been highlighted. During the symptom-free stage, multiple HIV-1 epitope specific CTLs can be detected in peripheral blood. This activity decreases with the onset of symptoms. Several plasmid-borne HIV genes have been tested in experimental animals. A recent study has investigated the immunogenicity of DNA constructs of the regulatory HIV genes, *nef*, *rev* and *tat*, in human beings[7]. These three regulatory genes are expressed early in the life cycle of the virus, and, therefore, it is possible that an immune response to these genes can eliminate the infected cells before the release of viral particles. The study carried out with 9 symptom-free, HIV-infected patients indicates that DNA vaccination induced detectable memory cell in all patients and MHC-class I restricted CTLs of CD8(+) origin in 8 patients. While the

study cannot be generalized in view of the small number of patients used, it indicates the possibility that a combination of DNA constructs that encode the HIV-1 regulatory genes might induce a full immune response in individuals who are already infected. In addition to being a therapeutic vaccine, it might also work as a prophylactic vaccine in non-infected individuals.

Yet another option is to examine whether DNA vaccine can serve as an adjunct to chemotherapy. This approach would be very valuable both in AIDS and tuberculosis, for which satisfactory vaccines are still not available. The only vaccine available against tuberculosis is BCG with all its limitations. An effective chemotherapy against tuberculosis is available, but it involves treatment with large doses of drugs for at least 6 months after diagnosis. In a recent study[8], it has been shown that a DNA vaccine coding for a mycobacterial heat shock protein of Mr 65000 (Hsp 65), when administered in 4 doses to mice 8 weeks after intravenous injection of virulent *M. tuberculosis* H37RV, leads to a dramatic decrease in the numbers of live bacteria in spleen and lungs 2 months and 5 months after the first dose of DNA. Certain other mycobacterial antigens and BCG did not have this effect. The Hsp 65 plasmid DNA was also effective against a drug (isoniazid)-resistant isolate of *M. tuberculosis*. This therapeutic effect was associated with a switch from a type 2 to a predominantly type 1 response, leading to CTLs that can be traced to antigen-specific adjuvant effect of the plasmid DNA. Much of the adjuvant effect of DNA vaccines is traced to unmethylated CpG motifs that induce IL-12 secretion by antigen presenting cells. In this study, a plasmid coding for IL-12 was able to bring about the greatest reduction in bacterial numbers in 11 weeks, although a 50:50 mixture of IL-12 and Hsp 65 plasmids led to antagonism! In another experiment, DNA vaccine (Hsp 65) given in 3 injections towards the end of chemotherapy (pyrazinamide + isoniazid) in infected mice completely eliminated the residual bacteria, precluding the possibility of regrowth of residual bacteria into drug-resistant forms, a real problem in developing countries. The authors state in the note added in proof that the Hsp 65 DNA therapy given 8 weeks after aerosol infection of mice or guinea pigs indicates a substantial therapeutic benefit and this would be very relevant to the human situation, since transmission of most human tuberculosis is airborne. Besides, guinea pig is a better model than mice, the latter exhibiting a relatively higher innate resistance to tuberculosis. It would be a significant advance in therapeutic strategy if the DNA vaccine would decrease the dose and duration of drug treatment in the case of patients with tuberculosis.

The first published reports from India indicate modest success in the development of DNA vaccines against rabies[9] and Japanese Encephalitis Virus[10] in experimental animals. Interestingly, the efficacy of DNA vaccine (G protein) against rabies is correlated to levels of neutralizing antibodies, whereas in the case of JEV (envelope protein), cell-mediated immunity appears to be the major mechanism of protection.

There is a great expectation that DNA vaccines can offer protection against dreadful infectious diseases in developing countries in view of their temperature stability, besides being affordable for the poor when compared to recombinant/cell culture vaccines. DNA vaccine would not need a cold chain for storage and transportation, making it an ideal product for villages and remote areas. But, as a human vaccine, genetic immunization has several hurdles to cross in view of the variable immune responses observed as influenced by the nature of the encoded antigen, dosage, route of administration, animal species, duration and type of immune response, that can be further compounded in the outbred human population. The concern of chromosomal integration and insertional inactivation by the administered naked DNA appears to have receded to the background in view of results obtained from safety studies. Ultimately, DNA vaccine as such or in combination with recombinant/cell culture vaccine or as an adjunct to chemotherapy is most likely to become a tool to benefit mankind in the 21st century.

1. Wolff, J. A., Malone, R. W., Williams, P., Chong, W., Acsadi, G., Jani, A. and Felgner, P. L., *Science*, 1990, **247**, 1465–1468.
2. Ertl, H. C. J. and Xiang, Z. J., *Immunology*, 1996, **156**, 3579–3582.
3. Good, M. F., Kaslow, D. C. and Miller, L. H., *Annu. Rev. Immunol.*, 1998, **16**, 57–87.
4. Shi, Y. A. P., Hasnain, S. E., Sacci, J. B., Holloway, B. P., Fujioka, H., Kumar, N., Wholhueter, R., Hoffman, S. L., Collins, W. E. and Lal, A. A., *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 1615–1620.
5. Wang, R., Doolan, D. L., Charoenvit, Y., Hedstrom, R. C., Gardner, M. J., Hobart, P., Tine, J., Sedegah, M., Fellarme, V., Sacci, J. B. Jr., Kaur, M., Klinman, D. M., Hoffman, S. L. and Weiss, W. R., *Infect. Immun.*, 1998, **66**, 4193–4202.
6. Hoffman, S. L., Rogers, W. O., Crucci, D. J. and Venter, J. C., *Nat. Med.*, 1998, **4**, 1351–1353.
7. Calarota, S., Bratt, G., Nordlund, S., Hinkula, J., Leaandersson, A.-C., Sandstorm, E. and Wahren, B., *Lancet*, 1998, **351**, 1320–1325.
8. Lowrie, D. B., Tascon, R., Bonato, V. L. D., Lima, V. M. E., Faccioli, L. H., Stavoupoulos, Colstan, M. J., Hewinson, R. G., Moelling, K. and Silva, C. L., *Nature*, 1999, **400**, 269–271.
9. Biswas, S., Ashok, M. S., Reddy, G. S., Srinivasan, V. A. and Rangarajan, P. N., *Curr. Sci.*, 1998, **76**, 1012–1016.
10. Ashok, M. S. and Rangarajan, P. N., *Vaccine*, 1999, **18**, 68–75.

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