Editorial

Naked DNA – the poor man's gene therapy?

Vaccination with naked DNA was originally designed as a control experiment to test liposomes as DNA carriers. It proved to be a surprise that DNA alone was more effective in provoking an immune response than DNA packaged with liposomes. This was the first of many surprises about naked DNA. DNA, when injected intramuscularly, is taken up by some kind of cell, is transcribed into the actual mRNA and translated into protein antigens. Thus, the vaccine is produced by the vaccinee himself. The plasmid DNA contains, besides the appropriate gene, promoter–enhancer signals, for example, the immediate-early promoter of cytomegalovirus. The DNA vaccine mimics a natural infection or a live-attenuated vaccine. While such vaccines are not without risk, a DNA vaccine can be tailor-made avoiding any dangerous or undesired sequences, for example, sequences which favor integration of the DNA into the host chromosome such as long terminal repeats (LTRs) of HIV, or genes required for replication. Antigens, when expressed intracellularly, induce preferentially a cellular immune response and activate cytotoxic T lymphocytes (CTLs) by presentation of peptides from the inside of the cell. Peptide vaccines, in contrast, preferentially activate a humoral response.

One of the first DNA vaccines was directed against influenza A. Its internal structural protein NP was coded for by the plasmid DNA and resulted in protection against challenge virus – even against distantly related influenza virus strains. This result has however recently been challenged by some groups. Since then numerous alternative approaches or improvements have been designed, new adjuvants, tools for application such as guns, jets, electroporators, needles, various routes of injection have been compared such as intramuscular, intradermal, subcutaneous, intraperitoneally, through the mucosa, by osmotic shock or directly into the portal vein, instillation into the lung, by scratching or wounding of the skin. Finally, combinatorial approaches are being designed – proteins, peptides or conventional vaccines are being combined with DNA. Also, RNA instead of DNA is being tested as an alternative. In the case of HIV, antiviral therapy for reduction of the viral load can be combined with DNA vaccine. Last but not least, cocktails of various genes are the biggest attractions of DNA vaccines.^{1,2}

Another surprise concerning naked DNA is the uncertainty which still exists about the nature of the target cell. Intramuscular injection of DNA as a vaccination procedure suggested initially that muscle cells process the

DNA. However, they are not professional antigen-presenting cells (APCs) and lack costimulatory molecules such as B7.1. Studies on the removal of the tissue around the site of injection or cutting off pieces of the ear soon after application of the DNA by needle or gene gun – in a so-called van Gogh experiment – suggest that migrating cells are involved, for example, professional APCs such as dendritic cells. They present peptides to CD8⁺ cells after travelling to the lymph nodes or spleen and activate CD4⁺ cells. Thus, immune stimulation of both cellular and humoral response is raised by DNA vaccines. The skin is especially rich in dendritic cells. Therefore, the higher probability of DNA uptake by these cells may explain why the amount of DNA required for intradermal injection is much lower than for intramuscular application (eg 1 microgram instead of 100 micrograms per shot per mouse). Interestingly, the type of immune response also differs depending on the site and mode of injection.

Two major properties characterize a DNA vaccine – the low level of antigen production and the long-lasting expression. Protein levels may be 1000- to 10 000-fold lower than in conventional vaccines. The duration of expression for some indicator genes has been determined for up to 13 months. These two phenomena led classical immunologists to be more than sceptical about this approach. Now there may be the remote possibility that even nature itself exploits this approach for a continuous low-level expression of viral antigens. An RNA virus, lymphotropic choriomeningitis virus, may persist as DNA by an illegitimate reverse transcription inducing a long-lasting immune response.³

Other surprises spring from the fact that combinations of plasmid DNA with protein or peptide vaccines are superior and mount stronger immune responses than each component individually, as has recently been shown in monkeys with HIV–DNA vaccines. Furthermore, DNA vaccines coding for antigens of pathogens are being combined with cytokines or costimulatory genes. GM-CSF, IL-2 and most prominently IL-12, resulted in unexpectedly high immune responses. Nonspecific activation of the immune system increases the specific immune response against the antigen expressed from a plasmid DNA. GM-CSF increases the antibody response. IL-12 strongly activates CTLs and induces a strong antitumor response. Thus, there seems to be no limit for the imagination to mix and match DNA and design novel strategies for its use.

Enhancement of conventional vaccines with DNA-encoding cytokines may not even be due to the expressed gene product but to a property of the plasmid DNA,

which suddenly became the focus of attention, again by a surprising positive control: the plasmid DNA without inserted coding genes induced some protective immune response. It was concluded that DNA encodes immunogenic or mitogenic information distinct from the triplets that define amino acids. It can induce an immune response due to chemical modifications, for example, thioate groups, unusual conformations such as Z-DNA, or base composition, depending on the species of its origin. In a binary fashion bacterial DNA encodes a 'danger signal' which alerts the immune system to foreign invading species. DNA can raise killer instincts! The sequences involved are nonmethylated dinucleotides, C-Gs, linked by a phosphodiester bond (CpG) – flanked by two 5' purines and two 3' pyrimidines.⁴ These immune stimulatory sequences, ISS, are 20-fold more abundant in bacterial than in mammalian DNA, and exhibit powerful immunological activities by induction of cytokines such as IFN- α , - β , - γ , IL-6, IL-12 and B cell stimulation. Bacterial DNA may be the reason for the higher efficiency of the old-fashioned Freund's complete adjuvants in comparison to the incomplete one which lacks DNA. Bacterial DNA may even contribute to cystic fibrosis, where the DNA of superinfecting bacteria may contribute to the tissue damage in the lung, as demonstrated by instillation of bacterial DNA or short oligonucleotides into the lung. One may ask what induces the cytokines, the non-coding CpG clusters or the gene, or both. The route of injection matters a great deal – intramuscular injection is ineffective compared with intraperitoneal injection of CpG oligos which can induce a cytokine storm which may even be dangerous.

Recently IL-12 DNA has been injected into mice and resulted in a strong anti-metastatic effect. Long-lasting IL-12 expression was detectable in the serum – thus DNA is not only a vaccine but a gene therapy for gene products required at low concentrations. It may even help to fight cancer.

Whether bacterial DNA from decomposed bacteria or the newly injected plasmid DNA remains really naked has not been demonstrated. It may find basic proteins inside the cell for binding. Many pathogens code for nucleoproteins which protect their DNA from nucleases. The immunogenicity of DNA-protein complexes raises a concern – the danger of systemic lupus erythematosus which has been attributed to DNA-protein complexes or single-stranded DNA. So far, however, none of the ongoing

clinical trials have shown any sign of anti-DNA antibodies. Another concern of the regulating bodies is the danger of integration, which may cause cancer. Estimates about integration frequencies amounted to about one in 10^{16} per DNA molecule and one in 10^9 to induce an oncogenic event. Two hundred micrograms of a DNA vaccine corresponds to 3×10^{13} molecules which would lead to an integration frequency 10 000-fold below an oncogenic event. No integration was detected at a sensitivity of one to 7.5 plasmid copies for 150 000 nuclei, representing a frequency which is approximately three orders of magnitude below the spontaneous mutation frequency.⁵ Vaccines will be administered to healthy volunteers, especially children, which requires life-long safety. It is difficult to guarantee this with the present knowledge. Scepticism that all this may work only in small but not in large animals is warranted. Proof ultimately has to come from clinical trials with patients. Some trials are ongoing and many more are forthcoming since the regulations by the FDA have been relieved.

If naked DNA fulfils its promises it will abrogate some of the life-threatening infectious diseases and even cancer in the Third World, since it can be easily handled, transported, stored in spite of heat, produced with less effort and lower cost than all other vaccines. It may also turn out to be the poor man's version of gene therapy for the richer Western world, where health-care costs are exploding. Gene therapy with naked DNA may be the only treatment we can afford for everybody.

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