

## Protection Against Infectious Diseases by DNA-Based Vaccination Strategies – Lessons for Allergy Treatment?

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### DNA vaccines – A new approach?

The observation that intramuscular gene-transfer into skeletal muscle results in antigen expression was made in 1990 [1]. Three years later, Ulmer et al. published the proof of principle that intramuscular DNA injection results in *in situ* antigen expression and a protective immune response against heterologous influenza virus challenge [2]. Plasmid-based DNA vaccines were considered very promising antigen delivery candidates because they not only induced a humoral response, but also a cellular, most notably a MHC class I restricted CD8<sup>+</sup> T cell, response. This observation has been repeated in a large number of small animal models of infectious and malignant diseases [3]. Therefore studies in non-human primates and clinical trials were initiated. Studies focussed on infectious diseases (HIV, influenza, hepatitis B virus infection, malaria) and cancer (melanoma and lymphoma). For use in humans, the main safety concerns of DNA vaccines were the integration of plasmid DNA sequences into the host cell genome and the induction of anti-double-stranded DNA antibodies, potentially cross-reacting with autoantigens as seen in certain conditions of autoimmune diseases. Results from these clinical studies confirmed that intramuscular DNA vaccination is safe [4]. However, the broad humoral and cellular immune responses observed in small animal models were not seen in humans. With the exception of the hepatitis B surface antigen, DNA vaccines did not induce antibody responses in humans [5]. At best modest cellular immune responses were detected. This apparent difference of immunogenicity between man and mice can be explained by the different doses administered: with 10 – 50 µg doses, mice receive approximately 4000 times more DNA on a weight per weight basis as compared to humans. More recent data suggest that plasmid DNA is recognized by the Toll-like receptor 9 [6]. Mice and humans may have different expression patterns of this receptor resulting in the described species differences.

This lack of immunogenicity can be improved by the use of adjuvants or by the use of other delivery systems.

More recently, the use of viral delivery systems have been proposed.

Table 1. Strategies for DNA vaccine development

#### Objective

Increase in target antigen expression in muscle

Optimize route of administration

Facilitate antigen presentation to immune system

Employ adjuvants to enhance immune response

#### Heterologous prime-boosting strategy for DNA vaccine development

Several groups have shown that a heterologous prime-boosting strategy delivers a more robust primary response with a more sustained response compared to a single dose. Such as vaccination with a DNA vaccine followed by a booster with a protein antigen, a moderate response is observed. Over time these responses wane and a booster phase is required to maintain and will be stimulated.

The superiority of the heterologous prime-boosting strategy has been confirmed in many studies in small animal models and non-human primates.

This lack of immunogenicity in humans leads to the development of strategies to improve DNA vaccines. The strategies aim at improving *in vivo* expression levels, optimization of the route of DNA injection and antigen presentation; finally a number of adjuvants are being tested (Table 1).

More recently, different strategies that aim to exploit mechanisms of the immune system have been developed.

**Table 1.** *Strategies for increasing DNA vaccine potency.*

| Objective                                            | Approach                                                                                                                                                                                           |
|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Increase in target antigen expression <i>in vivo</i> | Test different promoters, use of replicons to amplify mRNA, use of lipids and electroporation to increase <i>in vivo</i> transfection                                                              |
| Optimize route of DNA delivery                       | Intramuscularly (needle or jet injection systems), particle-mediated delivery in Langerhans cell-rich epidermis ("gene gun"), intralymphatic administration of pDNA vaccine                        |
| Facilitate antigen presentation to immune system     | Use of T cell epitope strings, secretion of antigen, introduction of antigen into MHC class I and II presentation pathways, increase intracellular turnover of expressed antigen by ubiquitination |
| Employ adjuvants to enhance immune responses         | Cytokines (GM-CSF, IL-12), block polymers, lipids, inclusion of immunostimulatory sequences (ISS)                                                                                                  |

***Heterologous prime boost – A novel approach to improve immunogenicity of DNA vaccines.***

Several groups have now established that heterologous prime-boost regimen are amongst the most potent strategies to induce strong immune responses [7-13]. The strategy delivers the same antigen in sequence using different vectors. For example, a primary response is induced by plasmid DNA vaccines. Boosting of this primary response with a heterologous (viral) vector will result in 4-10 fold higher T cell responses compared to homologous boosts with the same DNA vaccine. Viral vectors such as vaccinia virus [7] or adenovirus [14] proved to be particularly effective at boosting immune responses. During the priming phase of the heterologous prime-boost regimen, a moderate but focussed T cell response against the antigen of interest is induced. Over time these antigen-specific T cells transform into memory T cells. During the boosting phase antigen-specific memory T cells are present in relatively large numbers and will be stimulated and expand rapidly upon recognition of the same antigen [15].

The superiority of heterologous prime-boost immunization regimen has been confirmed in many pre-clinical models including small rodents [7,16-19], ruminants, and non-human primates [20-23]. In humans, heterologous prime-boost immunization reg-

imen are currently being tested for cancer (melanoma, colon carcinoma, prostate carcinoma), and infectious diseases (hepatitis B virus infection, HIV, malaria, and tuberculosis).

### Lessons for allergy treatment

DNA-based vaccines are currently being evaluated for the treatment of allergies. Pre-clinical and clinical trials in allergies could benefit from the information obtained in studies using DNA-based vaccines against cancer and infectious diseases. One of the key aims of DNA-based therapies for the treatment of allergies is to shift Th2-type allergen-specific responses to Th1-type responses [24]. Clinical studies of DNA vaccines suggest that plasmid DNA vaccines alone are not potent enough to achieve this shift. It is therefore justified to test immunization strategies for allergies with strategies such as heterologous prime-boost that have shown improved potency.

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### Discussion

**Larché:** We have seen a Th1 response when you prime with DNA and boost with MVA. Do you want to see a Th1 response or a Th2 response?

**Schneider:** No, we want to see a Th1 response and a Th2 response. We have done in mice these ELISPOT assays and we would really be interested in seeing a Th1 response.

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## Discussion

**Larché:** We have been discussing the issue of whether you really want to induce a Th1 response when you have an existing Th2 response to allergens. The question is whether you want to rectify the Th1/Th2 balance or is it better to induce some kind of regulation? Do you have the feeling from your results that you are driving strictly towards Th1 responses or have you looked for elements of regulation, as well?

**Schneider:** No, we have not. We and others have just looked at Th1 type immune responses and of course there are others looking at Th2 type cytokines. Again, this has been done in mice and of course you get high amounts of interferon- $\gamma$  and no IL-5 in these ELISPOT assays. But no one has looked for regulatory mechanisms so far, but I would really be interested to learn more about that.