

# Rational vaccine design through the use of molecular adjuvants

## Review Article

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## Summary

Nucleic acid immunization is an important vaccination strategy which delivers DNA constructs encoding for a specific immunogen into the host. These expression cassettes transfect the host cells, which become the *in vivo* protein source for the production of antigen. This antigen then is the focus of the resulting immune response. This vaccination technique is being explored as an immunization strategy against a variety of infectious diseases as well as cancer. The first generation DNA immunization experiments have shown that the DNA vaccines' ability to elicit humoral and cellular responses *in vivo* in a safe and well-tolerated manner in various model systems, including humans. As we explore the next generation of DNA vaccines, our goal is to refine the current strategy to elicit more clinically efficacious immune responses. A more clinically effective vaccine may need to elicit a more specific immune response against the targeted pathogen. It would be a distinct advantage to design immunization strategies which can be "focused" according to the correlates of protection known for the particular pathogen.

In order to focus the immune responses induced from DNA immunization, we have investigated the co-delivery of genes for immunologically important molecules, such as costimulatory molecules and cytokines which play critical regulatory and signaling roles in immunity. We and others have shown that the use of these molecular adjuvants could enhance and modulate immune responses induced by DNA immunogens. Co-administration of costimulatory molecules (CD80 and CD86), proinflammatory cytokines (IL-1 $\alpha$ , TNF- $\alpha$ , and TNF- $\beta$ ), Th1 cytokines (IL-2, IL-12, IL-15, and IL-18), Th2 cytokine (IL-4, IL-5 and IL-10), and GM-CSF with DNA vaccine constructs led to modulation of the magnitude and direction (humoral or cellular) of the immune responses. These studies demonstrate the potential utility of molecular adjuvant strategy as an important tool for the development of more rationally designed vaccines.

## I. Introduction

Although the injection of DNA into tissues was originally reported in the 1950s, the technology has gained more attention in recent years as a safe means of mimicking *in vivo* protein production normally associated with natural infection (Stasney et al, 1955; Paschkis et al, 1955; Ito, 1960). Nucleic acid or DNA inoculation is an

important vaccination technique which delivers DNA constructs encoding specific immunogens directly into the host (Wolff et al, 1990; Tang et al, 1992; Wang et al, 1993; Ulmer et al 1993; Kim et al, 1997a; Agadjanyan et al, 1997, Tascon et al, 1996, Conry et al, 1996). This injection results in the subsequent expression of the foreign gene in that host and the presentation of the specific encoded proteins to the immune system. DNA

vaccine constructs are produced as small circular vehicles or plasmids. These plasmids are constructed with a promoter site which starts the transcription process, an antigenic DNA sequence and a messenger RNA stop site containing the poly A tract necessary for conversion of the messenger RNA sequence into the antigen protein by the ribosomal protein manufacturing machinery (**Figure 1**). This antigen then is the focus of the resulting immune response. This vaccination technique is being explored as an immunization strategy against cancer as well as a variety of infectious diseases including AIDS.

## II. Potential advantages of DNA vaccines

Nucleic acid immunization may afford several potential advantages over traditional vaccination strategies such as whole killed or live attenuated virus and recombinant protein-based vaccines (Kim and Weiner, 1997; Chattergoon et al, 1997). Since DNA vaccines are non replicating and the vaccine components are produced

within the host cells, they can be constructed to function safely with the specificity of a subunit vaccine. However, DNA vaccine cassettes produce immunological responses that are more similar to live vaccine preparations. By directly introducing DNA into the host cell, the host cell is essentially directed to produce the antigenic protein, mimicking viral replication or tumor cell marker presentation in the host. This process has been reported to generate both antibody and cell mediated, particularly cytotoxic T cell-mediated, immunity (**Figure 2**). Unlike a live attenuated vaccine, conceptually there is little risk from reversion to a disease-causing pathogen from the injected DNA, and there is no risk for secondary infection as the material injected is not-living and not-infectious. In addition, genes which lead to undesired immunologic inhibition or cross-reactivity (autoimmunity) may be either altered or deleted altogether. Finally, DNA vaccines can be manipulated to present a particular genome of the pathogen or display specific tumor antigens in non-replicating vectors (**Figure 1**).

**Figure 1.** Potential immunologic targets for DNA vaccination against HIV-1. These targets include *env*, *gag*, and *pol* genes as well as the four accessory genes.

**Figure 2.** Induction of antigen-specific humoral and cellular immune responses.

### III. Molecular adjuvants as a immune modulation strategy

The overall objective of any immunization strategy is to induce specific immune responses which protect the immunized individual from a given pathogen over his or her lifetime. One major challenge in meeting this goal is that the correlates of protection from an individual pathogen vary from one infectious agent to the next. The first generation DNA immunization experiments have shown that the DNA vaccines' ability to elicit humoral and cellular responses *in vivo* in a safe and well-tolerated manner in various model systems, including humans. As we explore the next generation of DNA vaccines, our goal is to refine the current strategy to elicit more clinically efficacious immune responses. A more clinically effective vaccine may need to elicit a more specific immune response against the targeted pathogen. It would be a distinct advantage to design immunization strategies which can be targeted according to the correlates of protection known for the particular pathogen (**Figure 3**).

Such refinement could be accomplished by co-delivering genes for immunologically important molecules, such as costimulatory molecules and cytokines which play critical regulatory and signaling roles in immunity (Kim and Weiner, 1997). These molecular adjuvant constructs could be co-administered along with immunogen constructs to modulate the magnitude and direction (humoral or cellular) of the immune responses induced (**Figure 4**).

There has been several reports of immune modulation by protein delivered cytokines. However, the results in general appeared marginal. More recently, we and others have focused on analyzing immune responses induced to such gene delivery. Raz et al. observed that intramuscular injections of plasmids encoding IL-2, IL-4, or TGF- $\beta$ 1 modestly modulated immune responses to transferrin protein delivered at a separate site (Raz et al, 1993). IL-2 immunization resulted in an enhancement of antibody and T helper proliferative responses while TGF- $\beta$ 1 immunization reduced anti-transferrin responses.

**Figure 3.** The potential utility of the molecular adjuvant network. Tailoring the induction of specific immune responses by vaccination programs against viral, bacterial, or parasitic diseases could be beneficial.

**Figure 4.**

Cytokines as immune response regulators. Cytokines play critical roles in the immune and inflammatory responses. Based upon their specific function in the immune system these cytokines could be further grouped as proinflammatory, Th1, and Th2 cytokines. Along with costimulatory molecules, these cytokines also play important roles in the activation and proliferation of T and B cells.

#### **IV. Modulation of immune responses using cytokine molecular adjuvants**

In order to focus the immune responses induced from DNA immunization, we have investigated the co-delivery of molecular adjuvants. We first reported that co-immunization of GM-CSF genes with DNA vaccine

constructs increases antigen-specific antibody and T helper cell proliferation responses while co-immunization with IL-12 genes results in weaker antibody responses and enhanced T helper cell proliferation (Kim et al, 1997b,c). In addition, IL-12 co-immunization resulted in a significant enhancement of CTL responses. Importantly, we observed a significant enhancement of CTL response

in vivo with the co-administration of murine IL-12 genes with four different HIV-1 DNA immunogens (*gag/pol*, *envelope*, *vif*, and *nef*) which were CD8<sup>+</sup> T cell- and MHC class I-restricted. In contrast, almost no effect on CTL induction was observed with the genes for GM-CSF in these studies. Moreover, Iwasaki et al. (1997) reported that GM-CSF and IL-12 co-delivery with DNA immunogen encoding for influenza NP resulted in enhanced cellular immune responses. Moreover, Agadjanyan et al. (1997) reported that co-administration of IL-12 genes with HIV-2 DNA immunogen resulted in a dramatic enhancement of both Th and CTL responses. Furthermore, co-administration of IL-12 genes with DNA immunogens strongly directed the antigen specific immune response towards a Th1 type immunity and induced delayed type hypersensitivity (DTH) to contact allergens as an in vivo model of the Th1 response (Kim et al, 1998a). In addition to these reports, Chow et al. reported that either injection of plasmid co-expressing hepatitis B surface antigen (HBsAg) and IL-2 or co-injection of IL-2 genes with plasmid expressing HBsAg resulted in the enhancement of both antibody and T helper cell responses (Chow et al, 1997).

More recently, we investigated the induction and regulation of immune responses from the co-delivery of proinflammatory cytokines (IL-1 $\alpha$ , TNF- $\alpha$ , and TNF- $\beta$ ), T<sub>h</sub>1 cytokines (IL-2, IL-15, and IL-18), and T<sub>h</sub>2 cytokines (IL-4, IL-5 and IL-10) (**Figure 5**) (Kim et al, 1998b). We observed enhancement of antigen-specific humoral response with the co-delivery of T<sub>h</sub>2 cytokines IL-4, IL-5, and IL-10 as well as that of IL-2 and IL-18. A dramatic increase in antigen-specific T helper cell proliferation was seen with IL-2 and TNF- $\alpha$  co-injections. In addition, we observed a significant enhancement of the cytotoxic response with the co-administration of TNF- $\alpha$  and IL-15 genes with HIV-1 DNA immunogens. These increases in CTL response were both MHC class I-restricted and CD8<sup>+</sup> T cell-dependent. We also investigated whether the Th1 or Th2-type immune responses are more important for protection from HSV-2 infection (Sin et al, 1998). We co-delivered DNA expression construct encoding for HSV-2 gD protein with the gene plasmids encoding for Th1-type (IL-2, 12, 15, 18) and Th2-type (IL-4, IL-10) cytokines in an effort to drive immunity induced by vaccination. We then analyzed the vaccine modulatory effects on resulting immune phenotype and on the mortality and the morbidity of the immunized animals following HSV lethal challenge.

We observed Th1 cytokine gene co-administration not only enhanced survival rate, but also reduced the frequency and severity of herpetic lesions following intravaginal HSV challenge (**Figure 6**). On the other hand, co-injection with Th2 cytokine genes increased the rate of mortality and morbidity of the challenged mice. Again, among the Th1 type cytokine genes tested IL-12 was particularly a potent adjuvant for the gD DNA vaccination.

## V. Modulation of immune responses using costimulatory molecule adjuvants

The generation of the T cell immune response is a complex process that requires the engagement of T cells with professional APCs such as dendritic cells, macrophages, and B cells. These professional APCs possess large surface areas for interaction with T cells. They also express high levels of MHC class I and II molecules, adhesion molecules, and costimulatory molecules which are critical for efficient antigen presentation and T cell activation. Professional APCs initiate T cell activation by binding antigenic peptide-MHC complexes to T cell receptor molecules. In addition, the APCs provide secondary signals through the ligation of costimulatory molecules with their receptors (CD28/CTLA-4) present on T cells. These costimulatory signals are required for the clonal expansion and differentiation of T cells. The blocking of this additional costimulatory signal leads to T cell anergy (Schwartz et al, 1992). Among different costimulatory molecules, CD80 and CD86 have been observed to provide potent immune signals (Lanier et al, 1995, Linsley et al, 1990).

The CD80 and CD86 molecules are surface glycoproteins and members of immunoglobulin superfamily which are expressed only on professional APCs (Lanier et al, 1995, Linsley et al, 1990, June et al, 1994). Although both CD80 and CD86 molecules interact with either CD28 or CTLA-4 molecules on T cells, CD80 and CD86 expression seem to be differentially regulated. CD86 is constitutively expressed by the APCs whereas CD80 is expressed only after activation of these cells (Freeman et al, 1989; Azuma et al, 1993; Freedman et al, 1991). Thus, CD86 may be important in the early interactions between APCs and T cells during the induction phase of the immune response.

**Figure 5.** Each cytokine gene was cloned into expression plasmids under the control of a CMV promoter.

**Figure 6.**

Protection from lethal HSV-2 challenge. Each group of mice (n=10) was immunized with gD DNA vaccines (60 µg), and/or cytokine genes (40 µg) at 0 and 2 weeks. Three weeks after the second immunization, mice (n=8) were challenged i.v. with 200 x LD<sub>50</sub> of HSV-2 strain 186 (7 x 10<sup>7</sup> pfu).

We recently reported that CD86 molecules play a prominent role in the antigen-specific induction of CD8<sup>+</sup> cytotoxic T lymphocytes when delivered as vaccine adjuvants (**Figure 7**) (Kim et al, 1997a). Co-administration of CD86 cDNA along with DNA encoding HIV-1 antigens intramuscularly dramatically increased antigen-specific T-cell responses without a significant change to the level of the humoral response. This enhancement of cytotoxic T lymphocyte (CTL) response was both major histocompatibility complex (MHC) class I-restricted and CD8<sup>+</sup> T cell-dependent. Similar results have been obtained by other investigators who also found that CD86, not CD80 co-expression results in the enhancement of T cell-mediated immune responses (Tsuji et al, 1997; Iwasaki et al, 1997). Accordingly, we

speculate that engineering of non-professional APCs such as muscle cells to express CD86 costimulatory molecules could empower them to prime CTL precursors. On the other hand, the enhancement effect of CD86 co-delivery could also have been mediated through the direct transfection of a small number of professional APCs residing within the muscle tissue. Subsequently, these cells could have greater expression of costimulatory molecules and could in theory become more potent.

## VI. Future directions

As summarized in **Figure 8**, we observed that significant modulation was possible using molecular adjuvants. This cytokine gene adjuvant network

underscores an important level of control in the induction of specific immune responses to tailor vaccination programs more closely to the correlates of protection which vary from disease to disease. This type of fine control of vaccine and immune therapies was previously very difficult to obtain. Controlling the magnitude and direction of the immune response could be advantageous in a wide variety of vaccine strategies. For instance, in a case where T cell mediated response is paramount, but the humoral response may not be needed or even be harmful, IL-12 genes could be chosen as the immune modulator to be co-delivered with a specific DNA immunogen. On the other hand, for building vaccines to target extracellular bacteria, for example, IL-4, IL-5 or IL-10 genes could be co-injected. Furthermore, in cases where both CD4<sup>+</sup> T helper cells and antibodies play more important roles in protection, GM-CSF as well as IL-2 could be co-delivered. Lastly, in cases where all three arms of immune responses are critical, TNF- $\alpha$  could be co-injected to give a combined enhancement of antibody, T helper cell, and CTL responses. In this regard it will be important to examine combination delivery in the presence or the absence of costimulatory genes to further control the immune responses. Furthermore, additional molecular adjuvant candidates, such as chemokines, should be further developed and tested. Cumulatively, these studies demonstrate the potential utility of molecular adjuvant strategy as an important tool for the development of more rationally designed vaccines.

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**Figure 7.** Co-expression of HIV-1 envelope gp120 protein with CD86 on muscle cells. Frozen muscle sections were prepared from DNA injected animals and stained with FITC-labeled (green)  $\alpha$ -CD86 antibodies and Texas Red-labeled (red)  $\alpha$ -gp120 antibodies. (A) A slide from a leg immunized with pCDNA3 (control vector) was stained with  $\alpha$ -CD86 and  $\alpha$ -gp120.

(B) A slide from a leg immunized with pCEnv+pCD86 was stained with  $\alpha$ -CD86 and  $\alpha$ -gp120 antibodies.

**Figure 8.** A summary of the each cytokine co-administration effects on antibody (y-axis), T helper (x-axis), and cytotoxic T lymphocyte responses (z-axis). Each cytokine is plotted on the 3-D axis according to its effects on the three modes of immune response.

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