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DNA vaccines: developing new strategies to enhance immune responses

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Abstract We have focused our research on understanding the basic biology of and developing novel therapeutic and prophylactic DNA vaccines. We have among others three distinct primary areas of interest which include: 1. Enhancing in vivo delivery and transfection of DNA vaccine vectors 2. Improving DNA vaccine construct immunogenicity 3. Using molecular adjuvants to modulate and skew immune responses. Key to the immunogenicity of DNA vaccines is the presentation of expressed antigen to antigen-presenting cells. To improve expression and presentation of antigen, we have investigated various immunization methods with current focus on a combination of intramuscular injection and electroporation. To improve our vaccine constructs, we also employed methods such as RNA/codon optimization and antigen consensus to enhance expression and cellular/humoral cross-reactivity, respectively. Our lab also researches the potential of various molecular adjuvants to skew Th1/Th2 responses, enhance cellular/humoral responses, and improve protection in various animal models. Through improving our understanding of basic immunology as it is related to DNA vaccine technology, our goal is to develop the technology to the point of utility for human and animal health.

Keywords DNA vaccination · Electroporation · Plasmid · Consensus immunogens · Codon/RNA optimization · Chemokines · Cytokines

The beginnings of DNA vaccines

Although DNA vaccines are a relatively new vaccination strategy, the science behind DNA vaccination had its beginnings over a half century ago with early tumorigenesis studies. Both Stasney et al. [1] and Ito [2] in separate studies showed that injections of mouse-derived tumor DNA not only induced tumors but also precipitated seroconversion in injected mice. In the 1980s, studies into the in vivo expression of injected DNA, both linear and plasmid,

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expanded [3]. These studies demonstrated in vivo activity in a variety of animal models, including studies that showed that Hepatitis B Virus DNA could induce hepatitis in chimpanzees [4] and that injection of insulin and growth hormone genes could cause the production of these hormones in rats [5]. One of these studies of the 1980s even demonstrated that injection of DNA alone could elicit immune responses. Studies by Seeger et al. [6] found that following intrahepatic injection of Ground Squirrel Hepatitis Virus (GSHV) genomic DNA, experimental animals tested positive for antibodies against GSHV surface antigen [6] supporting the immune induction arm of this in vivo delivery platform.

While these early studies were important in demonstrating in vivo expression of injected DNA, they most often relied on special DNA preparations, such as liposome encapsulation [7, 8] or calcium phosphate precipitation [5, 9], to facilitate transfection. Wolff et al. [10] were one of the first groups to demonstrate that intramuscular (IM) injection of a pure DNA plasmid encoding a reporter gene alone could lead to the transfection of murine muscle cells.

These early studies demonstrating the efficacy of in vivo transfection led to early studies in the area of applied DNA vaccination. Tang et al. [11] reported the production of Human Growth Hormone-specific antibodies after biolistic or high-speed particle-mediated "genetic immunization" of the human-derived Growth Hormone gene (hGH) in mice. Simultaneous with this report, the Annual Cold Spring Harbor Vaccines meeting featured presentations on DNA vaccines for infectious diseases or cancer therapies by Margaret Liu (Merck) [12], Harriet Robinson (University of Massachusetts) [13], and David Weiner (University of Pennsylvania) [14]. Following these initial reports the field of DNA vaccines took hold with numerous studies investigating various DNA constructs in a wide range of animal disease models [15]. Subsequently, DNA vaccines were moved to clinical examination where they have been studied for their ability to drive relevant immune responses against a host of human diseases.

How do DNA vaccines prime immune responses?

After IM injection of a plasmid, myocytes are the predominant cells transfected; however, other cells located within the muscle including dendritic cells (DCs) and to a high degree monocytes are also transfected [16, 17]. Although the exact mechanism of immune induction has yet to be elucidated, the transfection of these cell types including DCs allows immune induction through MHC I and/or MHC II pathways. In either case, DCs and other APCs play a critical role in activating adaptive immunity and must eventually acquire the antigen to be delivered to the tissue-draining lymph node for priming of CD8+ cytotoxic T cells and the activation of CD4+ helper T-lymphocytes. Direct priming of cytotoxic T-lymphocytes (CTL) may be facilitated through DCs endogenously expressing antigen. DCs may also acquire antigen exogenously through MHC I cross-presentation by various means including direct acquisition from dead or dying cells by phagocytosis [18, 19] and acquisition of antigen from actively expressing myocytes through a process termed "nibbling" [20]. DCs may also acquire secreted antigen to be displayed through either MHC class I and/or II pathways [21].

What are the key advantages and disadvantages of DNA vaccination?

DNA vaccines have several conceptual advantages over traditional vaccination platforms, such as protein subunit, live attenuated, or inactivated viral vaccines. First, DNA vaccines

are inherently safer than live attenuated vaccines or inactivated viral vaccines, which pose the risk of causing pathogenic infection in vivo. DNA plasmids are relatively simple and inexpensive to design and create. This combined with their high stability and relative temperature insensitivity, lacking the necessity of a cold chain, makes them highly suitable for mass production and distribution in both industrialized and unindustrialized nations. Also, the DNA plasmid is amenable to the introduction of several open reading frames from one or more genes from a desired infectious agent. The incorporation of whole genes also allows for proper conformation of the protein during assembly within the cytoplasm potentially producing more native immunogenicity. Additionally, DNA plasmids in and of themselves are not immunogenic. Thus, it is possible to successfully boost after DNA vaccination without producing a heterologous immune response to the chosen vector, in contrast to the case with viral or bacterial vectors [22].

Beyond these implicit advantages, DNA vaccine plasmids may also possess an inherent adjuvant capacity due to the incorporation of cytosine-phosphate-guanine oligonucleotide sequences (CpG). These unmethylated regions of DNA are common in bacterial genomes and maybe found in the antibacterial resistance genes placed within DNA plasmids. CpGs are recognized by Toll-like receptor 9 (TLR9), a receptor found on APCs [23]. Activation of TLR9 can result in the induction of proinflammatory cytokines by APCs, such as IL-12 and Type I interferon, which helps drive CTL differentiation and priming [24]. Although CpG motifs are not essential for the induction of immune response, they likely play a role, especially in mice, in the adjuvanting of immune response to DNA vaccines [24]. Their role as adjuvants in primates including humans appears more complex. This is in part due to the differential expression of TLR9 in mice and primate immune cells.

One of the most significant hurdles of DNA vaccine development has been transferring the success of inducing protective immunity in small animal models [25–29] to larger animal models. For example, in a macaque study comparing cellular responses induced by IM injection of DNA plasmid-based vaccines and recombinant adenovirus serotype 5 (Ad5) viral vector vaccines, the DNA vaccine was found to be only one-third as immunogenic as the recombinant Ad5 model [30]; similar results have been observed in clinical studies as well.

In order to increase the immunogenicity of DNA vaccines in large animal models, various methods have been researched including plasmid design, delivery techniques, and various molecular adjuvants. Some of these approaches that we have focused on will be reviewed below. For a more in-depth review of these and other strategies, please refer to [31–33].

Improving immunogenicity: construct design and delivery

The appropriate design of the plasmid is central to high level expression of its encoded antigen and subsequent immune response to that antigen. Although all life shares the same genetic code, there does exist differences between the frequency of use of certain codons in various organisms [34]. This is due to the fact that not all transfer DNAs (tDNA) exist at equal levels within cells; this negatively selects against certain codons within species. Therefore, by designing an antigenic sequence, which encodes codons that target more abundant tDNAs within the cell, it is believed that a given antigenic sequence is more efficiently translated. This results in increased expression of the antigenic sequence and thus a higher "dose of antigen," which can translate to increased immunogenicity of the resulting protein product [35, 36]. Another essential step in achieving maximum plasmid

expression, RNA optimization, takes into account other important factors that may have a deleterious impact on mammalian expression such as RNA secondary structures which impair mRNA transport, high CG values, splice sites, and other instability elements resulting in premature destruction of mRNA and thus limiting immunogenicity. In addition, optimization of the mRNA leader stretch appears particularly important. Optimizing all of these functions is an important part of the RNA optimization process.

Construct design is also essential for eliciting immune responses to highly variable viral diseases. Due to genetic drift, circulating influenza strains may change enough in a single season to make standard vaccine preparations ineffective [37]. HIV-1 is noted for its genetic diversity with 9 different subtypes within its main group alone. Differences between subtypes may also be vast with sequence differences within Env as high as 35%. Hepatitis C virus (HCV) is also a highly variable virus with 6 different genotypes and more than 50 different subtypes [38]. Traditional means of vaccination produces the greatest amount of immunity to autologous infection. Importantly, a significant improvement and an advantage of DNA vaccines is the ability to place consensus immunogens within the plasmid vector. Consensus immunogens select the most common amino acids across several different strains, and thus may elicit far greater cross-reactive cellular immunity and possibly more cross reactive humoral responses as well. These and other strategies such as removal of glycosylation sites that block antigen access to the effector arm of the immune response are important in construct design.

Electroporation

Beyond plasmid design, plasmid delivery is also an essential step in promoting DNA vaccine-mediated immune response. As noted above, cellular transfection and expression of the DNA plasmid are essential for MHC class I or II presentation. Many different strategies have been in focus for improved in vivo DNA delivery. These include formulations such as lipids [39], salts [40], local anesthetics [41], sugars [42], and devices such as the gene gun [43] and the Biojet [44].

Electroporation (EP) is a method in which an electrical pulse is applied to the vaccination site to increase cellular transfection of a plasmid construct to aid the overall immunogenicity of a DNA vaccine. Although the exact mechanism by which EP facilitates greater vaccination efficiency is unknown, it is believed to function in two distinct ways. One, the application of a brief electrical pulse to a cell membrane creates transient pores, which facilitates the entry of plasmids into the cell [45, 46]. Two, the tissue damage caused by the application of EP causes inflammation and recruits DCs, macrophages, and lymphocytes to the injection site [47, 48].

However, application of EP also requires a precise balance of voltage and current so as to prevent excessive tissue damage. EP devices which rely on constant voltage regardless of decreasing tissue resistance during an applied pulse also respectively increase the current applied to the tissue, causing electrical shock to cells and may have some deleterious affects if not well monitored. Other EP devices rely on the application of constant-current square wave pulses which do not apply increasing voltage to tissues, mitigating tissue damage and thus preventing loss of plasmid expression during tissue repair.

In the case of DNA vaccines, EP can boost both cellular expression of DNA plasmids and immunogenicity in mice [16]. We have examined this approach in several animal species. For example, immune responses in C57BL/6 mice were examined comparing IM

injection plasmids encoding consensus NS3/NS4A HCV proteins (pConNS3/4A) both with and without EP. Specific T-lymphocyte responses were measured by IFN-g ELISpot using collected splenocytes. Mice receiving IM/EP had responses (1,042 SFU/10^6 splenocytes) nearly 10 times higher than mice receiving IM alone (134 SFU/10^6 splenocytes). More importantly, in rhesus macaques, EP administered immediately upon vaccination can boost cellular responses, enhance proliferation, promote cross-reactivity, and increase T-lymphocyte proliferative responses [49–52]. In these studies, we demonstrated that the resulting T cell responses mimic those induced by live vector systems. These are some of the first reports of a DNA vaccine exhibiting such a potent immune profile in this important primate species.

Molecular adjuvants for DNA vaccines

To further increase immunogenicity of DNA vaccine, the use of molecular adjuvants such as chemokine and cytokines has been employed. Molecular adjuvants are administered typically as plasmids encoding chemokines, cytokines, or costimulatory molecules. They are administered in conjunction with a given DNA vaccine and serve as immune modulators. Studies have included proinflammatory cytokines, such as GM-CSF, IL-1alpha, TNF-alpha; Th1 cytokines, such as IFN-g, IL-2, and IL-18; Th2 cytokines, such as IL-4, IL-6, and IL-10; and chemokines, such as CCL5 or CCL21 [53].

Our lab is particularly interested in how the addition of molecular adjuvants to DNA vaccination modulates the immune response (Table 1). Findings from our laboratory have shown that the addition of molecular adjuvants may not only increase the overall breadth and magnitude of immune responses but may also skew the type of immune response seen in vivo.

Table 1 Selected molecular adjuvants reported from the Weiner lab

Molecular adjuvant	Type	Animal model(s)	Adjuvant effect	Reference(s)
RANTES	Chemokine	Mice	Cellular	[60, 61]
MIP-1a	Chemokine	Mice	Humoral	[60]
IL-8	Chemokine	Mice	Cellular/humoral	[60, 61]
SDF-1	Chemokine	Mice	Cellular	[60]
MCP-1	Chemokine	Mice	Humoral	[60]
IL-2	Cytokine	Mice	Cellular/humoral	[77, 78]
IL-4	Cytokine	Mice, nonhuman primates	Humoral	[68, 78]
IL-7	Cytokine	Mice	Cellular/humoral	[79]
IL-10	Cytokine	Mice	Cellular	[78]
IL-12	Cytokine	Mice, nonhuman primates	Cellular	[49, 80, 81]
IL-15	Cytokine	Mice, nonhuman primates	Cellular	[74, 80]
IL-18	Cytokine	Mice, nonhuman primates	Cellular	[78, 82]
M-CSF	Cytokine	Mice	Cellular	[83]
GM-CSF	Cytokine	Mice	Humoral	[83]
IFN-g	Cytokine	Mice, nonhuman primates	Cellular	[77]
ICAM-1	Co-Stimulatory	Mice	Cellular	[84]
CD40L	Co-Stimulatory	Mice	Cellular	[85]
CD80/86	Co-Stimulatory	Mice, nonhuman primates	Cellular	[86, 87]

One of the cytokines we initially reported on is interleukin 12 (IL-12), a pro-inflammatory cytokine mainly secreted by DCs. Other immune cells such as macrophages and B-cells may also secrete IL-12. IL-12 is a potent inducer of Th1 responses [54]. When expressed early during clonal expansion, both CD4+ and CD8+ T-cells are permanently primed for the production of high levels of IFN-g upon restimulation [55]. In one of the first reports showing the potential of IL-12 as a DNA vaccine adjuvant, Kim et al. [56] found that coimmunization of mice with a HIV-1 DNA vaccine and IL-12 genes resulted in increased Th1 responses while Th2 responses were correspondingly reduced. In a Herpes (HSV2) model [57], Sin reported that coimmunization of mice with HSV DNA vaccine and IL-12 genes inhibited antibody responses while promoting cellular proliferation and significantly increasing the secretion of chemokines (CCL3 and CCL5) and Th1 cytokines. Furthermore, coimmunization with IL-12 resulted in reduced mortality and morbidity in mice receiving lethal challenge with HSV than mice receiving HSV DNA vaccine alone. More recently, Hirao et al. [49] found that macaques receiving the IL-12 plasmid in addition to a HIV DNA vaccine had at least double the cellular responses of those macaques receiving IM alone. Additionally, co-immunization with IL-12 resulted in memory responses nearly 10 times higher than in macaques receiving IM alone.

Another cytokine of importance is Interleukin-15 (IL-15). It is also produced by phagocytic cells, such as DCs. The expression of this cytokine is critically important for the development and maintenance of memory CD8+ T-cells [58]. We have observed that when IL-15 is used as a molecular adjuvant in DNA vaccination experiments, it is able to induce and maintain protective memory immune responses in macaques.

In a recent study, IL-15 was administered in conjunction with simian/HIV (SHIV) DNA vaccines. In this study, Boyer et al. [59] vaccinated macaques with SIV gag plasmid (pSIVgag) with and without co-vaccination of a macaque-derived IL-15 plasmid (pmacIL-15). After a 92-week rest, the macaques were vaccinated an additional three times with the inclusion of SIV pol plasmid (pSIVpol) and HIV env plasmid (pHIVenv) plus or minus the IL-15 plasmids. CD8+ and CD4+ T-lymphocyte responses as measured by IFN-g ELISpot were similar amongst macaques immunized with the antigen plasmid alone and antigen plasmid+pmacIL-15. The macaques were challenged with the chimeric SHIV89.6p. Macaques receiving coimmunization with pmacIL-15 were able to control viremia faster within an average time of 12 weeks as compared to 25 weeks for those receiving SIV plasmid DNA alone. Moreover, animals vaccinated with pmacIL-15 also had greater T-cell proliferation in CFSE assays than those receiving SIV DNA plasmid alone. This result is promising for cytokine adjuvants in the area of DNA vaccines.

Another very interesting molecular adjuvant area that our laboratory has been developing is the use of chemokines to modulate vaccine-specific immune responses. Our first report on the use of chemokines focused on IL-8 (CXCL8), SDF-1 (CXCL12), MIP-1alpha (CCL3), RANTES (CCL5), and MCP-1 (CCL2) [60]. Kim et al. reported that mice co-immunized with a HIV DNA vaccine and IL-8 had increased T-Helper cell proliferation and increased antibody responses. SDF-1 coimmunization minimally increased T-helper cell proliferation, MIP-1alpha dramatically increased antigen-specific humoral responses, RANTES enhanced cytotoxic T lymphocyte (CTL) responses, and MCP-1 was the most potent activator of CD8 + CTLs. Further developing chemokines as adjuvant platform, Sin et al. found that mice coimmunized with a HSV DNA vaccine and either RANTES or IL-8 had enhanced Th1 cellular responses and reduced morbidity and mortality after HSV-2 challenge [61]. More recently, we have begun studies attempting to use chemokine adjuvants to traffic immune cells to sites of primary infection in various viral diseases with promising initial results.

Molecular adjuvants show great promise in promoting and increasing the longevity of immune responses. Our lab continues to investigate not only currently used molecular adjuvants but also novel adjuvants.

Learning much from both small animal and non-human primate models of DNA vaccination, we have applied both DNA vaccination and the varied methods mentioned above to enhance DNA vaccine immunogenicity to various viruses including HIV/SIV, Avian Influenza, Chikungunya, and Hepatitis C. These will be discussed below.

HIV/SIV

Our lab has a long history in the study and application of DNA vaccines for HIV. During the infancy of HIV DNA vaccine research, one of our first vaccine studies demonstrated that a HIV-1 envelope DNA construct (pM160) could induce in mice specific humoral responses as measured by ELISA and cellular responses as measured by proliferation assays [14]. Moving to non-human primate models in the 1990s, we demonstrated that vaccination with HIV-1 gene constructs could induce humoral and cellular responses which lowered viremia during heterologous challenge in macaques and chimpanzees [62–65]. This was followed by the first human trial of a HIV DNA vaccine, which, although did not significantly reduce viral load in patients or induce high cellular responses did demonstrate the long-term safety of DNA constructs in humans [66, 67].

Since then we have continued to focus on improving the immunogenicity of DNA vaccines employing combination techniques as described above [56]. Hirao et al. [49] found that co-vaccinating with HIV-1 DNA constructs encoding codon optimized gag and env plasmid with an IL-12 construct followed by EP had positive effect on cellular responses. As measured by IFN-g ELISpot, the first immunization resulted in 1,030 $\pm$ 494 SFU/10^6 PBMCs, second immunization 2,819 $\pm$ 872 SFU/10^6, and the final immunization 7,228 $\pm$ 2227 SFU/10^6. Each result was at least a log higher than IM alone. Memory responses in the vaccinated animals were similarly enhanced with IFN-g results of 3,795 $\pm$ 1336 SFU/10^6 PBMCs, more than 2 logs higher than IM alone and double that of macaques receiving IM/EP without IL-12.

Beyond focusing on strategies of increasing immune responses in non-human primate models, we have also focused on understanding the importance of Th1 over Th2 responses in the Rhesus macaque SIV model. In this study, Boyer et al. [68] examined macaques infected with SIVmac239 and treated with 9-2-(phosphonomethoxy)propyl-adenine (PMPA) to control viral load in a therapeutic vaccine model. The macaques were subsequently vaccinated with SIV antigen expressing DNA constructs with one group being given IL-4 plasmid construct while another group was not given IL-4. After the third immunization, PMPA treatment was terminated. Macaques given IL-4 were biased to Th2 responses with higher antibody titers, but exhibited a much more limited ability to control viral replication following removal of PMPA therapy. Viremia was increased compared to macaques not receiving IL-4 construct and more rapid disease progression was observed. This study reinforced the importance of Th1 responses in contributing to cellular immunity for controlling HIV immunity.

Chikungunya

Chikungunya virus (CHIKV) is a zoonotic alphavirus endemic to tropical areas of Africa and Asia. Mosquitoes of the genus *Aedes* are its typical vector [69]. Chikungunya virus

causes an acute febrile disease associated with anthralgic joint pain which may persist for months [70]. Currently, there exists no vaccine for this virus. Muthumani et al. [51] developed a novel consensus construct which encoded both envelope (E1 and E2) and capsid genes from CHIKV. Mice were given IM injection of the DNA construct with EP 3 times in a 6-week period. After a 1-week rest period, the mice were killed, and sera and spleens were collected and immune responses monitored.

As determined by antibody ELISA, mice became seropositive for both the capsid and envelope proteins from CHIKV. Furthermore, strong cellular responses were detected by IFN-g ELISpots with the strongest responses induced to E2 (>2,000 SFU/10^6 splenocytes); however, both E1 and capsid peptides generated strong responses over 1,000 SFU/10^6 splenocytes. The positive results of this study support that the DNA approach deserves further study as a strategy for controlling CHIKV [51].

Avian influenza

In recent years, Avian Influenza A subtype H5N1 has become a serious health concern as a potential agent of a pandemic. Originally confined to birds, the virus first made the jump to humans in 1997 [71]. Since then it has developed an international profile as a highly pathogenic virus. In fact, of the more than 385 known cases worldwide, 243 infections resulted in mortality. Importantly, cases of human-to-human transmission of the H5N1 have been few and isolated [72, 73], but have raised fears of potential pandemic. Conventional vaccination strategies for Influenza focus on prophylactic antibody vaccines. These have some limitations due to potential escape from neutralizing antibody responses of a changing pandemic. DNA vaccines are capable of eliciting both cellular and humoral responses and may represent an avenue to prevent high morbidity and mortality from pandemic Influenza. We have studied DNA vaccines in the influenza model. In an earlier study, mice were coimmunized with a DNA vaccine encoding influenza A PR8/34 hemagglutinin and an IL-15 plasmid optimized to increase its in vivo expression. Kutzler et al. [74] reported that mice receiving coimmunization with the IL-15 plasmid adjuvant exhibited greater immune responses and were protected against both morbidity and mortality during influenza challenge as opposed to mice vaccinated with the influenza plasmid alone.

Building on this work, we initiated development of a set of synthetic consensus DNA constructs encoding Avian Influenza A antigens and began a pilot study in C57BL/6 and BALB/c mice. The immunization schedule was comparable to that used for CHIKV. Laddy et al. [32] reported that immunization with a synthetic pH5HA induced T-lymphocyte cellular responses of approximately 400 SFU/10^6 splenocytes in IFN-g ELISpots, as well as clear HI titers. In recently reported studies, Laddy et al. [50] further demonstrated the ability of consensus Avian Influenza constructs (pH5HA, pH1NA, and pNP) to induce cross-protective immunity in mice, ferrets, and macaques. Both C57BL/6 and BALB/c mice vaccinated and then challenged with Influenza strains not included in the various consensus constructs had lower mortality and morbidity rates. In fact, pH5HA offered complete protection from both morbidity and mortality. Challenge studies in vaccinated CD8+ and/or CD4+ T cell-depleted C57BL/6 mice demonstrated that protection was mediated by cellular immunity with depleted mice showing significantly higher mortality rates than undepleted mice. Ferrets immunized with all three constructs exhibited protective humoral immune responses by their second immunization. After Influenza challenge, ferrets receiving the combined constructs, which conferred both humoral and

broad cellular immune responses, had the highest protection from weight loss. Animals only receiving pNP, which induced strictly cellular immune responses, were protected against weight loss to a significantly lesser degree. Interestingly, Macaques immunized with the constructs also had high cellular responses as measured by IFN-g ELISpots and demonstrated Hemagglutination inhibition activity against divergent strains of Avian Influenza.

These studies demonstrate the effectiveness of consensus DNA immunogens in raising potent cross-protective immune responses to Avian Influenza in various important model organisms. Moreover, the ability of these constructs to induce strong protective correlates of immunity in non-human primates not only represents an important advance for this technology but also raises the prospect of an effective and rapid vaccination platform for pathogenic influenza.

Hepatitis C

Hepatitis C is a global pandemic with approximately 3% of the world's population currently infected. Hepatitis C virus (HCV), the causative agent of Hepatitis C, preferentially infects hepatocytes. Approximately 70% of individuals with acute infection will progress to the chronic infection state, which is associated with extreme morbidity. In fact, approximately 30% of chronically infected individuals will develop progressive liver diseases, such as cirrhosis or hepatocellular carcinoma. There is no vaccine for HCV, and responses to the standard treatments of IFN-alpha and ribavirin are highly dependent on genotype. Genotype 1, the most common genotype in both Europe and North America, typically has response rates of 42% to treatment [75].

DNA vaccines, which induce strong cellular immunity, may help to protect against chronic infection, and its associated morbidity. To this end, a DNA consensus construct of the NS3/NS4A genes of HCV genotypes 1a/1b (pConNS3/4A) was designed [76]. Mice were IM immunized followed by EP on a similar schedule as described above with 5 ug, 12.5 ug, 25 ug, and 50 ug doses of the construct. Immunization with the construct was able to induce strong cellular responses in the three mice groups receiving the highest levels of construct with approximately 800 SFU/10^6 splenocytes responding to antigen stimulation as measured by IFN-g ELISpot.

Encouraged by positive results in mouse studies, the construct was further assessed in a non-human primate model. Macaques were IM immunized followed by EP with the construct two times, four weeks apart. Blood was drawn from the macaques before immunization and two weeks after each immunization. After peripheral blood mononuclear cells (PBMC) isolation, antigen-specific cellular responses were assessed by IFN-g ELISpot. Responses were not detected until after the second immunization. The highest responder after the second immunization exhibited nearly 1,000 SFU/10^6 PBMCs with an average response of approximately 555 $\pm$ 280 SFU/10^6 PBMCs.

Currently, studies continue in both mice and macaques to both improve this vaccine model and further characterize the immune responses induced by HCV constructs.

Conclusions

DNA vaccines have come a long way since the first specific focus on "DNA immunization" over 16 years ago. As a vaccination platform it has often proved its ability to elicit

potent cellular and humoral immune responses to a variety of pathogens in small animal models. This combined with the inherent advantages provided by DNA vaccine models, such as low cost, simple design/administration, insignificant construct immunogenicity, and relatively fast production, has created much excitement for this potential vaccination platform. The last significant hurdle for implementation and use of DNA vaccines as therapeutics or potential prophylactics has been the difficulty of translating small animal success to larger models including in clinical studies. Research by our lab and others has slowly chipped at this wall. Research into plasmid design strategies, such as consensus antigens or codon/RNA optimization, has increased cross-reactivity and expression in vivo. A plethora of studies into various delivery strategies has also been promising with an important focus on EP, which not only increases transfection but also enhances immune responses. Various molecular adjuvants have also shown much promise as methods of enhancing immune responses both soon after immunization and postinfection. With these and other advances, a new generation of DNA vaccines shows great promise. Future studies of this important platform in humans will determine the utility of this platform for treatment of human diseases.

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