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The VesiVax system: a method for rapid vaccine development

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1. ABSTRACT

The VesiVax® system is based upon the concept that highly potent vaccines can be designed by engineering proteins that are capable of stably inserting themselves into liposomes. Such a nanoscale liposomal particle can then serve as an immunogen for vaccine development. The VesiVax® vaccine technology platform is designed to make it relatively easy to engineer and produce new vaccines quickly. Vaccines based on the VesiVax®system have been designed against the influenza virus and herpes simplex type 2 virus, the causative agents of the "flu" and genital herpes, respectively. Both vaccines have been tested in animal models and have demonstrated significant protective efficacy from challenge with lethal doses of virus. Assays of the immunological parameters suggest that both T and B cell responses can be elicited by VesiVax® vaccines. The safety profile of the VesiVax® vaccines is expected to be much better than that of vaccines prepared by conventional techniques. Taken together, the inherent flexibility of the VesiVax platform is expected to facilitate the rapid development of new vaccines which are effective at stimulating protective immune responses.

2. INTRODUCTION

Immunization against biological agents has a long history of being a safe and effective method of protecting large populations against infectious diseases. The most successful of these vaccines have been based on immunization with the intact pathogen. Since many of the most important human pathogens exhibit minimal antigenic variation, little emphasis has been placed on flexibility to respond quickly to new, rapidly emerging pathogens. However, the possibility of the appearance of a highly virulent pathogen either through a natural outbreak or the deliberate release of an engineered microbe has exposed the underlying weaknesses associated with the classical methods of vaccine development and production that rely primarily on the use of the pathogen itself or a similar agent as the immunogen. For example, development of microbial-based vaccines generally requires a lengthy period of time and often results in a unique set of production conditions that are not broadly applicable to other pathogens, which makes it difficult to streamline the vaccine development process (1). Classical vaccines may also suffer from a number of additional problems including

low efficacy, severe adverse side effects and protective immunity of limited duration. Thus, although vaccination is regarded as one of the greatest achievements in medicine, improvements in the form of new vaccine technologies are greatly needed.

While generally safe, vaccines that employ intact organisms can be associated with serious toxic reactions. For instance, the smallpox vaccine is based on a strain of vaccinia virus that has a very well documented history of causing significant morbidity and mortality (2). Occasionally, immunization can precipitate the development of autoimmune diseases directed against normal tissues (*e.g.,* Guillain-Barre' syndrome) (3). In addition to the agents themselves, vaccine production within cells or complex artificial media may allow for the induction of severe allergic reactions to foreign proteins incorporated in the vaccine preparation (4). Bacterial vaccines can also have undesirable side effects: certain pertussis vaccines cause some very severe complications in children (*e.g.,* vomiting, anorexia, convulsions, pain, fever) because of endotoxins present in the vaccine (5) while the widely used BCG vaccine for tuberculosis has been shown to cause a number of adverse reactions, including local abscesses at the site of injection, lymphadenitis, pulmonary lesions and even bone lesions (6). Since effective vaccination procedures frequently require multiple immunizations, these adverse events can seriously compromise the effectiveness of the vaccine and may reduce public acceptance of the vaccination procedure.

Modern molecular biologic techniques promise to provide increased safety and efficacy for non-pathogen based vaccine technologies. Some of the more popular strategies for immunization that have been under investigation include: vaccines based upon recombinant proteins (7,8), generation of simple synthetic peptides as antigens (9-12) and the direct injection of genetic material into tissues to stimulate protective immune responses (13-15). In particular, the use of protein or peptide antigens to induce specific immune responses has been the focus of many studies. This strategy is attractive because it has the potential to provide immunological specificity, tighter control of manufacturing processes, and elimination of most of the secondary sources of materials or contaminants associated with the production of the immunogen.

The use of purified proteins or peptide fragments for vaccination, however, depends upon the delivery of the antigens to the immune system by an effective carrier system. Protein and peptide antigens are typically ineffective in stimulating host immune responses when used as soluble antigens. Zinkernagel and colleagues have proposed that the important variables that determine the effectiveness of an immune response are antigen localization and concentration (16), conditions that are difficult to achieve with small soluble molecules. Immune responses require both exposure to the appropriate responding cells and sufficient antigen to stimulate an effective response. Soluble antigens can, however, be manipulated to more effectively stimulate immune responses, either by incorporation into a carrier system that distributes the antigen more efficiently to the cells of the immune system, thereby facilitating better uptake by antigen presenting cells (APC), or by attracting more APC to the site of antigen localization (17). Alternatively, carriers that release soluble antigen over time can enhance the response by extending exposure of immune cells to antigen. The choice of a carrier system that effectively promotes antigen presentation is nearly as important as selecting the target antigen for vaccine development.

One potential method to improve the immunogenicity of soluble antigens is to incorporate them into a carrier system such as a liposome. The ability of liposome/antigen complexes to efficiently stimulate the cells of the immune system (*e.g.,* macrophages) can be a highly effective delivery system for well-defined protein epitopes. Small unilamellar liposomes (<200nm) administered *in vivo* can circulate widely throughout the body and their particulate structure stimulates removal by the monocyte/macrophage phagocytic system, especially in the liver, spleen, lymph nodes, and lungs (18-20). Vaccination studies using these constructs have shown that lipid and liposome-based antigen complexes promise to be safe and effective carriers for a variety of vaccine applications (21-28). However, while formulations based on these strategies have been shown to be immunologically active, technical difficulties associated with the large-scale production of proteins with lipophilic domains that allow for efficient interaction with liposomes have made them less attractive for commercial vaccine applications. Clearly, an improved system or method for incorporating protein antigens in a liposome is required to take advantage of the potential offered by liposomes in vaccine development.

3. THE *VESIVAX®* SYSTEM

One recently developed strategy for addressing the problems associated with liposomal delivery of antigens has been to design and engineer proteins that are capable of being stably inserted into lipid bilayers. This approach requires a fundamental understanding of the interactions of proteins and/or peptides with lipid bilayers and an in-depth knowledge of the processes used in the commercial preparation of liposomes (29). Structural and functional studies on the interactions of proteins and peptides with lipid bilayers has, for instance, identified structural amphiphilicity and hydrophobicity as important parameters that control the nature of the interaction between proteins and membranes (30-32). By taking advantage of this information, the *VesiVax®* vaccine system was created which, in the most optimal version, employs a flexible and easily modified gene cassette designed to rapidly engineer and produce antigenic proteins that are compatible with liposomal bilayer membranes. These specially-engineered proteins possess an aqueous soluble hydrophobic domain (HD) that makes purification simple and allows stable insertion of the immunogen within the lipid membrane for use as a vaccine. The *VesiVax®* system has been extensively tested and shown to be highly effective for immunizing against several different bacterial and viral infections. This technology eliminates one of the most

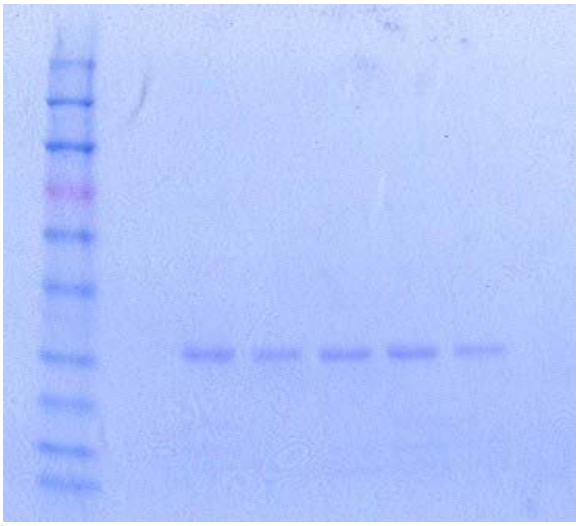

Figure 1. Coomassie stained SDS-PAGE of M2e-HD recombinant proteins. Molecular weight marker (lane 1), M2e1-HD, M2e2-HD, M2e3-HD, M2e4-HD, M2e5-HD, (Lanes 3-7).

critical issues associated with hydrophobic protein domains that make large-scale vaccine development difficult, *i.e.*, the production and purification of proteins with hydrophobic regions using standard preparative techniques.

Several prototypic *VesiVax*® vaccines have been constructed, evaluated and demonstrated to provide protection against a number of different pathogens. The procedures for preparing each antigen protein and formulating the liposomes are essentially the same for each vaccine. As described here, epitopes from the N-terminus of the gD envelope protein of herpes simplex virus type 2 (HSV2) and the M2 ectodomain (M2e) from influenza virus type A (IAV) were prepared as fusion proteins coupled to an HD segment and purified. It has been demonstrated in experimental animal (HSV2; IAV) models that the vaccines incorporating the HSV2 and IAV HD constructs provide protection against challenge with viral pathogens administered by several different routes of infection (*e.g.*, respiratory, vaginal, rectal) with no evidence of vaccine-related adverse side effects (33,34). *VesiVax*® vaccines are highly immunogenic, stimulating protective immune responses in both male and female as well as in adult and young animals. Because the VesiVax® system is designed to be flexible, antigens ranging from epitope segments as small as 10-15 amino acids to larger proteins of several hundred amino acids can easily be accommodated. Taken together, the results suggest that the application of modern molecular biologic techniques to quickly identify new antigenic determinants from emerging pathogens in combination with an antigen/lipid carrier designed to stimulate potent protective immune responses could potentially facilitate the rapid development of new vaccines.

4. CONSTRUCTION OF A *VESIVAX*® VACCINE

The construction of a *VesiVax*® vaccine begins with the identification of an appropriate target antigen or epitope. Examples of promising target antigens include the M2e protein from IAV and the gD envelope protein from HSV2. The M2 antigen has been proposed as a potential "universal" antigen to induce protection against a broad range of influenza viral subtypes since Zebedee and Lamb first raised a monoclonal antibody (14C2) to the N-terminus of M2 that restricted viral growth (35) and was subsequently shown in passive transfer experiments to inhibit viral replication in mice (36). A recombinant M2 protein expressed in baculovirus stimulated antibodies to M2 (37) and provided protection as an immunogen in mice against both homologous and heterologous influenza challenge (38). Others have expressed fusion proteins containing segments of M2 coupled to the Hepatitis B core protein (HBc) (39) or deletion mutants (40) and elicited protective responses to both homologous and heterologous challenge with IAV. The gD envelope protein of HSV has been the focus of many vaccine studies because immunization using gD from either HSV1 or HSV2 viruses protects animals from viral challenge (41-45) and has been shown to be an important target of the host humoral and cellular immune responses (46,47). Studies with monoclonal antibodies specific for gD indicate that this protein plays a role in recognition and binding (48,49), penetration (48) and cell fusion (50) of the virus. Within the gD protein, a number of epitopes have emerged as potential targets for vaccine development. Amino acids 1 to 23 of the mature form of gD (gD23) stimulate both humoral (51) and cell-mediated responses (52-54) and mice immunized with synthetic peptides within this region are protected from lethal or paralytic viral challenge (52). In this review, *VesiVax*® vaccines based on M2e and gD23 will be discussed.

Once a target antigen has been selected, a *VesiVax*® vaccine can be prepared either by peptide synthesis or by recombinant methods. While peptide synthesis is relatively straightforward, it is limited to smaller antigen or epitope segments. In addition, the requirement of a hydrophobic domain segment makes purification extremely difficult. Thus, a recombinant approach has recently been developed. In this method, a gene encoding the selected antigen is then prepared by directly cloning it from the pathogen of interest or by chemical synthetic methods. With the recent development of techniques for chemically synthesizing larger genes, it is becoming much more convenient to use this method, since the synthesized gene can be codon optimized for the recombinant expression system (*e.g., E. coli*) to be used. Once the gene encoding the antigen has been obtained, it is then inserted into a plasmid that has been engineered with the appropriate restriction sites adjacent to sequences for the HD gene (AG-HD). The construct is then transformed into *E. coli*. The resulting AG-HD construct is expressed and the aqueous soluble AG-HD protein is purified using standard affinity and ion exchange column chromatographic methods. As an example, shown in Figure 1 is a SDS-PAGE analysis of several purified M2e-HD constructs that correspond to different strains of influenza. Each was expressed and purified following the same procedure. The technique has been designed to be compatible with large scale manufacturing process

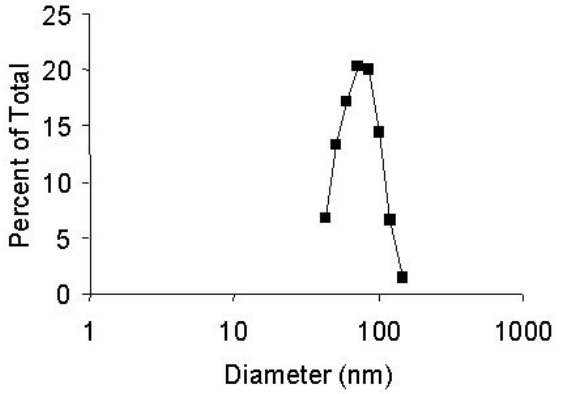

Figure 2. Particle size distribution of L-M2e-HD. Samples of the L-M2e-HD were sized by dynamic light scattering on a Microtrac UPA 150.

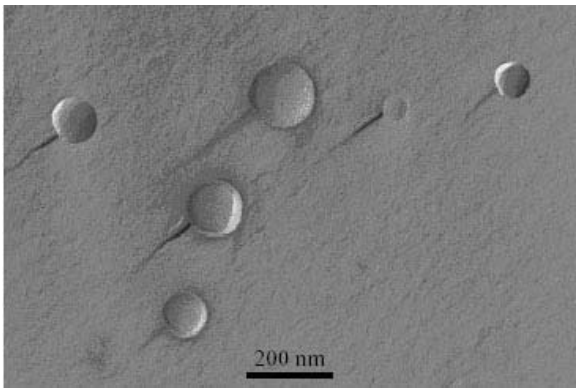

Figure 3. Freeze-fracture electron micrograph of L-M2e-HD. Magnified 125,000 times. Adapted with permission from (34).

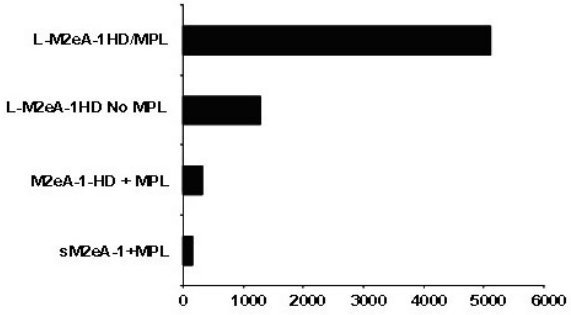

Figure 4. IgG antibody response of mice immunized with different formulations of M2e. BALB/c (n=5) were immunized two times; s.c. (day 0) and i.n. boost (week 8) with different forms of M2e. Mice were sacrificed one week after the boost and tested for IgG response by ELISA. There was statistical difference between L-M2e-HD and all other groups ($p<0.05$). Adapted with permission from (34).

technology, which is an important consideration for commercial production of the AG-HD proteins.

Liposome formulation studies were conducted to establish the most immunogenic composition to carry the AG-HD proteins (L-AG-HD). A broad range of lipid combinations were evaluated as mixtures of phosphatidylcholines (PC), cholesterol (CH), phosphatidylglycerols (PG) and adjuvants (*e.g.,* monophosphoryl lipid A (MPL)). Upon processing, as previously described (34), each liposome preparation is required to meet certain physicochemical parameters in order to advance to immunological screening in animals. This ensures that any formulation selected for clinical development will be capable of being manufactured in commercial quantities. As part of the primary selection criteria, the liposome preparation must form small unilamellar vesicles that remain stable (*i.e.*, no aggregation or precipitation) for an extended period of time. Formulations meeting these specifications were typically found to have mean diameters of approximately 100nm as judged by dynamic light scattering (Figure 2). The size distribution and unilamellarity of the liposomes has also been confirmed by electron microscopic analysis (Figure 3) (34).

Liposome formulations meeting the above physicochemical criteria were then screened in mice for their ability to stimulate a strong immune response. For example, the M2e segment from influenza virus type A was prepared and groups of BALB/c mice were immunized using different routes of administration (subcutaneously and intranasally), with or without MPL adjuvant, and with different combinations of the liposomes, M2e-HD and adjuvant. Serum was collected one week after the last vaccination and M2e specific antibody titers measured. The results (Figure 4) (34) show that the liposomal form of the M2e-HD protein with MPL (L-M2e-HD) elicited the highest immune response as judged by elevation in antibody titers. It was also found, in general, that M2e liposomes containing MPL performed much better than liposomes without the MPL. These results demonstrated the overall validity of the *VesiVax*® system as a potentially useful vaccine technology that could be used as a platform for rapid vaccine development.

5. *VESIVAX*® VACCINES ELICIT PROTECTIVE IMMUNE RESPONSES

Having identified L-AG-HD formulations that stimulate potent immune responses, several studies were conducted in animal challenge models (55). In an influenza protection study, groups of mice were given a subcutaneous (s.c.) priming vaccination on day 0 followed by an intranasal (i.n.) booster at week 8 with 10-15μg of L-M2e-HD. The vaccinated animals were challenged intranasally with 25, 50 or 150 LD_{50} of the X-88 strain of influenza virus. X-88 is an H6N2, recombined viral strain (HA-A/Turkey/Massachusettes/3740/76 x NA-A/Aichi/6/68 and all other genes from A/PR8/34) and contains the M2e target sequence used in the vaccine (55). To increase the virulence of the virus in the mouse model, this strain has been adapted in mice by successive passaging through mouse lungs. The mice vaccinated with the L-M2e-HD vaccine were able to withstand exposure to viral challenges of up to 150 LD_{50} of the X-88 strain whereas all the mice vaccinated with the liposomes without the peptide (control

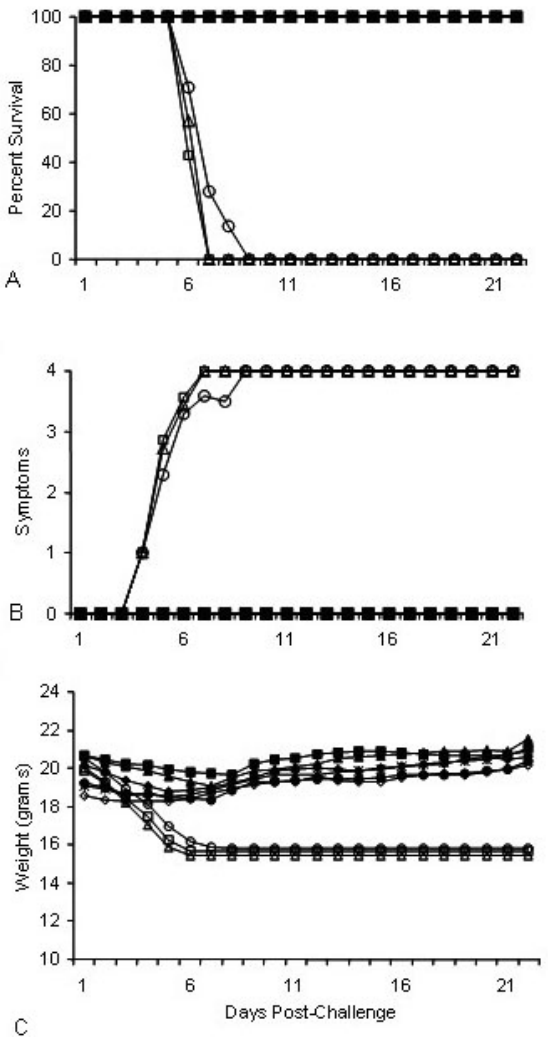

Figure 5. Efficacy of L-M2e-HD or UV inactivated X-88 immunization with increasing challenge doses of X-88 influenza. BALB/c mice (n=7/group) were immunized s.c. (day 0) and i.n. boost (week 8) with L-M2e-HD (15µg M2e/dose), UV-inactivated X-88 (dose=10 LD_{50} non-inactivated X-88) or control liposomes with MPL. Mice were challenged intranasally one week post-boost with 25, 50 or 150 LD_{50} X-88 and evaluated for survival (Panel A), symptoms, 0=healthy to 4=moribund (Panel B) and weight loss (Panel C). The mice immunized with either L-M2e-HD or UV inactivated X-88 showed significantly better survival than the control liposomes at all viral challenge doses ($p<0.05$). Symbol legend: 150 LD_{50} L-M2e-HD (-■-); 50 LD_{50} L-M2e-HD (-♦-); 25 LD_{50} L-M2e-HD (-▲-); 150 LD_{50} X-88 (-x-); 50 LD_{50} X-88 (-●-); 25 LD_{50} X-88 (-○-); 150 LD_{50} control (-□-); 50 LD_{50} control (-◊-) and; 25 LD_{50} control (-Δ-).Adapted with permission from (34).

liposomes) died following viral challenge even at the lowest LD_{50} challenge dose (Figure 5). In comparison, mice vaccinated with UV inactivated whole virus showed similar protection to the L-M2e-HD vaccine. In another study aimed at demonstrating cross-protection against different H and N strains of influenza, groups of mice were immunized with the L-M2e-HD and tested at the Centers for Disease Control and Prevention against an H1N1 influenza virus (Strain A/PR/8/34) that has an M2e that is homologous with the X-88 (H6N2) virus. The mice were immunized twice (day 0 s.c. and week 8 i.n.) and challenged approximately 10 days after the boost. Mice immunized with L-M2e-HD showed 100% survival compared to 0% survival for the liposome control group (Figure 6) (34). The lung viral titers of the immunized and challenged mice were also analyzed and found to be reduced by an average of 2.5 log or over 300-fold compared to the control vaccinated mice.

In vaccination studies conducted on HSV2, the causative agent of genital herpes, similar results were obtained that paralleled the influenza results in terms of protective efficacy. These studies were conducted with a chemically synthesized AG-HD corresponding to the N-terminal 23 amino acid segment from the gD envelope glycoprotein (gD23) of HSV2 fused to the HD (gD23-HD). The vaccine efficacy of liposomal gD23-HD containing MPL, gD23-HD mixed 1:1 with alum, gD23-HD mixed with MPL, gD23-HD without adjuvant and control liposomes (no gD23-HD) containing MPL (25µg/dose) was tested in BALB/c female mice. The animals were subcutaneously vaccinated on day 0, week 4 and week 8 and intravaginally challenged week 9 with 10 LD_{50} HSV2. No irritation, reddening or swelling occurred at the vaccine injection sites of the mice, except for minor swelling in the group receiving the alum adjuvanted gD23-HD. Maximum protection (100% survival) was observed with the L-gD23-HD containing MPL group while vaccination of gD23-HD without adjuvant or gD23-HD mixed with alum or MPL produced survival rates of only 18% (Figure 7). None of the mice given the control liposomes survived beyond day 13. L-gD23-HD with MPL was significantly more protective than any of the other formulations ($P<0.005$).

Having determined that the L-gD23-HD provided significant protective efficacy, BALB/c female mice were vaccinated with L-gD23-HD to compare the protection generated one week (week 9) and twelve weeks (week 20) post-vaccination. At week 9, L-gD23-HD produced 86% survival following challenge with 10 LD_{50} of HSV2, with no survivors in the buffer treated group ($P<0.05$) (Figure 8A). When the viral challenge was delayed until week 20, more than half of the L-gD23-HD vaccinated mice were still protected, with a survival rate of 57% (Figure 8D) and none of the buffer treated mice survived the challenge ($P<0.05$). The clinical signs of infection in the BALB/c mice paralleled the survival data with severe symptoms for the control mice at both timepoints (Figures 8 B, C, E, and F). Minimal vaginal and neurological signs were observed for the mice given L-gD23-HD and challenged one week post-vaccination (Figures 8 B, C). However, when the viral challenge was delayed for 12 weeks post-vaccination, the initial symptoms in the L-gD23-HD immunized mice were more severe than at 1 week post-vaccination, with signs resolving by the end of the observation period (Figures 8 E, F).

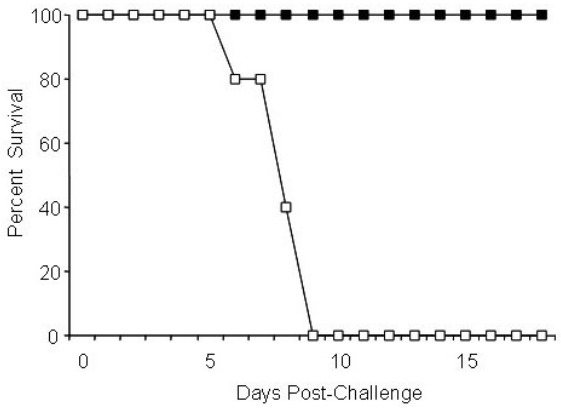

Figure 6. Efficacy of L-M2e-HD upon challenge with influenza virus strain A/PR/8/34 (H1N1). Mice (N=10) were immunized day 0 (subcutaneous) and week 8 (intranasal) and then challenged intranasally at week 9. Symbol legend: L-M2e-HD (-■-); control liposomes (-□-). Data courtesy of Dr. Terrence Tumpey, Centers for Disease Control and Prevention.

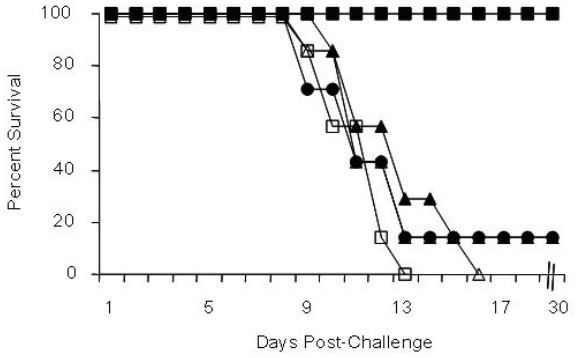

Figure 7. Protection of mice immunized with gD23-HD formulations. Groups of BALB/c mice (n=7/group) were immunized with three subcutaneous injections of L-gD23-HD (-■-), gD23-HD with MPL (-▲-), gD23-HD with alum (-●-), gD23-HD (-Δ-) and control liposomes (-□-) at day 0, week 4 and week 8. All mice were primed with depoprovera on day -7 and day -1 prior to intravaginal challenge. One week later (week 9) mice were intravaginally challenged with 10 LD_{50} HSV2. $P<0.005$, for L-gD23-HD/MPL compared to other groups (Kaplan Meier Log Rank test).

6. *VESIVAX*® VACCINES STIMULATE B AND T CELL RESPONSES

Immunological assays were conducted to determine the nature of the immune response elicited by the L-AG-HD constructs. Antibody titers were measured by ELISA from the serum of mice immunized with the L-M2e-HD vaccine and the role of these antibodies in protection were further characterized by passive transfer of serum from immunized mice to naïve mice. For the L-gD23-HD vaccine, virus neutralization assays from the serum of immunized mice were performed and this serum was tested for protection of naïve mice against HSV-2 challenge. Evaluation of the cellular immune response for the L-M2e-HD vaccine included an adoptive splenocyte transfer study and analysis of the cytokine profiles was done for both the L-M2e-HD and L-gD23-HD vaccines. The results demonstrated that L-AG-HD vaccines can stimulate both humoral and cellular immune responses.

6.1. Humoral responses to the liposomal M2e-HD and gD23-HD vaccines

The M2e antigen has previously been shown to stimulate antibody mediated protective immune responses (56). High M2e specific antibody titers were reported to be elicited in mice vaccinated with L-M2e-HD as judged by ELISA (34). To determine if the antibodies generated by the L-M2e-HD vaccine would mediate protection, a passive transfer experiment was conducted in which BALB/c mice were immunized with L-M2e-HD, UV inactivated influenza virus (strain X-88) or control liposomes and the serum collected one week after the last vaccination. Naïve BALB/c mice were given pooled immune serum prior to challenge with influenza virus. Animals receiving sera with titers greater than 13,300 (Table 1) obtained from the mice vaccinated with the L-M2e-HD or UV inactivated influenza virus displayed 100% survival whereas mice given sera from mice vaccinated with control liposomes died (Figure 9). Preliminary antibody isotype data by ELISA indicated that an IgG_1 response was stimulated in the L-M2e-HD vaccinated animals, suggesting that a more humoral Th2 response (rather than cell and antibody Th1 response) was elicited by this vaccine. These results support the hypothesis that anti-M2e antibodies can provide protection against influenza infection.

In a comparable study, neutralizing antibodies in the serum of L-gD23-HD vaccinated mice were titered in a neutralization assay. Female BALB/c mice were vaccinated subcutaneously at weeks 0, 4 and 8, with L-gD23-HD or control liposomes. At one week and 12 weeks post-vaccination, blood was collected and the serum assayed for neutralizing antibodies. The neutralizing antibody titer was expressed as the reciprocal of the serum dilution that produced 50% reduction in pfu of Vero cells compared to pfu in wells treated with virus alone. The results (Table 2) showed that one week and twelve weeks after their last subcutaneous vaccination, BALB/c female mice vaccinated with L-gD23-HD produced elevated levels of serum neutralizing antibodies compared to buffer treated controls (BALB/c, p=0.0002 (9wks), 0.0092 (12wks)).

6.2. Cellular responses to the liposomal M2e-HD and gD23-HD vaccines

To assess the role of the cellular response in the protective immunity provided by the liposomal M2e-HD vaccine, an adoptive transfer study was performed by isolating splenocytes from BALB/c mice vaccinated with liposomal M2e-HD or control liposomes. Four days after the last vaccination, splenocytes were harvested from the vaccinated mice and transferred to naïve mice at a dose of at least 5 $x10^7$ splenocytes per mouse. Four hours post-transfer, the animals were intranasally challenged with 10 LD_{50} of influenza virus and the morbidity and mortality

Table 1. Anti-M2e and anti-X-88 antibody titers[1]

Immunized Mouse Sera	Coating Antigen	IgG Titer
L-M2e-HD[2]	M2e	13,312
L-M2e-HD[2]	M2e	5,120
Control Liposomes	M2e	0
UV Irradiated X-88	1:32 Irr. X-88	81,920
UV Irradiated X-88	M2e	256
PBS	1:32 Irr. X-88	0
PBS	M2e	0

[1] Adapted with permission from (34). [2] Sera gathered from two separate experiments

Table 2. Neutralizing antibody titers of serum collected one week and twelve weeks post-vaccination

Treatment	Titer	
	One week	Twelve weeks
L-gD23-HD	96 (±34)	184 (±142)
Control	24 (±7)	4 (0)

followed for 21 days. Naïve mice that received splenocytes from mice vaccinated with the liposomal M2e-HD vaccine started dying 6 days after the influenza challenge, as well as the group of mice that received splenocytes from mice vaccinated with the control liposomes. By day 10 post infection, all the mice from both groups had died (Figure 10). These results are consistent with previous studies (56) indicating that the mechanism of the protective effect of M2 is dependent primarily on an antibody response.

To determine which type of T cell response (Th1 or Th2) was upregulated in the immunized mice following vaccination with the L-gD23-HD vaccine, splenocytes were isolated from L-gD23-HD and control (PBS buffer) vaccinated mice and these cells were incubated with the gD23 epitope. Evaluation of the gD23 specific cytokine response by splenocytes from female BALB/c at one week post-vaccination with L-gD23-HD (Figure 11) showed increased levels of IL-2, IL-4 and γ-IFN when compared with splenocytes from buffer treated mice. Notably, γ-IFN levels were more than twice that of the other cytokines. Although the γ-IFN levels remained higher than that of the other cytokines at 12 weeks post-vaccination, the γ-IFN levels were lower than they had been at one week post-vaccination (Figure 11). The production of IL-2 and IL-4 by the splenocytes from the liposome vaccinated mice in most cases remained about the same 12 weeks post-vaccination. Thus, in contrast to the L-M2e-HD influenza vaccine, the L-gD23-HD vaccine appears to stimulate more of a cellular immune response.

To further study the contribution of the T cell response to the protective effect of the L-gD23-HD vaccine, several mouse strains were selected with different T cell immunological deficiencies, including β-2 microglobulin knockout mice, IL-12 knockout mice and γ-IFN knockout mice. β-2 microglobulin knockout mice are unable to mount a CTL response and IL-12 and γ-IFN knockout mice cannot produce these Th1 cytokines. Since NK cells can also play a role in controlling certain viral infections, the vaccine's efficacy was tested in female Beige mice which are deficient in NK cell activity. Lastly, IL-4 knockout mice were immunized with the L-gD23-HD vaccine to determine if the absence of this Th2 cytokine had any effect on the vaccinated animal's ability to respond to HSV2 challenge.

The immunodeficient mice were vaccinated subcutaneously with the L-gD23-HD vaccine on day 0, week 4 and week 8 or on day 0 and at week 8, and challenged one week post-vaccination with 10 LD_{50} HSV2. The results showed that protection was only observed in the IL-4 knockout mice and the Beige mice, which had 67% and 71% survival, respectively (Figure 12). The liposome control vaccinated groups for these two strains were not protected and had severe signs of infection. In contrast, neither the L-gD23-HD nor the liposome control treated, γ-IFN, IL-12 or β-2 microglobulin knockout mice were protected from intravaginal HSV2 challenge (Figure 13). These mice had severe vaginal lesions and neurological signs of infection. These data support the conclusion that the protective immune responses elicited by the liposomal gD epitope vaccine primarily involves Th1 type CD4 cells, as well as CD8 cells, and that NK cells do not contribute significantly to immune control of this infection.

From this data, several important observations can be made. First, the presentation of the target antigens in the liposomal format elicits a strong, protective immune response. Second, L-AG-HD vaccines provide a level of protection at least as good as traditional whole virus vaccines even though the immune response is targeted to one specific antigen. Third, production of protective antigen specific antibodies and T-cell mediated immune responses can be stimulated by the L-AG-HD vaccines. Taken together, the *VesiVax®* vaccine platform technology facilitates the rapid design and engineering of safe and effective vaccines by eliciting protective immune responses whether they are mediated by B or T cells or a combination of both.

7. PERSPECTIVE

VesiVax® vaccines represent a breakthrough improvement in liposome-based vaccine technology compared to more traditional liposome approaches for several reasons. First, the pathogen specific antigen(s) or epitope(s) can be chemically synthesized or genetically fused using standard molecular biology methods to a proprietary HD to form a protein that promotes stable association of the epitope with the membrane bilayer structure of the liposome. This novel innovation eliminates the need for covalent conjugation of the antigen to the lipids in the bilayer, a process that is often plagued with problems such as inefficient and unpredictable coupling efficiencies and extra processing steps. Second, adjuvant molecules of various types can be incorporated into the liposomes to direct and further amplify the immune response. Following administration, the nanoscale size of the liposomes which are produced (<200 nm), probably allows them to circulate in the body long enough to enter the lymph nodes and spleen and be taken up by the appropriate macrophages, thereby

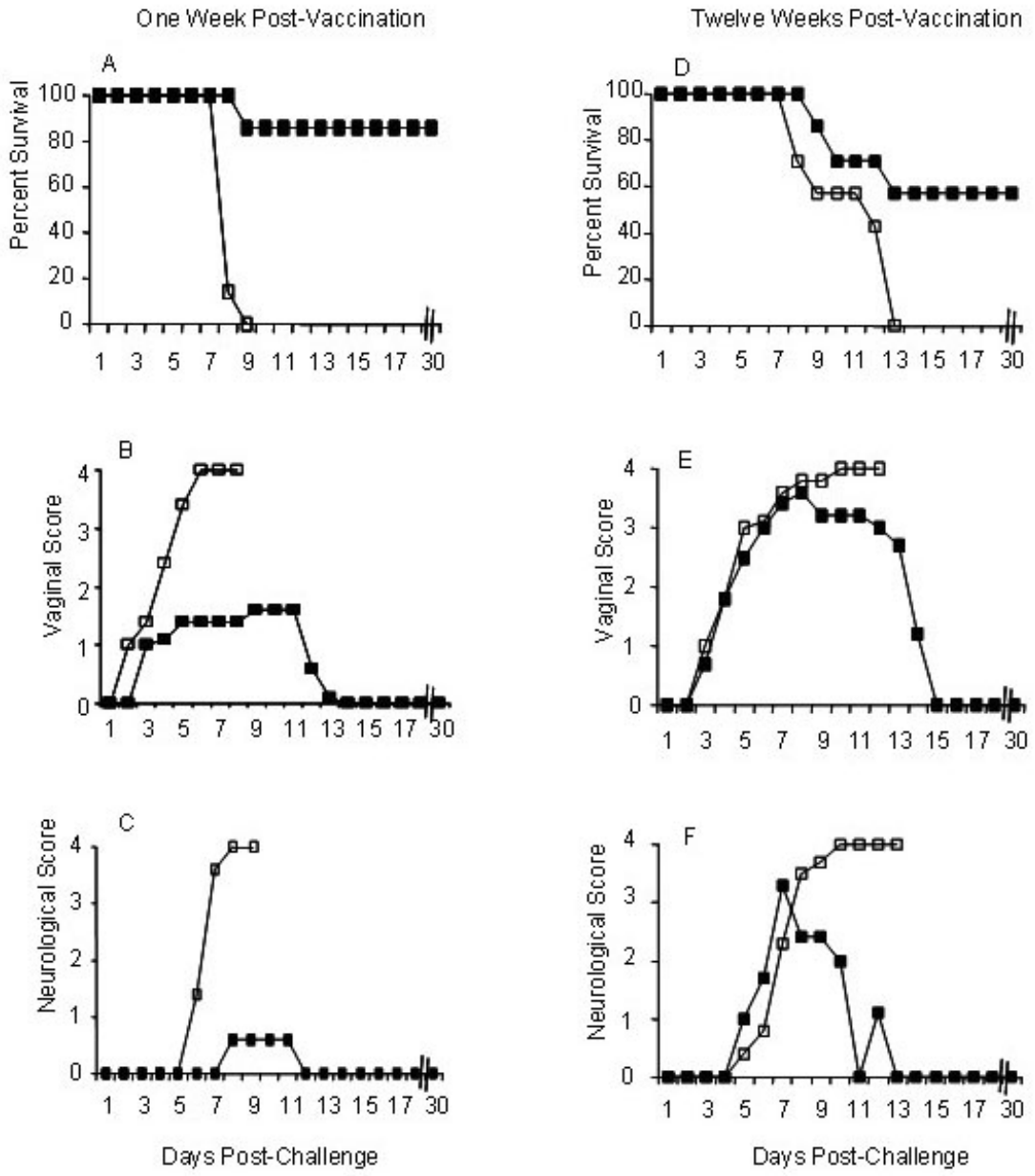

Figure 8. Immune protection from HSV-2 challenge of female BALB/c mice one week and 12 weeks post-vaccination with L-gD23-HD. Groups of BALB/c mice (n=7/group) were immunized with three subcutaneous injections of L-gD23-HD (80µg/mouse gD23/dose) or buffer at day 0, week 4 and week 8. All mice were primed with depoprovera on day -7 and day -1 prior to intravaginal challenge. One week (Figure 8 A,B,C) or 12 weeks (Figure 8 D,E,F) post-vaccination, mice were intravaginally challenged with 10 LD_{50} HSV2, and monitored for survival, vaginal and neurological signs based on a scoring system as follows: Vaginal Score, 0-4 scale with 0 = no lesions and 4 = severe lesions; Neurological Score, 0-4 scale with 0 = no paralysis to 4 = paralysis of both hind limbs. $P<0.05$, L-gD23-HD survival compared to buffer control (Kaplan Meier Log Rank test). Symbol legend: L-gD23-HD, -■-; Buffer control, -□-.

stimulating an effective systemic immune response rather than the localized, restricted response associated with larger, multilamellar liposomes. In addition, the components of *VesiVax®* vaccines are derived from non-pathogenic sources and hence, safety concerns are not expected to be an issue. Finally, *VesiVax®* vaccines are amenable to alternative routes of administration such as intranasal, which may prove to be a more acceptable approach for mass distribution and administration of vaccines to protect the public from a natural outbreak of an emerging pathogen as well as an attack with a biological weapon.

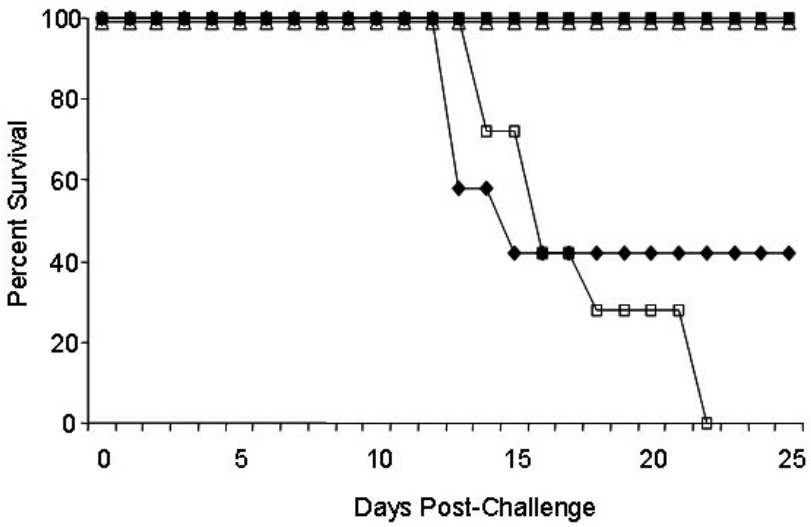

Figure 9. Passive protection of naïve mice from influenza X-88 challenge by M2e immune sera. Naïve BALB/c mice (n=7/group) were injected intraperitoneally with 0.3ml of immune sera at -12 hours and -2 hours prior to i.n. challenge with 10 LD_{50} of the X-88 strain of influenza virus. The immune sera were obtained from mice immunized s.c. day 0 and i.n. week 8 with L-M2e-HD with MPL (15µg M2e/dose), UV-inactivated X-88 (dose=10 LD_{50} non-inactivated X-88) or control liposomes with MPL. Groups of mice given serum from mice immunized with L-M2e-HD (anti-M2e ELISA titer = 13,120) or from mice immunized with UV inactivated X-88 (anti-X-88 ELISA titer = 81,920) had significantly better survival than the liposome control ($p<0.05$). Symbol legend: L-M2-HD (13,312 titer), -■-; L-M2-HD (5,120 titer), -♦-; UV inactivated X-88 (81,920 titer), -Δ-; Control liposome, -□-. Adapted with permission from (34).

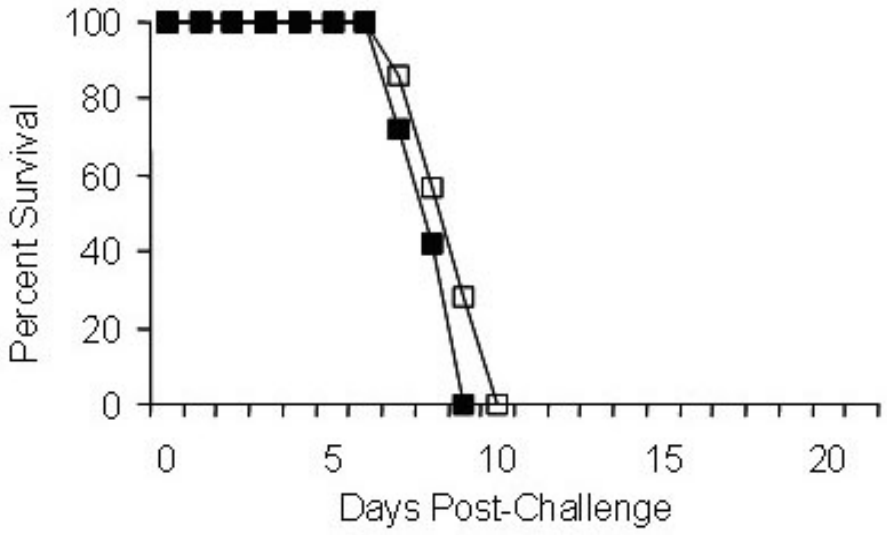

Figure 10. Adoptive transfer of splenocytes from L-M2e-HD vaccinated mice to naïve mice followed by challenge with the X-88 strain of influenza virus. Naïve BALB/c mice (n=7/group) were injected intraperitoneally with 0.3ml of a cellular suspension of splenocytes isolated from mice vaccinated with L-M2e-HD at -12 hours and -2 hours prior to i.n. challenge with 10 LD_{50} of the X-88 strain of influenza virus. The splenocytes were obtained from mice immunized s.c. day 0 and i.n. week 8 with L-M2e-HD with MPL (15µg M2e/dose), UV-inactivated X-88 (dose=10 LD_{50} non-inactivated X-88) or control liposomes with MPL. Groups of mice immunized with L-M2e-HD or mice immunized with the liposome control did not show significantly better survival ($p<0.05$). Symbol legend: L-M2e-HD (-■-); control liposomes (-□-).

VesiVax® vaccines possess a number of other significant advantages over classical pathogen-based approaches to vaccine development. The *VesiVax*® system is extremely flexible and in principle, as long as a target antigen or epitope is known, a vaccine against a specific pathogen can quickly and easily be generated. The

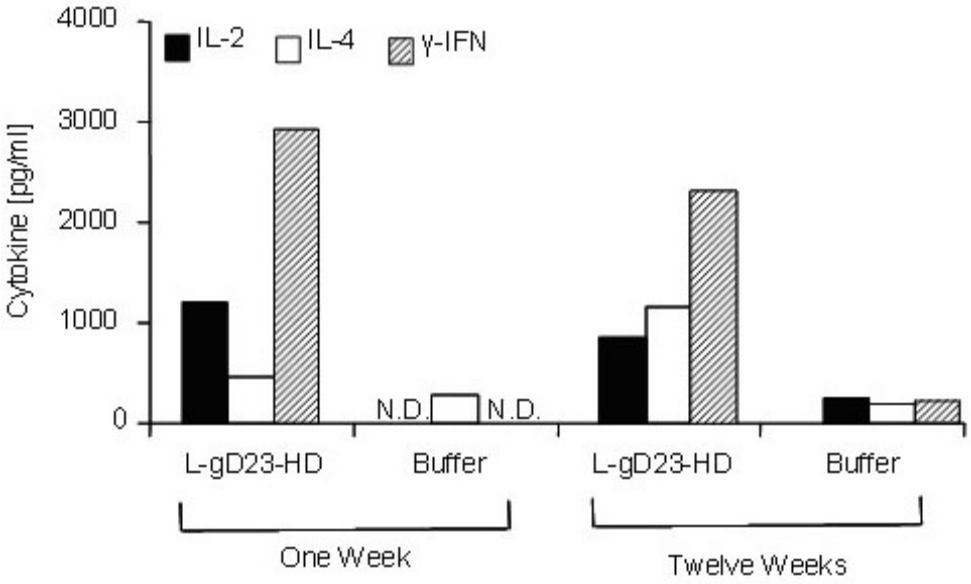

Figure 11. Cytokine analysis by ELISA. Splenocytes from BALB/c female mice subcutaneously vaccinated on day 0, at weeks 4 and 8 with L-gD23-HD (60µg gD23) or PBS buffer (n=5/group), were collected on wk 9. The spleens from 2 or 3 mice were pooled to give a total of two spleen preparations/treatment group. Each spleen preparation ($3x10^6$cells/well) was incubated in triplicate with gD23 (20mg/well) for 3 days and the supernates tested for IL-4, IL-2 and γ-IFN by ELISA.

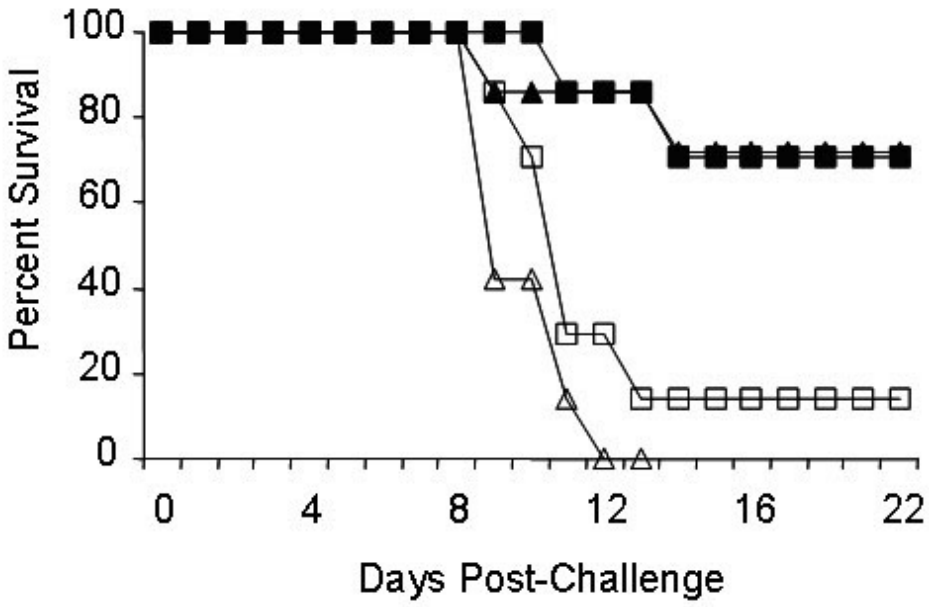

Figure 12. Beige and IL-4 knockout mouse study. IL-4 Knockout female mice (n=6/group) were vaccinated subcutaneously with L-gD23-HD (60µg/dose) or control liposomes on day 0 and at week 8. Beige female mice (n=7/group) were vaccinated subcutaneously with L-gD23-HD or buffer day 0, week 4 and week 8. All mice were primed with depoprovera on day -7 and day -1 prior to intravaginal challenge on week 9 with 10 LD_{50} HSV2 determined for each strain by infection dose studies in the intravaginal model. Mice were monitored for morbidity for 22 days. Symbol legend: L-gD23-HD IL-4 Knockout group (-■-); L-gD23-HD Beige group (-▲-); Beige control group (-Δ-) and; IL-4 Knockout control group (-□-).

advantages of *VesiVax®* technology for the rapid production of new vaccines are: 1) different epitopes can be readily exchanged by "cutting and pasting" them into a recombinant vector that has been engineered to provide for maximal flexibility in vaccine design; 2) larger antigen sequences containing many target epitopes (*i.e.,* whole proteins) or subunits (*e.g.,* envelope proteins or receptor domains) can also be inserted if required; 3) the antigenic

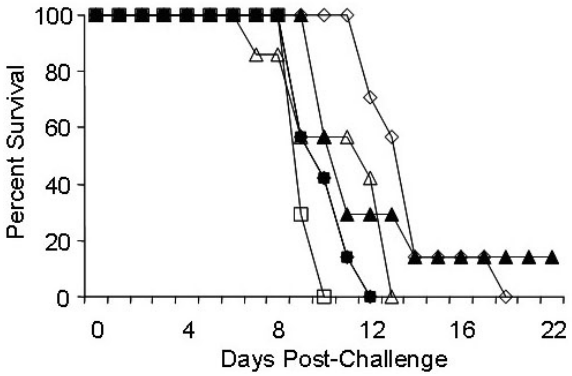

Figure 13. IL-12, γ-IFN and ß-2 microglobulin knockout mouse study. γ-IFN knockout (n=6/group), IL-12 knockout (n=6/group) and β-2 microglobulin knockout (n=7/group) female mice were vaccinated subcutaneously with L-gD23-HD or control liposomes on day 0 and at week 8. All mice were primed with depoprovera on day -7 and day -1 prior to intravaginal challenge on week 9 with 10 LD_{50} HSV2 determined for each strain by LD_{50} dose studies in the intravaginal model. There was no significant difference between any of the immunized and the corresponding control groups. Symbol legend: L-gD23-HD γ-IFN group (-■-); L-gD23-HD IL-12 group (-▲-); L-gD23-HD β-2 microglobulin group (-♦-); γ-IFN control group (-□-); IL-12 control group (-Δ-) and; β-2 microglobulin control group (-◊-).

construct is produced by standard methods; 4) intricate, time consuming and, in the case of vaccines against biological weapons, potentially hazardous and expensive production methods associated with whole pathogen vaccines are eliminated; 5) the shelf life stability of the liposomes is likely to be much longer than that of microbial vaccines; 6) precise control over the processes used to manufacture *VesiVax®* vaccines is possible, resulting in a commercially viable approach to rapid vaccine development and large scale production and; 7) adverse side effects linked to the use of certain adjuvants, such as alum or associated with the use of intact agents, such as killed or attenuated viruses or bacteria, are minimized. Because of these advantages, the *VesiVax®* system should provide a new method for responding readily to the emergence of new pathogens.

8. ACKNOWLEDGMENTS

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9. REFERENCES

1. R. W. Ellis: Product development plan for new vaccine technologies. *Vaccine* 19, 1559-1566 (2001)
2. J. M. Lane, F. L. Ruben, E. Abrutyn and J.D. Millar: Deaths attributable to smallpox vaccination, 1959 to 1966, and 1968. *JAMA* 212, 441-444 (1970)
3. A. D. Langmuir, D. J. Bregman, L. T. Kurland, N. Nathanson and M. Victor: An epidemiologic and clinical evaluation of guillain-barre syndrome reported in association with the administration of swine influenza vaccines. *Am J Epidemiol* 119, 841-879 (1984)
4. N. Yamane and H. Uemura: Serological examination of IgE- and IgG- specific antibodies to egg protein during influenza virus immunization. *Epidemiol Infect* 100, 291-299 (1988)
5. L. J. Baraff, C. R. Manclark, J. D. Cherry, P. Christenson and S. M. Marcy: Analyses of adverse reactions to diphtheria and tetanus toxoids and pertussis vaccine by vaccine lot, endotoxin content, pertussis vaccine potency and percentage of mouse weight gain. *Pediatr Infect Dis J* 8, 502-507 (1989)
6. A. Lotte, O. Wasz-Hockert, N. Poisson, N. Dumitrescu, M. Verron and E. Couvet: BCG complications. Estimates of the risks among vaccinated subjects and statistical analysis of their main characteristics. *Adv Tuberc Res* 21, 107-193 (1984)
7. N. Bourne, F. J. Bravo, M. Francotte, D. I. Bernstein,M. G. Myers, M. Slaoui and L. R. Stanberry: Herpes simplex virus (HSV) type 2 glycoprotein D subunit vaccines and protection against genital HSV-1 or HSV-2 disease in guinea pigs. *J Infect Dis* 187, 542-549 (2003)
8. S. Hu, G. D. Plowman, P. Sridhar, U. S. Stevenson, J. P. Brown and C. D. Estin: Characterization of a recombinant vaccinia virus expressing human melanoma-associated antigen p97. *J Virol* 62, 176-180 (1988)
9. B. Nardelli, J. P. Defoort, W. Huang and J. P. Tam: Design of a complete synthetic peptide-based aids vaccine with a built-in adjuvant. *Vaccine* 8, 1405-1407 (1992)
10. J. P. Briand, S. Muller and M. H. van Regenmortel: Synthetic peptides as antigens: pitfalls of conjugation methods. *J Immunol Meth* 78, 59-69 (1985)
11. R. Levi, E. Aboud-Pirak, C. Leclerc, G. H. Lowell and R. Arnon: Intranasal immunization of mice against influenza with synthetic peptides anchored to proteosomes. *Vaccine* 13, 1353-1359 (2002)
12. E. Watari, B. Dietzschold, G. Szokan and E. Heber-Katz: A synthetic peptide induces long-term protection from lethal infection with herpes simplex virus 2. *J Exp Med* 165, 459-470 (1987)
13. J. B. Ulmer, J. J. Donnelly, S. E. Parker, G. H. Rhodes, P. L. Felgner, V. J. Dwarki, S. H. Gromkowski, R. R. Deck, C. M. DeWitt, A. Friedman, L. A. Hawe, K. R. Leander, D. Martinez, H. C. Perry, J. W. Shiver, D. L. Montgomery and M. A. Liu: Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* 259, 1745-1749 (1993)
14. S. L. Epstein, T. M. Tumpey, J. A. Misplon, C. Y. Lo, L. A. Cooper, K. Subbarao, M Renshaw, S. Sambhara and J. M. Katz: DNA vaccine expressing conserved influenza virus proteins protective against H5N1 challenge infection in mice. *Emerg Infect Dis* 8, 796-801 (2002)

15. N. Bourne, L. R. Stanberry, D. I. Bernstein and D. Lew: DNA immunization against experimental genital herpes simplex virus infection. *J Infect Dis* 173, 800-807 (1996)
16. R. M. Zinkernagel, S. Ehl, P. Aichele, S. Oehen, T. Kundig and H. Hengarten: Antigen localization regulates immune responses in a dose- and time-dependent fashion: a geographical view of immune reactivity. *Immunol Rev* 156, 199-209 (1997)
17. V. E. J. C. Schijns: Induction and direction of immune responses by vaccine adjuvants. *Crit Rev Immunol* 21, 75-85 (2001)
18. E. Claassen, Liposomes in in vivo immunology. *Vesicles*, Rosoff M, ed. Marcel Dekker, Inc., New York (1996)
19. I. J. Fidler, S. Sone, W. E. Fogler and Z. L. Barnes: Eradication of spontaneous metastases and activation of alveolar macrophages by intravenous injection of liposomes containing muramyl dipeptide. *Proc Natl Acad Sci USA* 78, 1680-1684 (1981)
20. M. J. Gilbreath, C. A. Nacy, D. L. Hoover, C. R. Alving, G. M. Swartz and M. S. Meltzer: Macrophage activation for microbicidal activity against *leishmania major:* Inhibition of lymphokine activation by phosphatidylcholine-phosphatidylserine liposomes. *J Immunol* 134, 3420-3425 (1985)
21. M. Garcia, M. A. Alsina, F. Reig and I. Haro: Liposomes as vehicles for the presentation of a synthetic peptide containing an epitope of hepatitis A virus. *Vaccine* 18, 276-283 (1999)
22. B. Morein and K. Simons: Subunit vaccines against enveloped viruses: virosomes, micelles and other protein complexes. *Vaccine* 3, 83-93 (1985)
23. G. Gregoriadis: Engineering liposomes for drug delivery: progress and problems. *Trends Biotechnol* 13, 527-537 (1995)
24. H. M. Therien and E. Shahum: Importance of physical association between antigen and liposomes in liposomes adjuvanticity. *Immunol Lett* 22, 253-258 (1989)
25. C. R. Alving, R. L. Richards, J. Moss, L. I. Alving, J. D. Clements, T. Shiba, S. Kotani, R. A. Wirtz and W. T. Hockmeyer: Effectiveness of liposomes as potential carriers of vaccines: applications to cholera toxin and human malaria sporozoite antigen. *Vaccine* 4, 166-172 (1986).
26. C. R. Alving, V. Koulchin, G. M. Glenn and M. Rao: Liposomes as carriers of peptide antigens: induction of antibodies and cytotoxic T lymphocytes to conjugated and unconjugated peptides. *Immunol Rev* 145, 5-31 (1995)
27. K. Brynestad, B. Babbitt, L. Huang and B. T. Rouse: Influence of peptide acylation, liposome incorporation, and synthetic immunomodulators on the immunogenicity of a 1-23 peptide of glycoprotein D of herpes simplex virus: Implications for subunit vaccines. *J Virol* 64, 680-685 (1990)
28. P. T. Naylor, H. S. Larsen, L. Huang and B. T. Rouse: In vivo induction of anti-herpes simplex virus immune response by type 1 antigens and lipid A incorporated into liposomes. *Infect Immun* 36, 1209-1216 (1982)
29. G. Fujii, Liposomal amphotericin B (AmBisome): realization of the drug delivery concept. *Vesicles*, Rosoff M, ed. Marcel Dekker, Inc., New York (1996)
30. G. Fujii, S. Horvath, S. Woodward, F. Eiserling and D. Eisenberg: A molecular model for membrane fusion based on solution studies of an amphiphilic peptide from HIV gp41. *Protein Science* 1, 1454-1464 (1992)
31. G. Fujii, M. E. Selsted and D. Eisenberg: Defensins promote fusion and lysis of negatively charged membranes. *Protein Science* 2, 1301-1312 (1993)
32. G. Fujii: To fuse or not to fuse: the effects of electrostatic interactions, hydrophobic forces, and structural amphiphilicity on protein-mediated membrane destabilization. *Adv Drug Deliv Rev* 38, 257-277 (1999)
33. S. V. Avalos, M. Reid, W. A. Ernst, A. Alvarado, L. BenMohamed, G. Fujii and J. P. Adler-Moore: Role of CD4 and CD8 cell in protection of mice against Herpes Simplex II (HSV-2) challenge following immunization with a novel liposomal gD epitope Vaccine (L-gDe). American Society of Virology 2005
34. W. A. Ernst, H. J. Kim, T. M. Tumpey, A. D. A. Jansen, W. Tai, D. V. Cramer, J. P. Adler-Moore and G. Fujii: Protection against H1, H5, H6 and H9 influenza A infection with liposomal matrix 2 epitope vaccines. *Vaccine* 24, 5158-5168 (2006)
35. S. L. Zebedee and R. A. Lamb: Influenza A virus M2 protein: monoclonal antibody restriction of virus growth and detection of M2 in virions. *J Virol* 62, 2762-2772 (1988)
36. J. J. Treanor, E. L. Tierney, S. L. Zebedee, R. A. Lamb and B. R. Murphy: Passively transferred monoclonal antibody to the M2 protein inhibits influenza A virus replication in mice. *J Virol* 64, 1375-1377 (1990)
37. R. A. Black, P. A. Rota, N. Gorodkova, H. D. Klenk and A. P. Kendal: Antibody response to the M2 protein of influenza A virus expressed in insect cells. *J Gen Virol* 74, 143-146 (1993)
38. V. A. Slepushkin, J. M. Katz, R. A. Black, W. C. Gamble, P. A. Rota and N. J. Cox: Protection of mice against influenza A virus challenge by vaccination with baculovirus-expressed M2 protein. *Vaccine* 13, 1399-1402 (1995)
39. S. Neirynck, T. Deroo, X. Saelens, P. Vanlandschoot, W. M. Jou and W. Fiers: A universal influenza A vaccine based on the extracellular domain of the M2 protein. *Nature Med* 5, 1157-1163 (1999)
40. A. M. Frace, A. I. Klimov, T. Rowe, R. A. Black and J. M. Cox: Modified M2 proteins produce heterotypic immunity against influenza A virus. *Vaccine* 17, 2237-2244 (1999)
41. P. W. Berman, T. Gregory, D. Crase and L. A. Lasky: Protection from genital herpes simplex virus type 2 infection by vaccination with cloned type 1 glycoprotein D. *Science* 227, 1490-1492 (1985)
42. R. J. Eisenberg, C. P. Cerini, C. J. Heilman, A. D. Joseph, B. Dietzschold, E. Golub, D. Long, M. Ponce de Leon and G. H. Cohen: Synthetic glycoprotein D-related peptides protect mice against herpes simplex virus challenge. *J Virol* 56, 1014-1017 (1985)
43. D. Long, T. J. Madara, M. Ponce de Leon, G. H. Cohen, P. C. Montgomery and R. J. Eisenberg: Glycoprotein D protects mice against lethal challenge with herpes simplex virus types 1 and 2. *Infect Immun* 37, 761-764 (1984)
44. E. Paoletti, B. R. Lipinskas, C. Samsonoff, S. Mercer and D. Panicali: Construction of live vaccines using

genetically engineered poxviruses: biological activity of vaccinia virus recombinants expressing the hepatitis B virus surface antigen and the herpes simplex virus glycoprotein D. *Proc Natl Acad Sci USA* 81, 193-197 (1984)
45. L. J. York, D. P. Giorgio and E. M. Mishkin: Immunomodulatory effects of HSV2 glycoprotein D in HSV1 infected mice: implications for immunotherapy of recurrent HSV infection. *Vaccine* 13, 1706-1712 (1995)
46. S. Martin, B. Moss, P. W. Berman, L. A. Laskey and B. T. Rouse: Mechanisms of antiviral immunity induced by a vaccinia virus recombinant expressing herpes simplex virus type 1 glycoprotein D: Cytotoxic T cells. *J Virol* 61, 726-734 (1987)
47. J. W. Torseth, G. H. Cohen, R. J. Eisenberg, P. W. Berman, L. A. Lasky, C. P. Cerini, C. J. Heilman, S. Kerwar and T. C. Merigan: Native and recombinant herpes simplex virus type 1 envelope proteins induce human immune T-Lymphocyte responses. *J Virol* 61, 1532-1539 (1987)
48. A. O. Fuller and P. G. Spear: Anti-glycoprotein D antibodies that permit adsorption but block infection by herpes simplex virus 1 prevent virion-cell fusion at the cell surface. *Proc Natl Acad Sci USA* 84, 5454-5458 (1987)
49. A. O. Fuller and P. G. Spear: Specificities of monoclonal and polyclonal antibodies that inhibit adsorption of herpes simplex virus to cells and lack of inhibition by potent neutralizing antibodies. *J Virol* 55, 475-482 (1985)
50. A. G. Noble, G. T. Lee, R. Sprague, M. L. Parish and P. G. Spear: Anti-gD monoclonal antibodies inhibit cell fusion induced by herpes simplex virus type 1. *Virology* 129, 218-224 (1983)
51. G. H. Cohen, B. Dietzschold, M. Ponce de Leon, D. Long, E. Golub, A. Varrichio, L. Pereira and R. J. Eisenberg: Localization and synthesis of an antigenic determinant of herpes simplex virus glycoprotein D that stimulated the production of neutralizing antibody. *J Virol* 49, 102-108 (1984)
52. E. C. DeFreitas, B. Dietzschold and H. Koprowski: Human T-lymphocyte response in vitro to synthetic peptides of herpes simplex virus glycoprotein D. *Proc Natl Acad Sci USA* 82, 3425-3429 (1985)
53. E. Heber-Katz, M. Hollosi, B. Dietzschold, F. Hudecz and G. D. Fasman: The T cell response to the glycoprotein D of the herpes simplex virus: the significance of antigen conformation. *J Immunol* 135, 1385-1390 (1985)
54. J. H. Wyckoff III, A. P. Osmand, R. J. Eisenberg, G. H. Cohen and B. T. Rouse: Functional T cell recognition of synthetic peptides corresponding to continuous antibody epitopes of herpes simplex virus type 1 glycoprotein D. *Immunobiology* 177, 134-148 (1988)
55. B. E. Johansson, B. Grajower and E. D. Kilbourne: Infection-permissive immunization with influenza virus neuraminidase prevents weight loss in infected mice. *Vaccine* 11, 1037-1039 (1993)
56. A. Jegerlehner, N. Schmitz, T. Storni and M. F. Bachmann: Influenza A vaccine based on the extracellular domain of M2: weak protection mediated via antibody-dependent NK cell activity. *J Immunol* 172, 5598-5605 (2004)

Key Words: Liposome, vaccine, VesiVax®, influenza, genital herpes, HSV2, antigen, epitope, M2, gD, Review

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