Protection Generated Against Influenza in Mice Vaccinated with Different Dosage Regimens of Liposomes Containing Only Pam3CAG and No Influenza Proteins

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Abstract

Background: Several commercial influenza vaccines stimulate an adaptive immune response to yearly mutating influenza proteins. Previous experiments with a liposomal (Lp) influenza vaccine containing Pam3CAG only and no proteins generated a protective innate immune response against H1N1 virus. In this study, we examined the protection provided by different vaccination dosage regimens with liposomes containing only Pam3CAG.

Methods: Different doses of a liposomal VesiVax® vaccine, Provided by Molecular Express Inc., containing the adjuvant Pam3CAG, were used to vaccinate mice (n=9/group) and included: intranasal (IN) d4, d2 pre-challenge; subcutaneous (SC) d2 pre-challenge; IN d2, d4 post-challenge; SC d2 post challenge; SC buffer d2 pre-challenge. Mice were challenged d0 IN with influenza and monitored for morbidity 2X/day to d21.

Results: Using the Pam3CAG liposomes, the dosing regimen that was significantly better than all other groups (89% survival) was the IN d4 and d2 pre-challenge ($p \leq 0.0006$).

Conclusions: The innate immune response generated by the VesiVax liposomes with just Pam3CAG and no proteins provided significant protection against the influenza viral challenge, suggesting that this approach could be used to protect animals regardless of the influenza protein mutations.

Introduction

The surface antigens (hemagglutinin (HA) and neuroaminidase (NA)) of the influenza viruses can randomly and frequently change, which help the virus escape the memory response of the adaptive immune system generated by previous infection with influenza. This results in re-infection of the same host, making the virus more virulent and as a result, this more virulent virus could cause an extensive epidemic or even pandemic. Therefore, it is important to formulate a universal vaccine that utilizes immunostimulatory adjuvant molecules (IAMs) to stimulate the innate immune response, which is faster, non-specific, and produces cytokines that stimulate macrophages, neutrophils and natural killer cells[1-5].

Previous experiments have shown that Pam3CAG, which is a synthetic TLR2 agonist, stimulates antibody mediated responses and the production of pro-inflammatory cytokines such as TNF-alpha[6]. This adjuvant when entrapped within CMI VesiVax liposomes and given subcutaneously day 0, and intranasally day 24/26 and day 56/58, was very effective at eliciting a protective innate immune response in BALB/c mice challenged day 70 with10X LD50 H1N1 influenza virus. Therefore, Pam3CAG was the adjuvant used in the present experiment to determine the vaccination dose regimen that would provide the maximum protection against 10X LD50 H1N1 challenge if the vaccine was administered either within a few days before or after the viral challenge. This would eliminate the need for vaccinating the mice over a period of 8 weeks and potentially provide protection when there is known exposure to the influenza virus.

The objective of this experiment was to determine the Pam3CAG dose regimen that would protect BALB/c mice against H1N1 challenge primarily through an innate immune response. The mice were vaccinated using four different dose regimens of the adjuvant Pam3CAG in VesiVax liposomes to stimulate an innate immune response. The four regimens were as follows: Intranasally (IN) 4 and 2 days pre-challenge, subcutaneously (SC) 2 days pre-challenge, IN 2 and 4 days post challenge, SC 2 days post challenge. The nine mice in each of the 5 groups (4 experimental and 1 control) were followed for survival and morbidity over time.

The advantage of the present approach is that it would allow us to quickly administer the adjuvant vaccine in the case of an influenza pandemic for which the HA and NA vaccine was not available. This approach could also be used to protect individuals from possible viral infection, for example for airplane travelers who are often exposed to other passengers infected with influenza or even other viruses.

Results

Previous Study: Comparison of Efficacy Against Influenza Challenge Using Liposomes with Different Adjuvants and no M2e Peptide

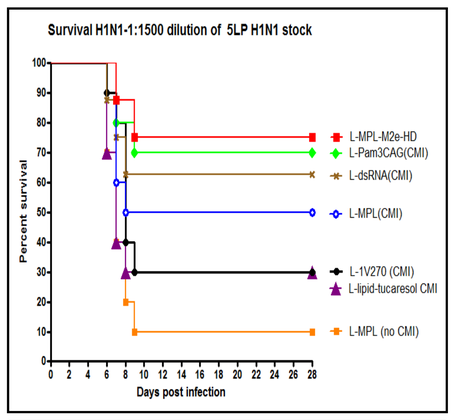

Figure 1A: Survival following H1N1 challenge was 75%, 70%, and 63% for L-MPL-M2e-HD (positive control), L-Pam3CAG (CMI), L-mycoviral dsRNA (CMI), respectively, which was significantly higher than that of the negative control, MPL in non-CMI liposomes (L-MPL) (10% survival, $P<0.02$) and higher than that of the liposomes with the other adjuvants.

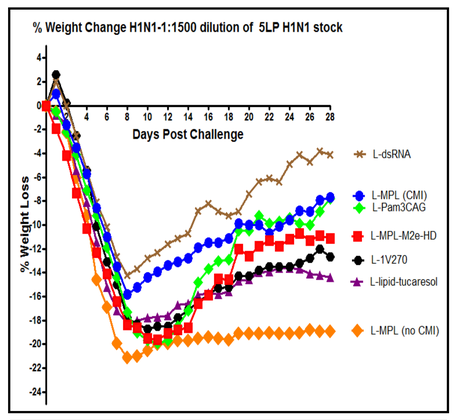

Figure 1B: Weight loss following H1N1 challenge was significantly greater for the negative control L-MPL (no CMI) compared to all other groups. L-mycoviral dsRNA (CMI) produced significantly less weight loss than the other adjuvant groups ($P \leq 0.04$). L-MPL (CMI) produced the second least weight loss which was significantly lower than the other adjuvant groups, except for L-Pam3CAG (CMI) ($P \leq 0.005$).

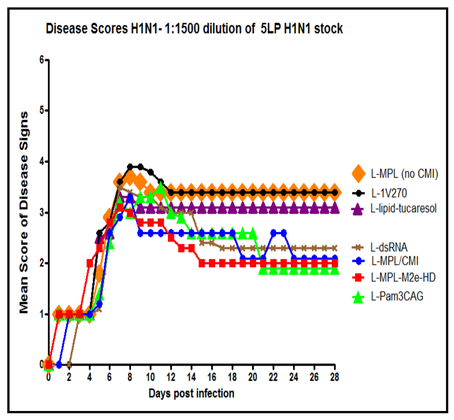

Figure 1C: H1N1-challanged SW mice vaccinated with L-Pam3CAG, L-MPL-M2e-HD, L-MPL (CMI), and L-mycoviral dsRNA had the lowest disease scores. The scores were not significantly different between L-Pam3CAG, L-MPL (CMI), and L-mycoviral dsRNA, but these groups had significantly lower disease scores compared to the other groups with higher scores ($P \leq 0.005$ between these groups and L-lipid-tucaresol).

Current Study: Comparison of Efficacy for H1N1 Influenza Challenge Using Liposomes Containing the Pam3CAG Adjuvant Only with Different Dosage Regimens

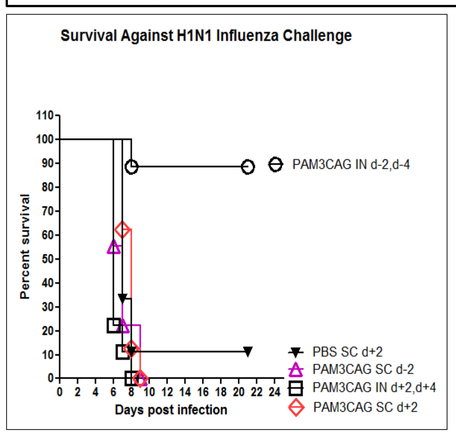

Figure 2: Survival following H1N1 challenge was 89% for the PAM3CAG IN d-2,d-4 treatment group, which was significantly higher than survival for all other groups ($p \leq 0.0006$) (n=9 mice/group).

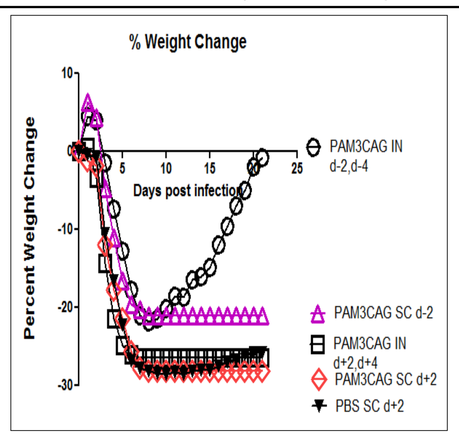

Figure 3A: Weight change following H1N1 challenge closely paralleled the survival data with the PAM3CAG IN d-2,d-4 being the only treatment that resulted in the animals recovering their initial weight by day 21.

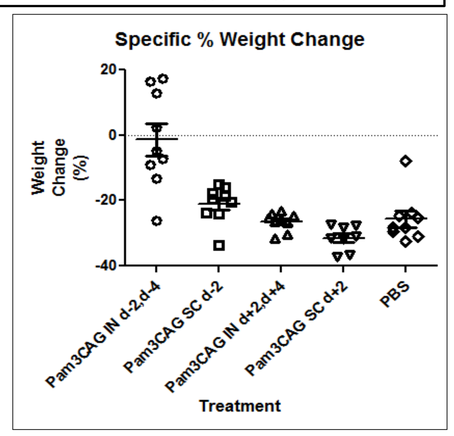

Figure 3B: Weight change for each mouse on the last day of the study (day 21) showed that mice given PAM3CAG IN d-2,d-4 had significantly less weight loss than all other treatment groups ($p \leq 0.0093$). The group given SC pre-challenge d-2 had significantly less weight loss than the groups that received the treatment post-challenge ($p \leq 0.0106$). For the groups that were given the treatment post-challenge, the mice given the treatment IN had significantly less weight loss than the mice given the post-challenge treatment SC ($p \leq 0.0069$).

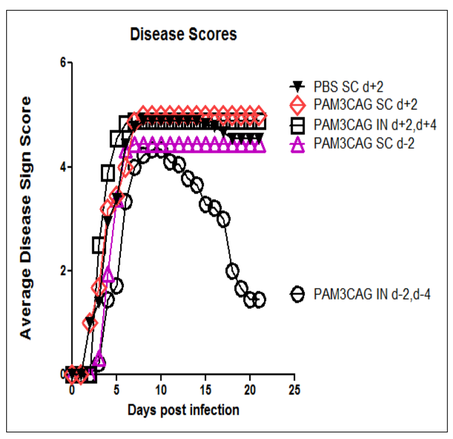

Figure 4A: Disease scores following H1N1 challenge closely paralleled the survival data with the PAM3CAG IN d-2,d-4 being the only treatment that resulted in a marked decrease in disease scores by day 21.

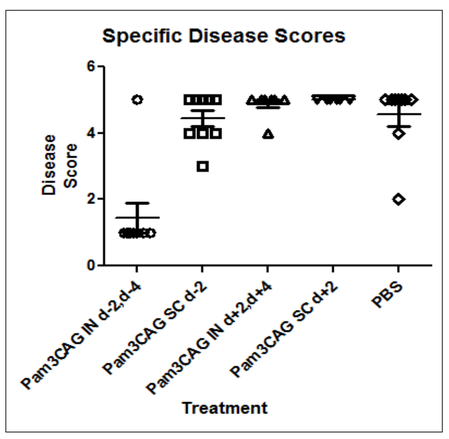

Figure 4B: Disease score for each mouse on the last day of the study (day 21) showed that mice given PAM3CAG IN d-2,d-4 had significantly lower disease scores than all other treatment groups ($p \leq 0.0015$). The group given SC pre-challenge d-2 had significantly lower disease scores than the group that received SC treatment post-challenge ($p = 0.0441$).

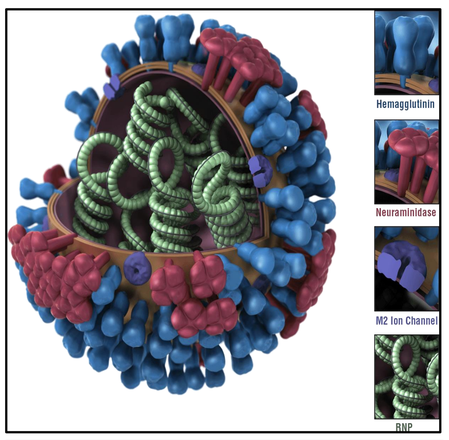

Figure 5: The influenza virus.

Conclusions

- The innate immune response produced by vaccinating the mice intranasally day -4 and day -2 prior to challenge with liposomes containing just the adjuvant Pam3CAG without viral proteins was protective against a lethal H1N1 influenza challenge.
- This approach eliminates the need for weeks of prior vaccination with a vaccine to stimulate a protective response to a specific influenza protein.
- This vaccine formulation by stimulating the innate immune response can potentially be used to protect individuals against an influenza pandemic when there is no time to produce a vaccine targeted to a specific influenza HA protein.
- Future studies

-Vaccinating the mice with other adjuvants such as dsRNA or MPL in the VesiVax CMI liposomes

-Examining the mechanism of this innate immune protection and finding out how long the protection lasts

-Determining if there is an increase in anti-influenza antibodies following challenge due to the cytokines generated by the innate immune response

Materials & Methods

Timeline:
Day -4 and Day -2: IN vaccination with VesiVax liposomes (n=9 mice.group).
Day -2: SC vaccination with VesiVax liposomes (n=9 mice/group)
Day -2: SC vaccination with PBS (n=9 mice/group).
Day 0: H1N1 IN challenge (all groups)(n=45 mice).
Day +2 and Day +4" IN vaccination with VesiVax liposomes (n=9 mice.group)
Day +2: SC vaccination with VesiVax liposomes (n=9 mice/group)..
Day +20: End of experiment.

Group	Vaccine Formulation	Dosage	µg/dose	Dose Volume (µL)	Vaccination Days	Number of Mice
1	Pam3CAG	1 IN	12.5 µg /dose/day=25 µg total over 2 days	40 µL /day= 80 µL total	4 days and 2 days before challenge	9
2	Pam3CAG	1 SC	25 µg	80 µL	2 days before challenge	9
3	Pam3CAG	1 IN	12.5 µg /dose/day=25 µg total over 2 days	40 µL /day =80 µL total	2 days and 4days post-challenge	9
4	Pam3CAG	1 SC	25 µg	80 µL	2 days post-challenge	9
5	PBS	1 SC	N/A	80 µL	2 days before challenge	9

-Mice were sedated prior to the IN doses and the IN challenge with ketamine (80 mg/kg) and xylazine (16 mg/kg) intraperitoneally.
-IN Solutions were applids to the nares slowly using a micropipettor infor inhalation.
-IN doses had to be divided into 2 days because mice can only take in 40 µL per day IN
.Solutions to be administered SC were given in a total volume of 80ul
-Mice were moniyotrf for survival and morbidity to day 20 - Surviving mice were euthanized using CO2 on day 20 post challenge.

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