ABSTRACT

Long term protection in Swiss Webster (SW) mice using a liposomal M2e Influenza A (L-M2e) vaccine

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Introduction: Previously we reported that L-M2e vaccine provided significant protection against viral challenge one week post-vaccination in SW mice. This study focused on how long that protection would last, with or without an additional L-M2e boost.

Methods: Mice were vaccinated with L-M2e (100ug M2e/dose, Groups A and C) or liposome control (Con, Groups B and D) subcutaneously d0 and intranasally (IN) d28 and d56 (n=21/Group). Only Groups A (M2e) and B (Con) were given an additional IN boost d84. Day 88, serum and spleens (n=6/group) were collected and 15 mice/group were challenged day 91 with 10XLD50 Influenza (PR8). Spleens were tested for cytokine production (multi-bead and EliSpot assays) and serum assayed for antiviral precipitating antibodies and anti-M2e IgG levels. Day 97 lungs were collected (n=5/group) for viral burden and remaining mice (n=10/group) were followed for morbidity.

Results: Mice given the extra boost (Group A) had less weight loss (P<0.0001), less disease signs (P<0.01) and prolonged survival (90%) versus mice not given the extra boost (50% survival, Group C), along with higher antiviral antibody titers versus Con (P<0.009). However, both Groups A and C had prolonged survival versus Con (0% survival) (P≤0.019), higher IFN-γ (P<0.02) and higher IgG1 and IgG2A levels versus Con (P<0.001).
**Conclusion:** Long term protection in outbred SW mice against lethal influenza virus infection could be achieved by L-M2e vaccination, with or without an extra IN boost. The additional IN boost, however, provides an enhanced level of protection with a more elevated antiviral antibody response.

**NARRATIVE**

Influenza A is responsible for both seasonal outbreaks (epidemics) as well as pandemics. The Spanish Flu (H1N1) pandemic of 1918 caused the deaths of between 25 and 50 million people [2]. In recent years, mortality from flu pandemics has decreased due to better diagnostic procedures and improved medical care. However, the Asian Flu (1957-1958) pandemic caused by H2N2 resulted in 2 million deaths, and the Hong Kong Flu (1968-1969) pandemic caused by H3N2 produced 1 million deaths. More recently, in the Avian Flu (2003-2009) pandemic caused by H5N1, there were 424 reported cases with 261 deaths (62% mortality), while the Swine Flu (2009-2010) pandemic caused by H1N1 resulted in 622,482 cases and at least 20,000 deaths (3% mortality) [3]. Other seasonal flu epidemics between 1960 and 2006 caused by H1N1, H2N2 or H3N2 strains caused 4,000 to 39,000 deaths with a mortality rate of 6-9% [4]. All of these pandemics and epidemics arise from the antigenic drift or shift of the viral hemagglutinin(HA) and viral neuraminidase(NA of the influenza type A virus. This situation creates the need for seasonal flu vaccines since the HA and NA targets of the vaccines keep changing [6].

There have been several recent attempts to develop a universal flu vaccine one of which focuses on the conserved regions of the M2 protein [6,7]. In our laboratory in collaboration with Molecular Express Inc., we have worked on the development of a liposomal M2 vaccine (L-M2e/MPL) which incorporates the M2e epitope (a 15 amino acid (aa) residue at the amino end of
the highly conserved region of the M2 protein) into unilamellar particles less than 100nm in size. The TLR4 adjuvant, monophosphoryl lipid A, is also incorporated into these liposomes. The 15aa residue of the M2 protein is not only highly conserved between H1N1 influenza strains, but also among strains of other A type influenza viruses including the bird flu (H5N1) [7].

Previous experiments in our laboratory with the L-M2e/MPL vaccine have shown that this vaccine generates protection in Swiss Webster mice against a severe viral challenge (250X LD50) when the mice are vaccinated with 100ug/dose of L-M2e using a vaccination regimen consisting of a subcutaneous (SC) prime on day 0 and intranasal (IN) boosts on days 28 and 56 followed one week later (day 63) by an IN Influenza A PR8 H1N1 challenge. In the present study we wanted to determine 1) if the protection generated by the L-M2e/MPL vaccine administered according to the regimen described above would last for up to five weeks post-vaccination and 2) if an additional IN boost at four weeks post-vaccination would be required to maintain protection up to five weeks post-vaccination.

We found that significant long-term protection could be achieved in Swiss Webster outbred mice vaccinated with 100ug M2e and challenged with influenza virus at five weeks after the initial vaccination regimen was completed, whether or not the mice received an additional boost at four weeks after the initial vaccination regimen was completed (90% and 50% survival, respectively, versus 0% survival with Liposomal controls without M2e). However, to maintain the initial 90% protection observed in mice challenged one week after the initial vaccination regimen was completed, the mice did require an additional L-M2e/MPL vaccine boost. Despite the differences in the extent of \textit{in vivo} efficacy, L-M2e/MPL vaccination lead to similar elevated levels of anti-M2e IgG antibodies in the mice, with or without the additional boost. In this ELISA assay, the anti-M2e antibodies were incubated with the M2e peptide alone,
suggesting a lack of direct correlation between anti-M2e IgG antibodies and the level of protection. In comparison, when the anti-M2e antibodies were reacted with the whole influenza virus, the precipitating antiviral antibody titer was significantly higher in the L-M2e/MPL group that received the additional boost compared to all other groups. This suggests that precipitation of the whole influenza virus by the anti-M2e antibodies provides a better correlate with the level of *in vivo* protection than the anti-M2e antibodies binding with just the M2e peptide.

Future studies are needed to determine the type of antibody associated with the precipitation of the whole virus, the mechanism for viral clearance from the body, and further testing of different vaccination regimens to maximize on the long-term protection provided by the L-M2e/MPL vaccine. Additionally, testing the L-M2e/MPL vaccine's efficacy against other strains of influenza (e.g. H3N2, H6N2) would support its use as a “universal” Influenza A vaccine.


8. Ernst, W. et all. Protection against H1, H5, H6 and H9 influenza A infection with liposomal matrix 2 epitope vaccines. Vaccine; 2006; 04.008; 6160-6171

Figure 1A – Survival of Swiss Webster mice vaccinated day 0, day 28, day 56 with 100ug of LM2e/HD-MPL vaccine with or without an additional boost on day 84 and challenged with 10XLD50 H1N1 PR8. Both vaccinated groups (A & C) had significantly higher survival than their respective controls ($P < 0.0001$ & $P=0.0096$) and there was a strong trend for higher survival in Group A vs Group C ($P=0.0625$).

Figure 1B – Percent weight change of vaccinated and non-vaccinated mice following H1N1 PR8 challenge (see Figure 1A for procedure). Mice from group A showed significantly less weight loss than both controls or test group C ($P<0.0001$); weight loss was not significantly different for group C compared to the controls during the first 8 days post challenge (prior to death of all controls).
Figure 2 – Precipitating Antibody Titer Assay. After incubating serum with whole virus (1:5 PR8 H1N1 virus), Group A mouse sera had a higher precipitating anti-viral titer compared to all other groups (P = 0.009) while the mouse sera from group C did not show an increase in precipitating anti-viral titer.

Figure 3 – Anti-M2e antibody concentration and immunoglobulin Isotype determined by ELISA. A: Both vaccinated groups, A and C, showed significantly elevated levels of IgG1 anti-M2e antibodies versus their respective controls (P=0.0022 and P=0.0043) but were not statistically significantly different from each other. B: Vaccinated groups A and C showed significantly higher IgG2A anti-M2e antibodies versus their respective controls (P=0.0022 and P=0.0043) but were not statistically significantly different from each other different. Additionally, there were no statistical differences when comparing the serum concentrations of IgG1 and IgG2A within groups A and C.