A mumps virus genotype G vaccine candidate displays enhanced neutralization of circulating variants over the current genotype A Jeryl Lynn vaccine

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Abstract

Despite widespread childhood mumps vaccination, outbreaks continue to occur, particularly among adults, which is believed to be due to a combination of waning immunity and antigenic drift. The mumps vaccine, in the currently approved Measles, Mumps, and Rubella (MMR), was developed in the 1960's as a live attenuated Jeryl Lynn virus belonging to the genotype A group of mumps virus based on a genetic classification system. Since the 1980's however, genotype A strains have ceased circulation. Although serum from Jeryl Lynn vaccinees can neutralize mumps viruses of all other genotypes in vitro, the potency of sera against heterologous genotypes is greatly reduced. This has resulted in calls for development of new, more effective mumps vaccines better representative of circulating strains of the virus. To this end, we created an attenuated mumps vaccine candidate expressing the consensus viral fusion (F) and hemagglutinin (HN) protein sequences of the genotype G strains (circulating globally for over a decade) on the backbone of the current Jeryl Lynn (JL) vaccine and named this vaccine candidate JL-G.

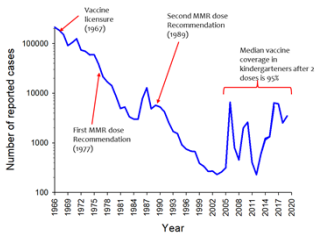
Methods: The immunogenicity of JL-G versus JL was assessed in rhesus macaques (n=5 per group) by testing serum collected at various time points following a second dose of each vaccine by plaque reduction neutralization assay against a panel of Mumps virus genotypes.

Results: The neutralizing geometric mean titers (GMT) of sera from the JL-G vaccinated animals against the JL-G virus was 18-fold higher as compared to sera from JL vaccinated animals. GMTs were also higher with JL-G sera when tested against JL and other genotypes (B, C, G, H, K, and N) as compared to JL sera. No fever or other adverse reaction was observed in any animal.

Conclusion: We successfully produced a mumps vaccine candidate that provided superior neutralization across all genotypes tested in this study when compared to the current JL vaccine. This enhanced ability to neutralize current circulating mumps strains may mitigate the effects of waning immunity in vaccinated individuals.

Introduction

Mumps is a relatively benign disease-causing flu like symptoms and most characteristically orchitis. In severe cases it can have neurological effects, such as meningitis and encephalitis. Historically, mumps has been a disease of childhood, but in recent years despite 95% median two dose MMR vaccine coverage among kindergarteners in the US, a resurgence has occurred primarily among young adults. A similar resurgence has been observed world-wide. Studies have demonstrated evidence of waning antibody immunity (Rubin et. al. JID 2008), although similar rates of antibody decline have been observed with measles and rubella without a resurgence in cases. Our studies have shown that while the current genotype A Jeryl Lynn (JL) mumps vaccine can neutralize other genotypes, these neutralizing titers are greatly reduced (Rubin et al JVI 2012). Genotype A has not been seen in circulation for about 40 years. Over the past 15 years, genotype G has been the predominant circulating strain in the US and most of the Western countries.



We propose that a resurgence may be a result of the differential humoral response due to antigenic differences coupled with waning antibody levels over time. We compare the cross-neutralizing antibody response of a circulating genotype G vaccine to the Jeryl Lynn genotype A vaccine.

Figure 1. Mumps resurgence in the United States

Material and Methods

Recombinant mumps vaccine candidate to match circulating genotype G strain

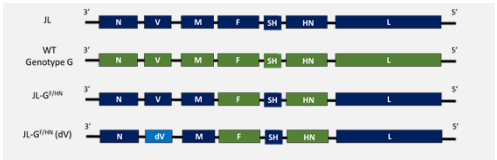
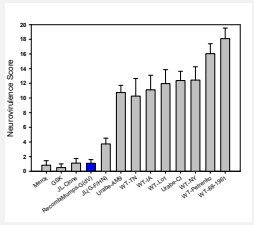


Figure 2. To make the Jeryl Lynn G (JL-G) vaccine candidate expressing the fusion (F)and hemagglutinin neuraminidase (HN) proteins of genotype G strains Genbank sequences of 120 F and 214 HN genes from genotype G were used to create a consensus F and HN sequence and cloned into the Jeryl Lynn backbone (JL-G$^{F/HN}$). To attenuate JL-G$^{F/HN}$ the sequence was modified with a defective V (dV) gene by introducing three stop mutations sequences into the portion of the V-gene that uniquely codes for the V protein without disrupting the reading frame to code for the P and I proteins.

Neuroattenuation of JL-G$^{F/HN}$ (dV) vaccine candidate

Figure 3. Lewis rat pups were intracranially injected with 100pfu/100ul into the left or right parietal area of the skull. About a month later the brains were dissected, fixed, sectioned, and stained to measure the hydrocephalus score as a ratio of the ventricle to brain area. Neuroattenuation is defined by a score less than 2.



Animal Immunization

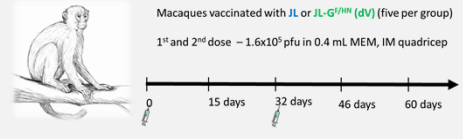


Figure 4. Two groups of macaques (n=5) were intramuscularly primed with 1.6X10^5 pfu of JL or JL-G$^{F/HN}$(dV) virus, then similarly boosted 32 days later. Serum was collected at 0, 15-, 31-, 46- and 60-day time points to assess the mumps specific neutralizing antibodies.

Results and Discussion

JL-G$^{F/HN}$ (dV) vaccine has enhanced neutralization for wild-type genotype G without loss for genotype A vaccine virus

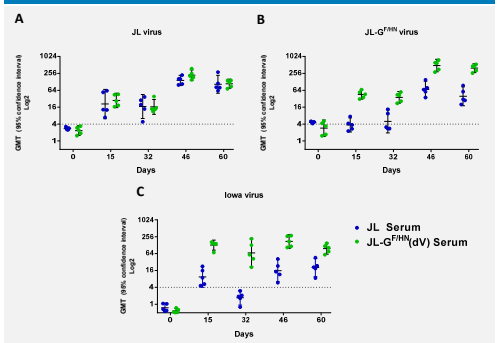
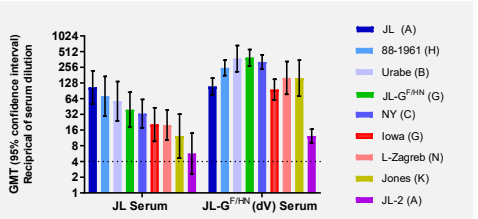


Figure 5. Serum from macaques immunized with either JL (blue) or JL-G$^{F/HN}$ (dV) (green) were collected at 0-, 15-, 32-, 46-, and 60-days. The mumps specific neutralizing titer was assessed by plaque reduction neutralization assay against A) genotype A JL virus B) genotype G recombinant JL-G$^{F/HN}$ (dV) virus and C) wild type genotype G Iowa virus. Dashed line represents the limit of neutralizing detection.

JL-G$^{F/HN}$ (dV) vaccine candidate provides enhanced neutralizing titers across genotypes



Average fold difference is 6.5-fold with a range between 2.2 and 13.0-fold

Figure 6. Serum from macaques immunized with two doses of either JL or JL-G$^{F/HN}$ (dV) were collected at 60-days post immunization and the neutralizing titer across mumps genotype A, B, C, G, H, K, and N viruses was assessed by plaque reduction neutralization. Dashed line represents the limit of neutralizing detection.

Enhanced neutralization by JL-G$^{F/HN}$ (dV) vaccine is not driven by viral replication

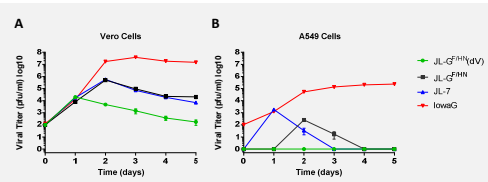
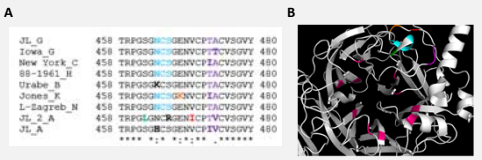


Figure 7. ***In vitro*** **growth kinetics of mumps vaccine candidate and wild type strain**. Confluent monolayer of Vero (A) and A549 cells (B) were infected with JL-G$^{F/HN}$(dV), JL-G$^{F/HN}$, JL-7, or Iowa G virus at an MOI of 0.05 and cultivated viral titer determined. The data represents mean values and standard error of the mean for two independent experiments.

Could a single N-glycosylation site explain difference in immune response?



N- glycosylation sites are known to be important in immunogenicity. Therefore, if this region represents an antigenic epitope, the current Jeryl Lynn vaccine would not be predicted to induce antibodies to this site which appears to be broadly present across wild-type viruses

Figure 8. A) Sequence alignment of mumps hemagglutinin neuraminidase (HN) amino acids between 458 and 480 across various mumps genotypes. B) The ribbon structure of the HN protein highlighting the same-colored amino acids represented in A. Blue represents a putative N-glycosylation site and fuchsia represent amino acids involved in receptor binding.

Conclusion

- Developed an attenuated vaccine candidate that induces higher antibody titers with enhanced cross neutralization against most circulating genotypes
- Mismatches in sequence between Jeryl Lynn and circulating mumps strains, such as the one predicted at the putative glycosylation site at amino acid 464 of HN, may play a role in Jeryl Lynn vaccine effectiveness