



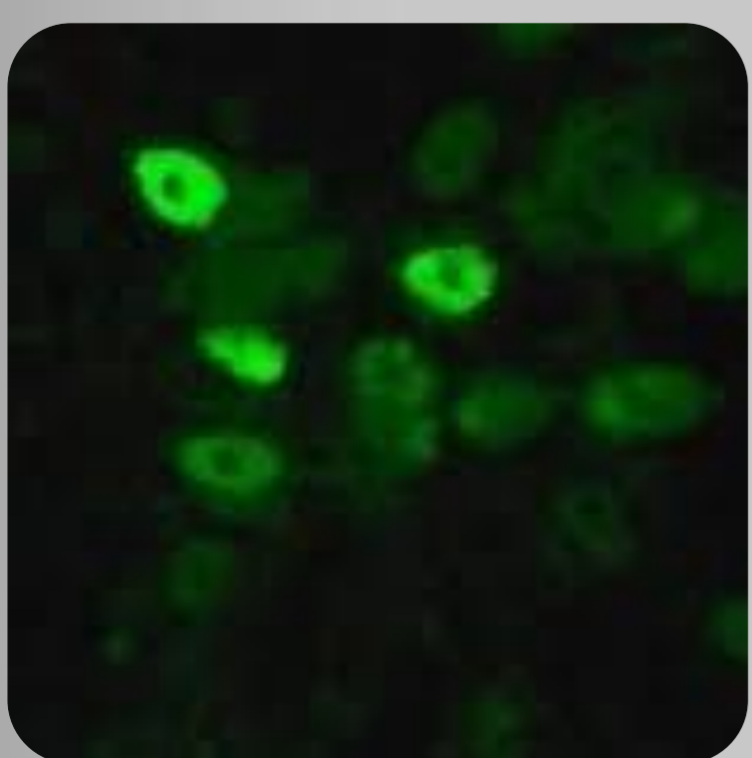
Analysis of Serological Assays, cross reactivity among subtypes and a better vaccine for human influenza A

Koushal Kumar Gopi
Supervisor: Jorma Hinkula

Master Thesis: Molecular Genetics and Physiology 2011

Background: Influenza virus is a respiratory pathogen well known for epidemic & pandemic. The vaccine induced immunity is often determined by immunological assays invitro. In Influenza vaccine field, Haemagglutination Inhibition assay is used which is not a true virus neutralization test and sometimes regarded as a poor analysis assay. Thus it would be better to use functional cell neutralization assay (NT). The other issue is often to understand how to decide which vaccine candidates (Protein or DNA) with different adjuvant or vaccination modes (Nasal Vs. intramuscular) are the most efficient in inducing good protective immunity. Also, the role of cross reactive antibodies in immune response is not well studied.

Method: The BALB/c mice were immunized with Haemagglutinin (HA) protein as well as pDNA-HA separately with different adjuvants such as saline, L3B, N3 and squalene. The mice were bled on day7, 28 and 56 and the HI, NT and ELISA test were done on the blood serum as per protocol.



Influenza virus infected MDCK cells

Objective: To study the accuracy and variability within HI and NT, evaluate a promising vaccine, DNA based or protein based, vaccination mode and how far the cross reactive antibodies provides immunity, if present.

Results:

protein vs. DNA based vaccine: The protein based vaccine with high antigen content showed high titer as shown in graph.

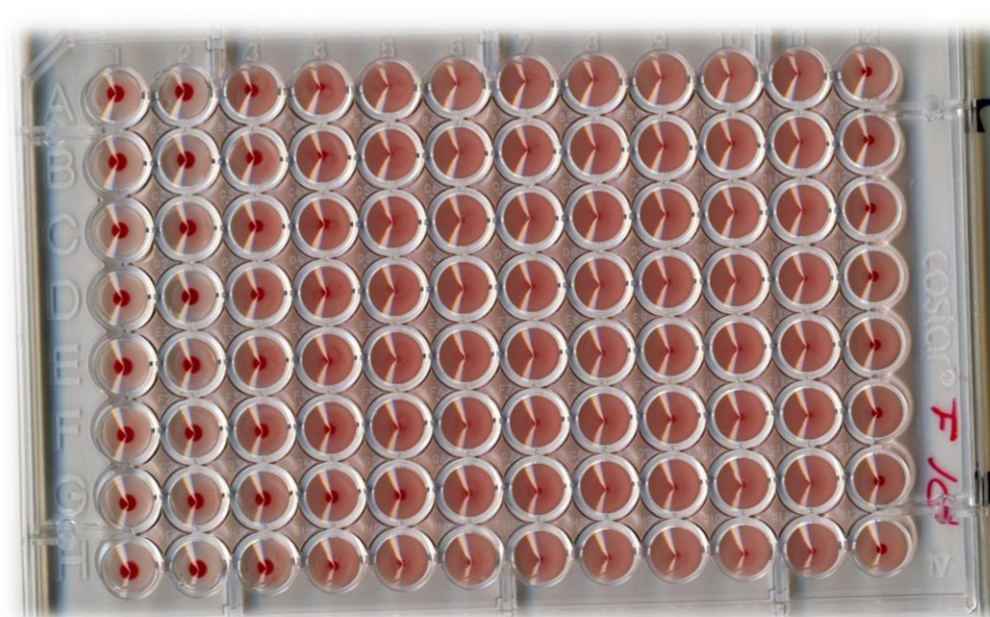
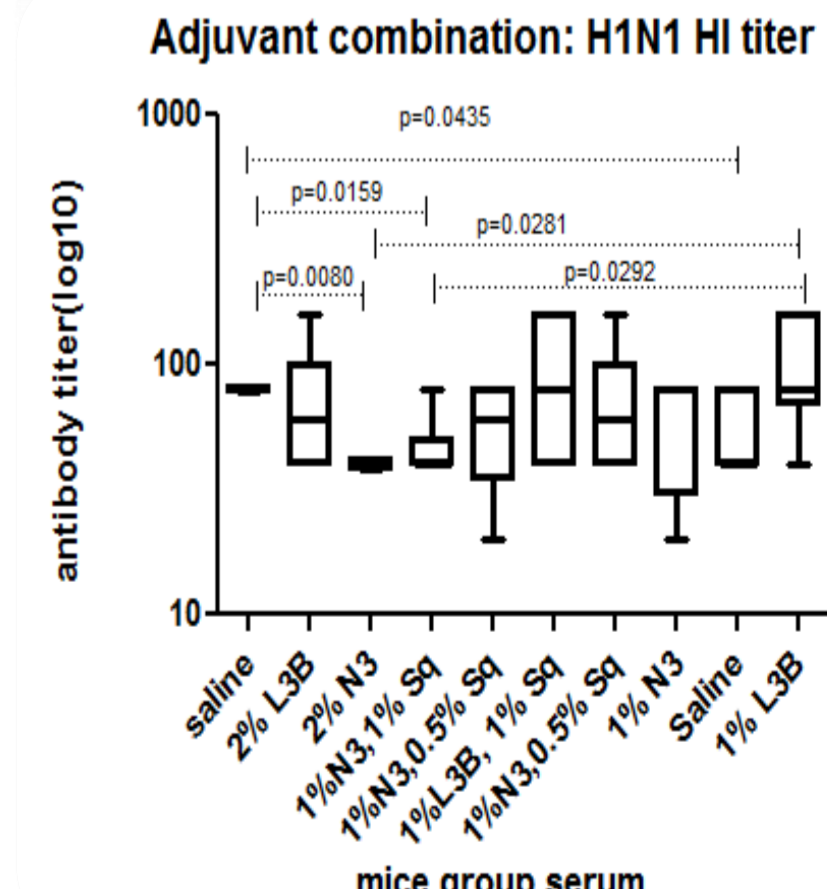
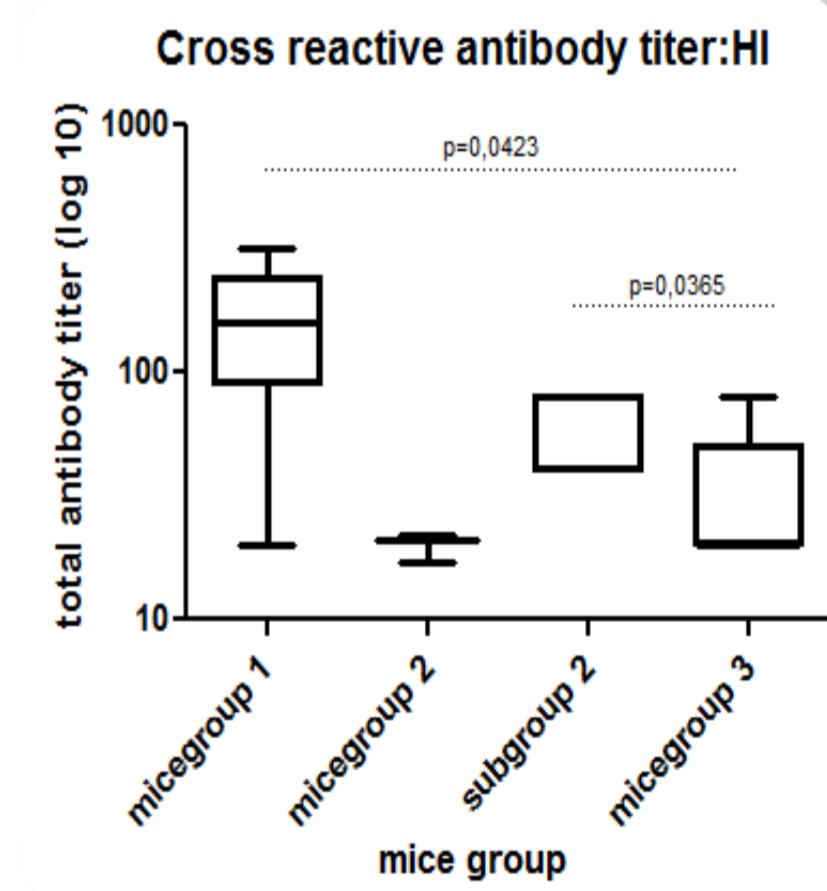
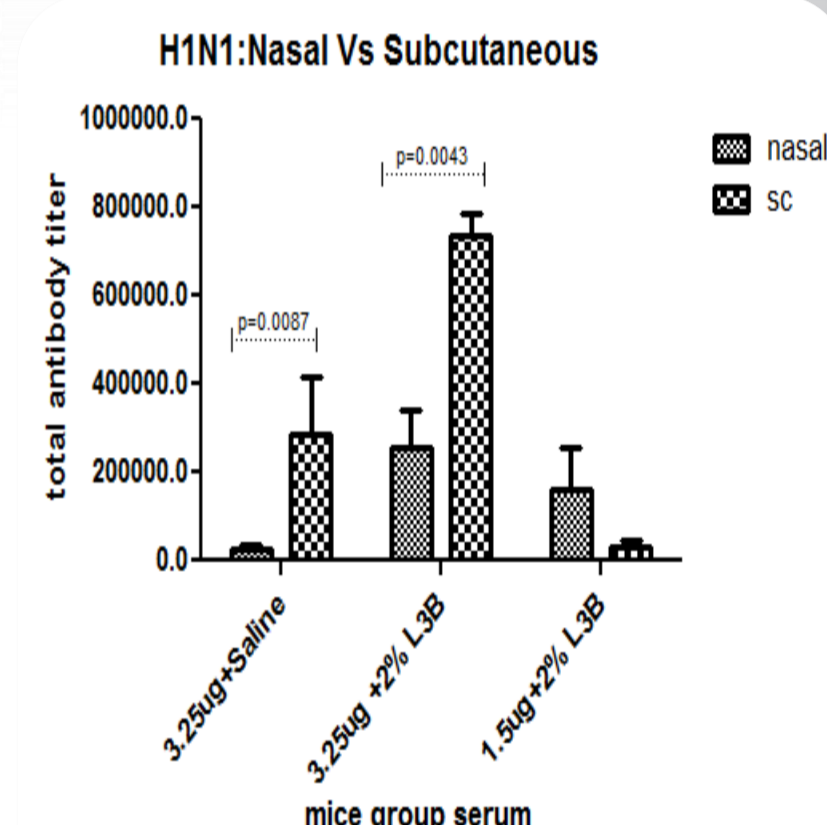
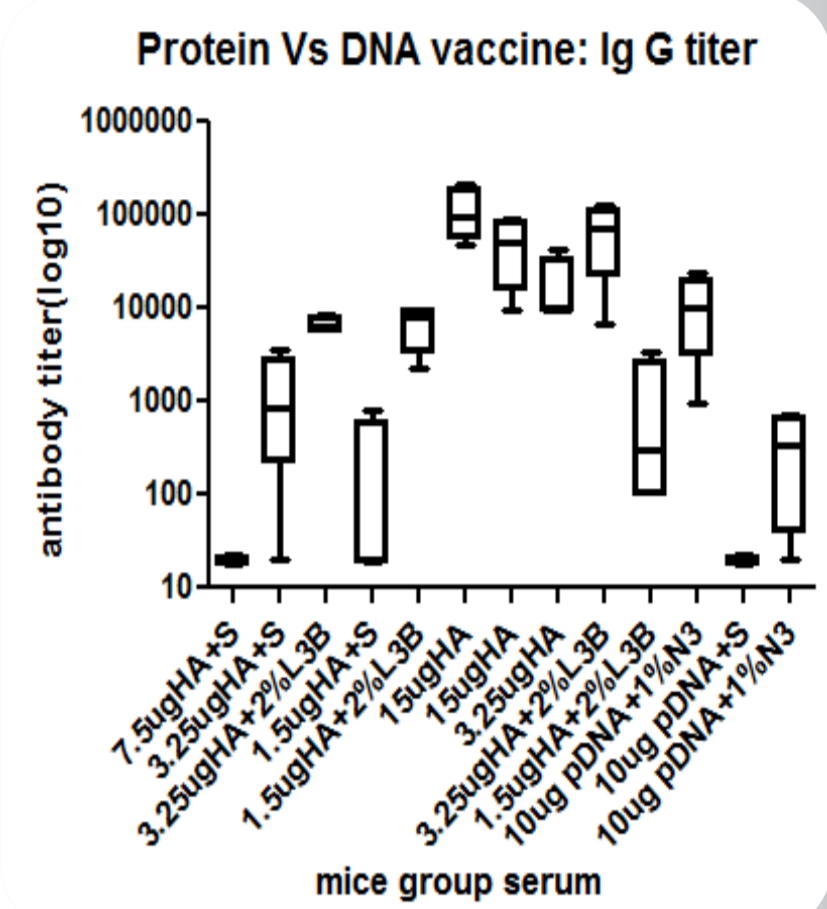
Nasal vs. subcutaneous: The subcutaneously administered vaccine induced high antibodies production.

Cross Reactivity among subtypes: Vietnam strain immunized serum antibodies were enough to give immunity against Brisbane strain.

Adjuvant combination: As shown in graph, 1% L3B was best among other adjuvant combination to induce immunity.

Conclusion: The NT assay has some technical problems, otherwise best for Influenza. The protein based vaccine with L3B when given subcutaneously induced good immune response. The cross reactive antibodies, if present may provide immunity depending on the subtype antibodies it has.

Acknowledgement: I am very much thankful to my supervisor for all the support and ideas.



HI assay: H1N1