Broadly Reactive Monoclonal Antibodies to Conserved Hemagglutinin, Neuraminidase, and Matrix Ectodomain Influenza Epitopes May Provide New Therapeutic Strategies

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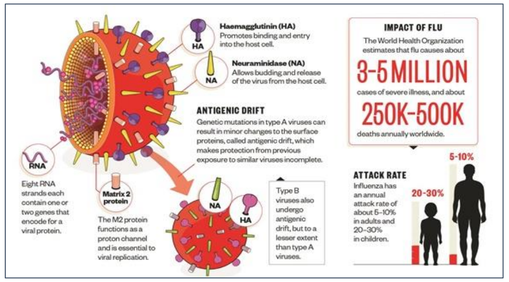
Background: Rapidly evolving influenza strains and the emergence of variants resistant to antiviral therapeutics in both human and animal hosts continue to present significant challenges in the clinical management of seasonal outbreaks and pandemics.[1] Our studies investigated new therapeutic strategies against influenza that utilize broadly reactive monoclonal antibodies (MABs) directed against composite peptides derived from hemagglutinin (HA), neuraminidase (NA), and Matrix ectodomain (M2e) viral surface proteins.



Objective: To examine our vaccine and therapeutic approaches by evaluating the binding activity of MABs to target influenza epitopes and their functional capacities against live contemporary influenza A strains.

Methods: Mice were immunized with small composite peptide conjugate vaccines (Table 1) and serum antibody titers were analyzed using an Antisera ELISA (e.g. M2e, Fig.1). The binding activity of selected MABs to composite peptides and live influenza virus was examined by ELISA. Functional capacity against contemporary influenza A H3N2 & H1N1 strains was evaluated *in vitro* using: (a) Microneutralization, (b) Hemagglutination Inhibition (HAI), and (c) Antibody-Dependent Cellular Cytotoxicity (ADCC) assays.

Results: MABs EA9, LD9 (both IgG1), NB5 (IgG2a), and GA4 (IgG1) were identified and demonstrated good binding activity to HA, NA and M2e peptides, respectively (Fig.2), and bound to live influenza viruses (Fig.3). Presence of neutralizing MABs against influenza A (H3N2 & H1N1) contemporary strains was exhibited (Fig.4). Additionally, MABs EA9, LD9 and NB5 inhibited hemagglutination of red blood cells using live influenza A/Hong Kong and A/Michigan viruses (Fig.5). Preliminary antisera ADCC activity was shown (Fig.6).

PEPTIDE	CONJUGATE	ADJUVANT	DOSE & REGIMEN	MODE OF ADMIN	PEPTIDE SOURCE PROTEIN	ANTISERA TITERS ON PEPTIDE (OD @ 450nM)	MOUSE, MAB ID & ISOTYPE	MAB BINDING PROFILE
Pep 11 Composite	CRM	TITERMAX GOLD	Prime: H3N2, 10^6 Boost: Pep: 20 µg D14, 30, 42, 70	H3N2: IM Peptide: SQ	Hemagglutinin Neuraminidase	MS 2209 Ag: Pep 11 D49: OD 2.8 D77: OD 3.1	MAB EA9 (IgG1) MAB NB5 (IgG2a) MAB LD9 (IgG1)	EA9 & LD9: HA NB5: NA Flu/A viruses (cont) H3N2 and H1N1
Pep 5906 Composite	CRM	FREUNDS	Primary, Boost 50 µg: D21, 35, 41	SQ	Matrix Ectodomain (M2e)	MS 1443 Ag: Pep 5906 D21: OD 3.4 D42: OD 4.0	MAB GA4 (IgG1)	Matrix M2e Flu/A viruses (cont) H3N2 and H1N1

Table 1. Composite peptide conjugate vaccines and MAB identification.

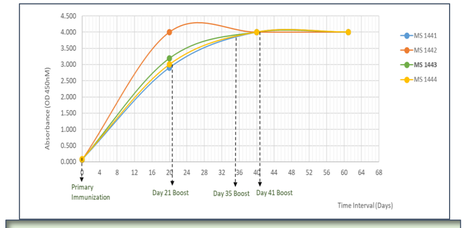


Figure 1. MS 1443 Antisera titers on M2e.

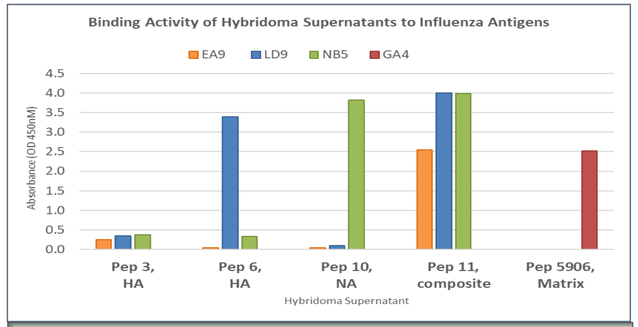


Figure 2. Binding activity of Hybridoma Supernatants EA9, LD9, NB5 & GA4 to influenza antigens (HA, NA and M2e).

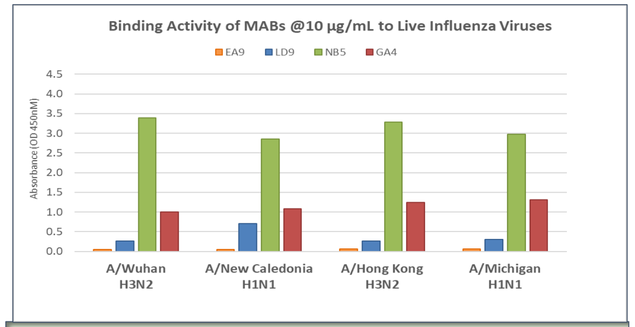


Figure 3. Binding activity of MABs EA9, LD9, NB5 & GA4 to live influenza A viruses (H3N2 & H1N1).

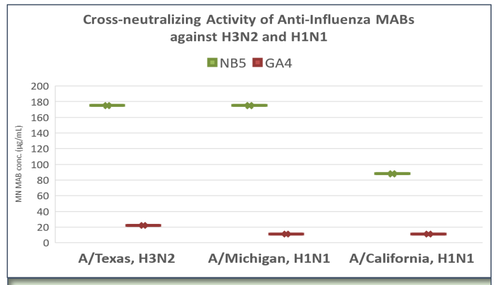


Figure 4. Cross-neutralizing activity (µg/mL) of MABs NB5 & GA4 against contemporary influenza A H3N2 & H1N1 strains.

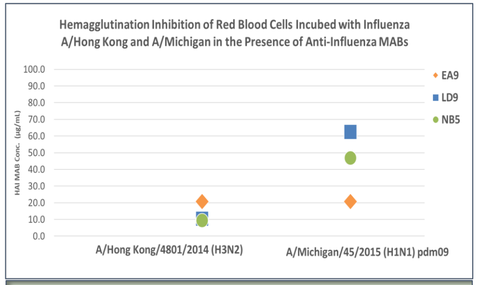


Figure 5. Hemagglutination inhibition activity (µg/mL) of MABs EA9, LD9 & NB5 against influenza A H3N2 and H1N1 strains.

ADCC Activity	
Preliminary Data	
MS 1443 Anti-M2e Serum	
against A/Michigan (H1N1)	
1:120	**93%**
1:360	**41%**

Figure 6. ADCC activity against influenza A/Michigan using target A-549 cells and PBMC effector cells. Percent cytotoxicity for MS 1443 anti-M2e serum is shown.

Conclusions

- Composite peptide vaccines were highly immunogenic eliciting strong humoral responses.
- MABs were identified that bind to target epitopes on live influenza.
- Cross-neutralizing MABs against H3N2 and H1N1 were confirmed: Highest activity, anti-M2e MAB GA4.
- Selected MABs demonstrated HAI using contemporary influenza strains.
- Preliminary data showed ADCC activity of anti-M2e (serum antibodies) against influenza A/Michigan.
- These broadly reactive MABs could provide new strategies for influenza epidemic and pandemic control.

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2. The Pharmaceutical Journal, October 2015, Vol 295, No 7882, online | DOI: 10.1211/PJ.2015.20069647. Available: https://www.pharmaceutical-journal.com/news-and-analysis/infographics/effectiveness-of-the-seasonal-flu-vaccine-and-new-production-methods/20069647.article.

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