Letter to the Editor (JVI)

Should not exceed 500 words with a couple of figures

**An H1 COBRA influenza vaccine elicits antibodies endowed with different breadth against H1Nx swine viruses**

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Computationally Optimized Broadly Reactive Antigens (COBRA) designed for different influenza virus subtypes (H1N1, H3N2 and H5N1) elicit a subtype-specific broad antibody (Ab) response in naïve as well as in pre-immune influenza virus animal models [1-8].

In particular, an H1 COBRA candidate, named P1, has been designed by a multiple sequence alignment of HA sequences belonging to H1 swine and human strains [7]. Importantly, immunization with COBRA P1 HA elicit a broad neutralizing polyclonal Ab response against H1 human and swine viruses [5].

Recently, we generated a panel of monoclonal antibodies (mAbs) against the COBRA P1 with the aim to dissect the generated Ab response following the immunization of influenza virus naïve mice (BALB/c). As previously described, these mAbs feature different functional activities, spanning from narrowly to broadly reactive binders and neutralizers (REF).

In this study we investigated the breadth of hemagglutination inhibition featured by representative P1-specific mAbs, along with those specific for wild-type historic H1N1 human vaccine strains, in order to dissect at the mAb level, the breadth of HAI activity of the P1-elicited humoral response against H1 swine viruses.

P1- and A/California/04/2009 (H1N1)pdm09 (CA/09)-specific mAbs have been expressed and purified as previously described (REF), while A/South Carolina/1/1918 (SC/18)-, A/Solomon Islands/3/2006 (SI/06)- and A/Brisbane/59/2007 (Brisb/07)-specific mouse mAbs have been provided by the Biodefense and Emerging Infections (BEI) Resources and the Influenza Reagent Resource (IRR).

The generation of virus-like particles (VLP) and influenza viruses belonging to the strains listed in Table 1 and 2 and the protocol for performing hemagglutination inhibition activity (HAI) assays have been previously described [5].

Similarly, as previously described, COBRA P1-specific mAbs featured a differentiating breadth of HAI activity, spanning from narrowly to broadly reactive mAbs against H1N1 and H1N2 swine viruses. However, contrarily, mAbs endowed with a broad HAI activity against a panel of human H1N1 viruses (REF), featured a narrower HAI profile against swine viruses. Comparatively, those endowed with a narrower HAI activity against human viruses, featured a broader HAI profile against swine viruses belonging to the Eurasian, classical, and human seasonal-like lineages.

Unsurprisingly due to its swine origin, CA/09-specific mAbs, previously classified to have a narrow profile of neutralization activity against pandemic and pandemic-like viruses, exhibited broad HAI activity against swine viruses belonging to all three of the lineages as well.

Interestingly, a class of P1-specific mAbs previously demonstrated HAI activity only against the P1 virus and none of the human H1N1 strains, showed detectable HAI activity against some H1N1 and H1N2 swine viruses, suggesting their HA recognition epitopes are particular to swine viruses and not present in pandemic and pandemic-like HA proteins.

Further investigation aimed at determining the amino acid contact residues of these mAbs will improve the resolution of the recognized epitopes, clarify distinctions between human and swine specific H1 epitopes, and elucidate the mechanism of breadth conferred by COBRA immunogens.

**Acknowledgements**

The following mouse monoclonal antibodies: NR-15170; monoclonal anti-influenza virus H1 hemagglutinin (HA), A/South Carolina/1/1918 (H1N1), clone 5D3 (produced in vitro), NR-13451; clone 6B9 (produced in vitro), NR-13452; clone 39E4 (produced in vitro), NR-13453; monoclonal anti-influenza A virus hemagglutinin (HA): clone IC5-4F8 (produced in vitro), NR-48783; monoclonal anti-influenza A virus hemagglutinin domain 2 (HA2), clone RA5-22 (produced in vitro), NR-44222; monoclonal anti-Influenza virus H1 hemagglutinin (HA), A/California/04/2009 (H1N1)pdm09, clone 1C5 (produced in vitro), NR-42015; clone 5C12 (produced in vitro), NR-42019; clone CA09-02 (ascites, Mouse), NR-28665; clone CA09-09 (ascites, Mouse), NR-28666; clone CA09-11 (ascites, Mouse), NR-28667; clone CA09-15 (ascites, Mouse), NR-28668 were all obtained from Biodefense and Emerging Infections (BEI) Resources, NIAID/NIH (Manassas, VA, USA).

The mouse monoclonal antibodies to recombinant H1 HA from: influenza A/Solomon Islands/3/2006 (H1N1), clone AT170.558.146 (FR-499), clone AT170.119.5 (FR-503) and influenza A/Brisbane/59/2007 (H1N1), clone AT163.272.54 (FR-494), clone AT163.210.182 (FR-495), clone AT163.333.93 (FR-496), clone AT163.104.93 (FR-497), clone AT163.329.189 (FR-498) were obtained through the Influenza Reagent Resource (IRR), Influenza Division, WHO Collaborating Center for Surveillance, Epidemiology and Control of Influenza, Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA.

The A/Swine/North Carolina/152702/2015, …, viruses were graciously provided by Dr. Mark Tompkins at the University of Georgia.

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