IMMUNE ENGINEERING ENHANCES H7N9 VACCINE IMMUNOGENICITY BY REGULATORY T CELL EPITOPE DELETION IN HEMAGGLUTININ

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Avian-origin H7N9 influenza is a novel influenza group A virus that emerged in humans in China in 2013. H7N9 influenza hemagglutinin (HA) elicits weak neutralizing antibody responses in natural infection and vaccination. Limited helper T cell response could explain the poor immunogenicity observed [1]. We hypothesize a T cell epitope in H7-HA stimulates regulatory T cells (Tregs) capable of suppressing crucial signals needed for protective antibody production. Furthermore, deletion of the epitope without perturbing neutralizing B cell epitope structures may increase H7-HA immunogenicity to produce an optimized vaccine.

Immunoinformatics tools were used to identify H7N9 class II HLA epitopes with high potential to cross-react with Tregs educated on human antigens. In peripheral blood leukocyte cultures, H7N9 epitopes with significant human homology expanded CD4+CD25+FoxP3+CD39+ Tregs and reduced IFNγ secretion when co-incubated with other H7N9 epitopes with low potential cross-reactivity [2]. We applied this finding to design an antigenically improved H7-HA based on Anhui/01 by introducing three modifications to recombinant HA (rHA) that delete a highly conserved Treg activating epitope. Engineered rHA (Opt1 rH7-HA) demonstrated both preserved antigenicity and improved immunogenicity in humanized mice. Monoclonal antibodies raised against wild type H7-HA recognized Opt1 rH7-HA with affinity equivalent to the wild type protein, suggesting that modifications did not induce significant structural perturbations. Similarly, human polyclonal sera demonstrated identical binding profiles against Opt1 and wild type rH7-HA. Vaccination of immunodeficient mice reconstituted with human PBMCs (N=8) using non-adjuvanted Opt1 rH7-HA stimulated higher anti-H7-HA IgG titers and higher frequencies of anti-H7-HA plasma cells than mice immunized with wild-type protein. In a related study, HLA-DR3 transgenic mice were immunized with Alum-formulated H7N9 virus-like particles containing either Opt1 or wild-type H7-HA and hemagglutinin inhibition (HAI) titers were measured. Opt1 rH7-HA stimulated protective levels of HAI antibodies suggesting that modifications of H7-HA preserved neutralizing epitopes. The Opt1 H7N9 VLP vaccine raised HAI antibodies sooner and at lower doses than wild-type vaccine.

Epitope-driven approaches to vaccine design that carefully consider T cell subsets primed in immunization promise to enhance vaccine efficacy.

1. De Groot AS, Ardito M, Terry F, Levitz L, Ross T, Moise L, Martin W. Low immunogenicity predicted for emerging avian-origin H7N9: implication for influenza vaccine design. Hum Vaccin Immunother. 2013 May;9(5):950-6.

2. Liu R, Moise L, Tassone R, Gutierrez AH, Terry FE, Sangare K, Ardito MT, Martin WD, De Groot AS. H7N9 T-cell epitopes that mimic human sequences are less immunogenic and may induce Treg-mediated tolerance. Hum Vaccin Immunother. 2015;11(9):2241-52.