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GENERATION OF AN INTERNATIONAL STANDARD SERUM TO MEASURE INFLUENZA VIRUS HEMAGGLUTININ STALK-REACTIVE ANTIBODIES

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Current efforts towards the development of a universal influenza virus vaccine rely on induction of effective long-term antibody responses against conserved regions of the influenza virus glycoproteins. The stalk domain of the hemagglutinin has been targeted for this purpose due to its high degree of conservation among numerous influenza subtypes and strains. Several vaccine candidates targeting this domain are in late pre-clinical or early clinical stage of development. Currently assays to measure stalk-based immunity are not standardized and no international standard is available. As various vaccine developers are generating data from pre-clinical and clinical studies, harmonization of assay read-outs will help in the comparison of experiments conducted in different laboratories and increase confidence in results. Moreover, researchers will aim to define correlates of protection for new vaccines targeting the hemagglutinin stalk domain. Joint efforts between the Krammer Laboratory at the Icahn School of Medicine at Mount Sinai and the National Institute for Biological Standards and Control (NIBSC) led to a collaborative project to generate an international standard human serum to measure hemagglutinin stalk-reactive antibodies. The WHO Expert Committee on Biological Standardization (ECBS) is currently evaluating this project for its endorsement to generate the first international standard for antibodies to the influenza virus hemagglutinin stalk domain.

In this work, serum samples from healthy individuals (n=110) were purchased from a commercial vendor and screened initially using a cH6/1 hemagglutinin enzyme-linked immunosorbent assay (ELISA) that measures stalk-reactive antibodies. We were able to identify samples with moderate to high IgG stalk-reactivity. Likewise, screening of the samples using the mini-HA headless construct 4900 and analysis of the correlation between the two assays confirmed the presence and specificity of anti-stalk antibodies. Furthermore, samples from low, intermediate and high responders in the cH6/1 ELISA were subjected to an antibody-dependent cell-mediated cytotoxicity (ADCC) assay to determine the correlation of binding with the effector functions of the stalk-reactive antibodies. Additionally, the neutralization potential of the antibodies contained in the serum was assessed using a cH6/1N5 virus-based neutralization assay. Finally, to characterize the specificity of the stalk-reactive antibodies, we performed competition ELISAs using the stalk-reactive monoclonal antibodies KB2 (mouse) and CR9114 (human) against samples from high responders in the cH6/1 ELISA. We detected a high percentage of competition using either monoclonal antibody. Overall, these assays reflect a wide variety of tools that are currently available to detect stalk-reactive antibodies. Importantly, for 10 individuals with the highest titers of stalk-specific IgG, the “full bleeds” were obtained (approximately 400ml of serum per individual). A “pooled serum” (PS) consisting of a mixture of these 10 samples in equal proportions was generated and characterized. The PS exhibited high levels of stalk-reactive antibodies as measured by ELISA, a cH6/1N5 neutralization titer of 320 and contains high levels of antibodies with ADCC activity. This serum is currently being evaluated in an international collaborative study for its usefulness as a potential international standard serum to measure stalk-reactive antibodies.

The generation of the first international standard to measure influenza virus hemagglutinin stalk-reactive antibodies is currently under development as a joint effort among different partners/institutions. Given the promising potential of stalk-based vaccine candidates and the increasing research into antibody responses against these conserved regions of influenza viruses, this valuable tool will potentially contribute to the harmonization of results worldwide.