DESIGN AND DEVELOPMENT OF MULTIVALENT PEPTIDE VACCINE CANDIDATE AGAINST INFLUENZA A VIRUS

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INTRODUCTION.

Influenza virus is a major pathogen with global spread which infects birds and mammals, including humans. The virus is constantly changing, thus influenza stays in the centre of vaccine research. Effective vaccine should have a wide spectrum of protection from the most circulating and dangerous subtypes of the virus. Our goals are identifying the most immunogenic peptide epitopes among three subtypes of Influenza A virus (H1N1, H3N2 и H5N1) according to affinity to MHC class I & II molecules, and finding epitopes localized in conservative regions of the viral proteome. We propose to screen synthetic oligopeptides emulating conservative and immunogenic regions for the strongest humoral and cell mediated responses in mice to select several epitopes/peptides as multivalent peptide vaccine candidate.

MATERIALS AND METHODS.

T and B cell epitopes of hemagglutinin (HA), neuraminidase (NA), and proton channel (M2) proteins of influenza were predicted using the Immune Epitope Database (IEDB) and analysis resource. Multiple alignment of amino acid sequences were built using MUSCLE tool. Peptides of 7-22-mer in length representing conservative epitopes were synthesized with solid-phase method. BALB/c mice were immunized subcutaneously either with peptide or with KLH-peptide conjugate emulsified in Complete Freund's Adjuvant (CFA). In two weeks, second immunization was performed, and mice were sacrificed ten days later. Blood was collected for assaying peptide-specific serum antibodies levels. Splenocytes were stimulated *in vitro* to measure IFN-γ and IL-4 cytokine responses.

RESULTS AND DISCUSSION.

Thousands of sequences representing different isolates of influenza A virus subtypes H1N1, H3N2 и H5N1 available at IEDB were analyzed. At 90% threshold for amino acid sequence conservancy, 53 viral epitopes showed high immunogenicity and likelihood to be presented for B and T lymphocytes were identified. Immunization with peptides or/and their KLH conjugates did not exhibit excessive mice mortality. In pilot immunization, we compared three peptides (p1, p2, p3) and three corresponding KLH-conjugates (pc1, pc2, pc3). P1 stimulated serum Ab and IFN-γ in lymphocytes, and the corresponding conjugate pc1 showed similar but stronger responses. Neither p2 nor pc2 produce any serum Ab, but both p2 and pc2 stimulated lymphocyte IFN-γ production. P3 and pc3 stimulated both Ab and lymphocyte IFN-γ production. Global screening of all selected peptides for antibody, Th1, Th2 cytokine responses is in progress.

CONCLUSIONS.

Immunization showed that mouse immune responses to KLH-conjugated and unconjugated peptides varied greatly, which was further complicated by the peptide charge and solubility. We showed that among n=53 viral epitopes, there were epitopes that stimulated only T cell branch of the immune responses to influenza, and there were epitopes that induced both B and T cells. Accordingly, we showed principal feasibility to design complex peptide vaccine candidate tackling different viral epitopes responsible for different phases of influenza pathogenesis.

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