DEVELOPMENT OF A MULTIVALENT VACCINE AGAINST HUMAN HERPESVIRUSES

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INTRODUCTION.

Herpesviridae comprise a large family of DNA viruses including herpes simplex viruses 1 and 2, Epstein-Barr (EBV) virus and cytomegalovirus (CMV), which can lead to a number of diseases in humans. Depending on population, 40% to 100% of humans are infected with one or several HHV. CMV and EBV can be major cause of death in immunodeficient patients (HIV-infected, transplant recipients, infected newborns), can cause neurodegenerative diseases and cancer and lead to "immune risk phenotype".

Currently, treatment of HHV infections is primarily based on antiviral therapy; however, this way of treatment is not completely effective. Vaccines being developed include subunit vaccines based on glycoproteins. The main problem is that the development of vaccines is focused only on known target proteins that have been extensively studied. Meanwhile, due to the complexity of HHVs, most of the proteins are not well characterized. The new approach of reverse vaccinology, which employs computer-based selection of vaccine candidates can be used for exploiting the full immunogenic potential of the pathogen, even if most of the proteins are not characterized.

The objective of this work is to develop a multivalent vaccine against human herpesviruses based on conservative epitope domains. The main stages will be the peptide synthesis of epitope library and testing it for immunogenicity in mouse models.

MATERIALS AND METHODS.

Twenty one peptides predicted for immunogenicity using bioinformatics software were chemically synthesized, conjugated to KLH carrier and used for mouse immunization. Immunized mice sera were tested for the presence of virus-neutralizing antibodies. ELISA was performed on the sera of immunized mice to monitor the peptide-specific antibody titer. After third immunization (2nd boost), the mice were challenged with LD_{50} of HSV-2 (strain BH) to B-cell response and protection against HSV-2 infection.

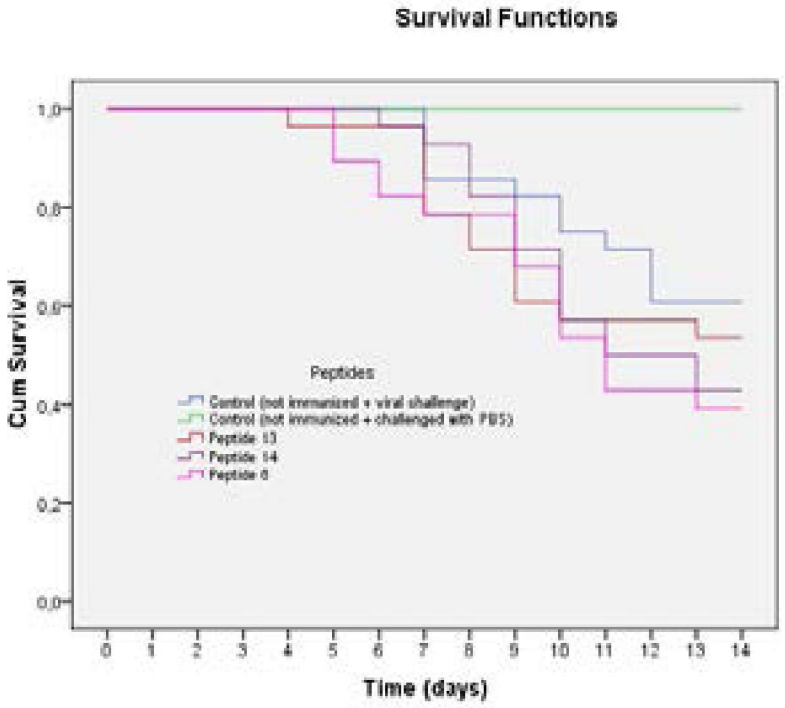
RESULTS.

All designed peptides induced high titers of antibody production in mouse model. However, only peptides 3, 6, 13 and 14 (Table 1) produced virus neutralizing effect against HSV-2 in the sera samples. Briefly, serum from each group was heat-inactivated at 56 C for 30 min. A 100 ml of serum was added into a 96-well plate for serial dilution. A 100 ml of 50x TICD50 of the live BH strain of HSV-2 was added to each well and incubated at 37 C for 3 days. Then 10 ml of $3x10^5$ viable MDBK cells were added to the plate, sealed and incubated for 2 h at 37 C. The serum dilution factor that neutralized 50% of the virus was determined as the titer.

Table 1. The list of selected peptides

Peptide No.	Peptide sequence	Source protein	Neutralizing antibody titer (serum dilution)
3	SFCDTSFFL	Major Capsid Protein	1:50
6	GYINNLFGTI	Capsid portal protein	1:1000
13	PVGPYHHPHADTETPAQ PPRYPAKAVYLPPPHIPP GPPL	Capsid Maturation Protease	1:800
14	HRYGTTVNCIVEEVDARS VYPYDEFVLATGDFVYMS PFYGYR	gB	1:1600

However, viral challenge of immunized mice showed no significant protection (Figure 1) compared to control group. All of the peptides showed no signs of cytotoxicity which demonstrates their safety.



CONCLUSIONS

Current work demonstrates use of modern *in silico* tools for predicting peptide-based vaccine candidates. Four out of twenty one showed good HSV-2 neutralization activity. Further investigation is needed to obtain effective anti-HSV protective vaccine candidates. Future directions of this work will be further optimization of epitope selection strategy and use of other search algorithms.

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