Talk

Search and characterization of viral antigens able to stimulate a protective immune response against CMV infection for vaccine design

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ABSTRACT

Motivation: Despite improvements in detection and treatment, human cytomegalovirus (CMV) infection is the main cause of morbi-mortality in solid organ transplant (SOT) recipients. Several efforts have been made to develop a vaccine to prevent CMV infection. However, no vaccine had been licenced to date. Efforts are necessary to identify antigens able to stimulate an immune response to protect against CMV infection.

Methods: We used serum (from SOT recipients with previous CMV infection and neutralizing antibody titers >480) as primary antibody to identify proteins from CMV-infected cell lysates, not recognized by the serum from the same patient with no neutralizing antibodies or in the mock-infected lysate. The identified bands were sequenced by mass spectrometry. The gene of the identified candidates was amplified by PCR using specific primers and cloned into the expression plasmids PTYB21 (fused to intein tail to purify the recombinant protein) and into pCDNA plasmid (for DNA vaccine). The PTYB21 construct was transformed into the E. coli BL21 Rosetta strain for protein expression. Protein purification was performed using the IMPACT kit (BioLabs) using a chitin affinity column. The pcDNA construct was transformed into the E. coli TOP-10 strain. All constructs were sequenced. Protein antigenicity was analyzed using VAxiJen informatic tool (threshold 0.4).

Results: We identified three proteins with 135, 70 and 50 kDa approximately. The 48 kDa candidate predicted to be from the tegument and with an antigenicity score of 0.520 was selected. A 1200pb band corresponding to the predicted size of the gene was amplified. Due to difficulties of blunt ligations we introduced a dephosphorylation step of the insert before ligation. We obtained 3 positive clones with the following pattern: a band of 1280 bp corresponding to the insert and a 7514 bp for the PTYB21 plasmid and 5400 bp for the pCDNA plasmid. Sequencing of positive clones showed a 100% homology. The 48 kDa protein had the highest expression using 0.5 mM IPTG for 6 hours, and was isolated in the insoluble fraction. We are in the process of optimizing the conditions for protein purification.

Conclusions: The use of patient sera is an optimal approach to identify proteins that induce an specific immune response against CMV that may be candidates for vaccine design. The process for protein purification must be optimized for each candidate since depends on multiple factors such as size and solubility.

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