

ABSTRACT

The success of cancer vaccines depends on factors associated with the vaccine, which define the main parameters of effective immune responses such as its size and quality, as well as on factors related with the host, represented by the immunosuppressive mechanisms that allow the tumor to escape recognition by the immune system or negatively influence the function of effector T-cells.

Attenuated, non-replicating viruses are at present preferred as VACV for safety reasons. A problem may arise concerning their lack of immunogenicity. Through the deletions of non-essential genes, vaccination vectors are therefore developed based on attenuated rVACV capable of replication, which induce a strong immune response. Genes of various immunological adjuvants (e.g., genes for cytokines and costimulatory molecules) are inserted into the vectors for the purpose of eliminating the influence of the immunosuppressive mechanisms of tumors.

The first part of the work describes our study of the influence of vCCI on biological properties of rVACV derived from the Prague strain. Testing of vCCI deletion and insertion mutants expressing tumor associated protein HPV16 E7 has shown that secreted vCCI attenuated the virus *in vivo*, which correlated with reduced levels of the corresponding CC chemokines in the blood compared with the parental virus. Examination of the specific CTL response by ELISPOT IFN- γ method showed that the immunogenicity of the rVACV producing secretory vCCI was similar to that of the parental virus or deletion mutant in the C23L/B29R locus. Immunization with the secretory vCCI-producing recombinant virus had a lower therapeutic effect against TC-1 tumors. Viral CCI downregulated the E7-specific response induced by gene-gun-mediated immunization with the DNA vaccines pBSC-SigE7 LAMP and pBSC-vCCI.

The second part of the work describes our study of the influence of GM-CSF on the immunization of mice with highly immunogenic DNA vaccines in the course of its local production by MVA virus injected into tumors with reduced expression of MHC molecules. Two doses of the DNA vaccine in combination with at least two consecutive i.t. doses of MVA-GM-CSF were able to inhibit significantly the growth of tumors. The analysis of the cellular immune response to HPV16 E7 protein by ELISPOT IFN- γ revealed that the *in situ* expression of GM-CSF gene did not enhance systemic specific T cell response to E7. Furthermore we found that the local injections of MVA-GM-CSF induced an increase of intratumoral CD3 + T cell counts and that the DNA vaccination resulted in up-regulation of MHC type I molecules on tumor cells *in vivo*. The final part of the work contains a description of the preparation of the model line of cancer cells suitable for testing different methods of immunotherapy of tumors expressing WT1.