[SV-3]

# Prophylactic and Therapeutic Applications of Genetic Materials Carrying Viral Apoptotic Function

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## Abstract

Genetic materials including DNA plasmid are effective delivery vehicle to express interesting gene efficiently and safely not to generate replication competent virus. Moreover, it has advantages to design a better vector and to simplify manufacturing and storage condition. To understand a possible pathogenic mechanism by a flavivirus, West Nile virus (WNV), WNV genome sequence was aligned to other pathogenic viral genome. Interestingly, WNV capsid (Cp) amino acid sequence has some homology to HIV-1 Vpr protein. These proteins induce apoptosis in human cell lines as well as in vivo and cell cycle arrest. Therefore, DNA plasmid carrying apoptosis-inducing and cell cycle arresting viral proteins including a HIV-1 Vpr and a WNV Cp protein can be useful for anti-cancer therapeutic applications. This WNV Cp protein is an early expressed protein which can be a reasonable target antigen (Ag) for vaccine design. Immunization of a DNA construct encoding WNV Cp protein induces a strong Ag-specific humoral and Th1-type immune responses in animal. Therefore, DNA plasmid encoding apoptotic viral proteins can be useful tool for therapeutic and prophylactic applications.

West Nile virus(WNV) is a member of the *Flaviviridae* Family of vector borne human and animal pathogens. Originally identified in the West Nile region of Uganda in 1937, WNV has recently been identified on the East Coast of North America where it has resulted in 7 deaths and over 62 hospitalizations. In 2002 summer, this mosquito borne outbreak has left 4 deaths and 54 infected.

In order to obtain a better understanding of possible mechanisms of WNV pathogenesis, the sequence of WNV-NY1999 (GenBank accession no. AF202541) was analyzed by alignment with other viral genome. The WNV capsid (Cp) amino acid sequence has some homology to HIV-1 Vpr protein. HIV-1 Vpr gene encodes a 96 amino acid protein with multiple functions involved in the HIV-1 viral life cycle, including cell cycle arrest ( Chen *et al.* 1999; Di *et al.* 1995; Mahalingam *et al.* 1997; Zhou *et al.* 1998) and apoptosis (Ayyavoo *et al.* 1997; Chen *et al.* 1999; Muthumani *et al.* 2002a; Muthumani *et al.* 2002b; Nishizawa *et al.* 2000) of host cells. The Cp gene in the absence of other viral gene products induces rapid nuclear condensation and cell death in tissue culture. Apoptosis is induced through the mitochondrial pathway as observed changes in mitochodrial membrane potential accompanied by Caspase9 activation and downstream Caspase3 activation. In

order to study the ability of the WNV Cp to induce apoptosis in vivo, a plasmid gene delivery system was used. Following i.m. injection of a WNVCp expression cassette, apoptosis as well as inflammation in mouse muscle tissue were clearly observed. More importantly, direct in vivo expression of WNVCp protein in mouse brain resulted in an induction of apoptosis in the brain similar to what is observed in natural infection.

In spite of an intense research to find appropriate agents to treat WNV infection, there is no current specific therapy for WNV infection (Jordan *et al.* 2000). There also is an increasing demand for the development of immunization strategies to prevent WNV infection in North America and elsewhere. In this regard, DNA vaccination is an important immunization strategy that delivers DNA constructs that encode specific immunogens directly into the host (reviewed in Donnelly *et al.* 1997; Weiner and Kennedy 1999). A DNA vaccine encoding for the WNV Cp protein was used for investigation of the in vivo immune responses generated in DNA vaccine-immunized mice. Antigen-specific humoral and cellular immune responses were observed, including a potent induction of antigen-specific Th1 and cytotoxic T lymphocytes responses (Yang *et al.* 2001). Strong induction of Th1-type immune responses included high levels of antigen-specific elaboration of the Th1-type cytokines interferon-gamma and interleukin-2 and beta-chemokines RANTES (regulated upon activation, normal T cell-expressed and secreted) and macrophage inflammatory protein (MIP)-1beta. Dramatic infiltration of CD4 and CD8 T cells and macrophages also was observed at the muscle injection site. These results support the potential utility of this method as a tool for developing immunization strategies for WNV and other emerging pathogens.

Therefore, this apoptosis-inducing viral protein including WNV Cp and HIV-1 Vpr proteins can be useful for cancer therapy and also WNV Cp protein is a suitable vaccine antigen for inducing inflammation and strong Th-1 type immune responses.

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