Immunization with Cytomegalovirus Envelope Glycoprotein M and Glycoprotein N DNA Vaccines can Provide Mice with Complete Protection against a Lethal Murine Cytomegalovirus Challenge

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Human cytomegalovirus virions contain three major glycoprotein complexes (gC I, II, III), all of which are required for CMV infectivity. These complexes also represent major antigenic targets for anti-viral immune responses. The gC II complex consists of two glycoproteins, gM and gN. In the current study, DNA vaccines expressing the murine cytomegalovirus (MCMV) homologs of the gM and gN proteins were evaluated for protection against lethal MCMV infection in a mouse model. Humoral and cellular immune responses, spleen viral titers, and mice survival and body-weight changes were examined. The results showed that immunization with gM or gN DNA vaccine alone was not able to offer good protection, whereas co-immunization with both gM and gN induced an effective neutralizing antibody response and cellular immune response, and provided mice with complete protection against a lethal MCMV challenge. This study provides the first in vivo evidence that the gC II (gM-gN) complex may be able to serve as a protective subunit antigen for future HCMV vaccine development.

Cytomegalovirus; Envelope glycoprotein complex; gM/gN; DNA vaccine

Human cytomegalovirus (HCMV), a member of the Betaherpesvirinae subfamily, is a ubiquitous pathogen that infects approximately 60 to 80% of the adult population worldwide (Boeckh M, et al, 2011). It generally causes asymptomatic latent infection in immunocompetent populations, but leads to serious illness and even death among immunocompromised populations, such as transplant recipients, infants with an immature immune system, and patients infected with human immunodeficiency virus (HIV) (Lazzarotto T, et al, 2011; Sung H, et al, 2010). Thus, HCMV infection is an important public-health problem. There are some anti-viral drugs currently used to treat HCMV infection in clinical practice, but their curative rates are not satisfactory, and there are issues such as severe side effects and emergence of drug-resistant HCMV strains (Griffiths PD, 2002; Manley K, et al., 2011). The most effective preventive measure against HCMV infection will therefore be vaccination; unfortunately, more than 30 years of research efforts have not yielded a usable HCMV vaccine (Schleiss MR, et al, 2005). Hence, the development of a CMV vaccine has been assigned the highest priority by the US Institute of Medicine (Arvin AM, et al, 2004; Zhong J, et al, 2007).

HCMV has broad cell tropism, and the pathway for HCMV entry into cells is very complex, involving interactions of multiple viral envelope glycoproteins and a series of cell receptors (Britt WJ, et al, 1996). The HCMV envelope mainly contains three glycoprotein complexes (gC I, II, and III), which are all necessary for virus infection (Wang D, et al, 2005). Of these, the gC II complex, consisting of the gM (also known as gpUL100) and gN (gpUL73) proteins, is the most abundant...
glycoprotein complex. The gM protein accounts for 10% of the net weight of the virus, and studies have shown that knockout of either the gM or gN genes is a lethal mutation (Britt WJ, et al., 2004; Krzyzaniak M, et al., 2007). The gC II complex binds to the heparan sulfate proteoglycan and is inferred to play a role in the initial steps of virus entry into cells. In addition, the gM protein also participates in virus replication and assembly (Krzyzaniak M et al, 2007), and the carboxyl terminal of the gN protein plays a key role in virus envelopment (Mach M, et al, 2007). It has been reported that natural HCMV infection can induce antibody responses against the gC II complex (Mach M, et al, 2000; Shimamura M et al, 2006), and that monoclonal antibodies against gM or gN are capable of neutralizing HCMV infection, indicating that, similar to the gB and gH proteins, the gM and gN proteins are also major target antigens in anti-viral immune responses.

Currently, HCMV vaccine development is focusing mainly on the gC I antigen gB, and the related vaccine has entered clinical Phase III trials. However, clinical studies have shown that the protection offered by the gB protein is only about 50% (Pass RF, et al, 2009), therefore, it is necessary for research and development to be carried out into new antigens that give greater protection. As not only is the gM/gN complex highly conserved in the Herpesviridae family and capable of inducing neutralizing antibody (NAb) response, but it is also the most abundant glycoprotein of HCMV and has become an attractive candidate vaccine antigen. Shen et al. reported that HCMV gM and gN DNA vaccines induced neutralizing antibodies that, in in vitro tests, demonstrated neutralizing activity against multiple HCMV strains (Shen S, et al., 2007). However, as CMV infection has strict species specificity, no animal model is available for studying the mechanisms of HCMV infection and immunity, and so far no in vivo protective study using gM-gN as vaccine antigens has been reported. Mice infected with murine cytomegalovirus (MCMV) has been the most commonly used animal model for simulating HCMV infection (Brune W, et al, 2001; Qureshi, MH, et al, 2005). So far there have been few reports describing the gM/gN proteins of MCMV. The M100 and M73 open reading frames of the MCMV genome encode the homologs of gM and gN, respectively. MCMV gM is speculated to comprise eight transmembrane domains and four N-linked glycosylation sites. Anthony et al. found that MCMV gM was completely conserved between six MCMV strains, suggesting that gM is an antigenically conserved protein (Scalzo A A, et al., 1995).

Generation of a DNA vaccine has long been an important direction for CMV vaccine research and development. Currently, a number of DNA vaccines have entered into clinical trials, such as VCL-CB01 (Phase II) and VCL-CT02 (Phase I) (both Vical Inc., San Diego, CA, USA), in which the protective antigens selected are gB/pp65 and gB/pp65/IE1, respectively. In this study, we prepared gM and gN DNA vaccines based on sequences of the MCMV Smith strain, and the two DNA vaccines were then tested separately and in combination in a lethal MCMV infection mouse model. The results showed that gM-gN antigens have good immunogenicity, and co-immunization with gM and gN DNA vaccines was able to provide the mice with complete protection against a lethal MCMV infection.

MATERIALS AND METHODS

Virus and mice

The MCMV Smith strain was used and propagated in NIH 3T3 cells. MCMV from such cell-culture propagation is referred to as tissue culture-derived MCMV (TC-MCMV), MCMV isolated from mouse salivary glands (SG) followed by in vivo passage for virulence enhancement is referred to as salivary gland (SG)-MCMV, and in this study was used in challenge experiments. The high-virulence SG-MCMV stock had a 50% lethal dose (LD$_{50}$) of approximately 10$^5$ plaque-forming units (PFU) in BALB/c mice, and challenge was performed with 5$\times$LD$_{50}$ virus stock.

Female BALB/c mice were purchased from the Center for Disease Control in Hubei Province, China, and kept under specific-pathogen-free conditions in the Animal Resource Center at Wuhan Institute of Virology, CAS. All procedures were inspected and ratified by the Animal Care Committee of the Wuhan Institute of Virology.

Plasmid construction

DNA vaccines were constructed by cloning the complete open reading frames of gM and gN genes from the MCMV Smith strain into the plasmid expression vector pcDNA3.1. We also constructed a gB DNA vaccine, which encoded only the extracellular domain of gB. All constructs were sequenced in full. The plasmids were cultivated in Escherichia coli DH5α bacteria and purified using NucleoBond® Xtra kits (MACHEREY-NAGEL GmbH & Co. KG, Duren, Germany).

DNA transfection and immunoblotting analysis

The 293T cells were seeded into six-well plates, then 24 hours after plating, cells were transfected with the