Identification and characterization of the guinea-pig cytomegalovirus glycoprotein H gene*

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Accepted June 16, 1996

Summary. Subunit vaccines which target viral envelope glycoproteins offer promise for the prevention of congenital cytomegalovirus (CMV) infection. The guinea pig model of CMV infection is uniquely well suited to testing vaccines for prevention of congenital infection, since, in contrast to other animal cytomegaloviruses, the guinea pig CMV (GPCMV) crosses the placenta, producing intrauterine infection. Antibody to the CMV glycoproteins B (gB) and H (gH) appears to be important in conferring protective immunity. Unfortunately, little is known about specific GPCMV envelope glycoproteins. Sequencing of GPCMV genome fragments was therefore undertaken to test whether GPCMV encodes a gH homologue. Partial sequencing of the Hind III A fragment of the GPCMV genome revealed an open reading frame of 2,169 nucleotides capable of encoding a protein of 723 amino acids. Computer matrix analyses demonstrated identity between this ORF and the gH coding sequences of other herpesviruses. The GPCMV gH ORF encodes 12 highly conserved cysteine residues, contains 9 potential N-linked glycosylation sites, and has a predicted M_r of 81.6 kDa. Northern blot hybridizations with gH-specific probes identified an abundant 5.1 kb mRNA with expression kinetics of an “early” gene. A polyclonal antiserum raised against a synthetic peptide derived from the deduced amino acid sequence of the gH ORF identified a virion-associated protein with an approximate M_r of 85-kDa, the putative GPCMV gH, in immunoblot assays.

* The nucleotide sequences reported in this paper have been submitted to the GenBank database and assigned the accession number U49361.

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Introduction

Infection with human cytomegalovirus (HCMV), although generally asymptomatic in healthy individuals, can have devastating consequences in immunocompromised individuals and newborn infants [2]. One important manifestation of HCMV-associated illness is congenital infection. Congenital CMV infection, occurring in 0.5% to 2.0% of all human births, is the most common perinatally acquired viral infection, and infected infants commonly suffer neurological handicaps, including sensorineural hearing loss [5, 10]. Currently no preventative strategies are available for congenital CMV infection. Epidemiologic evidence, however, indicates that congenital CMV infections which occur in the setting of nonprimary maternal infection are only rarely associated with sequelae, suggesting that preexisting maternal immunity protects the fetus against symptomatic disease [12]. These observations suggest that prenatal maternal immunization against CMV might substantially reduce the morbidity associated with congenital CMV infection. Indeed, if a safe, effective CMV vaccine were available for administration during pregnancy, routine immunization would likely be highly cost-effective and prevent considerable neonatal morbidity [32].

A live, attenuated strain of HCMV (Towne) used as a vaccine is capable of eliciting protective immune responses in immunocompromised transplant recipients [30]. However, immunization of immunologically normal young women with the Towne strain in one study induced virus-neutralizing antibody responses considerably lower than those induced by wild type infection [1]. Furthermore, theoretical considerations about the potential for establishment of latent CMV infection with live attenuated vaccines and concerns about possible oncogenicity of the CMV genome have limited development of live CMV vaccines [31]. Therefore considerable interest has centered around the concept of subunit vaccines using viral envelope glycoproteins as immunogens, particularly the glycoprotein B (gB, gpUL55) and glycoprotein H (gH, gpUL75). The majority of virus-neutralizing antibodies in sera following natural infection recognize gB [7]. However, antibodies are also made against gH during natural infection, and monoclonal antibodies against gH are capable of neutralizing viral infectivity in vitro, suggesting that gH is an important target in the host immune response to CMV infection [9, 25, 35, 36]. Therefore gH may represent an important constituent of a multicomponent subunit HCMV glycoprotein vaccine.

Ideally, a CMV vaccine should be tested in an animal model prior to trials in humans. Unfortunately, the strict species specificity of cytomegaloviruses has rendered such studies difficult. The most relevant small animal model of congenital CMV infection is the guinea pig model. In contrast to the cytomegaloviruses of other small mammals, GPCMV crosses the placenta, causing infection in utero, and affected newborn guinea pigs manifest signs of illness similar to those seen in infants with congenital HCMV infection [18]. The relatively long gestation of the guinea pig (60–70 days) and the structural and histologic