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CYTOMEGALOVIRUS VACCINES AND METHODS OF PRODUCTION - WO2009049138

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Abstract

Human cytomegalovirus infection (CMV) is the most common cause of congenital viral infection in the developed world. Approximately 40,000 congenitally infected infants are born in the United States each year. Congenital CMV infection is responsible for a wide range of neurodevelopmental disabilities, and is the most common infectious cause of hearing loss in children. The significant public health impact of congenital CMV has led the Institute of Medicine to rank development of a CMV vaccine as a top priority. Vaccine development has been ongoing; however there is no licensed CMV vaccine currently available. Before vaccines can be optimized, a better understanding of how CMV infects the host is required. Recently, it has been demonstrated that CMV enters epithelial and endothelial cells by different pathways than those employed for entry into fibroblasts, and that a recently described complex of CMV proteins, the gH/gL/UL128/130/131 complex, is essential for this process to occur. This discovery has allowed identification of novel, heretofore unexplored potential CMV vaccine targets, provides the basis for the patent, "Cytomegalovirus Vaccines and Methods of Production - WO2009049138". In this patent evaluation, the basis for this patent is reviewed. The potential application of this discovery to future CMV vaccine design is discussed.

Keywords

CMV vaccines; Congenital CMV; UL128-131: CMV epithelial and endothelial cell entry

1. Introduction

Human Cytomegalovirus (CMV) is a ubiquitous beta-herpes virus that leads to congenital infection in 0.5–2% of all pregnancies [1]. The risk of intrauterine transmission is highest when primary infection occurs during pregnancy, with a significantly increased risk of adverse fetal effects if primary CMV infection occurs during the first half of pregnancy [2]. Among congenitally infected infants, approximately 10% are symptomatic at birth. Of the remaining 90% of infants who are asymptomatic at birth, 10–15% will subsequently develop permanent sequelae [3], including sensorineural hearing loss (SNHL) and mental retardation. The public health impact of congenital CMV infection is large: indeed, more children suffer from long-term sequelae as a result of congenital CMV infection than either Down syndrome or fetal alcohol syndrome [4].

Congenital CMV infection can occur as the result of a primary CMV infection, reinfection with a new strain of CMV, or by reactivation of a latent infection [5–7]. As noted,

preconception maternal immunity to CMV provides some protection against vertical transmission of the virus. Reports in the literature regarding transmission of CMV to the fetus in the setting of primary maternal infection range from 24–75%, with an overall risk of transmission of approximately 30% reported in a recent meta-analysis [8]. In contrast, the risk of CMV transmission to the fetus after a recurrent maternal infection is only 0.15 to 2% [9, 10]. Fowler *et al.* demonstrated a 69% reduction in the risk of congenital CMV infection in future pregnancies in women who were seropositive for CMV when compared to seronegative women [10]. Women with preconception immunity to CMV have a significant reduction in transmission of CMV to the fetus, as well as decreased severity of disease in infected infants, compared to women with primary infections in pregnancy [9]. In that study, 25% of the infants whose mother had a primary infection had at least one sequela, compared with 8% with recurrent infection.

These observations regarding the protective effect of preconception maternal immunity have provided an impetus for the development of a vaccine against congenital CMV. Although a CMV vaccine could benefit other patient populations, particularly immunocompromised individuals, the public health impact of congenital CMV infection gives such a vaccine a uniquely high theoretical benefit. A recent Institute of Medicine (IOM) report, "Vaccines for the 21st Century", characterized a CMV vaccine *as the highest-level (Level 1) priority for vaccine development in the new millennium* [11, 12]. CMV vaccines evaluated to date in clinical trials have included live, attenuated vaccines as well as subunit vaccine based on key immunogenic proteins [13–15]. One live attenuated CMV vaccine candidate, the Towne vaccine, was found to have a good safety profile and was immunogenic in phase 1 studies, but failed to prevent CMV infection in seronegative women who had children attending group day care when tested in a placebo-controlled trial [16]. Since the laboratory-adapted Towne strain of CMV has genomic deletions that may affect immunogenicity, four "chimeric" vaccines were subsequently created which represented hybrids of the Towne strain and the less attenuated Toledo strain of CMV, and tested in a phase 1 study [17], but have not undergone efficacy evaluation for prevention of CMV infection. Among subunit vaccines for CMV, the envelope glycoprotein B (gB) has been the most extensively studied candidate, since neutralizing antibodies to gB are observed in all CMV-seropositive patients. Results of a phase II, placebo-controlled, randomized, double-blind trial of recombinant gB with MF59 adjuvant were recently published [18]. In this study, three doses of the CMV vaccine or placebo were administered at 0, 1, and 6 months to healthy women within 12 months postpartum. Women in the vaccine group were less likely to become infected with CMV than the placebo group ($p=0.02$), with a vaccine efficacy in this study of 50%. This was the first report of efficacy of any vaccine for prevention of CMV infection.

2. Biology

For decades, the majority of laboratory studies of the cellular biology of CMV infection were performed using human fibroblast cells. Primary clinical isolates of CMV, which are highly cell-associated, grow poorly in fibroblasts, but replication kinetics and viral yield improve upon serial passage. Following laboratory adaption, strains of CMV such as the Towne strain are able to generate high titers of extracellular virus, making them convenient for *in vitro* studies requiring large quantities of input virus. Correlates of protective immunity induced by vaccination have included assessment of the ability of antibody to neutralize the infectivity of adapted strains of CMV in fibroblast-based assays. *However, recent insights into the biology of attachment and entry of CMV into epithelial and endothelial cells have demonstrated that the host neutralizing response is more complex than previously thought.* Moreover, the pathways by which CMV enters fibroblasts versus endothelial/epithelial cells are different, suggesting that vaccine design may need to anticipate and prevent these distinct pathways of infection.

In the early part of this decade, work with herpes simplex virus (HSV) demonstrated that this virus can enter cells by at least two distinct pathways. Although HSV entry into Vero cells, fibroblasts, and neurons was shown to occur by the mechanism of pH-independent receptor-mediated fusion at the cell surface, entry into epithelial cells was demonstrated to occur via a pH-dependent "endocytic entry" pathway [19]. In this model, HSV first attaches to the cell surface, is endocytosed, and ultimately achieves fusion and cytoplasmic entry following endosome acidification. The possibility that CMV might employ similar cell type-specific mechanisms of entry was initially suggested by the work of Hahn and colleagues [20]. This work, using an endotheliotropic strain of CMV cloned as a bacterial artificial chromosome (FIX-BAC), demonstrated that three CMV genes, *UL128*, *UL130*, and *UL131*, were critical for endothelial cell tropism. Detailed genetic analyses revealed that attenuated, non-endotheliotropic strains of CMV, such as the Towne strain and another laboratory-adapted strain, AD169, contain mutations in these genes that modified their tropism in cell culture. Importantly, both Towne and AD169 could be "repaired" for growth in endothelial cells by correcting frame shift mutations in the *UL130* or *UL131* genes, respectively. These genetic determinants of endothelial tropism were subsequently found to also occur for CMV entry into dendritic and epithelial cells [21, 22]. Entry of CMV into endothelial and epithelial cells was subsequently shown to be mediated by endocytosis in a pH-dependent fashion; in contrast, entry into fibroblasts was shown to be non-endocytic and pH-independent [23], analogous to the mechanisms previously identified for HSV [19].

The current working understanding the endocytic and non-endocytic pathways of CMV entry are schematically reviewed in Fig. 1. CMV fibroblast entry is believed to be initiated by binding of virion-associated gB to an as-yet incompletely characterized cell surface receptor, followed by fusion with the cell membrane in a process that requires a complex of three other glycoproteins: gH, gL, and gO. The role gO plays in this setting is incompletely understood; although a gH/gL/gO complex is formed in laboratory adapted strains capable of high-level infection of fibroblasts, recent evidence from work with a primary clinical isolate suggests that it may not be present in the envelope, and may primarily function as a gH/gL chaperone protein [24, 25]. In contrast to the non-endocytic model of CMV entry, endocytic entry of CMV requires a complex of gH, gL, and UL128, 130, and 131. The mechanisms by which UL128, UL130, and UL131 mediate endocytic entry continue to be examined by a number of laboratories. The Shenk laboratory demonstrated that UL131 facilitates formation of the novel complex consisting of at gH, gL, UL128, and UL130 but not gO. In the absence of UL131, UL128 and UL130 are expressed intracellularly, but do not become incorporated into virions [22]. Additional, independent interactions between gH/gL and UL128, UL130, and UL131 have been identified by the Johnson laboratory, as well as evidence for epithelial cell type-specific receptors for this complex [26, 27]. A heteropentameric species containing all five proteins has been described in cells coinfecting with adenoviruses expressing these proteins [26], providing further evidence for the role that these protein-protein interactions play in epithelial cell entry of CMV via this complex.

3. Expert Opinion

What is the relevance of the endocytic and non-endocytic pathways of CMV cellular entry to vaccine design? *The invention claimed in this patent centers around the concept that an increased diversity of CMV vaccine targets can be engendered by propagation of CMV in non-fibroblast cell types.* The emphasis is on propagation in epithelial cells, but the invention is not limited to this cell type. Potential application of this approach is claimed for live, attenuated vaccine design; formalin-inactivated whole viral vaccines; and adjuvanted subunit protein vaccines containing proteins (e.g., the gH/gL/UL128/130/131 complex) from CMV propagated in epithelial cells. The possibility of producing cell-type conditioned CMV

in two or more different cell types, followed by combining the CMVs to produce a single vaccine, is also claimed.

Is there evidence to suggest that a CMV vaccine targeting the endocytic pathway of entry would provide advantages over existing CMV vaccine strategies in clinical trials? Several lines of evidence suggest that the answer to this question may be “yes”. The work of the McVoy [28] and Gerna [29] laboratories has demonstrated that the majority of the neutralizing activity of convalescent human sera from CMV-seropositive individuals targets the endocytic pathway of entry. In this context, conventional determination of the neutralizing activity of human sera in fibroblasts may be highly misleading. Work from the McVoy laboratory comparing sera from naturally infected subjects to sera from recipients of the Towne vaccine or the gB/MF59 vaccine showed that Towne recipient sera had epithelial entry neutralizing titers on average 28-fold lower than natural infection, while gB/MF59 recipient sera were 15-fold lower [28]. These data suggest significant concerns for the potential efficacy of either vaccine for prevention of CMV infection, the encouraging results of the recent phase II study of gB/MF59 [18] notwithstanding. Recently, a panel of human monoclonal antibodies was isolated that neutralized, at extremely low concentrations, CMV infection of endothelial, epithelial, and myeloid cells [30]. All but one of the antibodies in this panel was found to bind to conformational epitopes that required expression of two or more proteins of the gH/gL/UL128-131A complex. Antibodies generated against gB were able to neutralize infection of both endothelial-epithelial cells and fibroblasts, but with decreased potency compared to those that targeted the pentameric complex. Thus, these proteins represent highly intriguing targets for future subunit vaccine design.

What are the practical implications of a CMV vaccine strategy that primarily targets epithelial cell modes of entry? *Key to this analysis is the knowledge that the majority of CMV infections are acquired at mucosal surfaces.* Common modes of transmission include acquisition of primary infection in the oropharyngeal epithelium, or sexual transmission via the genitourinary tract epithelium [31]. Thus, the invention claimed in this patent may be uniquely well suited to the interruption of CMV transmission at the most route of acquisition of infection, the mucosal surface. It remains to be determined what will be the ideal expression system for eliciting antibodies capable of neutralizing viral infectivity at epithelial surfaces. Repair and restoration of wild-type coding sequences for the *UL128*, *130* and *131* genes in highly attenuated tissue culture-adapted strains of CMV is one option, but it runs the risk of conferring an unacceptable safety profile for a live virus vaccine, particularly if such a CMV vaccine had restoration of replication competence in epithelial and endothelial cells *in vivo*. Subunit expression of the gH/gL/UL128/130/131 proteins, either as a pentameric complex or individually, merits consideration for future preclinical and clinical development. Another potential strategy would be the therapeutic administration of monoclonal antibodies targeting these proteins in a passive vaccination strategy toward the goal of interrupting CMV transmission to the fetus in pregnancies at high risk for congenital CMV transmission: such an approach has been shown to be successfully using pooled, polyclonal anti-CMV antibody [32]. Irrespective of the eventually vaccine strategies studies, the identification of novel pathway of CMV infectivity and neutralization is an important advance in our understanding of potential vaccine strategies, and the invention described in patent WO2009049138 is highly likely to lead to successful, novel approaches to the eventual control of congenital CMV infection.

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Figure 1. Distinct pathways exist for entry of CMV into fibroblasts and epithelial/endothelial cells

A CMV-encoded gH/gL-containing complex, gH/gL/gO, has been described for fibroblasts. This pathway of infection is non-endosomal, pH independent, and infection can be neutralized by antibodies engendered to gB or Towne vaccines. In contrast, CMV depends upon a five protein complex, gH/gL/UL128-UL131, to enter epithelial and endothelial cells; this pathway is endosomal, pH dependent, and infectivity can be neutralized by antibodies to this pentameric complex. The current prevailing model is that gH/gL/UL128-131 is required for entry into biologically important epithelial and endothelial cells and gH/gL/gO is required for infection of fibroblasts.