CMV vaccine progress - the gH pentamer story

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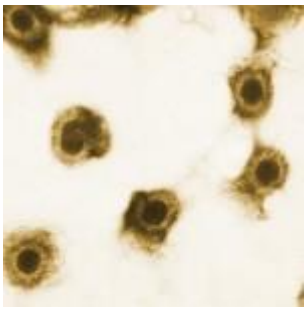
What is CMV?



Human cytomegalovirus (HCMV) is a member of the β-herpesviruses. It is a global, species-specific, highly successful pathogen with a seroprevalence of 50-90%, depending on socio-economic status (1). The virus can be transmitted both vertically and horizontally by bodily fluids, establishing a life-long latent infection with intermittent virus re-activation.

Transmission from mother to foetus is the most common cause of infectious central nervous system damage, including sensorineural hearing loss and/or motor and cognitive deficits (2) (3). In addition, HCMV infection or re-activation can lead to death or major sequelae in immunocompromised individuals (4).

To date there is no effective vaccine on the market although there have been vaccine attempts in the past, and at least 3 major pharma companies have vaccine candidates in clinical trials.

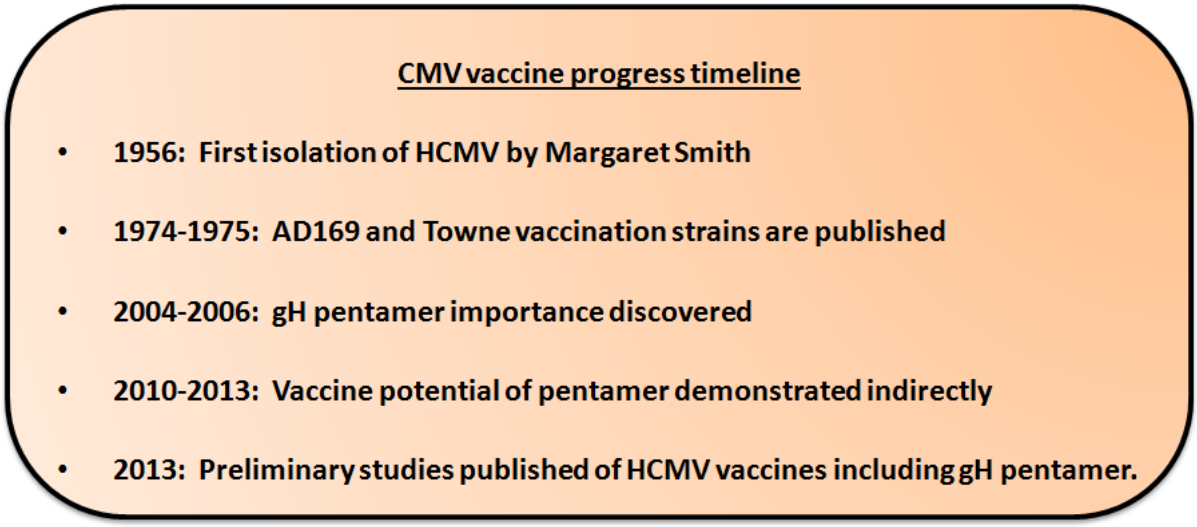
Discovery of the pentamer

HCMV can infect a variety of cell types *in vivo* such as peripheral blood monocytes, hematopoietic cells, and epithelial and endothelial cells (5) (6) (7). *In vitro* an even wider variety of cells can be used to propagate HCMV: smooth muscle cells, retinal pigment epithelial cells, trophoblasts, hepatocytes, kidney glomerular visceral cells and brain cells (8) (9) (10) (11) (12) (13).

However, as early as 1991 it was shown that clinical isolates of HCMV serially passaged in fibroblasts could not produce cytopathic effect in endothelial cells whereas the same isolate was able to produce cytopathic effect in endothelial cells when serially passaged in endothelial cells (14). Further, it was shown that HCMV strains extensively passaged in fibroblast cells such as the early vaccine candidates and widely used laboratory strains AD169 or Towne had lost their ability to infect endothelial cells or leukocytes, whereas the low-passage strain Toledo retained this property (15) (16) (17).

Early comparisons of the genomes of AD169, Towne and Toledo strains revealed a 13-15 kpb region missing in AD169 and Towne that was present in Toledo (18). Aided by the development of a bacterial artificial chromosome harbouring the full genome of clinical isolate VR1814 in 2002 and the possibility of molecular manipulation (19) this region was elegantly shown to contain the three open reading frames (ORFs) UL128, UL130 and UL131A that each are indispensable for productive replication of HCMV in endothelial cells and virus transfer to leukocytes as shown by endothelial infectivity loss-of-function and gain-of-function studies (20) (21). This was followed by the discovery of complex formation of UL128 and UL130 with glycoproteins H (gH) and L (gL) in 2005 (22), and the report of its molecular

characterisation in 2008 (23). By using protein-protein interaction studies Ryckman et al. could show that the proteins gH, gL, UL128, UL130 and UL131A assemble into a pentameric complex, and only when completely assembled can the pentameric complex be transported from the endoplasmic reticulum (ER) to the Golgi complex (23).



Initial vaccine candidates

The ramifications of these findings became clear when two HCMV vaccine candidates, Towne strain and recombinant glycoprotein B (gB/MF59), were tested for their ability to induce neutralising antibodies against fibroblast and epithelial/endothelial cell entry: Both vaccine candidates produced neutralising antibody titers 28-fold or 15-fold, respectively, lower than a natural HCMV infection when tested for epithelial/endothelial cell entry (24). In addition, it was shown that a panel of patient immune sera had 128-fold higher titers of neutralising antibodies when the same clinical HCMV isolate was used to infect endothelial cells rather than fibroblasts, and that monoclonal antibodies raised against UL130 and UL130A gene products displayed major neutralising activity (25).

In 2010 Macagno et al. reported the isolation of potently neutralising antibodies from human serum targeted against epitopes on the pentameric complex (26). In 2011 a panning of patient sera taken at different stages of infection showed that neutralising antibodies to UL128, UL130 and UL131A are produced within 2-4 weeks post infection and last up to 12 months, whereas antibodies against gH were delayed (27). This also suggested use of neutralising antibody titers elicited against the pentameric complex for evaluation of vaccine efficacy. Interestingly, a similar later study using recombinant gH pentameric complex found that high antibody titers against pentameric complex correlated with lower rates of transmission of HCMV from mother to foetus by the congenital route (28). In fact, it was found that the majority of antibodies in hyperimmunoglobulin used to treat organ transplant patients who contracted HCMV targeted the gH pentameric complex (29). All these studies

strongly suggested that future HCMV vaccines need to elicit antibodies against the gH pentameric complex, and that these antibodies could be able to control HCMV infection and spread.

Latest approaches

To this end, short peptides of UL130 and UL131A gene products were used to immunise rabbits, and resulting antibodies could block HCMV entry into epithelial cells *in vitro* (30). The next step was to immunise rhesus macaques with a vaccinia virus encoding the rhesus macaque CMV gH pentameric complex. All 8 animals used in this study developed antibodies that could neutralise entry of virus into epithelial cells and fibroblasts, and subcutaneous challenge 8 weeks post immunisation resulted in markedly reduced viral load (31). This was followed by construction of a vaccinia virus expressing the HCMV pentameric complex, and mice and rhesus macaques were vaccinated with recombinant vaccinia virus. Again, elicited antibodies were able to block entry of a clinical HCMV isolate into placental macrophages, a possible vehicle for congenital virus transfer (32). Recent studies on their CMV vaccine progress published by Novartis and Merck show that their respective vaccine candidates based on either viral replicon particles expressing gH pentameric complex, recombinantly produced pentameric complex or an attenuated HCMV strain with reconstituted gH pentamer complex expression all elicited high titers of neutralising antibodies in either mice or rabbits (33) (34).

All these studies strongly suggest that the HCMV gH pentameric complex is the missing piece of the HCMV vaccine. To aid pharma companies and academic researchers achieve this goal, The Native Antigen Company decided to produce a soluble version of the HCMV gH pentameric complex expressed in human cells.

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