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Exploiting 2A peptides to elicit potent neutralizing antibodies by a multi-subunit herpesvirus glycoprotein complex

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ABSTRACT

Neutralizing antibodies (NAb) interfering with glycoprotein complex-mediated virus entry into host cells are thought to contribute to the protection against herpesvirus infection. However, using herpesvirus glycoprotein complexes as vaccine antigens can be complicated by the necessity of expressing multiple subunits simultaneously to allow efficient complex assembly and formation of conformational NAb epitopes. By using a novel bacterial artificial chromosome (BAC) clone of the clinically deployable Modified Vaccinia Ankara (MVA) vector and exploiting ribosomal skipping mediated by 2A peptides, MVA vectors were generated that expressed self-processing subunits of the human cytomegalovirus (HCMV) pentamer complex (PC) composed of gH, gL, UL128, UL130, and UL131A. These MVA vectors expressed 2A-linked HCMV PC subunits that were efficiently cleaved and transported to the cell surface as protein complexes forming conformational neutralizing epitopes. In addition, vaccination of mice by only two immunizations with these MVA vectors resulted in potent HCMV NAb responses that remained stable over a period of at least six months. This method of eliciting NAb by 2A-linked, self-processing HCMV PC subunits could contribute to develop a HCMV vaccine candidate and may serve as a template to facilitate the development of subunit vaccine strategies against other herpesviruses.

1. Introduction

Herpesviruses include a number of ubiquitous and highly-adapted human pathogens that can cause severe illnesses in individuals with impaired immune system such as transplant recipients or AIDS patients or in congenitally infected fetuses (Arvin et al., 2007). While licensed vaccines are available for varicella-zoster virus (VZV), vaccines candidates for other human herpesviruses including herpes-simplex viruses (HSV), human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), and Kaposi sarcoma-associated herpesvirus (KSHV) remain elusive (Arvin et al., 2007; Stratton et al., 2000). Considerable efforts to develop herpesvirus vaccines have been made using approaches based on selected humoral or cellular immunodominant antigens as safer and more cost-effective alternatives to live-attenuated, inactivated, or replication-defective herpesvirus vaccines. These subunit vaccine approaches using plasmids, viral vectors, purified protein, or virus-like particles showed promising results in different animal models and clinical trials (Cohen, 2015; Dasgupta et al., 2009; Johnston et al., 2016; Pass et al., 2009; Schleiss, 2008; Schleiss, 2016).

Eliciting neutralizing antibodies (NAb) that interfere with glycoprotein complex-mediated virus entry into host cells is thought to be important for a vaccine formulation to prevent or control herpesvirus infection (Nelson et al., 2017; Plotkin, 2013). While the essential and highly-conserved herpesvirus envelope glycoprotein complexes composed of gB and gH/gL are principal targets for NAb, additional glycoprotein complexes or accessory glycoproteins associating with gH/gL can represent critical immune targets that contribute to the stimulation of herpesvirus neutralizing activity (Heldwein, 2016; Macagno et al., 2010; Sathiyamoorthy et al., 2017; Vanarsdall and Johnson, 2012). Over the past years it has been discovered for HCMV that NAb blocking infection of many biologically relevant host cells recognize in majority three accessory glycoproteins called UL128, UL130, and UL131A that form a pentamer complex (PC) with gH/gL (Fouts et al., 2012; Macagno et al., 2010; Wang and Shenk, 2005). While this complex is dispensable for HCMV infection of fibroblasts (FB), it is required for efficient infection of epithelial cells (EC), endothelial cells, and other cells thought to be important for HCMV dissemination and transmission (Hahn et al., 2004; Sinzger et al., 2008; Vanarsdall and Johnson, 2012; Wang and

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Shenk, 2005). In contrast to NAb interfering with the essential entry function of gB and gH/gL complexes, NAb interfering with PC-mediated entry are unable to block FB infection, though they are substantially more potent than NAb targeting gB or gH/gL epitopes in preventing infection of non-FB cell types such as EC (Chiuppesi et al., 2015; Macagno et al., 2010). These results suggest that the PC could be an important vaccine component to prevent or control HCMV infection.

Since NAb targeting the HCMV PC recognize mainly quaternary conformational epitopes that are formed effectively only upon assembly of UL128/130/131A with gH/gL, vaccine-mediated induction of HCMV NAb is promoted by simultaneous expression of all five PC subunits (Chandramouli et al., 2017; Chiuppesi et al., 2017a; Chiuppesi et al., 2015; Chiuppesi et al., 2017b; Ciferri et al., 2015a; Ciferri et al., 2015b; Macagno et al., 2010; Wussow et al., 2014; Wussow et al., 2013). Ribosomal skipping mediated by 2A peptides of picornaviruses is a widely used mechanism to express multiple proteins via a single transcript because of the relative small size of the 2A peptides (18–22 amino acids) and the potential stoichiometric expression of the co-expressed proteins (de Felipe, 2004; Kim et al., 2011; Szymczak et al., 2004). By using a novel bacterial artificial chromosome (BAC) clone of the well-characterized and clinically-deployable Modified Vaccinia Ankara (MVA) vector (Cottingham et al., 2008; Gilbert, 2013; Verheust et al., 2012), the use of 2A-mediated ribosomal skipping was exploited to stimulate NAb by self-processing subunits of the HCMV PC. The results indicate that recombinant MVA vectors either expressing 2A-linked polyproteins composed of all five PC subunits or co-expressing 2A-linked polyproteins composed of UL128/130/131A and gH/gL stimulate robust HCMV NAb responses in mice. This method of eliciting HCMV NAb by 2A-linked polycistronic expression constructs could contribute to develop a HCMV vaccine candidate and may serve as template to facilitate the development of vaccine approaches based on multi-subunit glycoprotein complexes of other herpesvirus.

2. Materials and methods

2.1. Cells and viruses

Baby hamster kidney (BHK-21) cells, ARPE-19, MRC-5 (American Type Culture Collection [ATCC]) and chicken embryo fibroblasts (CEF; Charles River) were maintained by standard procedures. MVA was propagated in BHK-21 and CEF cells as described previously (Wussow et al., 2014). HCMV and MVA virus stocks were prepared following virus propagation in ARPE-19 or BHK-21 cells, respectively, and titrated as described (Chiuppesi et al., 2017a; Chiuppesi et al., 2015; Wussow et al., 2014). HCMV TB40/E expressing GFP was derived from TB40/Ewt-GFP BAC DNA (O'Connor and Murphy, 2012). MVA 1974/NIH clone 1 was kindly provided by Dr. Bernard Moss (NIAID) (Mayr and Malicki, 1966). The construction of MVA-PC has been described previously (Wussow et al., 2014).

2.2. Plasmids

Transfer plasmids for generating a novel MVA BAC, termed MVABAC-TK (Fig. 1), or MVABAC-TK-derived recombinants with 2A-linked HCMV PC subunits were constructed by standard molecular biology cloning techniques. For generating MVABAC-TK, a transfer vector was generated in which pBeloBAC11 sequences (New England Biolabs) and a GFP expression cassette with vaccinia P11 late promoter were flanked by DNA sequences homologues to the MVA Thymidine kinase (TK) gene locus (Fig. 1). MVA TK homology flanks were derived by PCR from MVA 1974/NIH clone 1 and corresponded to base pairs 69313–70000 and 70001–70703 (MVA Acambis, Accession Nr. AY603355.1). The GFP marker with P11 promoter was derived by PCR from plasmid pLW73 (Wyatt et al., 2009). A unique *Avr*II restriction site was introduced between the ends of the TK homology flanks to allow linearization of the entire transfer construct (Fig. 1). Transfer constructs

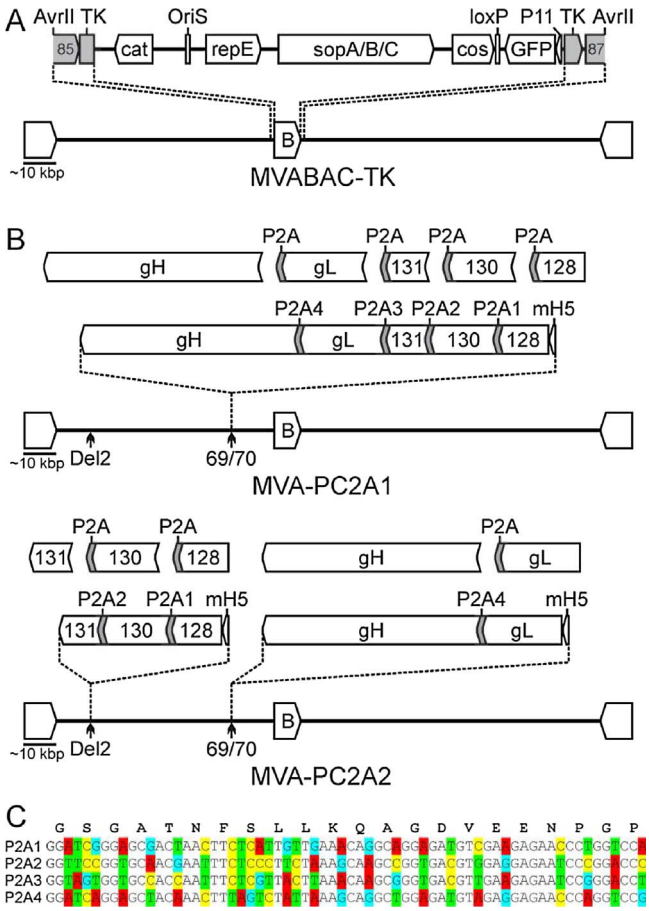

Fig. 1. Construction of MVA expressing self-processing HCMV PC subunits. A) MVABAC-TK construction. pBeloBAC11 vector sequences (B = cat, OriS, repE, sopA/B/C, cos, loxP site) and a GFP expression cassette (GFP, Vaccinia P11 promoter) were inserted into the Thymidine kinase (TK) gene MVA utilizing ~700 bp homologous sequences (gray filled elements). 85 and 87 = MVA ORFs 85 and 87 (Accession Nr. U94848). B) Construction of MVA-PC2A1 and MVAPC2A2. HCMV PC subunits (gH, gL, UL128, UL130, UL131A) linked by different P2A sequences (P2A1-P2A4) were inserted into MVABAC-TK either all together into the MVA intergenic region 69/70 (IGR69/70) to generate MVA-PC2A1, or separately as UL128/130/131A and gH/gL subunit subsets into the MVA deletion 2 (Del2) site and IGR69/70 to generate MVA-PC2A2. B = BAC vector; mH5 = modified H5 promoter. C) P2A sequences (P2A1-P2A4) with different codon usage were used to link the HCMV PC subunits within the MVA constructs as indicated in B. Lower 4 lines indicate DNA sequences with mutated nucleotides (marked in colors) that were used to encode the different P2A peptides between the HCMV subunits. Upper line shows the amino acid sequences of the P2A peptide.

for inserting P2A-linked HCMV PC subunits into MVABAC-TK by *En passant mutagenesis* were generated by cloning codon-optimized and P2A-linked UL128/UL130/UL131A or gH/gL subunit subset gene sequences between the vaccinia modified H5 early/late promoter (mH5) and vaccinia transcription termination signal (TTTTAT) of pGEM-T-mH5 (Wussow et al., 2013). A kanamycin expression cassettes with adjacent I-SceI homing endonuclease restriction sites and flanking 50 bp gene duplication was subsequently introduced into the pGEM-T-mH5-cloned HCMV PC subunit subset genes sequences (Tischer et al., 2010; Wussow et al., 2013). All HCMV PC subunit gene sequences were based on HCMV strain TB40/E (TB40/E-BAC; Accession Nr. EF999921). Codon-optimized and P2A-linked PC gene sequences of HCMV TB40/E were synthesized by Genescript. Optimization of the HCMV TB40/E gene sequences for Vaccinia codon usage was performed using the Codon Optimization Tool from Integrated DNA Technologies. Runs of more than three nucleotides of the same type in a row within the codon-optimized P2A-linked HCMV gene sequences were silently mutated to enhance the stability of HCMV genes within MVA (Wyatt et al., 2009). Gene internal Kanamycin/I-SceI cassettes flanked by 50 bp gene