Virology 483 (2015) 253–263

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Structural comparison of four different antibodies interacting with human papillomavirus 16 and mechanisms of neutralization

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ARTICLE INFO

Article history:
Received 20 March 2015
Returned to author for revisions
13 April 2015
Accepted 17 April 2015
Available online 19 May 2015

Keywords:
Cryo-EM
Virus Fab complex
HPV16
Neutralization
mAb
Crosslinking
Stabilization
Epitope
Human papillomavirus
Conformation

ABSTRACT

Cryo-electron microscopy (cryo-EM) was used to solve the structures of human papillomavirus type 16 (HPV16) complexed with fragments of antibody (Fab) from three different neutralizing monoclonals (mAbs): H16.1A, H16.14J, and H263.A2. The structure-function analysis revealed predominantly monovalent binding of each Fab with capsid interactions that involved multiple loops from symmetry related copies of the major capsid protein. The residues identified in each Fab-virus interface map to a conformational groove on the surface of the capsomer. In addition to the known involvement of the FG and HI loops, the DE loop was also found to constitute the core of each epitope. Surprisingly, the epitope mapping also identified minor contributions by EF and BC loops. Complementary immunological assays included mAb and Fab neutralization. The specific binding characteristics of mAbs correlated with different neutralizing behaviors in pre- and post-attachment neutralization assays.

Introduction

Human papillomaviruses (HPVs) cause epithelial tumors and are the etiologic agents of numerous anogenital and oropharyngeal cancers (Bosch et al., 1995; Walboomers et al., 1999; Crow, 2012). Identification of neutralization-sensitive epitopes on the capsid protein structures (conformational epitopes) support investigations to develop improved recombinant vaccines that maximize effective and long-term antibody-mediated protection against multiple HPV types (Culp et al., 2007). As one of the major cancer-causing HPV types, HPV16 is extensively studied (Bosch et al., 1995; Crow, 2012; Castillo, 2013; Marur et al., 2010; Burk et al., 2009), and together with HPV18 comprises a major target for vaccine development (Zhao et al., 2012; Monie et al., 2009). Since the life cycle of HPVs relies on differentiation of basal cells into keratinocytes, purifying high titer virus stocks for structural studies is difficult. Therefore, other production methods have been developed as an alternative for studies of the native virions. Virus-like particles (VLPs) are comprised of only the major structural protein, L1, and are not infectious since they are devoid of viral genome (Kirnbauer et al., 1992). Quasivirions (QV16) and pseudovirions (PsV16) were used for our structural analysis and neutralization assays (Christensen, 2005; Buck et al., 2005) as both types of HPV 16 particles contain a mock genome.

Papillomaviruses form a $T=7$ icosahedral, non-enveloped ~55 to 60 nm diameter capsid containing a circular dsDNA genome of 8 kb. The capsid is comprised of 360 copies of the L1 structural protein and up to 72 copies of the L2 minor structural protein (Buck et al., 2005; Baker et al., 1991). Five L1 proteins intertwine to form each capsomer, 72 of which make up one capsid. Twelve of the 72 capsomers lie on an icosahedral five fold vertex and are described as pentavalent capsomers. The remaining 60 capsomers are each surrounded by six other capsomers and are consequently referred to as hexavalent capsomers. The C-terminus, or "C-terminal arm," of each L1 protein extends along the capsid floor to interact with the neighboring capsomer and then returns to the original donor capsomer (Wolf et al., 2010; Cardone et al., 2014; Lee et al., 2014). Inter-capsomer disulfide bonds are formed between cysteine C428 and C175, which stabilize the capsid

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http://dx.doi.org/10.1016/j.virol.2015.04.016

structure and play an important role in virus maturation (Cardone et al., 2014; Buck et al., 2005). The core of the capsomer is composed of the common viral structural motif, the antiparallel β-strands BIDG and CHEF (Rossmann and Johnson, 1989), which are connected by surface loops of BC, DE, EF, FG, and HI. Nearly all conformational epitopes are located on one or more of these outwardly facing surface-exposed loops (Carter et al., 2003). Our knowledge of these epitopes has been largely obtained from mAb/Fab binding and neutralization assays (Culp et al., 2007; Christensen et al., 1996; Day et al., 2007; Day et al., 2008), hybrid virus loop exchange studies (Christensen et al., 2001), and previous structural analysis (Lee et al., 2014; Bishop et al., 2007). These complementary studies represent an important approach to analyze the nature of conformational epitopes, neutralization mechanisms, and how the host immune system recognizes and responds to the virus.

H16.V5 is a well-characterized HPV16-specific neutralizing mAb induced by HPV16 L1 VLPs. This mAb has been extensively used in major HPV vaccination trials and is an especially important tool in inhibition-based HPV serological assays (Zhao et al., 2012; Carter et al., 2003; Christensen et al., 1996; Wang et al., 1997; Rizk et al., 2008; Deschuyteneer et al., 2010). The neutralizing antibodies of H16.1A, H16.14J, and H263.A2 were raised against HPV16 L1 VLP (Christensen et al., 1996) or hybrid capsids (Christensen et al., 1991). Like H16.V5, based on previous immunological studies, all three antibodies were thought to recognize portions of the FG and HI loops. The H16.V5 neutralization mechanism has been shown to be one of capsid stabilization that consequently inhibits the conformational changes required during entry (Zhao et al., 2012; Rizk et al., 2008; Deschuyteneer et al., 2010; Chen et al., 2011). Although many immunological studies of H16.V5 neutralization have been published, no information on H16.V5 Fab has been recorded. For the three antibodies H16.1A, H16.14J, and H263.A2, details of neutralization are unknown.

Previously, two HPV16-H16.V5 complex cryo-EM maps of 20 Å (Zhao et al., 2014) and 10 Å (Lee et al., 2014) resolution showed that H16.V5 Fab binding induced conformational changes and bound predominately to the hexavalent capsomers. Here we present three new cryo-EM structures of HPV16 complexed with the Fabs from the specific mAbs, H16.1A, H16.14J, and H263.A2 at ~12 Å resolution (Fig. 1). Atomic structures of the component parts, virus and Fab, were fitted into the cryo-EM complex maps using rigorous fitting algorithms developed for this purpose (Rossmann et al., 2005, 2001; Fabiola and Chapman, 2005). For each complex, the resulting pseudo-atomic model was used to define the Fab binding site and identify the amino acids that likely comprise the complex conformational epitope (Rossmann et al., 2005; Fabiola and Chapman, 2005; Hafenstein et al., 2009). We found that besides the well-known FG and HI loops, the DE loop also composes the core of each footprint; however, additional participation by BC and EF loops vary between the antibodies. The structural results were complemented with immunological studies that showed Fabs from all four neutralizing antibodies are also neutralizing, albeit at higher molar concentrations. H16.1A and H16.14J Fabs can distinguish conformational changes of the capsid after host cell attachment, whereas H16.V5 and H263.A2 do not. Differences in the antibody footprints correlate with these immunological differences. Thus, the structure-function study predicts the neutralization mechanisms to be a combination of stabilization and cross-linking.

Experimental procedures

Preparation of HPV16 quasivirus and pseudovirus

QV16 virions are comprised of HPV16 L1 and L2 proteins and encapsidate the cottontail rabbit papillomavirus genome (CRPV) containing the SV40 origin of replication. QV16 were prepared as described previously (Brendle et al., 2010; Mejia et al., 2006; Pyeon et al., 2005). PsV16 virions are comprised of the HPV16 L1 and L2 proteins and contain the pYSEAP (alkaline-phosphatase producing genome). Briefly, HPV16 sheLL plasmid (kindly provided by John Schiller, NIH) was transfected together with linear CRPV/SV40ori DNA (QV) or pYSEAP (PsV) into 293TT cells and prepared as described previously (Buck et al., 2005; Pastrana et al., 2004). PsVs were purified by Optiprep gradient centrifugation as previously described (Buck et al., 2004). QVs were allowed to mature overnight and then pelleted by centrifugation. The centrifuged pellet was re-suspended in 1 M NaCl 0.2 M Tris, pH 7.4. After CsCl gradient purification, the lower band was collected, concentrated, and dialyzed against PBS, as described previously (Lee et al., 2014). The concentrated QVs were applied to Formavar-coated copper grids, stained with 2% phosphotungstic acid, and analyzed for integrity and concentration on a JEOL JEM 1400 electron microscope.

Preparation of antibody and Fabs

Antibodies of H16.V5, H16.1A, H16.14J and H263.A2 were generated in Balb-C mice as described previously (Christensen et al., 1996; Christensen et al., 1991). Hybridomas were acclimated to animal component free media (BD) and supernatant was purified on Protein A or G columns (Pierce). Fab was prepared by digestion with papain in the presence of cysteine (Pierce). Purity of the Fab was assessed by the lack of the fragment crystallizable (Fc) portion and integrity of the Fab was determined by ELISA. Antibody and Fab protein concentrations were determined by absorbance spectrometry at a wavelength of 280 nm.

Sequencing of antibody heavy and light chains

The hybridoma cells were pelleted by centrifugation and RNA was extracted using TRIzol® Reagent (Life Technologies). Total RNA was treated with DNase I (RNase-free) (New England Biolabs) to digest potentially contaminating DNA in the sample. cDNAs were synthesized from treated RNA with the RevertAid First Strand cDNA synthesis kit (Thermo Scientific). The cDNAs were used as a template for PCR and amplified using Pfu Turbo DNA Polymerase (Agilent) or Choice Taq DNA polymerase (Denville). PCR amplification used primers previously described by Wang et al. (2000). Immunoglobulin heavy chains were amplified using the isotype specific constant region 3′ primer and two highly degenerate 5′ primers. The light chains were amplified using the 3′ degenerate kappa chain constant region primer and the 5′ kappa chain framework one region universal degenerate primer. Prior to sequencing, PCR products were purified using the QIAquick PCR Purification Kit (Qiagen). The same primers used for PCR amplification were also used as sequencing primers to obtain initial sequences. Resolution of the 5′ and 3′ ends of the sequence required sequence specific primers. The 263.A2 light chain required cloning into pUC19 to completely resolve the sequence.

Neutralization assays

The neutralization activity of mAbs and Fabs was investigated by pre- and post-virus attachment assays in 293TT cells. In the pre-attachment assay, PsVs were pre-incubated with mAb or Fabs at dilutions ranging from 66 nM to 0.067 pM for 1 h at 37 °C before adding to cells. For the post-attachment assay, PsVs were incubated with 293TT cells for 1 h at 4 °C to allow for attachment. Cells were washed 1x with media prior to incubation with mAbs or Fabs. 72 h later 30 μl of the cell culture supernatant was assayed with 4-Nitrophenyl phosphate disodium salt hexahydrate (pNPP) (Sigma) and the optical density was determined by absorbance