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Characterization of murine antibody responses to vaccinia virus envelope protein A14 reveals an immunodominant antigen lacking of effective neutralization targets

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ABSTRACT

Vaccinia virus (VACV) A14 is a major envelope protein and a dominant antibody target in the smallpox vaccine. However, the role of anti-A14 antibodies in immunity against orthopoxviruses is unclear. Here, we characterized 22 A14 monoclonal antibodies (mAb) from two mice immunized with VACV. Epitope mapping showed that 21 mAbs targeted the C-terminal hydrophilic region, while one mAb recognized the middle region predicted to be across the viral envelope from the C-terminus. However, none of the mAbs bound to virions in studies with electron microscopy. Interestingly, some mAbs showed low VACV neutralization activities in the presence of complement and provided protection to SCID mice challenged with VACV ACAM2000. Our data showed that, although A14 is an immunodominant antigen in smallpox vaccine, its B cell epitopes are either enclosed within the virions or are inaccessible on virion surface. Anti-A14 antibodies, however, could contribute to protection against VACV through a complement-dependent pathway.

1. Introduction

Smallpox was once a deadly disease afflicting millions of people before being eradicated through strategies that included immunization with live vaccinia virus (VACV), an orthopoxvirus closely related to variola virus (Moss, 2007). The cession of routine smallpox vaccination following the eradication led to a population that is largely immune naïve to orthopoxviruses, some of which still cause zoonotic infections in humans (Shchelkunov, 2013). Monkeypox virus (Parker et al., 2007), previously found only in Africa, caused a brief outbreak in the U.S. in 2003 (Reed et al., 2004). Cowpox virus and vaccinia virus have been reported to cause infection of domesticated animals and their human handlers in Europe, South America and the Indian subcontinent (Essbauer et al., 2010; Megid et al., 2012; Singh et al., 2012; Trindade et al., 2007).

Despite the success of VACV as the smallpox vaccine, the immunological basis of smallpox vaccine has only been studied in recent years with modern biology. In animal models and human vaccinees, neutralizing antibodies have been shown to play an essential role in protection against orthopoxvirus infection (Belyakov et al., 2003; Hopkins and Lane, 2004). VACV produces two antigenically different forms of virions (Condit et al., 2006; Moss, 2007; Smith et al., 2002), and antibodies against both virion forms are required for optimal protection against orthopoxviruses (Lustig et al., 2005). The intracellular mature virions (MVs) stay within the cells until cell lysis, while the extracellular enveloped viruses (EVs) exit the cells via exocytosis (Smith et al., 2002). MVs have an envelope embedded with more than 20 viral proteins, while EVs have an additional envelope with at least six viral proteins. VACV B5 is the major target of neutralizing antibodies against EV (Bell et al., 2004; Benhnia et al., 2009; Putz et al., 2006), as depletion of anti-B5 antibodies from sera of vaccinated individuals greatly reduced neutralization of EVs (Bell et al., 2004; Putz et al., 2006). In

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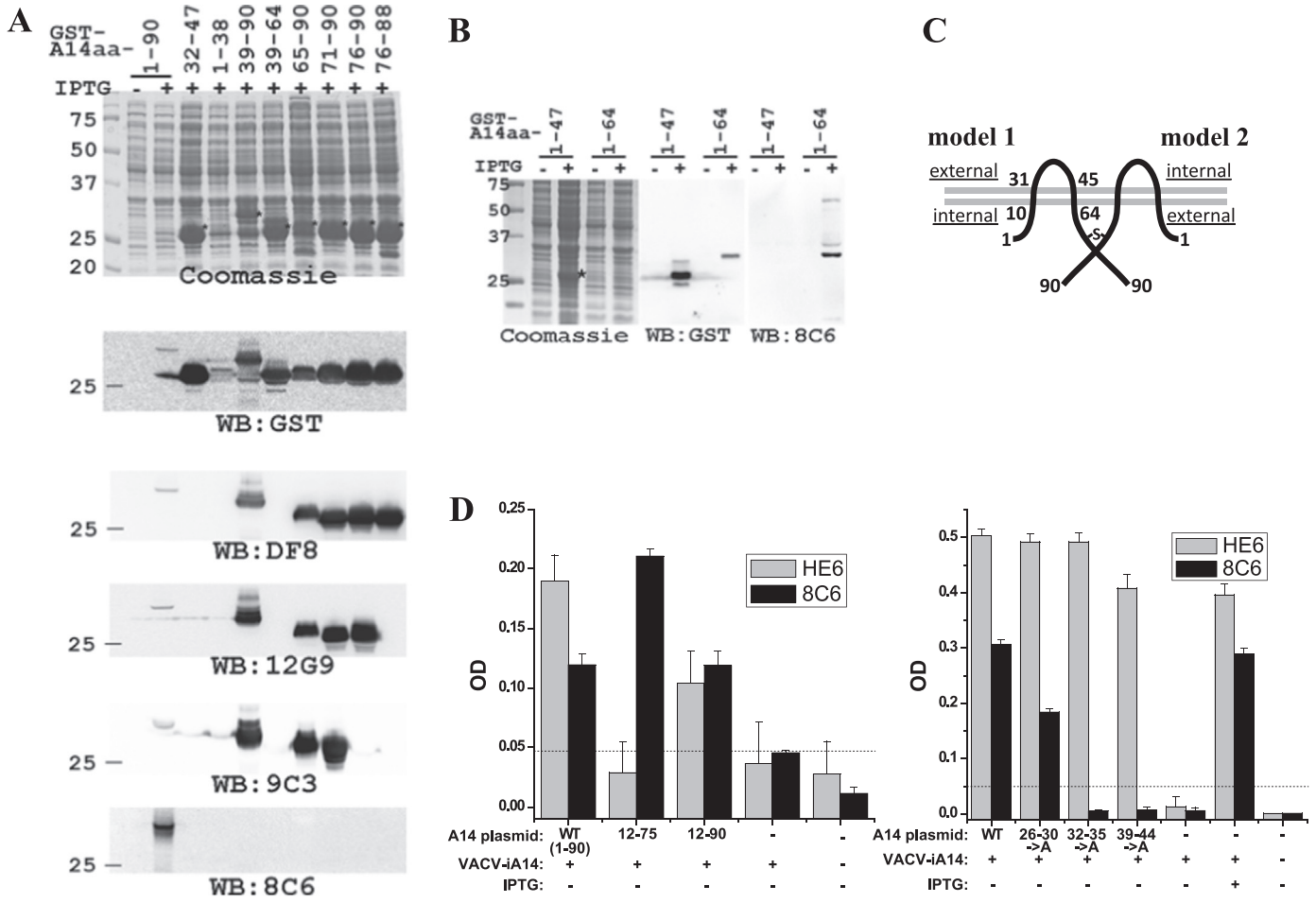

Fig. 1. Mapping the epitopes of A14 mAbs. A and B). Mapping the epitopes by Western blot of GST-A14 proteins. *E. coli* strains were either not induced (-) or induced with IPTG (+) to express GST fusion protein with the indicated A14 fragments. Proteins from the whole cell lysates were resolved by SDS-PAGE and analyzed by either Coomassie staining or by Western blot with the indicated antibodies. Prominent protein bands that are only present in induced samples are marked with * . Only Western blots with representative antibodies are shown. **C).** Predicted topology of A14 on MV with two possible orientations. The two grey lines represent the viral envelope, and the dark lines represent an A14 dimer. The amino acid residue numbers are indicated. The "-S-" denotes the disulfide bond via Cys71. The internal and external side of the virion are indicated in the two models. **D).** Further define the 8C6 epitope by ELISA of A14 mutants. 293T cells were transfected with plasmids encoding A14 alleles under the control of a VACV promoter and subsequently infected with an IPTG-inducible A14 mutant VACV (VACV-iA14) either in the presence or absence of IPTG. The cell lysates were used to coat a microtiter plate, and ELISA was performed with either 8C6 or HE6. The A14 plasmids are named after the A14 residues expressed (12–75, 12–90) or A14 residues substituted with alanines (26–30- > A, 32–35- > A,39–44- > A).

contrast, no single protein has been found to be the dominant MV-neutralizing target. Neutralizing antibody levels in at least subsets of vaccinated individuals correlate with human IgG responses to several MV proteins, including H3, A27, D8, L1 and A14 (Benhnia et al., 2008). H3 (Davies et al., 2005), A27 (Kaever et al., 2016; Rodriguez et al., 1985), D8 (Hsiao et al., 1999) and L1 (Ichihashi and Oie, 1996; Wolffe et al., 1995) are known to be the targets of MV-neutralizing antibodies, but whether A14 is a neutralizing target is unknown. Depletion of individual or a combination of the major MV-neutralizing antibodies from sera of the vaccinees did not significantly reduce neutralization of MV (Aldaz-Carroll et al., 2005; Benhnia et al., 2008; He et al., 2007), indicating that additional candidates such as A14 warrant further testing.

A14 is a major MV envelope protein and plays an essential role in viral assembly (Rodriguez et al., 1998; Salmons et al., 1997). It is a small protein of only 90 amino acids (aa) and predicted to have two transmembrane domains (residues 13–31 and 45–64) that are separated by a 13-aa hydrophilic loop (residues 32–44) (Mercer and Traktman, 2003) (Fig. 1C). The orientation of A14 protein in respect to MV envelope is unclear. The formation of an intermolecular disulfide bond involving a cysteine near the C-terminus suggested that the C-terminus was internal to the virion envelope (Mercer and Traktman, 2003). However, an opposite orientation of A14 was suggested by a more recent model of virion assembly (Maruri-Avidal et al., 2013; Weisberg et al., 2017), which involves the budding of ER membranes into ER lumen.

In this study, we isolated 22 anti-A14 monoclonal antibodies (mAb) from two mice that had been infected with VACV. We characterized this large panel of mAbs in terms of their epitope, antibody sequence, in vitro MV neutralization and in vivo protection efficacy.

2. Material and methods

2.1. Hybridoma generation and characterization

The A14 antibodies were developed in two batches. The generation of the first batch of A14 antibodies has been described (Meng et al., 2011). The second batch of A14 antibodies were generated similarly. Briefly, a BALB/c mouse was infected intranasally with 5×10^3 plaque-forming-unit (PFU) of VACV WR. Seven weeks after the infection, the mouse was injected intravenously with 7×10^7 PFU of UV-inactivated WR virus. Three days afterwards, the spleen of the mouse was harvested for hybridoma generation. The hybridomas secreting anti-VACV antibodies were identified with an immunofluorescence assay of VACV-infected cells as described (Meng et al., 2011). Those that are positive for anti-A14 were identified based on both immunoprecipitation and immunofluorescence results as described (Meng et al., 2011). Anti-H3