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An epitope conserved in orthopoxvirus A13 envelope protein is the target of neutralizing and protective antibodies

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ABSTRACT

Primary immunization of humans with smallpox vaccine (live vaccinia virus (VACV)) consistently elicits antibody responses to six VACV virion membrane proteins, including A13. However, whether anti-A13 antibody contributes to immune protection against orthopoxviruses was unknown. Here, we isolated a murine monoclonal antibody (mAb) against A13 from a mouse that had been infected with VACV. The anti-A13 mAb bound to recombinant A13 protein with an affinity of 3.4 nM and neutralized VACV mature virions. Passive immunization of mice with the anti-A13 mAb protected against intranasal VACV infection. The epitope of the anti-A13 mAb was mapped to a 10-amino acid sequence conserved in all orthopoxviruses, including viriola virus and monkeypox virus, suggesting that anti-A13 antibodies elicited by smallpox vaccine might contribute to immune protection against orthopoxviruses. In addition, our data demonstrates that anti-A13 mAbs are effective for treating orthopoxvirus infection.

Introduction

Smallpox, once a deadly infectious disease afflicting millions of people, was officially eradicated more than 30 years ago through a global immunization campaign with live vaccinia virus (VACV). Routine smallpox vaccination has since stopped, as the vaccine carries risk for a significant portion of the population (Fulginiti et al., 2003), including pregnant women and immunocompromised individuals. The current population largely lacks protective immunity to smallpox, which is now considered to be a potential bioterrorism agent, and to monkeypox, which is still endemic in parts of Africa. Monkeypox virus causes a smallpox-like disease in humans with approximately 10% mortality rate (Parker et al., 2007). It was accidentally imported to the U.S. in 2003, causing a brief outbreak in the Midwest. Currently, the only licensed therapeutics to treat infection by an orthopoxvirus is Vaccinia Immune Globulin (VIG) (Hopkins and Lane, 2004), a blood product derived from people immunized with smallpox vaccine. VIG contains neutralizing antibodies against VACV and is used to treat complications of VACV vaccination. However, the exact composition of VIG is not well defined and its supply depends on the availability of people vaccinated with smallpox vaccine, so there has been considerable interest in developing well-defined immunotherapies for treating orthopoxvirius infection.

VACV, the prototypical orthopoxvirus, produces two types of infectious virions that are biologically and antigenically different (Condit et al., 2006; Moss, 2007; Smith et al., 2002). The majority of the virions produced is intracellular mature virions (MVs), which remain inside the cell until cell lysis. MVs contain an envelope with more than 20 envelope proteins. A fraction of MVs gain additional membranes inside the cells and eventually exit the cells as the extracellular enveloped viruses (EVs) (Smith et al., 2002). EV contains an additional envelope with at least six envelope proteins. Antibodies against both MV and EV are required for optimal immune protection against orthopoxvirus. Among the EV proteins, B5 is the major target of neutralization antibodies (Bell et al., 2004; Benhnia et al., 2009; Putz et al., 2006), while A33 is the target of protective antibody (Galmiche et al., 1999). Depletion of ant-B5 antibodies from sera of vaccinated individuals greatly reduced in vitro neutralization of EVs (Bell et al., 2004; Putz et al., 2006). Among the MV envelop proteins, A27 (Rodriguez et al., 1985), L1 (Ichihashi and Oie, 1996; Wolffe et al., 1995), D8 (Hsiao et al., 1999), H3 (Davies et al., 2005), A28 (Nelson et al., 2008) and A17 (Wallengren et al., 2001) are known to be the targets of neutralizing antibodies. However, no single protein has been found to be the dominant MV-neutralizing target, as depletion of individual or a combination of the major MV-neutralizing antibodies from sera of vaccinated individuals did not significantly reduce neutralization of MV (Aldaz-Carroll et al., 2005; Benhnia et al., 2008; He et al., 2007).

Primary VACV immunization in humans consistently elicits antibody response to at least 12 antigens (Davies et al., 2007), including membrane proteins on MV (A13, A17, D8 and H3) and EV (B5 and A33). However, it was unknown whether anti-A13 antibodies play any role in immune protection against orthopoxvirus. In the current

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study, we isolated an anti-A13 monoclonal antibody (mAb) from a mouse that had been infected with VACV. The characterization of the anti-A13 mAb shows that anti-A13 antibodies can contribute to immune protection against orthopoxviruses and that anti-A13 mAbs are effective for treating orthopoxvirus infection.

Results

Identification and characterization of an anti-A13 mAb

Similar to what we reported recently (Meng et al., 2011), we developed anti-VACV mAbs from a BALB/c mouse that had been infected with WR strain of VACV. Among the mAbs, one (clone name 11F7) immunoprecipitated a 12-kda protein from HeLa cells that had been infected with VACV (Fig. 1A). The 11F7 mAb also recognized the 12-kda protein in a Western blot of VACV-infected HeLa cells (Fig. 1B). Mass spectrometry finger-printing analysis of the 12-kDa protein matched two peptides with that of VACV A13 protein (30% sequence coverage, data not shown). This identification was confirmed by an enzyme-linked immunosorbent assay (ELISA) in which 11F7 specifically recognized recombinant A13 protein expressed in *E. coli.* (Fig. 1C). The isotype of 11F7 was determined to be IgG2a (data not shown). A13 has a theoretical molecular mass of 8-kda, but it was previously shown to migrate as a 12-kda protein on SDS-PAGE (Unger and Traktman, 2004). It is phosphorylated at a serine residue, thus explaining the faint band that migrated slightly above the 12-kda band in Fig. 1A. 11F7 stained viral factories and virion-size particles in immunofluorescence analysis of infected cells (Fig. 1C), consistent with A13 being a MV membrane protein.

Anti-A13 11F7 neutralizes VACV MV

Since A13 is a dominant antibody target in smallpox vaccine (Davies et al., 2007), we were interested in finding out whether anti-A13 antibody contributes to protection against orthopoxviruses. We thus tested the ability of 11F7 to neutralize VACV MV with a plaque reduction assay. Purified VACV MVs were incubated in the presence or absence of the antibody for one hour and then inoculated to a cell monolayer. The inoculum was removed after one hour, and the number of plaques that appeared after 2 days was enumerated. To facilitate plaque counting, the amount of viruses that were initially used for inoculation were just enough to yield on average 55 plaques per well in a 6-well plate (Fig. 2A). Under this condition, 11F7, at a concentration from 4 to 100 μg/ml, reduced the plaque number by approximately 30-40%, similar to a murine monoclonal antibody against H3 (#41, IgG2a) (McCausland et al., 2010). Complement has been previously reported to enhance the neutralization of MVs in vitro (Isaacs et al., 1992). Indeed, 11F7 together with 2% rabbit complement reduced the plaque number by 90%, while complement alone reduced the plaque number only by 20% (Fig. 2A).

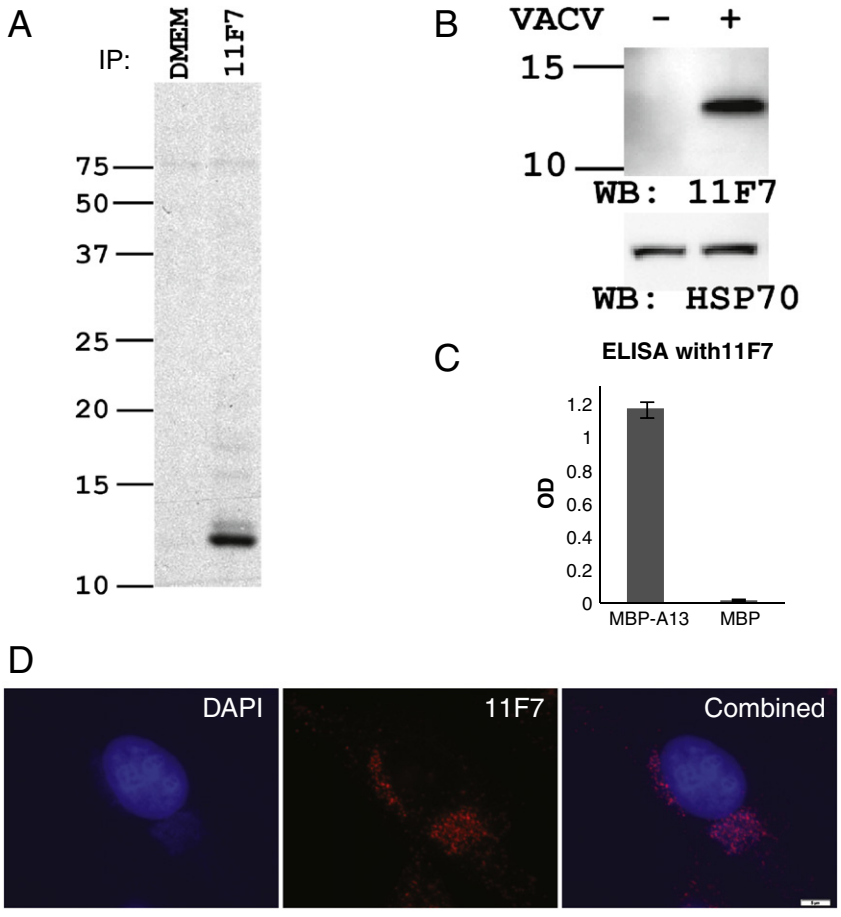

Fig. 1. Identification and characterization of an anti-A13 mAb 11F7. A). HeLa cells were infected with VACV WR at a MOI of 10 and metabolically labeled with ^{35}S-methionine and -cysteine from 8 to 16 hpi. The cells were lyzed and immunoprecipitated with either the culture supernatant from 11F7 hybridoma cells or DMEM medium only. The precipitated proteins were analyzed by SDS-PAGE, and the autoradiograph is shown. B). Proteins from uninfected (−) cells or cells infected with VACV WR (+) were analyzed by Western blot using the culture supernatant from 11F7. The same membranes were also blotted with anti-HSP70 antibody as a loading control. C). The same amount of purified recombinant MBP or MBP fused with VACV A13 were used to coat ELISA plates, and ELISA were performed with the culture supernatant from 11F7. Average OD from triplicate experiments is shown. D). BHK cells grown on cover-slips were infected with VACV WR at a MOI of 0.5 PFU/cell for 8 h and then analyzed by immunofluorescence with 11F7 supernatant. The primary antibody was stained with a Cy3-conjugated goat anti-mouse secondary antibody (red), and the DNA was stained with DAPI (blue).