Virology 464-465 (2014) 264–273

Contents lists available at ScienceDirect

Virology

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

A highly specific monoclonal antibody against monkeypox virus detects the heparin binding domain of A27

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ARTICLE INFO

Article history:
Received 22 April 2014
Returned to author for revisions
10 June 2014
Accepted 30 June 2014
Available online 9 August 2014

Keywords:
Monkeypox virus
Vaccinia virus
Orthopoxvirus
Antibody
Heparin binding

ABSTRACT

The eradication of smallpox and the cessation of global vaccination led to the increased prevalence of human infections in Central Africa. Serologic and protein-based diagnostic assay for MPXV detection is difficult due to cross-reactive antibodies that do not differentiate between diverse orthopoxvirus (OPXV) species. A previously characterized monoclonal antibody (mAb 69-126-3-7) against MPXV [1] was retested for cross-reactivity with various OPXVs. The 14.5 kDa band protein that reacted with mAb 69-126-3 was identified to be MPXV A29 protein (homolog of vaccinia virus Copenhagen A27). Amino acid sequence analysis of the MPXV A29 with other OPXV homologs identified four amino acid changes. Peptides corresponding to these regions were designed and evaluated for binding to mAb 69-126-3 by ELISA and BioLayer Interferometry (BLI). Further refinement and truncations mapped the specificity of this antibody to a single amino acid difference in a 30-mer peptide compared to other OPXV homologs. This particular residue is proposed to be essential for heparin binding by VACV A27 protein. Despite this substitution, MPXV A29 bound to heparin with similar affinity to that of VACV A27 protein, suggesting flexibility of this motif for heparin binding. Although binding of mAb 69-126-3-7 to MPXV A29 prevented interaction with heparin, it did not have any effect on the infectivity of MPXV. Characterization of 69-126-3-7 mAb antibody allows for the possibility of the generation of a serological based species-specific detection of OPXVs despite high proteomic homology.

Introduction

Orthopoxviruses are a genus of DNA viruses of which four species are known to cause disease in humans: vaccinia virus (VACV), cowpox virus (CPXV), variola virus (VARV), and monkeypox virus (MPXV). Monkeypox virus is a zoonotic virus, endemic to central and Western Africa that can cause smallpox-like symptoms in humans. The virus was initially identified in non-human primate rash lesions in 1958 and first identified in humans in 1970 during the smallpox eradication efforts. Incidence of MPXV infections in the Democratic Republic of the Congo (DRC), where the majority of cases occur, has increased as much as 20-fold since the end of smallpox vaccination in 1980 (Rimoin et al., 2010). The first case of human MPXV in the USA occurred in 2003 and was linked to the handling of prairie dogs that had been housed with imported African rodents (Reed et al., 2004). Similar to VARV or smallpox, MPXV disease presents with fever 10–12 days after exposure, followed by rash 2–3 days later. This rash progresses from macular to papular, vesicular, and finally pustular phases, similar to smallpox. This makes differentiation between these diseases based on clinical presentation difficult. While smallpox was declared eradicated by the World Health Organization in 1979, it is still a concern as a potential biological weapon or potential accidental release. The methods for laboratory confirmed diagnostics include electron microscopy, isolation of virus on chorioallantonic membranes (CAM), antibody-based assays, and viral DNA assays (Jezek and Fenner, 1988). Of these, only CAM and DNA assays are capable of providing a species-specific diagnosis (Li et al., 2006, 2010; Olson et al., 2004; Shchelkunov et al., 2011), which require sensitive reagents and equipment. An antibody based serological assay is ideal for diagnostics and surveillance, but due to antigen conservation within OPXV genomes, most serum is cross-reactive and cannot differentiate between individual species of OPXVs. Recent work utilizing animal and human sera samples have identified the primary immune targets within the poxvirus proteome and demonstrated that the antibody response is redundant, cross-reactive, and cross-protective, which is perfect for vaccine design, but far from optimal for

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http://dx.doi.org/10.1016/j.virol.2014.06.039

species-specific antibody assays (Benhnia et al., 2008; Davies et al., 2007; Jezek and Fenner, 1988; Keasey et al., 2010; Keckler et al., 2011; Townsend et al., 2013).

Previous work identified a MPXV-specific monoclonal antibody (Roumillat et al., 1984). This antibody, 69-126-3-7, was shown to be non-neutralizing and to bind to a 14.5 kD band under reducing conditions; however, the protein target was not identified. To explore this antibody's potential use as a diagnostic tool, it was imperative to identify its target protein and epitope. In this study, we have identified the protein target to be MPXV A29 protein (VACV A27 ortholog) and mapped its binding epitope.

Extensive work has been accomplished towards understanding the multiple functions and interactions of VACV A27. It has been reported that VACV A27 exists in complex with A17, A26, and A25, though A27 does not interact directly with A25 (Fig. 1) (Howard et al., 2008; Rodriguez et al., 1993; Wang et al., 2014). It binds heparin on the cell surface (Chung et al., 1998). It, in complex with A17, can mediate cell fusion (Gong et al., 1990; Kochan et al., 2008). This A27-mediated fusion requires binding to glycosaminoglycans (GAGs), such as heparin or heparan sulfate on the cell surface and fusion is inhibited by the presence of A26 (Ching et al., 2009; Hsiao et al., 1998). MPXV and some strains of VACV, like many other OPXVs, are resistant to heparin inhibition, indicating that binding to heparin is not required for entry and VACV A27 mediated fusion that is heparin/heparan sulfate dependent more likely to be involved in cell-to-cell spread via cell fusion (Bengali et al., 2012; Hsiao et al., 1998). A27 is also required for intracellular mature virus (IMV) trafficking on microtubules within the cell and the formation of intracellular enveloped virus (IEV) in the Golgi (Rodriguez and Smith, 1990; Sanderson et al., 2000). Additionally, the A27/A17 complex likely serves as an anchor to the viral membrane for A26 and A25 in the formation of A-type inclusion bodies in OPXV viruses that form inclusion bodies such as some strains of cowpox virus (Howard et al., 2010).

VACV A27 is a 110 amino acid protein that consists of a signal peptide, an attachment domain or heparin binding site, a fusion domain, a coiled-coil domain, and an anchoring domain as depicted in Fig. 1 (Rodriguez et al., 1991; Vázquez and Esteban, 1999). Structural studies indicate that in the monomer the N-terminal amino acids form a random and fairly flexible coil that is followed by a rigid α-helical region (Lin et al., 2002). The native protein exists as a hexamer and the α-helical region forms a coiled-coil in the self-assembling oligomerization region. Even though the heparin binding site (HBS) is found at the N-terminus, the oligomeric structure is required for heparin binding (Ho et al., 2005). The "KKPE" sequence within the HBS has been shown to be required for binding to heparin and this binding is sequence specific rather than a charge requirement (Shih et al., 2009). Extensive work has been done with VACV A27, but many questions still remain and similar studies have not been done with other OPXV orthologs. Identification of MPXV A29 specific monoclonal antibody and its epitope within the HBS demonstrate flexibility in amino acid requirement for heparin interaction.

Results

Characterization of the protein target of mAb 69-126-3-7

When originally isolated and identified, mAb 69-126-3-7 was observed to bind to an approximately 14.5 kD protein by Western blot analysis (Roumillat et al., 1984). Select MPXV proteins <50 kD were obtained and screened against 69-126-3-7 (Fig. 2A). Reactivity was found specific only for MPXV protein A29, and not to MPXV proteins A35, B6, or M1, or to vaccinia ortholog A27. As further confirmation, binding was measured using BLI technology on the forteBio Octet system. Binding to MPXV A29 was specific and little dissociation was observed while binding to the VACV ortholog was comparable to background (Fig. 2B). To ensure the specificity was, in fact, due to differences in the orthologous proteins and not an artifact caused by choice in expression system/conditions or alterations in protein structure triggered by the addition of a 6X-his tag,

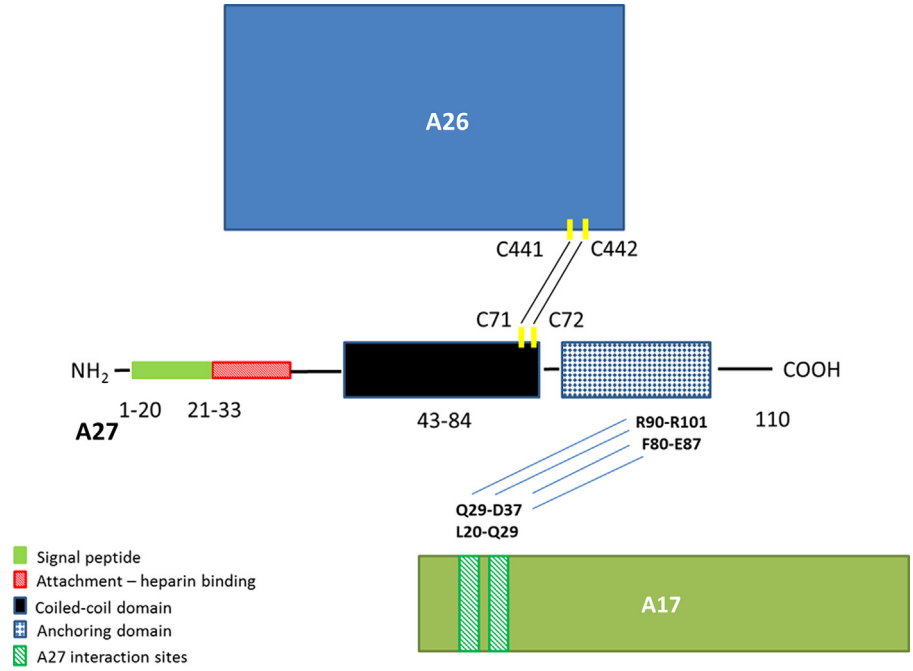

Fig. 1. Diagram of VACV A27 functional domains. Functional regions: signal peptide (29), HBS (4, 15), coiled-coil (34), and anchoring domains (37) are modeled highlighting the heparin binding domain in red. Interaction with VACV A26 and A17 are both shown. Interaction with VACV A26 is shown by the formation of two disulfide bonds between VACV A26 C441 and C442 with VACV A27 C71 and C72 respectively (3). Binding between VACV A27 and VACV A17 occurs at two binding sites on A17. VACV A27 amino acids R90 and R101 bind to one site on A17 through VACV A17 amino acids Q29 and D37. The other binding site is between VACV A27 F80 and E87 and VACV A17 L20 and Q29 (37).