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Research paper

Selection and characterization of scFv antibodies against the Sin Nombre hantavirus nucleocapsid protein

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Abstract

Rodent-borne hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) in the old world and hantavirus cardio-pulmonary syndrome (HCPS) in the new. Most cases of HCPS in North America are caused by Sin Nombre Virus (SNV). Current viral detection technologies depend upon the identification of anti-viral antibodies in patient serum. Detection of viral antigen may facilitate earlier detection of the pathogen. We describe here the characterization of two single-chain Fv antibodies (scFvs), selected from a large naïve phage antibody library, which are capable of identifying the Sin Nombre Virus nucleocapsid protein (SNV-N), with no cross reactivity with the nucleocapsid protein from other hantaviruses. The utility of such selected scFvs was increased by the creation of an scFv-alkaline phosphatase fusion protein which was able to directly detect virally produced material without the need for additional reagents.

Keywords: Hantavirus; Sin Nombre Virus; Phage display; scFv; Phage antibody; Nucleocapsid protein

1. Introduction

Hantaviruses are a group of enveloped negative-strand RNA viruses carried by numerous rodent species throughout the world. The hantavirus genome contains three segments, termed large, medium and small, encoding the polymerase (L segment), two surface glycoproteins, G1 and G2, (M segment) and the nucleocapsid protein (S segment). The nucleocapsid protein is responsible for encapsidating genomic RNA segments into ribonucleoprotein complexes, which participate in genome transcription and replication as well as in virus assembly.

These viruses cause hemorrhagic fever with renal syndrome in the Eastern hemisphere and an acute respiratory disease termed hantavirus cardio-pulmonary syndrome (HCPS)(Nichol et al., 1993) in the USA, a disease which arose for the first time in 1993 in the Four Corners region (Nichol et al., 1993). Hantavirus cardiopulmonary syndrome is a life threatening zoonotic disease with a mortality of 50%, as a result of which it is considered an NIAID category A bio-threat agent. Since

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the identification of hantaviruses as the causative agent of HCPS, many other novel hantaviruses have been discovered. Their ubiquity and the gravity of the diseases they cause make these viruses a serious global public health issue (Schmaljohn and Hjelle, 1997). Most cases of HCPS in the USA are caused by Sin Nombre Virus (SNV), carried asymptomatically by deer mice (*Peromyscus maniculatus*).

Diagnosis of infection is based on detection of either viral components or antibodies produced in the host in response to those components. At the time of presentation, patients often have strong serum antibody responses to the nucleocapsid protein and the G1 surface glycoprotein (Jenison et al., 1994; Vapalahti et al., 1996), as a result of which, the detection of antibodies recognizing the SNV nucleocapsid protein (SNV-N) has been shown to be both sensitive and specific for SNV infection (Schmidt et al., 2005), with clinical outcome often related to the levels of these antibodies (low antibody responses are associated with a poor outcome (Bharadwaj et al., 2000)). Reliable diagnostic assays for anti-SNV antibodies in the serum (Schmidt et al., 2005), with very high sensitivity and specificity for acute SNV infection (Green et al., 1998; Hjelle, 2002) are available, as is a robust strip blot assay to detect the presence of anti-SNV antibodies in rodent serum (Hjelle et al., 1997), allowing detection of infection in rodents under field conditions. However, it takes two to three weeks after infection to detect anti-SNV serum antibodies, indicating a need to detect SNV components directly, rather than the immune response to them. Monoclonal antibody based diagnostic kits are available to detect viral antigen in other infections, such as measles (Olding-Stenkvist and Bjorvatn, 1976), CMV (Rawlinson and Scott, 2003), HIV (Iweala, 2004), Hepatitis B (Hatzakis et al., 2006) and C (Seme et al., 2005), and some tissue culture isolated viruses. Such kits are increasingly used for diagnostic purposes due to their relatively low cost, rapid results and ability to detect infection at early time points (Grandien, 1996). Given the strong response to SNV-N, and the fact that recombinant SNV-N prepared in *E. coli* is effectively recognized by serum antibodies (Jenison et al., 1994), the nucleocapsid protein is a good target for direct antigen detection. This is in contrast to the use of recombinant G1 surface glycoprotein, which being glycosylated, is more difficult to prepare in large quantities.

Traditional methods to derive antibodies rely on the immunization of laboratory animals, with either the harvesting of polyclonal antibodies, or the creation of monoclonal antibodies. Phage display is an alternative in vitro method to develop antibodies which relies on the creation of large phage antibody libraries, from which monoclonal antibodies binding to a target of interest can be selected. In general two kinds of library can be used: naïve or immune. Naïve libraries are generally derived from natural unimmunized human rearranged V genes (Marks et al., 1991; Vaughan et al., 1996; Sheets et al., 1998; de Haard et al., 1999; Sblattero and Bradbury, 2000) or synthetic human V genes (Griffiths et al., 1994; Nissim et al., 1994; de Kruif et al., 1995; Knappik et al., 2000), while immune libraries are created from the V genes of immunized humans (Burton et al., 1991; Zebedee et al., 1992; Williamson et al., 1993; Amersdorfer and Marks, 2000; Amersdorfer et al., 2002) or mice (Orum et al., 1993; Ames et al., 1994, 1995b). Immune libraries have the advantage that they are composed of antibodies with a strong bias towards the antigen/organism used for immunization, resulting in higher affinities, but the disadvantage that new libraries need to be made for each immunogen. As antibody genes are cloned simultaneously with selection in these *in vitro* systems, antibody fragments can be subjected to downstream genetic engineering to increase affinity (Schier and Marks, 1996; Daugherty et al., 1998; Boder et al., 2000; Hanes et al., 2000; Coia et al., 2001) and multimericity (de Kruif and Logtenberg, 1996; Krebber et al., 1997; Kortt et al., 2001; Zhang et al., 2004), as well as being linked to desired effecter functions for downstream applications (Griep et al., 1999; Muller et al., 1999; Casey et al., 2000; Hink et al., 2000; Han et al., 2004), including recloning into the full length immunoglobulin format (Ames et al., 1995a; Persic et al., 1997; Nowakowski et al., 2002). Recently, both naïve and immune phage antibody libraries have been used to select specific high-affinity human antibodies against a number of infectious disease agents and toxins, including botulinum toxin (Amersdorfer et al., 2002), crotoxin (Cardoso et al., 2000), HIV (Burton et al., 1991; Barbas et al., 1994; Moulard et al., 2002; Zhang et al., 2003), herpes virus (Sanna et al., 1995), SARS (van den Brink et al., 2005), rabies (Kramer et al., 2005) and hepatitis B (Zebedee et al., 1992; Park et al., 2005), with the potential for both diagnosis and therapy.

In the work described here, we have expanded the use of large naïve phage antibody libraries to select single chain Fvs (scFvs) against recombinant SNV-N protein. A number of different antibodies were isolated, some of which were shown to be specific for the SNV nucleocapsid protein, and not recognizing a panel of related hantavirus nucleocapsid proteins. This confirms the utility of these in vitro methods in the study of