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Targeting Sindbis virus-based vectors to Fc receptor-positive cell types

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Abstract

Some viruses display enhanced infection for Fc receptor (FcR)-positive cell types when complexed with virus-specific immunoglobulin (Ig). This process has been termed antibody-dependent enhancement of viral infection (ADE). We reasoned that the mechanism of ADE could be exploited and adapted to target alphavirus-based vectors to FcR-positive cell types. Towards this goal, recombinant Sindbis viruses were constructed that express 1 to 4 immunoglobulin-binding domains of protein L (PpL) as N-terminal extensions of the E2 glycoprotein. PpL is a bacterial protein that binds the variable region of antibody kappa light chains from a range of mammalian species. The recombinant viruses incorporated PpL/E2 fusion proteins into the virion structure and recapitulated the species-specific Ig-binding phenotypes of native PpL. Virions reacted with non-immune serum or purified IgG displayed enhanced binding and ADE for several species-matched FcR-positive murine and human cell lines. ADE required virus expression of a functional PpL Ig-binding domain, and appeared to be FcγR-mediated. Specifically, ADE did not occur with FcγR-negative cells, did not require active complement proteins, and did not occur on FcγR-positive murine cell lines when virions were bound by murine IgG-derived $F(ab')_2$ fragments.

Keywords: Sindbis; Alphavirus; Fc receptor; Vector; Targeting; Protein L; ADE

Introduction

Alphaviruses are single-stranded, positive-sense RNA viruses, and are classified within the *Togaviridae* virus family (genus Alphavirus) (Strauss and Strauss, 1994). The alphavirus genome can accommodate foreign gene sequences and a variety of recombinant alphavirus-based expression vectors have been described (Frolov et al., 1996; Rayner et al., 2002; Ryman et al., 2004; Schlesinger and Dubensky, 1999; Thomas et al., 2003). Alphaviruses display tropisms for a broad range of cell/tissue types in vivo, and alphavirus-based vectors have been used for targeted transgene expression in dendritic cells (DCs) (Gardner et al., 2000; Ryman et al., 2002) neurons (Altman-Hamamdzic et al., 1997; van Marle et al., 2003), vascular smooth muscle (Roks et al., 2002), and tumors (Tseng et al., 2002, 2003). However, targeting alphavirus vectors to cell/tissue types not included in their natural viral tropism is more difficult as this requires strategies for ablating or minimizing the natural tropism of the virus, and for generating the desired tropism de novo. Dubuisson and Rice (Dubuisson and Rice, 1993) demonstrated that the normal receptor-binding properties of Sindbis virus could be disrupted by the insertion of short peptides (11 amino acids) into defined regions of the PE2 glycoprotein. Viruses containing these modifications displayed markedly reduced binding to CEF and BHK-21 cells, but retained all other replication functions. Efforts to redirect the cell/tissue tropism of alphaviruses have focused on generating novel virus affinities for specific receptors expressed on the surface of target cells. One approach has been to incorporate a receptor-specific ligand into the Sindbis virus E2 glycoprotein (Sawai and Meruelo, 1998). An alternative approach utilized receptor-specific antibodies as a bridge to link Sindbis virus-based replicons to receptors on target cells. This strategy involved insertion of an IgG-

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binding domain of *Staphylococcus aureus* protein A within the E2 glycoprotein, followed by formation of a replicon/IgG complex via interactions between the protein A domain and the Fc region of IgG. By design, the cell type that is targeted by the replicon particle/IgG complex is determined by the Fab regions of the immunoglobulin (Ig) molecule, which should be specific for a surface antigen that is expressed by the cell type of interest. Although replicon particles containing the receptor-specific ligand or protein A domain could be targeted to specific cell types in cell culture, the particles exhibited low infectious titer and appeared to exclude E1 from their structure (Iijima et al., 1999; Ohno et al., 1997; Sawai and Meruelo, 1998).

The objective of this project was to develop a new strategy for targeting alphaviruses and alphavirus-based replicon particles to cell types that express receptors for the Fc region of certain classes of Ig. Many cells of myeloid or lymphoid origin express FcRs that bind to the Fc region of IgG (FcγR), or other classes of Ig (Ravetch and Bolland, 2001). FcR-positive cells are able to capture microbes/antigens that are bound by Ig (immune complexes [ICs]), and for some cell types, including macrophages and DCs, this interaction can lead to internalization of the IC/FcR complex. IgG-bound virions can also interact with FcγRs, and a variety of viruses have been shown to exploit the FcγR-mediated endocytic pathway to infect FcγR-positive cells. This phenomenon is known as antibody-dependent enhancement of viral infection (ADE) (Takada and Kawaoka, 2003), and has been demonstrated for several alphaviruses (Chanas et al., 1982; Lidbury and Mahalingam, 2000; Linn et al., 1996), including Sindbis virus.

We reasoned that an adaptation of the ADE mechanism could be exploited to target alphavirus-based vectors to FcR-positive cell types. The ability to target gene expression to select FcR-positive cell types (e.g., DCs, macrophages, M cells, NK cells, mast cells) could facilitate the study of these cells, and could potentially be manipulated to enhance the efficacy of alphavirus-based vaccine vectors. Towards this goal we constructed Sindbis viruses that were designed to spontaneously bind IgG in an antigen-independent manner. This phenotype was achieved by exploiting the biological properties of protein L (PpL). PpL is an Ig-binding, cell wall protein expressed by the *Peptostreptococcus magnus* bacterium (Kastern et al., 1990; Myhre and Erntell, 1985). PpL contains 4 or 5 (depending of strain) highly homologous, repeated Ig-binding domains (designated B1–B5), 72 to 76 amino acids in length (Kastern et al., 1992; Murphy et al., 1994). Individual B domains possess 2 separate Ig-binding sites; a high-affinity binding site designated site 1, and a low-affinity binding site designated site 2 (Graille et al., 2001, 2002; Housden et al., 2004). The Ig-binding properties of PpL are distinctly different from those of protein A (derived from *Staphylococcus aureus*) (Forsgren and Sjöquist, 1966) and protein G (derived from group C and G *Streptococci*) (Björck and Kronvall, 1984; Reis et al., 1984), which predominantly bind to the Fc region of IgG. PpL binds with high affinity to the framework region of the variable domain of kappa (human subgroups κI, κIII, and κIV) light chains (V_L) (Enokizono et al., 1997; Nilson et al., 1992). Therefore, PpL binds Ig independently of its class or antigen-specificity (Åkerström and Björck, 1989; Björck, 1988). PpL binds Igs from a broad range of mammalian species, and displays particularly high affinity for Ig derived from human and non-human primates, mice, rats, and swine (De Château et al., 1993). The majority of human and murine Igs contain kappa light chains (Nilson et al., 1993; Solomon, 1976), and PpL is able to bind 50% or more of the polyclonal antibodies in human serum, and at least 40% of the antibodies present in mouse serum (Nilson et al., 1993).

In this report, we describe the construction and characterization of recombinant Sindbis viruses that express fusion proteins consisting of 1 to 4 PpL B domains with a novel location as N-terminal extensions of the viral E2 glycoprotein. E2 forms heterodimers with the second viral glycoprotein, designated E1, and each of the 80 spike structures that project from the surface of an alphavirus particle are composed of three E1/E2 heterodimers (Strauss and Strauss, 1994). The recombinant viruses incorporate the PpL/E2 fusion proteins into the virion structure, bind Ig in an antigen-independent manner, and recapitulate the species-specific Ig-binding phenotypes of native PpL. The Ig-binding viruses displayed FcγR-dependent ADE for several FcγR-positive murine and human cell lines. ADE was only demonstrated for viruses that expressed a functional PpL Ig-binding domain, and only occurred in the presence of intact Igs containing functional IgG Fc components.

Results and discussion

Recombinant viruses contain PpL/E2 fusion proteins and are viable

Targeting viral vectors to cell types that lie outside of their natural tropism usually requires physical alteration of the virus. Alterations can consist of pseudotyping virions with glycoproteins from heterologous viruses, or inserting receptor-specific ligands or single-chain antibodies into the viral receptor-binding proteins. These strategies have been applied most successfully using retrovirus- and adenovirus-based systems (Krasnykh et al., 2000; Sandrin et al., 2003; Wickham, 2003) for recent reviews. Sindbis virus has been shown to tolerate the insertion of short peptides (11 amino acids) within defined regions of the E3 and E2 proteins (Dubuisson and Rice, 1993; London et al., 1992); however, virus particles containing larger insertions within E2 were of low infectivity and failed to incorporate E1 into their structures (Ohno et al., 1997; Sawai and Meruelo, 1998). For this study, recombinant Sindbis viruses were constructed which expressed PpL/E2 fusion proteins containing 1, 2, 3, or 4 B domains of PpL as N-terminal extensions of