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Genetically delivered antibody protects against West Nile virus

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Abstract

Gene-based delivery of recombinant antibody genes is a promising therapeutic strategy offering numerous advantages including sustained antibody levels, better safety profile and lower production cost. Here we describe generation of a recombinant antibody Fc-9E2 comprising a fusion protein between human Fc of IgG1 and a single-chain Fv derived from a hybridoma 9E2 secreting a mAb neutralizing West Nile virus (WNV). Fc-9E2 was shown to retain parental mAb's specificity and WNV-neutralizing capacity. Adenovirus-mediated in vivo delivery of the antibody gene resulted in sustained Fc-9E2 serum levels leading to abrogation of lethal WNV infection in an animal model.
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1. Introduction

In 1999, West Nile flavivirus was introduced into the Western hemisphere and is considered an important emerging pathogen. While development of vaccines to control WNV and other flavivirus infections continues to attract considerable attention (Pugachev et al., 2003), even the most efficient flavivirus vaccines would provide a little help in treatment of ongoing infections. Transfer of specific antibodies (Ab) or immunoglobulins has been shown to abort or modify a number of *Flavivirus* infections (Phillpotts et al., 1987; Roehrig et al., 2001; Schlesinger et al., 1985). Passive immunization with Abs against WNV can prevent disease in animals infected with WNV (Ben-Nathan et al., 2003; Diamond et al., 2003; Engle and Diamond, 2003). While therapeutic use of human immune globulin obtained from pooled donor sera collected in regions with the high occurrence of WNV infections has been suggested (Ben-Nathan et al., 2003), such therapy is not yet widely available. Genetic engineering provides the means to generate recombinant antibodies (recAbs) in vitro by employing eukaryotic expression systems, thus expanding the therapeutic Ab repertoire. Soluble recAbs were demonstrated to bind and effectively neutralize various viral pathogens in vitro and in vivo (Prosniak et al., 2003; Wu et al., 2007). Effective protection against WNV was achieved after passive transfer of WNV specific recAbs (Gould et al., 2005; Oliphant et al., 2005; Throsby et al., 2006). However, production of clinical grade antibodies for passive immunization, either natural or engineered, is complicated. Due to high-quality standards applied to clinical grade Ab preparations, the cost of Ab production and/or purification is extremely high (Kasuya et al., 2005). An attractive alternative to passive inoculation of protective antibodies is in vivo production of therapeutic Abs by gene transfer.

Various delivery vectors, both non-viral and viral, have been developed for genetic transfer of antibody genes (Bakker et al., 2004). Although adenovirus (Ad) gene transfer vectors have been used to deliver recAbs for cancer applications (Alvarez et

Abbreviations: WNV, West Nile virus; Ad, adenovirus; recAb, recombinant antibody; scFv, single chain fragment variable; Fc, fragment crystallizable; WNVEC, C-terminal fragment of WNV E protein; PRNT, plaque reduction-neutralization test.

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al., 2000; Jiang et al., 2006; Yoshio-Hoshino et al., 2007), studies on the use of Ad vectors for Ab gene delivery are still underrepresented in the treatment of infectious diseases. Kasuya et al. (2005) reported substantial survival advantage from anthrax lethal toxin achieved by an Ad vector-mediated recAb gene transfer (Kasuya et al., 2005). Recombinant Ads are attractive as delivery vehicles for this application for many reasons. Ad vectors are stable; they can be easily engineered to incorporate large transgenes, their high-titer production and purification can easily be achieved using inexpensive methods. There is an extensive safety record for replication-deficient recombinant Ad in humans (NIH report, 2002). Transduction efficiency achieved using Ad vectors in vivo is unparalleled among gene delivery vectors, either non-viral or viral. The intrinsic property of replication-incompetent Ad vectors to mediate transient gene transfer looks especially attractive in the context of acute infectious disease treatment with recAbs. The vector is cleared from the organism when the disease is eliminated.

However, as an antibody molecule is a product of two gene expression, special attention should be given to design gene delivery vectors encoding recAbs. It is difficult to achieve balanced expression of two genes with a single vector. Single-chain variable fragment (scFv) is a genetically engineered, single polypeptide molecule that consists of the variable fragments of the heavy chain (VH) and the light chain (VL) of an immunoglobulin joined together by a flexible peptide linker. RecAbs can be engineered to possess new and useful properties by fusion of an scFv to other proteins, including immunoglobulin constant fragments, thereby introducing new therapeutic, physiological and/or biochemical functions. The functional activity of a successful therapeutic Ab is most likely dictated by its entire structural composition, meaning that both the antigen (Ag)-binding and the effector portions of a recombinant antibody should be preserved. Recently, Lo et al. described an approach that permits high level production of recombinant proteins in eukaryotic expression systems, whereby the protein of interest is produced as a carboxy-terminal fusion protein with an immunoglobulin constant fragment (fragment crystallizable, Fc) (Lo et al., 1998). By swapping the Fc portions of recAbs it is possible to tailor their effector functions. We further developed this approach by designing and producing a recombinant antibody molecule, that includes an scFv as the C-terminal fusion with the IgG Fc fragment. It has been demonstrated that the recombinant antibody in this configuration retains its binding properties (Korokhov et al., 2003).

In the current study we describe Ad-mediated gene transfer of a WNV-neutralizing recAb gene resulting in high and sustained levels of protective recAb in vivo. We demonstrate that a single injection of such engineered Ad prevents the development of lethal WNV infection in mice.

2. Materials and methods

2.1. Cells and viruses

WNV strains were grown on Vero cells (ATCC CRL-1586) at 37 °C in a humidified atmosphere containing 5% CO_2 in Dulbecco's modified Eagle medium (DMEM) supplemented with 5% FetalClone III (FCIII; Hyclone, Logan UT) and 1× antibiotic–antimycotic mixture (Invitrogen, Carlsbad, CA). Strain Vlg99-27889 (Lvov et al., 2000) was obtained from the State Collection of Virus Strains (Ivanovskii Institute of Virology, Russian Academy of Medical Sciences). Strain Eg101 (Melnick et al., 1951) was obtained from Dr. L. Zeng (CBER, Bethesda, MD). A 385–99 isolate of the NY99 strain (Tesh et al., 2002) was provided by Dr. R. Tesh (Galveston, TX). Recombinant Ad vectors were rescued and propagated on HEK-293 cells (ATCC CRL-1573). Human lung adenocarcinoma A549 cells (ATCC CCL-185) were used for in vitro production of the Fc-9E2 antibody. These cells were grown in DMEM/F12 medium supplemented with 10% FCS and 1× antibiotic–antimycotic mixture.

2.2. Gene engineering and Fc-9E2 production/purification

A fragment encoding the C-terminal portion of the envelope (E) protein of Vlg99 WNV (WNVEC; aa 551-743, GenBank #AAP22089) was PCR-amplified from a construct described in (Razumov et al., 2005) and cloned in the pET21a expression vector (Novagen, Madison, WI). The protein was produced in BL21(DE)plysS bacteria and purified by immobilized metal affinity chromatography (IMAC) using TALON resin (Clontech, Paolo Alto, CA) followed by dialysis against PBS.

To generate scFv 9E2, total RNA was isolated from a hybridoma cell line 9E2 secreting neutralizing monoclonal antibody (mAb) to WNV envelope protein using RNeasy kit (Qiagen, Valencia, CA). Monoclonal antibody produced by this hybridoma demonstrated a high neutralization activity against several WNV isolates. MAb 9E2 recognizes the C-terminal portion of WNV E protein (Domain III) (Razumov et al., 2005). Coding DNA was synthesized using Omniscript RT kit (Qiagen, Valencia, CA) and was used for PCR amplification of VH and VL gene fragments with a set of published primers (Breitling et al., 2001). The PCR products were ligated into a phagemid vector pSEX81 (Breitling et al., 1991). *Escherichia coli* cells bearing pSEX/9E2 were infected with M13KO7 helper phage resulting in bacteriophages displaying scFv 9E2 variants. This phage-scFv mini-library was screened once using a purified WNVEC, as described (Kontermann, 2001). A positive phage clone was identified by ELISA using purified WNVEC. Nucleotide sequence of scFv 9E2 was determined by DNA sequencing of phage replicative form DNA. The scFv gene was re-cloned into a bacterial expression vector pOPE101 (Dubel et al., 1993) featuring a c-myc detection tag and the His6 purification tag.

Assembly of Fc-9E2-coding DNA was performed as follows. The hinge, CH2 and CH3 domains (collectively called the Fc, or fragment crystallizable) of the human immunoglobulin gamma-1 gene was PCR-amplified from a cDNA clone (Open Biosystems, Huntsville, AL; GenBank #BC073782) introducing a single Cys(248)Ser (according to AAH73782) amino acid substitution in the hinge region. That cysteine residue normally forms a disulfide bond with the light chain. The Fc gene fragment was cloned into pSecTag2a vector (Invitrogen) in frame

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