Antiviral Research 97 (2013) 318–328

Contents lists available at SciVerse ScienceDirect

Antiviral Research

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

Passive immunization with a recombinant adenovirus expressing an HA (H5)-specific single-domain antibody protects mice from lethal influenza infection

Irina L. Tutykhina [a], Elena S. Sedova [a], Irina Y. Gribova [a], Tatiana I. Ivanova [b], Lev A. Vasilev [b], Marina V. Rutovskaya [b], Andrei A. Lysenko [a], Maxim M. Shmarov [a], Denis Y. Logunov [a], Boris S. Naroditsky [a,*], Sergei V. Tillib [b], Alexander L. Gintsburg [a]

[a] Gamaleya Research Institute for Epidemiology and Microbiology, 18, Gamaleya Street, Moscow 123098, Russia
[b] Institute of Gene Biology, Russian Academy of Sciences, 34/5, Vavilova Street, Moscow 119334, Russia

ARTICLE INFO

Article history:
Received 10 September 2012
Revised 3 December 2012
Accepted 12 December 2012
Available online 26 December 2012

Keywords:
Passive immunization
Influenza virus
Single-domain antibody
Adenoviral vector

ABSTRACT

One effective method for the prevention and treatment of influenza infection is passive immunization. In our study, we examined the feasibility of creating an antibody-based preparation with a prolonged protective effect against influenza virus. Single-domain antibodies (sdAbs) specific for influenza virus hemagglutinin were generated. Experiments in mouse models showed 100% survivability for both intranasal sdAbs administration 24 h prior to influenza challenge and 24 h after infection. sdAb-gene delivery by an adenoviral vector led to gene expression for up to 14 days. Protection by a recombinant adenovirus containing the sdAb gene was observed in cases of administration prior to influenza infection (14 d–24 h). We also demonstrated that the single administration of a combined preparation containing sdAb DNA and protein expanded the protection time window from 14 d prior to 48 h after influenza infection. This approach and the application of a broad-spectrum sdAbs will allow the development of efficient drugs for the prevention and treatment of viral infections produced by pandemic virus variants and other infections.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Influenza type A virus poses a serious threat to public health and causes repeated seasonal epidemics with increased morbidity and significant economic loss. At present, there are several methods for the prevention and treatment of influenza (www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1287147812045: Health Protection Agency, 2011; Mossad, 2009). Active immunization (vaccination) is the most efficient way to protect people against this infection (Mossad, 2009), but there are some potentially severe complications that pose certain restrictions, as well as a number of conditions where administration of influenza vaccines is not allowed (Musana et al., 2004).

Passive immunization is another method for the prevention and treatment of influenza infection (Ye et al., 2012; Luke et al., 2010). Hyperimmune sera with a high titer of virus-neutralizing antibodies against a defined influenza strain obtained from immunized animals or from patients who have been subject to infection previously have been used to prevent influenza infection in recipient patients (McGuire and Redden, 1918). Currently, drugs based on hyperimmune sera are being gradually replaced by monoclonal antibodies (mAb) because of their low immunogenicity and high level of neutralizing activity and specificity (Kohler and Milstein, 1975). However, creating a low cost scheme for pharmaceutical manufacturing mAb products is difficult due to their complex structure, which puts them at a disadvantage compared with classical drugs (Shukla and Thömmes, 2010; Peterson et al., 2006). Therefore, various strategies to generate mAb fragments (Fab, scFv, VH, VL) have become more widespread (Peterson et al., 2006).

Natural serum of camelids and sharks contains smaller Abs. They lack the first constant domain of the heavy-chain (CH1), as well as the whole light chain, so they represent single-domain antibodies (sdAbs) (Hamers-Casterman et al., 1993; Vanlandschoot et al., 2011; Arbabi Ghahroudi et al., 1997; Harmsen and De Haard,

Abbreviations: Ab, antibody; mAb, monoclonal antibody; sdAb, single-domain antibody; rAd, recombinant adenovirus; Ad5, human adenovirus serotype 5; IAV, influenza A virus; HA, influenza virus hemagglutinin; HA1, membrane-distal globular domain of hemagglutinin; aHAsdAb, anti-hemagglutinin formatted sdAb; psdAb, prokaryotically (*E. coli*) expressed aHAsdAb; esdAb, eukaryotically (adenoviral vector) expressed aHAsdAb; ILZ, isoleucine zipper; VNA, virus-neutralization assay; HIA, hemagglutination inhibition assay; WB, Western blotting; BALF, bronchoalveolar lavage fluid; NS, nasal swab; Cyk8, cytokeratin 8; PEG, polyethylene glycol; scFv, single-chain variable fragment; VHH, variable fragment of a camelid antibody.

* Corresponding author. Tel.: +7 4991936135.
E-mail address: bsnar1941@yahoo.com (B.S. Naroditsky).

0166-3542/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.antiviral.2012.12.021

2007; Wesolowski et al., 2009; Muyldermans et al., 2009; Ghassabeh et al., 2010; Tillib, 2011). It has been shown that these camel antigen-binding domains can be "humanized" without significant loss of their specific activity via a few amino acid substitutions (Vincke et al., 2009). The advantages of sdAbs include their smaller size, good solubility, novel structural features (i.e., better penetration into tissues and the ability to recognize epitopes inaccessible to conventional antibodies), simple genetic engineering and relatively economical production (Hamers-Casterman et al., 1993; Greenberg et al., 1996; Nuttall et al., 2001; Harmsen and De Haard, 2007; Wesolowski et al., 2009; Muyldermans et al., 2009; Ghassabeh et al., 2010; Tillib, 2011). Because the coding sequences for sdAbs are known, it is possible to produce the corresponding functionally active protein in different expression systems (prokaryotic and eukaryotic) (Harmsen and De Haard, 2007; Bazl et al., 2007). However, it should be noted that sdAbs have short half-lives *in vivo* (Harmsen et al., 2005). A promising solution is to elicit a prolonged expression of the sdAb gene in a recipient organism.

One of the most efficient methods for delivering genetic material (including genes of Abs) to target cells is viral vectors (Campana et al., 2009; Zuber et al., 2008). Adenoviral vectors are among the most commonly used tools used for delivery and expression of recombinant genes in mammalian cells (Wilson, 1996). It is known that recombinant adenoviruses are capable of efficiently transferring the genes of bacterial and viral antigens, cytokines, growth factors, and other proteins to target cells, ensuring a high level and duration of target gene expression (Lasaro and Ertl, 2009; Tani et al., 2011; Kesser et al., 2008; Kita et al., 2004). Furthermore, these vectors are safe, which has been confirmed by more than 150 clinical trials (Shirakawa, 2009).

This paper describes a novel strategy for the prevention of influenza infection through the use of a recombinant adenovirus (rAd) expressing a neutralizing camel sdAb against hemagglutinin (HA). We demonstrated that combined administration of sdAb and rAd expressing a sdAb specifically prevented lethal mouse infection for a prolonged period of time (14 d) and expanded the protection time window from 14 d prior to 2 d after influenza infection.

2. Materials and methods

2.1. Reagents

The following reagents were used in this work: Ad-Easy vector system (Stratagene, USA); bovine serum albumin (BSA), monoclonal anti-HA (aHA) antibody produced in mouse (Sigma–Aldrich, USA); recombinant influenza A virus H5N2 HA1, influenza A virus H5N2 HA/Hemagglutinin (Sino Biological Inc.) and HRP-labeled sheep anti-mouse IgG antibody (Amersham Bioscience, USA). A sdAb against mouse cytokeratin 8 (aCyk8sdAb) were obtained in our previous work (Gribova et al., 2011) and used here as a negative control.

2.2. Cell culture

The human embryonic kidney-293 (HEK-293), Madin–Darby canine kidney (MDCK) and H1299 cell lines were used in the experiments. Cells were propagated in DMEM supplemented with 10% fetal bovine serum (HyClone), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine at 37 °C in a humidified atmosphere with 5% CO_2.

2.3. Production of the cDNA clone encoding the sdAb for the specific recognition of influenza virus

The sdAb antibody capable of specifically recognizing A/Mallard duck/Pennsylvania/10218/84 (H5N2) IAV was obtained from the Tillib S.V. research group (Institute of Gene Biology, Moscow). The first stage of its generation included immunization of the Bactrian camel (*Camelus bactrianus*) with purified inactivated A/Mallard duck/Pennsylvania/10218/84 IAV (H5N2). The protein concentration of the purified virus was determined by a Bradford protein assay kit (Bio-Rad). The camel was immunized 5 times subcutaneously with 2.5 mg of virus preparation mixed with equal amounts of Freund adjuvant (complete only for the first injection). The second injection was made 3 weeks after the first and the remaining three injections were given in 10-day intervals. Blood samples (150 ml) were collected 5 d after the last injection. PBS (50 ml), heparin (100 U/ml) and EDTA (3 mM) were added to prevent clotting.

A nucleotide sequence library of sdAbs was constructed and sdAbs were selected by phage display as described (Tillib et al., 2010). Phages carrying the IAV-specific nanobodies were selected by 2 and 3 cycles of panning on the immobilized virus. Individual sequence variants of enriched nanobodies were identified by the fingerprinting-like PCR/restriction analysis ('HMR analysis') as described (Tillib et al., 2010; Arbabi Ghahroudi et al., 1997; Nguyen et al., 2001; ElsConrath et al., 2001; Saerens et al., 2004). The clones that showed high absorbance values reflecting specific binding to the immobilized IAV were considered positives. As a result, 16 different sdAbs were selected. To obtain high avidity sdAbs, initially selected sdAb sequences were modified (formatted). A special coiled-coil sequence (an isoleucine zipper domain, or ILZ (Harbury et al., 1993; Shiraishi et al., 2004), separated by a camel hinge-originated spacer sequence from the sdAb sequence was attached to the sdAb C-terminus (Tillib et al., 2010). Presumably, the ILZ domain containing peptides adopted a trimeric parallel (Harbury et al., 1993; Sorger and Nelson, 1989) conformation, which could lead to significantly increased biological activity for these peptides (Sorger and Nelson, 1989; Shiraishi et al., 2004). Two other peptidic tag sequences (HA-tag and (His)6-tag, HH) were also added to the C-terminal extremity of the formatted recombinant antibody to improve detection and purification.

cDNA sequences of selected sdAbs were subcloned (by conventional or PCR cloning) into the pHEN4 (expression plasmid (ElsConrath et al., 2001)) together with the pelB leader sequence (for periplasmic production), camel upper hinge (the longest hinge variant) and ILZ domain (Harbury et al., 1993) sequences (to obtain formatted sdAb–fsdAb), and two short tag sequences (HA-tag and (His)6-tag) at the C-terminus coding region. The plasmids were transformed into *Escherichia coli* BL21 (New England BioLabs) for bacterial expression and purification. Protein expression was induced by the addition of 1 mM isopropyl-d-1-thiogalactoside (IPTG). After 5–7 h induction at 37 °C, cells were harvested by centrifugation and the formatted sdAbs were purified from the periplasmic extract. The sdAbs were purified using Ni–NTA agarose and the QIAExpressionist purification system (QIAGEN, USA). All the purified antibodies were maintained in 1× PBS. The proteins were then sequentially concentrated (to a concentration of approximately 5 mg/ml) in Amicon 10 kDa ultrafiltration devices (Millipore, Billerica, MA), affinity purified from endotoxin using 'Detoxi-Gel Endotoxin Removing Gel' (Thermo Scientific), and sterilized by filtration using a cellulose acetate membrane filter (0.2 μm) (Nalgene Co.). Purified formatted sdAbs were stored in aliquots at 4 °C or, after addition of 50% glycerol, at −20 °C.

Degree of purity was evaluated by SDS–PAGE analysis (data not shown) and protein concentrations were determined spectrophotometrically using a protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA).

All 16 formatted sdAbs were assessed by ELISA (binding to the immobilized IAV), erythrocyte hemagglutination inhibition (HI) and virus neutralization (VN) *in vitro* assays. The results showed that sdAbs were able to efficiently bind IAV and H5 hemagglutinin,

Download English Version:

https://daneshyari.com/en/article/5822632

Download Persian Version:

https://daneshyari.com/article/5822632

Daneshyari.com