Virology 482 (2015) 60–66

Contents lists available at ScienceDirect

Virology

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

Mucosal vaccination by adenoviruses displaying reovirus sigma 1

Eric A. Weaver [a], Zenaido T. Camacho [b], Matthew L. Hillestad [c], Catherine M. Crosby [d], Mallory A. Turner [d], Adam J. Guenzel [d], Hind J. Fadel [d], George T. Mercier [e], Michael A. Barry [a,f,*]

[a] Department of Internal Medicine, Division of Infectious Diseases, Translational Immunovirology and Biodefense Program, Mayo Clinic, Rochester, MN 55902, USA
[b] Department of Cell Biology, Department of Natural Sciences, Western New Mexico University, Silver City, NM 88062, USA
[c] Nephrology Training Program, Mayo Clinic, Rochester, MN 55902, USA
[d] Virology and Gene Therapy Graduate Program, Mayo Clinic, Rochester, MN 55902, USA
[e] Department of Physics, University of Houston, Houston, TX 77004, USA
[f] Department of Immunology and Department of Molecular Medicine, Mayo Clinic, Rochester, MN 55902, USA

ARTICLE INFO

Article history:
Received 19 August 2014
Returned to author for revisions
17 February 2015
Accepted 25 February 2015
Available online 30 March 2015

Keywords:
Adenovirus
Reovirus
Sigma 1
Mucosal
Immunization

ABSTRACT

We developed adenovirus serotype 5 (Ad5) vectors displaying the sigma 1 protein from reovirus as mucosal vaccines. Ad5-sigma retargets to JAM-1 and sialic acid, but has 40-fold reduced gene delivery when compared to Ad5. While weaker at transduction, Ad5-sigma generates stronger T cell responses than Ad5 when used for mucosal immunization. In this work, new Ad5-fiber-sigma vectors were generated by varying the number of fiber β-spiral shaft repeats (R) between the fiber tail and sigma. Increasing chimera length led to decreasing insertion of these proteinsAd5 virions. Ad-R3 and R14 vectors effectively targeted JAM-1 *in vitro* while R20 did not. When wereused to immunize mice by the intranasal route, Ad5-R3-sigma produced higher serum and vaginal antibody responses than Ad5. These data suggest optimized Ad-sigma vectors may be useful vectors for mucosal vaccination.

Introduction

Most pathogens enter the body at mucosal surfaces. Generating robust "barrier protection" at mucosal surfaces may therefore be an ideal strategy to block infections before they become systemic (reviewed in Lycke, 2012).

Adenoviruses (Ads) are non-enveloped DNA viruses (reviewed in Campos and Barry, 2007). Ads are potent vectors for gene-based vaccination (Lasaro and Ertl, 2009). Adenovirus serotype 5 (Ad5) binds to the coxsackie and adenovirus receptor (CAR) using its trimeric fiber protein (Fig. 1 and reviewed in Khare et al., 2011). Thefiber protein consists of a 44 amino acid "tail" domain on its n-terminus that docks into the penton base of the viral icosahedron, a 21 β-spiral repeats in its shaft domain, and a c-terminal CAR-binding "knob" domain (Fig. 1).

Reoviruses are non-enveloped RNA viruses that infect gut mucosa by binding junctional adhesion molecule 1 (JAM-1) and sialic acid (Kirchner et al., 2008). Reoviruses display a trimeric protein for receptor binding that is called sigma 1. Despite having evolved separately and binding different receptors, sigma 1 has a shaft domain bearing β-spiral repeats that are remarkably similar to those in adenovirus fibers (Forrest et al. (2003) and Fig. 1).

Because Ads naturally cause a number of ocular, respiratory, and digestive infections, they can be one of the most robust vectors for vaccination at mucosal surfaces. While this is true, CAR-utilizing adenoviruses may not be optimal for mucosal vaccination because mucosal epithelial cells do not actually display CAR on their luminal surfaces (Grubb et al., 1994; Zabner et al., 1997). Instead, CAR is sequestered on the basolateral surface of mucosal cells making infection there less efficient. In contrast, the T3D reovirus sigma 1 protein binds to sialic acid that is expressed on nearly all mucosal epithelial cells. Sigma 1 also binds JAM-1, which is expressed on microfold cells (M cells) of Peyer's patches in the lumen of the gut. JAM-1 is also expressed on dendritic cells (Mercier et al., 2004). In contrast, CAR is not expressed on these professional antigen-presenting cells.

Given the desire to improve mucosal vaccination, we previously generated a chimeric adenovirus that displays the sigma 1 protein (Mercier et al. (2004) and Fig. 1). This was accomplished by replacing the virion docking motif of the sigma 1 protein from reovirus T3D with the 44 amino acid Ad5 fiber tail. When the

* Corresponding author at: Department of Internal MedicineDivision of Infectious Diseases, Mayo Clinic, 200 First Street SW, Rochester, MN, USA. Tel.: +507 266 9090; fax: +507 255 2811.
E-mail address: mab@mayo.edu (M.A. Barry).

http://dx.doi.org/10.1016/j.virol.2015.02.050
0042-6822/

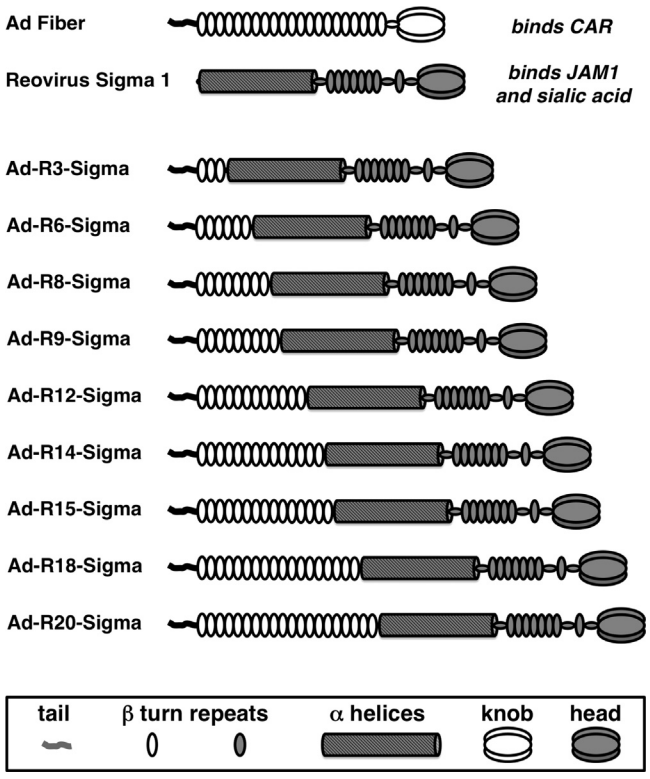

Fig. 1. Cartoon of fiber-sigma chimeric proteins.

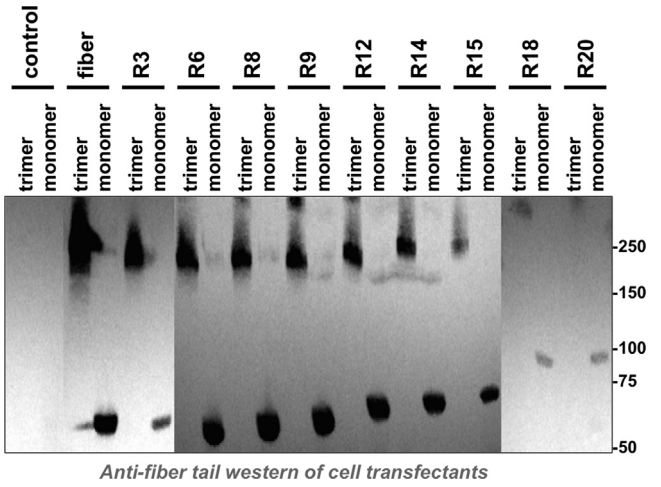

Fig. 2. Western blot of fiber-sigma chimeras. The indicated fiber chimeras shown in Fig. 1 were used to transfect cells and cell lysates were boiled in standard Laemli loading buffer to observe fiber monomers. To observe trimers, samples were mixed with loading buffer with reduced SDS and were not boiled prior to loading. After SDS-PAGE and transfer to PVDF membranes, the fiber chimeras were detected by western blot with an antibody against the Ad5 fiber tail.

tail-sigma 1 protein was engineered into Ad5 vectors in place of the fiber, sigma 1 was successfully displayed on Ad5 virions. When tested *in vitro,* Ad5-sigma 1 was shown to no longer bind CAR, but instead bind to sialic acid and JAM-1 (Mercier et al., 2004). When Ad5-sigma 1 was subsequently tested *in vivo* in mice, it was 40-fold less efficient at transducing muscle or nasal mucosa than Ad5 (Weaver et al., 2012). This weak transduction correlated to weak antibody production against its transgene product. This weak vector function could be due to defects in either end of the chimeric protein. The tail-sigma fusion might be inefficient at docking into the Ad5 penton base on the viral icosahedron. Alternately, this one fusion protein might not display sigma 1 in a fashion that allowed efficient use of its cognate receptors. In this work, we engineered a series of fiber-sigma fusion proteins and displayed them on Ad5 to test for *in vivo* transduction and antibody production.

Results

Construction of fiber-sigma chimeric proteins

To allow the reovirus sigma protein to dock into the Ad penton base, sigma 1 was originally fused to just the minimal 44 amino acid tail of the fiber tail (Fig. 1 and Mercier et al. (2004)). The Ad5 fiber has 21 β-turn repeats in its shaft that provides the bulk of the trimer's length. The third β-turn, repeat 3 (R3), contains a four amino acid insertion that provides flexibility to the shaft of fiber (Nicklin et al., 2005; Wu and Nemerow, 2004). We hypothesized that including tail-R1–R2–R3 with this flexibility motif might enhance Ad-sigma functionality. It was also possible that adding more fiber repeats to the fusions might optimize the protein for mucosal infection.

To test this, a series of fiber-sigma chimeras were generated and these were cloned into expression plasmids (Fig. 1). Each of the plasmids were transfected into 293 cells and cell lysates were evaluated by western blot using an antibody against the fiber tail (Fig. 2). Increasing the number of fiber shaft repeats from 3 (R3) to 20 (R20) led to the expression of increasing longer fiber-sigma chimeric proteins. Notably, all of the chimeras trimerized, suggesting that they had the potential to be incorporated into the penton base of Ad5 virions (Parrott et al., 2003).

Sigma 1 virus generation

R3, R14, and R20 fiber-sigma chimeric proteins were selected for further testing. Each was used to replace the fiber gene in replication-defective E1/E3 deleted Ad5 vectors expressing the *Aequorea victoria* green fluorescent protein fused to firefly luciferase (GFPLuc) by homologous recombination in bacteria as in (Mercier et al., 2004). The recombinants were verified by sequencing and then rescued in fiber-expressing 633 cells (Von Seggern et al., 2000) as in Mercier et al., 2004. In their final round of amplification, the viruses were produced in 293 cells for display only the virally-encoded sigma chimera (Mercier et al., 2004).

Virus composition

Ad5, R3, R14, and R20 fiber-sigma chimera viruses were purified by CsCl banding. These purified virions were separated by SDS-PAGE and stained with Sypro Ruby^{tm} to detect all virion proteins (Fig. 3A). All viruses had normal virion capsomers including hexon, penton, IIIa, V, VI, and VII. There are normally 36 copies of fiber per virion. Fiber and fiber-sigma chimeras are difficult to discern when staining for total protein, since the 60 copy IIIa protein overlaps these lower copy proteins. These were detected by western blot with an antibody directed against the fiber tail (Fig. 3B). Western blotting detected wild type fiber as well as the larger sigma chimeras in all the viruses. As chimera size increased, progressively less fiber-tail detected protein appeared to be incorporated. R3-sigma intensity appeared to be similar to that of the fiber, but there was markedly less R14 protein on the purified virus and R20 was barely detectable. Adenoviruses lacking fiber fail to mature and proteolytically cleave precursor proteins in the virion including processing pVI and pVII to VI and VII (Legrand et al., 1999). While Ad5, R3, and R14 virions appeared fully mature, R20 virions had pVI and pVII bands indicated they were immature in this slightly overloaded lane (Fig. 3A). This as well the observed