Published in final edited form as:

Vaccine. 2007 April 20; 25(16): 3215–3218.

Protection of Mice Against Rotavirus Challenge following Intradermal DNA Immunization by Biojector Needle-Free Injection

Anthony H.-C. Choi^{a,*}, Kristi Smiley^a, Mitali Basu^a, Monica M. McNeal^a, Mingyuan Shao^b, Judy A Bean^b, John D. Clements^c, Richard R. Stout^d, and Richard L. Ward^a

a Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229

b Department of Biostatistics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229

c Tulane University Medical Center, New Orleans, Louisiana 70112

d Bioject Inc., Portland, OR 97224, USA

Abstract

Mucosal administration (intranasal or oral) of a VP6 rotavirus vaccine to mice consistently elicits high levels of protection after rotavirus challenge (93 – >99% reductions in fecal rotavirus shedding) but only when co-administered with an effective adjuvant such as LT(R192G). Here, we showed that Biojector needle-free injection of VP6-encoded plasmids also induced protection (85 – 93%) when they were co-administrated with LT(R192G)-encoded plasmids. A reduction in the amount of VP6 plasmid from 50 to 10 µg reduced protection from 93 to 70%, but the immunized mice remained significantly ($P<0.05$) protected. Intramuscular needle injection of VP6/LT(R192G)-plasmids also induced significant protection (66%).

Keywords

Rotavirus; DNA vaccine; needle-free injection

1. Introduction

Rotavirus (RV) is the single most common cause of acute gastroenteritis among children from 3 to 36 months of age worldwide [1]. RV gastroenteritis usually starts with fever, an upset stomach and vomiting, followed by watery diarrhea. Severe dehydration caused by RV infection results in more than 600,000 deaths worldwide annually. In the USA, RV infection is responsible for approximately 600,000 physician visits, 55,000 hospitalizations, and 20 to 60 deaths each year. Two live, orally-deliverable RV vaccines (RotarixTM and RotaTeqTM) are being marketed in numerous countries throughout the world. However, live RV vaccine immunization can lead to adverse effects and the best example may be the association of RotashieldTM with intussusception [2]. The safety and efficacies of the two vaccines now being marketed will only be fully recognized in post-licensure studies. Therefore, development of second-generation non-living rotavirus vaccines is warranted.

*Corresponding author. Tel.: +1 513 636 7679; fax: +1 513 636 7655. E-mail address: anthony.choi@cchmc.org (A.H.-C. Choi).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Several non-living vaccines are being evaluated, including a recombinant protein vaccine based on the highly conserved inner-capsid VP6 protein of RV strain EDIM [3-7]. Studies have been performed to investigate the efficacy of *E. coli*-expressed VP6 proteins co-administered with an adjuvant chosen from several that have been tested in a mouse model [6]. Vaccine efficacy is measured by quantifying reductions in fecal rotavirus shedding in immunized animals relative to unimmunized mice following an oral challenge with a murine RV [3-7]. The highest levels of protection (93 – >99% reductions in fecal rotavirus shedding relative to those of unimmunized animals) were elicited by either intranasal (i.n.) or oral administration of VP6 co-administered with LT(R192G), a genetically attenuated *E. coli* heat-labile enterotoxin [3, 5-7]. By contrast, reduced or no protection was obtained following subcutaneous (73%), Biojector (26%), transcutaneous (13%) or intradermal (0%) administration of VP6/LT(R192G) proteins [7]. Furthermore, plasmids containing a RV VP6 gene also provided no protection of mice following gene gun immunization [4].

Although both i.n. and oral administration of VP6/LT(R192G) induced high levels of protection, it has not been determined whether co-administration of a VP6 DNA/LT(R192G) DNA vaccine would induce protection and whether the levels of protection would be affected by the routes of administration. Therefore, protection studies were conducted in mice after administration of plasmids encoding the VP6 gene of RV strain EDIM and LT(R192G) by Biojector 2000 needle-free injection (B2000), intramuscular (i.m.) needle injection, or i.n. administration.

2. Materials and Methods

2.1. Construction of rotavirus recombinant VP6- and LT(R192G)-plasmid vectors

The VP6 DNA vaccine tested in this study is composed of recombinant plasmids pVP6 and pLT(R192G). The VP6 gene of rotavirus mouse strain EDIM (Genbank Accession No. AF461757), and the *E. coli* heat-labile enterotoxin A (LTA) and B (LTB) gene sequences (Genbank Accession No. AB07668) contained in pVP6 and pLT(R192G) had been modified for optimal protein expression. The modifications included reducing rare-codon usage, minimizing local GC content; and removal of internal TATA-boxes, chi-sites, ribosomal entry sites, repeat sequences, RNA secondary structures, and cryptic splice donor and acceptor sites. In addition, the codon in LTA gene encoding the arginine residue in position 192 was changed to one encoding glycine to create LTA(R192G). The VP6, LTA(R192G) and LTB genes were synthesized by Geneart (Rogensburg, Germany). The VP6 gene was cloned into the plasmid pVIVO2 (InvivoGen, San Diego, CA) placing it under the control of the human Fer-L promoter. The LTA(R192G) and LTB DNAs were cloned into the plasmid pVIVO2, placing them under the control of the hFer-H and hFer-L promoters, respectively. The recombinant plasmids were transformed into *E. coli* strain DH5 α . The recombinant bacteria were grown in Fast Media Hygro according to the instructions of the supplier (InvivoGen). The recombinant plasmids were purified using EndoFree Plasmid Maxi kits (Qiagen, Valencia, CA).

2.2. Recombinant rotavirus VP6 and LT(R192G) proteins

The *E. coli*-expressed VP6 and LT(R192G) proteins were used for i.n. administration. The recombinant VP6, i.e., VP6::6 $\times$ His, was derived from the mouse rotavirus strain EDIM VP6 and contains the 6 $\times$ His affinity tag for facilitating purification of the protein using affinity resin. LT(R192G) is an *E. coli* heat-labile enterotoxin, which has its glycine at position 192 replaced by arginine to attenuate its toxicity. The methods employed for purification of VP6 and LT(R192G) proteins have already been described [3,8].

2.3. Immunization and RV challenge

Treatment groups contained 5 – 8 six-week old, rotavirus-free, female BALB/c mice. The mice were administered 3 immunizations, separated by 2 weeks, of VP6 and LT(R192G) proteins or pVP6 and pLT(R192G) plasmids. The VP6/LT(R192G) proteins (4 µg and 10 µg, respectively, in 20 µl of phosphate-buffered saline) were administered i.n. (10-µl per nostril) essentially as described previously [3,5-7]. For DNA immunization, pVP6/pLT(R192G) plasmids (10 or 50 µg of pVP6, and 10 µg of pLT(R192G)) in 100-µl volumes were administered intradermally (i.d.) using the B2000 injector according to the instructions of the manufacturer (Bioject Medical Technologies, Inc., Portland, OR), or 50 µg of pVP6 and 10 µg of pLT(R192G) in 100-µl volumes for i.m. immunization using 28 gauge needles. Four weeks after the last immunization, mice were orally (gavage) challenged with 10,000 shedding-dose-50 of RV strain EDIM.

2.4. Detection of RV antigen in stools specimens

Two to three fecal pellets were collected daily for 7 days from each mouse into Earl's balanced salt solution starting on day 1 following rotavirus challenge. Samples were frozen until analyzed, at which time they were homogenized and centrifuged ($1,500 \times g$, 5 min, 4°C) to remove debris. The quantities of rotavirus antigen in the fecal samples were determined by ELISA as ng/ml of stool specimen, using methods already described [3,5-7].

2.5. Statistical methods

Statistical analysis was performed to determine whether total fecal RV shedding over 7 days by mice in the vaccinated groups was significantly lower than that in the control group, and whether total RV shedding was significantly different among the vaccinated groups. A two-sample, one-sided *t*-test was applied to compare the control group with the vaccinated groups, and a general linear model was constructed to compare the vaccinated groups for each study. All the analyses were performed using SAS statistical software package Version 9.1.3, and the statistical significance level was set at 0.05.

3. Results

3.1. Rotavirus pVP6/pLT(R192G) DNA vaccine administered using B2000 injector induced significant protection against an oral RV challenge

We previously reported that i.d. administration of gold particles coated with the plasmid pcDNA1/EDIM6, encoding the mouse RV strain EDIM VP6 gene, using the Helios Gene Gun (Bio-Rad, Hercules, CA) did not elicit protection in mice [4]. VP6 protein, when administered i.n. or orally, also did not induce protection, but when co-administered with LT(R192G) protein, it elicited between 93 – >99% protection [3,5-7]. Thus, it was of interest to determine whether i.d. administration of the plasmid pVP6 that encodes EDIM VP6 together with the plasmid pLT(R192G) encoding LT(R192G) adjuvant would induce reductions in rotavirus shedding. A study was carried out to determine whether protection levels induced by administration of pVP6/pLT(R192G) plasmids using the B2000 injector would be affected by varying the dose of pVP6. For this, groups of mice were administered either 3 doses of pVP6 (50 µg)/pLT(R192G) (10 µg) or pVP6 (10 µg)/pLT(R192G) (10 µg). Control groups were administered i.n. with 3 doses of VP6 (4 µg)/LT(R192G) (10 µg) proteins or were left unimmunized. The DNA vaccines elicited 93 and 70% reductions in shedding of fecal rotavirus antigen, respectively, relative to the unimmunized group; while 96% reduction was found in the protein immunized group (Table 1). The quantities of antigen shed by the 3 immunized groups were significantly lower ($P < 0.05$) than those shed by the unimmunized group. However, the 10 µg-pVP6 dose induced significantly ($P < 0.05$) lower protection than the 50

μg -pVP6 dose, while the protection induced by the 50 μg pVP6 dose was not significantly ($P>0.05$) different from that obtained with VP6/LT(R192G) proteins.

3.2. Biojector-delivered rotavirus pVP6 induced protection against RV challenge only when it was co-administered with pLT(R192G)

A study was next conducted to determine whether the protection induced by pVP6/pLT(R192G) would be reduced by omitting pLT(R192G) from the injectant. Groups of mice were immunized with pVP6 alone (50 μg), pLT(R192G) alone (10 μg), or pVP6/pLT(R192G) (50 μg and 10 μg , respectively). In addition, a group of mice was immunized i.n. with VP6/LT(R192G) proteins (4 μg and 10 μg , respectively) and a mouse group was not immunized. Mice immunized with pVP6 or pLT(R192G) alone had only 25% and 19% reductions in shedding, respectively, while those administered pVP6/pLT(R192G) had an 85% reduction in shedding (Table 2). As expected, the quantities of antigen shed by pVP6- as well as pLT(R192G)-immunized mice were significantly higher than those shed by the pVP6/pLT(R192G)-immunized mice. I.n. administration of VP6/LT(R192G) proteins produced a 97% reduction in shedding in this experiment which, again, was not significantly different from that elicited in the pVP6/pLT(R192G)-immunized mouse group. Therefore, co-administration of the pLT(R192G) plasmid was required for pVP6-induced protection.

3.3. Rotavirus VP6 DNA vaccine administered i.m. induced protection against an oral RV challenge

We previously showed that i.m. injection of mice with VP6-encoding plasmids alone did not induce protection [4], but whether protection could be induced by injection of pVP6 together with pLT(R192G) has not been evaluated. Since the i.n. route is the most effective route for VP6/LT(R192G) protein-induced protection, it was also of interest to determine whether i.n. immunization of pVP6/pLT(R192G) plasmids would induce protection comparable to that obtained by VP6/LT(R192G) proteins. Mice immunized i.m. with pVP6/pLT(R192G) induced a 66% reduction in rotavirus antigen shedding relative to the unimmunized group, and the quantities shed were significantly ($P<0.05$) less than those shed by the unimmunized mice (Table 3). However, mice immunized i.n. had only a 10% reduction in rotavirus antigen shedding, and the amounts of RV antigen shed were not significantly ($P>0.05$) different from those shed by the unimmunized control mouse group.

4 Conclusion

In the studies described, we showed that i.d. administration of a DNA vaccine composed of plasmids encoding RV VP6 and LT(R192G) proteins using the B2000 needle-free injector induced significant protection levels (85 – 93% reductions in fecal rotavirus shedding). Induction of these protection levels was obtained only when the pLT(R192G) plasmid was co-administered, and the protection levels were comparable to those obtained following i.n. immunization with VP6 and LT(R192G) proteins ($\geq 96\%$). The induced protection is dose-dependent, since the reduction of pVP6 administered, from 50 μg to 10 μg , significantly reduced the protection levels from 85 – 93% to 70%. I.m. immunization also induced significant, albeit lower, protection (66%) than that obtained by B2000 administration. I.n. administration of the VP6/LT(R192G) DNA vaccine was found to induce essentially no protection (10%), even though VP6/LT(R192G) proteins consistently provide 93 – >99% protection. These studies indicate that the levels of protection induced against an oral rotavirus challenge in the mouse model depend on the compositions, the routes and the methods of vaccination.

Acknowledgements

This work was supported in part by Grant R43AI1051918 from the NIH/NIAID to AHC Choi.

References

1. Glass RI, Parashar UD, Bresee JS, et al. Rotavirus vaccines: current prospects and future challenges. *Lancet* 2006;368(9532):323–32. [PubMed: 16860702]
2. Bines JE. Rotavirus vaccines and intussusception risk. *Current Opinion in Gastroenterology* 2005;21(1):20–5. [PubMed: 15687880]
3. Choi AH, Basu M, McNeal MM, Bean JA, Clements JD, Ward RL. Intranasal administration of an *Escherichia coli*-expressed codon-optimized rotavirus VP6 protein induces protection in mice. *Protein Expression & Purification* 2004;38(2):205–16. [PubMed: 15555936]
4. Choi AH, Knowlton DR, McNeal MM, Ward RL. Particle bombardment-mediated DNA vaccination with rotavirus VP6 induces high levels of serum rotavirus IgG but fails to protect mice against challenge. *Virology* 1997;232(1):129–38. [PubMed: 9185596]
5. Choi AH, McNeal MM, Basu M, et al. Intranasal or oral immunization of inbred and outbred mice with murine or human rotavirus VP6 proteins protects against viral shedding after challenge with murine rotaviruses. *Vaccine* 2002;20(2728):3310–21. [PubMed: 12213401]
6. Choi AH, McNeal MM, Flint JA, et al. The level of protection against rotavirus shedding in mice following immunization with a chimeric VP6 protein is dependent on the route and the coadministered adjuvant. *Vaccine* 2002;20(1314):1733–40. [PubMed: 11906760]
7. Choi AH, Smiley K, Basu M. Induction of immune responses and partial protection in mice after skin immunization with rotavirus VP6 protein and the adjuvant LT(R192G). *Vaccine* 2005;23(1718):2290–3. [PubMed: 15755613]
8. Dickinson BL, Clements JD. Dissociation of *Escherichia coli* heat-labile enterotoxin adjuvanticity from ADP-ribosyltransferase activity. *Infection & Immunity* 1995;63(5):1617–23. [PubMed: 7729864]

Table 1

Biojector administration of plasmids encoding rotavirus VP6 and LT(R192G) proteins induce significant and dose-dependent protection in mice against an oral rotavirus challenge

Mouse group ^a	Immunogen	Quantities of RV antigen shed ^b	% Protection ^f
None (6)	None	24,794 ± 4,190	-
Biojector (6)	pVP6 (50 µg)	1,629 ± 437 ^{c d}	93
	pLT(R192G) (10 µg)		
Biojector (5)	pVP6 (10 µg)	7,364 ± 1,824 ^{c e}	70
	pLT(R192G) (10 µg)		
i.n. (6)	VP6 protein (4 µg)	958 ± 362 ^c	96
	LT(R192G) protein (10 µg)		

^aThe number of mice used in each treatment groups are shown in parenthesis.

^bRotavirus antigen shed in the 7-day stool collection period (ng/mouse) ± SEM (standard error of mean).

^cSignificantly ($P < 0.05$) lower than the unimmunized group.

^dNot significantly ($P > 0.05$) different from mice immunized i.n. with VP6/LT(R192G) proteins.

^eSignificantly ($P < 0.05$) greater than mice immunized with pVP6 (50 µg)/pLT(R192G) (10 µg) or VP6/LT(R192G) proteins.

^fPercent reductions in rotavirus antigen shed by the immunized relative to unimmunized group.

Table 2

Biojector administration of the LT(R192G)-encoding plasmid was required for protection induced by the VP6-encoding plasmid

Mouse group ^a	Immunogen	Quantities of RV antigen shed ^b	% Protection ^e
None (8)	None	20,298 ± 2,037	-
Biojector (8)	pVP6 (50 µg)	3,072 ± 636 ^{c d}	85
	pLT(R192G) (10 µg)		
Biojector (8)	pVP6 (50 µg)	15,150 ± 3,372	25
Biojector (8)	pLT(R192G) (10 µg)	16,461 ± 2702	19
i.n.	VP6 protein (4 µg)	654 ± 161 ^c	97
	LT(R192G) protein (10 µg)		

^aThe number of mice used in the treatment groups are shown in parenthesis.

^bRotavirus antigen shed in the 7-day stool collection period (ng/mouse) ± SEM.

^cSignificantly ($P < 0.05$) lower than unimmunized, pVP6- and pLT-immunized mice.

^dNot significantly ($P > 0.05$) different from i.n. immunized group.

^ePercent reductions in rotavirus antigen shed by the immunized relative to unimmunized group.

Table 3

Protection was induced by i.m. needle injection of plasmids encoding rotavirus VP6 and LT(R192G) but not by i.n. administration of these plasmids

Mouse group ^a	Immunogen	Quantities of RV shed ^b	% Protection ^e
None (8)	None	19,432 ± 1,048	-
i.m. (7)	pVP6 (50 µg)	7,523 ± 1,111 ^c	66
	pLT(R192G) (10 µg)		
i.n. (8)	pVP6 (50 µg)	17,500 ± 2,407 ^d	10
	pLT(R192G) (10 µg)		

^aThe number of mice used in the treatment groups are shown in parenthesis.

^bRotavirus antigen shed in the 7-day stool collection period (ng/mouse) ± SEM.

^cSignificantly ($P < 0.05$) lower than the unimmunized group.

^dNot significantly ($P > 0.05$) different from unimmunized group.

^ePercent reductions in rotavirus antigen shed by the immunized relative to unimmunized group.