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Induction of Protective Immunity in a Syrian Hamster Model Against a Cytopathogenic Strain of Andes Virus

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Andes virus (ANDV) is responsible for the Hantavirus Pulmonary Syndrome cases in Argentina and neighboring countries, with moderate to high case-fatality rates. ANDV has some particular features, which make it unique among other members of the *Hantavirus* genus such as person-to-person transmission and causing a disease similar to Hantavirus Pulmonary Syndrome in the hamster as an animal model. The kinetics of replication in Vero E6 cells of an ANDV strain isolated in Argentina, called Andes/ARG, was studied. Cytopathic effect and the formation of clear plaques were observed and therefore Andes/ARG could be quantified by classic plaque assay. The Andes/ARG strain was found to be highly lethal in Syrian hamsters allowing experiments to demonstrate the protective potential of vaccines. A recombinant nucleocapsid protein of ANDV induced a long lasting antibody response and protective immunity against a homologous challenge, but to a lower extent against heterologous challenge by the Seoul virus. ***J. Med. Virol. 9999:1–9, 2011.*** © 2011 Wiley-Liss, Inc.

KEY WORDS: hantavirus pulmonary syndrome; vaccine candidate; *Mesocricetus auratus*

INTRODUCTION

The genus[Q2] *Hantavirus* belongs to the family *Bunyaviridae*, which comprises trisegmented single-stranded RNA viruses. Hantaviruses are rodent-borne viruses of worldwide distribution that cause human diseases. Old World and New World hantaviruses have been associated with Hemorrhagic Fever with Renal Syndrome and Hantavirus Pulmonary Syndrome, respectively.

Andes virus (ANDV), characterized from the Hantavirus Pulmonary Syndrome cases in Argentina [Lopez et al., 1996] and several neighboring countries [Johnson et al., 1997; Johnson et al., 1999; Padula et al., 2000a], causes high case-fatality rates [Martinez et al., 2010]. ANDV had shown some particular features which makes it unique among other members of *Hantavirus* genus, such as its ability to establish person-to-person transmission, which has only been described in Argentina and Chile [Padula et al., 1998; Martinez et al., 2005; Ferres et al., 2007]. In addition, it was the only pathogenic hantavirus that could cause illness in an animal model reproducing a disease similar to Hantavirus Pulmonary Syndrome in hamsters [Hooper et al., 2001]. Other pulmonary hantaviruses, such as Sin Nombre and Choclo, failed to cause illness in hamsters [Hooper et al., 2001; Wahl-Jensen et al., 2007; Eyzaguirre et al., 2008]. Furthermore, hantaviruses are very difficult to isolate, only three pathogenic agents from South America have been successfully isolated in cell culture: Laguna Negra, Araucaria, and ANDV [Johnson et al., 1997; Padula et al., 2002; Machado et al., 2010].

To date there is no specific treatment for hantavirus infections, efforts have been focused on the development of hantavirus vaccines. Because Hantavirus Pulmonary Syndrome is a relatively rare disease, most vaccine attempts have been directed to Hemorrhagic Fever with Renal Syndrome. Because of the lack of suitable disease models for Hemorrhagic Fever with Renal Syndrome, protection has been usually measured by the ability to protect from infection. Several of these approaches include conventional vaccines, such as rodent brain and cell culture-derived inactivated vaccines, and molecular vaccines [Schmaljohn, 2009]. The nucleocapsid protein expressed by different

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systems has been used to protect rodents from infection with Hemorrhagic Fever with Renal Syndrome agents with different efficacy [Lundkvist et al., 1996; Dargeviciute et al., 2002; de Carvalho Nicacio et al., 2002; Klingstrom et al., 2004]. A successful approach for the protection of bank voles consisting in recombinant chimeric virus-like particles carrying a segment of the nucleocapsid protein of Puumala virus [Ulrich et al., 1998] helped to identify the protective regions within the nucleocapsid protein [Koletzki et al., 2000]. For Hantavirus Pulmonary Syndrome few vaccine approaches have been evaluated for Sin Nombre virus and ANDV Chile-9717869 strain. DNA vaccines based on M and S segment of Sin Nombre and Chile-9717869 viruses were proved in animal models [Bharadwaj et al., 1999; Bharadwaj et al., 2002; Custer et al., 2003; McElroy et al., 2004]. A DNA vaccine based on ANDV M segment resulted neither immunogenic nor protective in the ANDV Chile-971786/hamster lethal model of disease [Custer et al., 2003]. However, non-replicating adenovirus vectors expressing the nucleocapsid protein of ANDV Chile-971786, and both glycoproteins administered to hamsters individually or in combination, elicited a robust immune response that protected hamsters from disease [Safronetz et al., 2009].

In this study, an Argentinean strain of ANDV, Andes/ARG, showed cytopathic effect and formation of clear plaques in Vero E6 cells. Hamster's susceptibility to infection with this strain was evaluated in order to develop a model of disease useful for protection studies. The potential efficacy in protection of a recombinant vaccine based on the nucleocapsid protein expressed in *Escherichia coli* was analyzed with the Andes/ARG-hamster model.

MATERIALS AND METHODS

Virus and Hamsters

A strain of ANDV, called Andes/ARG, was isolated from the lung of an *Oligoryzomys longicaudatus* captured in the Patagonian Forests ecoregion [Padula et al., 2002]. The isolate was obtained in October 2000; it was confirmed by immunofluorescence with rabbit serum raised against ANDV nucleocapsid protein. The stock used in this study was the 9th viral passage in Vero E6 cells (ATCC, CRL 1586), and its infectious titer was 2×10^5 plaque forming units (PFU)/ml. Undiluted supernatants or dilutions in PBS were utilized as inoculums in challenge experiments and to infect Vero E6 cell monolayers. Twenty-one-week-old male Golden Syrian Hamsters (*Mesocricetus auratus*) were utilized in either infection or immunization experiments. They were provided by the National Institute of Biologic Production from the National Administration of Health Institutes and Laboratories "*Dr. C. G. Malbrán*". Animals were anesthetized by an intramuscular injection of ketamine (approximately 3 mg/100 g of body weight) prior inoculation.

Recombinant Nucleocapsid Proteins and ELISA

Recombinant nucleocapsid proteins from ANDV (ANDV-rNP) and SEOV (SEOV-rNP) and a negative control antigen (rCP) were obtained as described previously [Padula et al., 2000b; Padula et al., 2010]. Briefly, the nucleocapsid protein ORFs of ANDV and SEOV cloned in pRSET (R&D System Europe, Oxford, UK) were used to transform *E. coli* BL21 (DE3) cells. Cells were cultured for expression and the protein purified on a nickel resin affinity column (Ni-NTA agarose; Qiagen[Q3]). The rCP was expressed in the same manner and was used as control antigen. Gel electrophoresis on SDS–polyacrylamide gels revealed highly purified recombinant protein of the expected size (data not shown). The SEOV Sapporo rat strain stock virus was kindly provided by Jay Hooper and Connie Schmaljohn, United States Army Medical Reaserch Institute of Infectious Diseases (Fort Detrick, Frederick, MD).

The antigens were diluted in 0.1 M sodium carbonate buffer (pH 9.5) at concentrations of 4 μg/ml and applied to polystyrene plates overnight at 4°C. The coated plates were blocked with 5% skimmed milk in PBS, 0.1% Tween 20 (blocking buffer). Dilutions of samples and conjugates were done in blocking buffer. All the following incubations were done at 37°C for 1 hr and the plates were washed six times with 0.1% Tween-20 in PBS between each step. After blocking, serial fourfold dilutions of hamster serum (from 1:100) were added. Specific antibody binding was detected with horseradish peroxidase conjugated goat anti-hamster IgG antibodies diluted 1:4,000 (Kirkegaard[Q4] & Perry).

Establishment of Virus Replication Kinetics

Culture tubes were seeded with 2×10^4 Vero E6 cells per tube in 1 ml of Eagle's minimal essential medium containing 10% heat-inactivated fetal bovine serum (FBS), 10 mM HEPES, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml of streptomycin and 0.25 μg/ml amphotericin B (10% FBS cEMEM) and incubated at 37°C in an humidified atmosphere containing 5% CO_2 for 48 hr. Confluent monolayers were infected with Andes/ARG at a multiplicity of infection (moi) of 0.5 and maintained with 2% FBS cEMEM; mock-infected cells (PBS) were used as controls. Cells and supernatant were collected daily to perform plaque assay and RNA extraction.

Cell Viability Assay

Confluent monolayers infected in the same manner and simultaneously with the cells infected for the growth curve were used to determine cell viability by trypan blue dye exclusion assay. Cells adherent to culture tubes were treated with a solution of 0.25% trypsin and 1 mM EDTA, once detached were combined with the floating cells collected from the culture

media, washed and resuspended in PBS and mixed with an equal volume of 0.4% trypan blue. The cells that excluded the dye (viable cells) and the stained cells (death cells) were counted under a microscope using a hemocytometer chamber.

Plaque Assay and Plaque Reduction Neutralization Test (PNRT)

Dilutions of infected cell supernatants were added to 24-well plates containing 7-day-old Vero E6 cell monolayers. After adsorption of 1 hr, the wells were overlaid with 0.5% agarose in 10% FBS cEMEM. Plates were incubated 11 to 12 days at 37°C in a humidified atmosphere containing 5% CO_2, and cells were then fixed with 10% formaldehyde solution and stained with 0.2% crystal violet.

Neutralization activity of serum against Andes/ARG was analyzed by PNRT. Hamster serum samples previously heated for 30 min at 56°C, were serially diluted (twofold) and mixed with an equal volume containing 100 PFU of virus per 100 μl. The mixtures were incubated overnight at 4°C, inoculated into wells of 24-well plates containing 7-day-old Vero E6 cell monolayers and plaque assay was performed as described above. An >80% reduction in number of plaques was selected as criterion for virus neutralizing titers.

RNA Extraction and Real-Time RT-PCR

Fluids of infected monolayers were centrifugated to pellet floating cells and samples of 200 μl of supernatant were subjected to RNA extraction. Cell monolayers were trypsinized and resuspended in 200 μl of PBS; 100 μl of cell suspension were subjected to RNA extraction. RNA was extracted using Trizol (Invitrogen[Q5]) and purified by the RNAid kit (Bio 101[Q6]) following both manufacturer's recommendations. For ANDV RNA quantification real-time RT-PCR was performed using a MyiQ single color RT-PCR detection system (BioRad[Q7]). Primers and probe were designed to amplify the conserved region of the S-segment of ANDV from position 28 to 116 (GenBank accession number AF00460); sense primer 5′-GTCGCGAAAG-CTGGAATGAG-3′; antisense 5′-AGCTTTTGCCGA-GCAGTCA-3′, and the fluorescently labeled TaqMan probe 5′-(FAM)-AGAAAACATCACAGCACACGAACA-ACAGCT-(TAMRA)-3′. The PCR mixture contained the following: 1 μM of forward and reverse primers, 100 nM probe, 12.5 μl 2× Probe QuantiTect RT-PCR master mix (Qiagen), 2 μl of RNA, and RNAse free water to complete 25 μl of final volume. Reaction mixtures of 25 μl were analyzed in duplicate. vRNA was quantified using a standard curve generated using S-segment templates of known copy number (Bellomo, unpublished). Briefly, the nucleocapsid protein open reading frame of ANDV (AH1 strain) cloned in pGem (Promega[Q8]) was digested with *Spe* I and the RNA was transcribed in vitro with RiboMax Large scale RNA production T7 (Promega). The purified RNA was spectrophotometrically quantified and six 10-fold dilutions were aliquoted and frozen to use as standards.

Evaluation of Susceptibility of Hamster to Andes/ARG

Five Syrian Golden Hamsters (*Mesocricetus auratus*) were intramuscularly (i.m.) inoculated (caudal thigh) with Andes/ARG infected Vero E6 cell culture supernatant diluted in PBS (10^5 PFU). Control animals were inoculated with supernatants of uninfected cells. The development of illness was evaluated for lack of movement, no interest in food and dyspnea. After infection, two animals were placed per cage inside an aseptic air negative-pressure environmental cabinet (A130SN-Flufrance) placed in an animal facility equipped for that purpose and of exclusive use for these experiment.

Immunization Procedure and Experimental Challenge of Hamsters

Study design: The study consisted in animal groups for homologous challenge (12 hamsters sham-immunized and 13 hamsters immunized with ANDV-rNP) and for heterologous challenge (2 control hamsters sham-immunized and 4 immunized with SEOV-rNP). Ten-week-old hamsters (~100 g) were i.m. inoculated with ANDV-rNP, SEOV-rNP, rCP, or PBS. Proteins were emulsified in Freund's adjuvant. Two doses of 40 μg of ANDV-rNP or SEOV-rNP were administered; the interval between doses was 14–16 days. Sham-immunized animals used as controls were injected with PBS or rCP in the same adjuvant. All hamsters were bled before challenge to determine the development of humoral response against ANDV-rNP or SEOV-rNP. For Andes/ARG challenge hamsters were inoculated as described above. Time of challenge after immunization and viral doses are shown in Tables I and II for homologous and heterologous groups, respectively. Survivors were bled and sacrificed after 9 weeks post-challenge. Lung samples were removed and examined for the presence of viral RNA.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 with the level of significance set at 0.05. The correlation between cell viability and virus RNA accumulation within cells was assessed by linear regression analysis (Pearson's method). The statistical significance of differences in anti-NP response of vaccinated hamsters challenged at different time points was calculated by paired *t*-test. The efficiency of ANDV-rNP as vaccine was calculated by comparing survival between the experimental group (n = 6) and the control group (n = 5) challenged 22 weeks after vaccination was assessed by Fischer's exact test.

TABLE I. Time Point and Doses of Virus Challenge Following Immunization With ANDV-rNP

		Viral doses (PFU) at indicated time (weeks p.v.)		
Hamster no.	Immunogen	4	22	43
h28-h31	ANDV-rNP	2×10^3		1×10^4
h25-h27	PBS	2×10^3		
h63-h68	ANDV-rNP		1×10^4	
h57-h62	rCP		1×10^4	
h33-h35	ANDV-rNP			2×10^3
h36-h38	PBS			2×10^3

p.v., post vaccinated.

RESULTS

Andes/ARG Infection Produces Dramatic CPE on Vero E6 Cells

Vero E6 cells infected with Andes/ARG appeared normal in morphology during the first 2 days post infection (p.i.). At day 3, many cells were rounded up and had detached from the tube wall (Fig. 1). The proportion of detached cells increased up to day 7 p.i. in which almost all cells had detached from the tube. The uninfected control monolayer remained unaltered along the entire experiment as shown in Figure 1. This observation prompted a study to determine the ability of Andes/ARG to form visible plaques in Vero E6 cells by plaque assay. Infected monolayers stained with crystal violet showed clearly visible plaques (Fig. 2). This technique was used in the subsequent titration experiments of Andes/ARG.

Andes/ARG Replication Kinetics in Cell Culture

In order to analyze whether the CPE on Vero E6 infected monolayers was associated to Andes/ARG infection, cell viability, infectious virus, and level of viral genome were measured since days 1 to 7 p.i. As shown in Figure 3A, there was a gradual increment of infectious virus in supernatant up to day 5 p.i., day in which the maximum level was reached (3.4×10^4 PFU/ml). The infectious viral titer obtained in the present curve was less than one log lower than the titer of the viral stock used in this assay. The percentage of infected cells on day 2 and 6 was 10% and 90%, respectively (data not shown). An inverse correlation between cell viability and virus RNA accumulation within cells was found (Pearson r: -0.96; $P = 0.0006$) (Fig. 3B). Maximum molecule number of S-segment was reached at day 7 p.i. probably due to viral RNA accumulated within or associated with death of cells.

Andes/ARG is Highly Lethal in Hamsters

In a preliminary susceptibility testing of the hamsters, none of the five Andes/ARG inoculated animals presented any signs of disease up to day 8 p.i. but they were found all dead at day 10 p.i., while control animals remained healthy. Based on these results, a second study was then performed to determine the lethal doses needed to kill 50% of the animals (LD_{50}). Five groups of four hamsters were infected with different viral doses beginning with 2×10^4 PFU (10-fold dilutions). All animals died with the exception of one hamster inoculated with 2×10^3 PFU, so LD_{50} was estimated to be lower than 2 PFU. Signs of illness appeared evident 1 or 2 days before death and the time of survival increased with the decreasing input viral load. The only survivor, infected with 2,000 PFU, developed a robust IgG response (final titer >6,400 at day-35 p.i.).

The Nucleocapsid Protein Resulted Highly Immunogenic in Hamsters

The hamsters vaccinated with 40 μg of ANDV-rNP developed humoral response before challenge as it was evidenced by the IgG ELISA test. The response to

TABLE II. Protection and Immune Response to Andes/ARG Challenge Following SEOV-rNP Immunization

		IgG α-ANDV-NP titer		
Hamster no.	IgG α-SEOV-NP titer b.ch.[a]	b.ch.[a]	p.ch.[b]	Survived
h103	102400	<100	ND	Yes
h104	102400	<100	ND	No
h109	102400	<100	409600	Yes
h111	102400	<100	>409600	Yes

b.ch., before challenge; p.ch., post challenge.
[a]Hamsters were immunized with 40 μg of SEOV-rNP.
[b]Doses of Andes/ARG were 1×10^3 PFU; challenge was performed 22 weeks p.v.

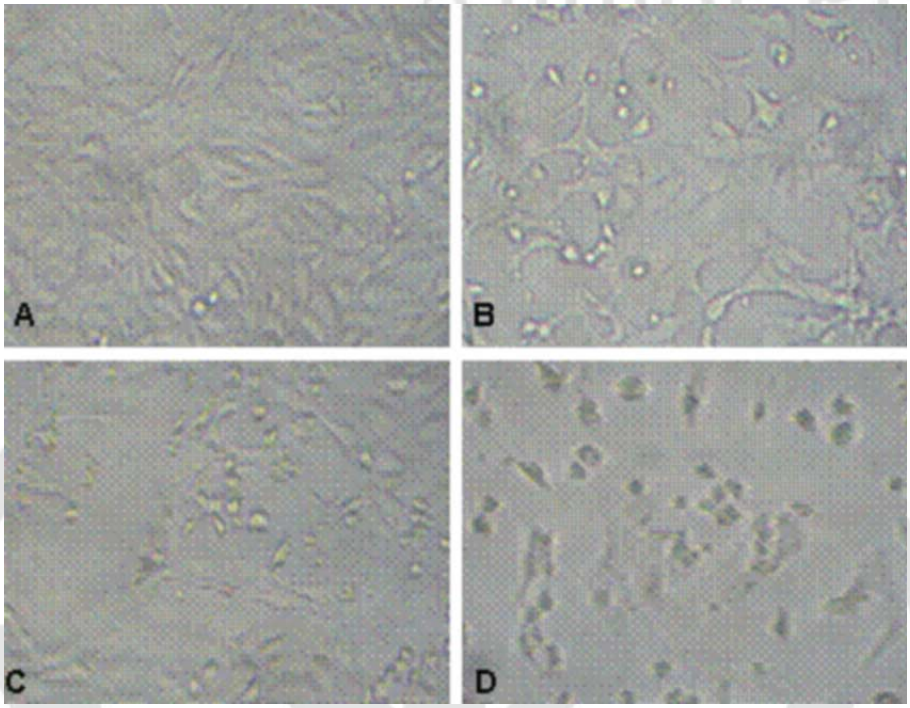

Fig. 1. ANDV infection in Vero E6 cells monolayers. **A**: non-infected control monolayer 3 days p.i.; (**B–D**) infected monolayes at 3, 5, and 6 days p.i (moi = 0.5), respectively.

the nucleocapsid protein was significantly higher in the group challenged 22 weeks post vaccination (p.v.) (paired *t*-test, $P = 0{,}017$) (Fig. 5). ANDV-rNP did not elicit ANDV neutralizing antibodies (nAbs) in the hamsters (Fig. 6; mean nAb titer = 20; N = 12). The hamsters vaccinated with SEOV-rNP or rCP showed negative titers against ANDV-rNP. The four hamsters vaccinated with SEOV-rNP developed high IgG titers to SEOV antigen (Table II).

ANDV-rNP Prevented the Lethal Disease Caused by Andes/ARG in Hamsters

Immunized hamsters of the homologous challenge group were divided in three groups to be challenged at different time after immunization and with two different doses (Table I). Almost all hamsters immunized with ANDV-rNP survived challenge without any apparent sign of illness, 12/13 (>92%) (Fig. 4). From

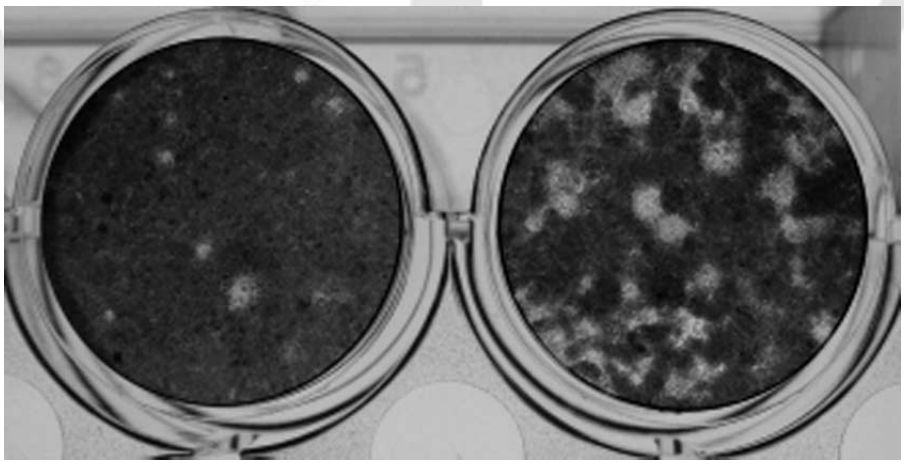

Fig. 2. Visualization of plaques in Andes/ARG infected Vero E6 infected monolayers. After incubation of 11–12 days infected monolayers were stained with the crystal violet procedure. The inoculum used to infect the left well was 1/10 of that used for the right well.

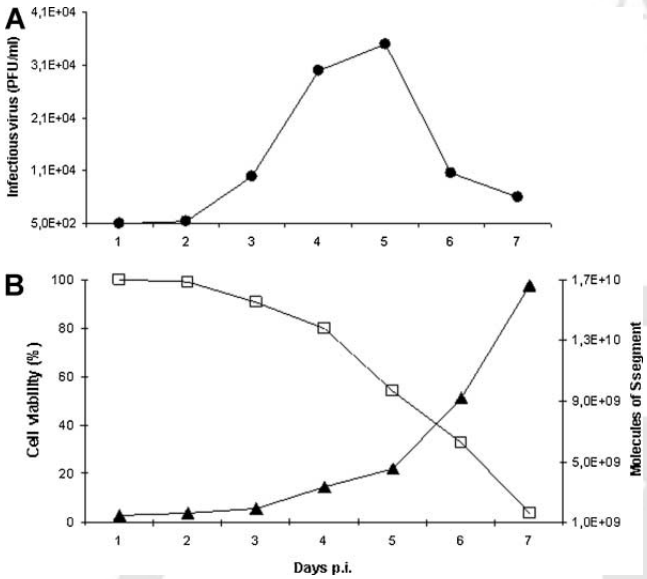

Fig. 3. Andes/ARG replication kinetics in Vero E6 cells. Cell monolayers were infected at 0.5 moi. **A**: Infectious virus in supernatants. **B**: Cell viability (squares) and viral RNA (triangles) were quantified by real time RT-PCR within cells.

the group challenged 4 weeks p.v., only one immunized hamster died at day 15 post challenge (p.ch.). The survivors of this group were challenged again with the highest viral dose (10^4 PFU) 43 weeks p.v. and they all survived (N = 3). Long duration of immune response could be achieved: the three hamsters challenged once at 43 weeks after immunization survived without any manifestation of illness. All hamsters from the control groups died, the mean day of death was 11. The challenge heterologous group was utilized as control to prove specificity of ANDV-rNP to Andes/ARG but only one of the four hamsters immunized with SEOV-rNP died (day 14 p.ch.); the remaining three hamsters showed signs of illness (lack of movement, no interest in food, breathing faster) but they recovered and survived.

Statistical analysis showed that ANDV-NP constitutes an efficient vaccine in the group challenged at 22 weeks p.v. (Fischer's exact test, $P = 0.0022$). For the other groups (challenged 4 and 43 weeks p.v.) in order to detect statistically significant differences in the proportion of animals vaccinated with nucleocapsid protein versus control, higher number of treated and control animals would be necessary.

Humoral immune responses were analyzed in serum samples of hamsters which survived to virus challenge. Changes in antibody titers to the nucleocapsid protein and the development of nAbs was determined. In the group challenged 4 weeks p.v., a significant increment in IgG titers to the nucleoprotein was observed (paired t-test $P = 0{,}001$), while this was not evidenced in the group challenged 22 weeks p.v. (Fig. 5). There were no detectable nAbs in hamsters prior to challenge, but moderate titers were found after challenge in all survivors tested, this increment could be consistent with Andes/ARG replication. Although two hamsters developed high nAb titers, there were no significant differences between groups (Fig. 6).

Evaluation of Lower Doses

The success in protection with 40 μg of ANDV-rNP prompted us to evaluate the immunization with lower doses of protein (Table III). Immunization with 4 and 0.8 μg of ANDV-rNP induced a robust response. Both doses resulted efficient in protection of hamsters to Andes/ARG challenge.

DISCUSSION

American hantaviruses constitute a growing group in the Americas. Unlike Hemorrhagic Fever with

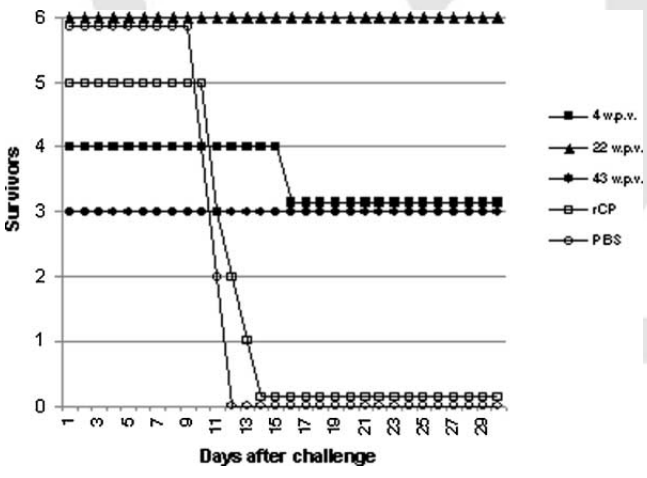

Fig. 4. Protective efficacy of ANDV-rNP against viral challenge. Hamsters were challenged with Andes/ARG 4, 22, and 43 weeks after the immunization with ANDV nucleoprotein. Five from six hamsters immunized with rCP were challenged 22 w.p.v.; one hamster died during bleeding procedure; two groups of three animals immunized with PBS were challenged at 4 and 43 w.p.v. References are shown in the graph. w.p.v.: weeks post vaccination.

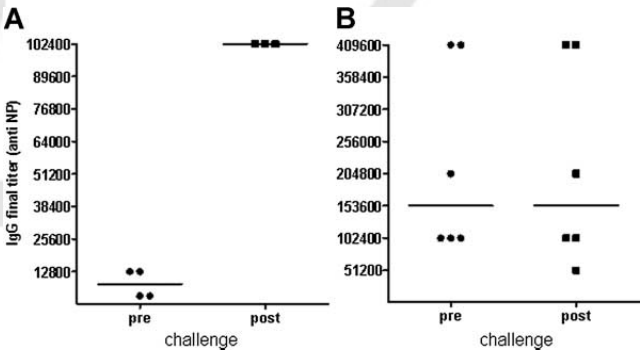

Fig. 5. Anti-nucleocapsid protein IgG response of hamsters following vaccination and challenge. Hamsters were challenged with Andes/ARG 4 and 22 weeks after the immunization procedure (**A** and **B**, respectively); one animal from the first group died after challenge (panel A); final titers of anti-NP IgG response before and after virus challenge are shown for each graph.

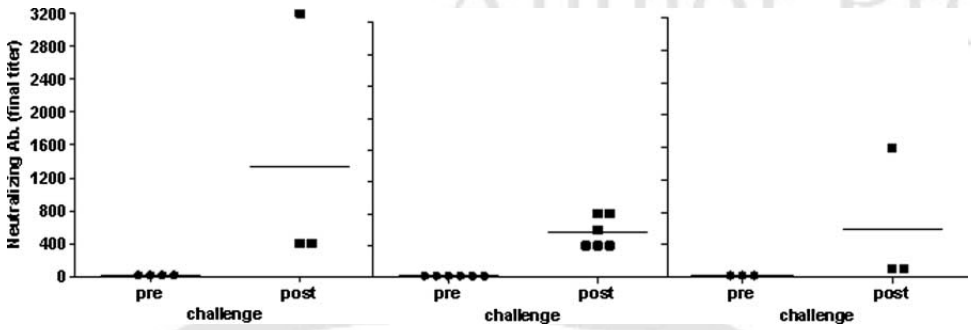

Fig. 6. nAbs in immunized hamsters before and after challenge. Final titers of nAbs in hamsters challenged 4, 22, and 43 weeks after the immunization procedure, from left to right, respectively. One animal from the first group died after challenge.

Renal Syndrome, which causes 1,50,000 cases annually in Eurasia but with low lethality, Hantavirus Pulmonary Syndrome has shown lesser incidence but higher lethality [Khaiboullina et al., 2005]. Several hundred cases of Hantavirus Pulmonary Syndrome are reported annually on the American continent. Consequently, there is a need for effective prevention of hantaviral infections.

One of the hallmarks of in vitro hantavirus infection is the absence of cytolysis or marked cytopathic effect and many investigators have reported difficulty in plaquing hantaviruses [Summers and McClain, 1999]. In the present study, as a consequence of the observation of a rapid loss of viability of Andes/ARG infected Vero E6 cell culture, a classic staining procedure with crystal violet on plaque assay was optimized and clear formation of plaques was obtained. Therefore, an accurate method for virus titration was established for this strain. In general for hantavirus plaque assay, the use of immunostaining was recommended for virus quantification. Other investigators could quantify the Chilean strain of ANDV by immunocytochemistry [Tischler et al., 2005; Valdivieso et al., 2006]. Visualization of ANDV strain Chile-9717869 plaques stained with neutral red was also possible but difficult, and it was suggested that it could be a consequence of an existing disturbance of the functions of the virus in the infected cells, which lead them to take-up neutral red in a different way than uninfected cells do [Wahl-Jensen et al., 2007]. For Andes/ARG, it could be possible to perform a staining procedure as is usual for cytolytic viruses.

Cell death could be triggered by the high rates of virus replication. In a previous study, ANDV strain Chile-9717869 infections tend to produce higher levels of RNA and do so more rapidly than Sin Nombre infections [McElroy et al., 2004]. In our study, an inverse correlation between cell viability and Andes/ARG RNA level within cells was found. Virus RNA could remain stable in non-infectious virus particles accumulated within or associated to unviable cells. The long stability of hantaviral particles has been reported; Hantaan virus could maintain its infectiousness up to 8 days at 37°C under humid conditions [Hardestam et al., 2007]. Future studies will be necessary to find out the viral mechanism which led cells to death.

No vaccines are available for any Hantavirus Pulmonary Syndrome agent and therefore, there is an urgent need for well-characterized hantavirus vaccine candidates. As it was proved for the Chilean strain [Hooper et al., 2001], Andes/ARG was highly lethal in hamsters. LD_{50} was estimated to be lower than 2 PFU. In the present study, it was found that ANDV-rNP was able to avoid death and illness in Andes/ARG challenge experiments using hamster as an animal model. The protection was long-lasting; it was proved up to 43 weeks after immunization. As low as 0.8 μg of ANDV-rNP were enough to provide protection. The development of moderate titers of nAbs only after challenge could mean that the virus could establish an asymptomatic infection indicating that the immunization was not sterile. A protective effect of non-nAb has been found in animal models for

TABLE III. Effect of ANDV-rNP Doses in Immunogenicity and Protection

No. of hamsters	Immunization	IgG α-Nucleocapsid protein titer		Number of survivors
		b.ch. (mean)	p.ch.[a] (mean)	
2	40 μg ANDV rNP	256100	256100	2/2
2	4 μg ANDV rNP	1638400	ND	2/2
2	0.8 μg ANDV rNP	1638400	409600	2/2
2	40 μg rCP	<100	ND	0/2

ND, not determined; b.ch., before challenge; p.ch., post challenge.
[a]Dose of Andes/ARG was 2×10^3 PFU; challenge was performed 4 weeks p.v.

hantavirus and other viruses [Schmaljohn et al., 1990; Takita-Sonoda et al., 1993; Yoshimatsu et al., 1993; Safronetz et al., 2009]. Cell-mediated immune responses such as antibody-dependent cellular cytotoxicity or direct cellular cytotoxicity have been considered as the most plausible mechanisms involved in the protection. Monoclonal antibodies to antigenic sites of the nucleoprotein inhibited the spread of Hantaan virus in cell culture, and it has been suggested that a humoral immune response alone should be considered a protective mechanism [Yoshimatsu et al., 1996].

In a previous work, hamsters administered with non-replicating adenovirus vector expressing the nucleoprotein of ANDV strain Chile-9717869 were successfully protected against 154 focus forming units (FFU) of the Chilean strain in challenge experiments [Safronetz et al., 2009]. In the present work, immunized hamsters survived at least to viral inoculums as high as 10,000 PFU. The immunization with recombinant nucleocapsid protein could have been avoided to[Q9] the asymptomatic infection in challenge experiments, if lower viral doses than 2,000 PFU had been used. In fact, it is not known which is the minimal viral input required to establish natural infections with ANDV, but it is probably lower than viral doses used in this study. SEOV-rNP was utilized as control to prove specificity of ANDV-NP but it also provided high degree of protection to Andes/ARG challenge. However, unlike ANDV-NP, SEOV-NP protein did not protect hamsters from illness, suggesting a poor cross-protective immunity for Hantavirus Pulmonary Syndrome. A vaccinia virus-vectored Hantaan virus vaccine protected hamsters from infection against closely related, Murindae-borne Seoul virus but not against more distantly related Cricetidae-borne hantavirus Puumala [Chu et al., 1995]. Different degree of cross protection could also be achieved with recombinant nucleoproteins from several hantaviruses in bank voles challenged with Puumala virus [de Carvalho Nicacio et al., 2002]. However, pre-existing cross-nAbs did not contribute to the protective immunity elicited by prior hantavirus infection [Hooper et al., 2001]. Furthermore, a study performed in Hantavirus Pulmonary Syndrome survivors from different geographic origin demonstrated the absence of heterologous nAb titers [Valdivieso et al., 2006]. These findings could be considered in broadly vaccine designs against hantavirus infections.

The present study has demonstrated the protective efficacy of a recombinant protein against the lethal ANDV Argentinean strain. The difficulties of in vitro hantaviruses propagation in cell culture constitute a disadvantage to obtain live vaccines. Producing large quantities of a Hantavirus Pulmonary Syndrome virus for inactivation would require specialized high-containment facilities. Molecular approaches to vaccines for Hantavirus Pulmonary Syndrome and Hemorrhagic Fever with Renal Syndrome circumvent problems associated with live vaccines such as rodent brain and cell culture-derived vaccines. [Schmaljohn, 2009]. Furthermore, the potential capacity of persistent infection of ANDV [Manigold et al., 2010] discourages the use of live vaccines for American hantaviruses. A vaccine designed and based on a defined and single peptide presents advantages because its production and control are simpler and safer. Extensive efficacy testing and the evaluation of additional components will be necessary in Hantavirus Pulmonary Syndrome vaccine designs.

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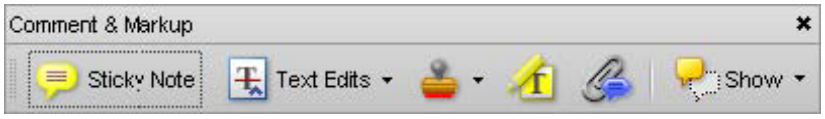

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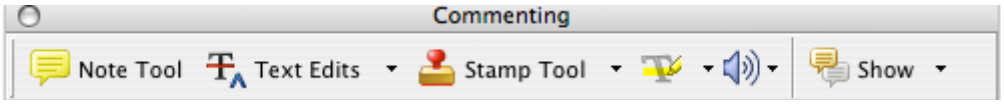

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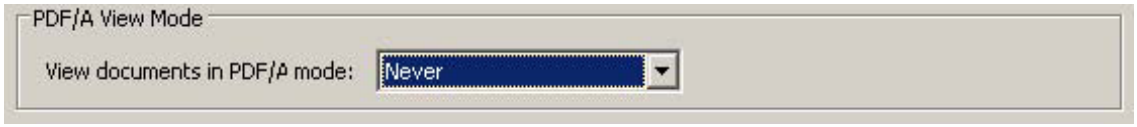

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