Characterization of a Bivalent Vaccine Capable of Inducing Protection Against Both Ebola and Cross-clade H5N1 Influenza in Mice

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Background. Ebola virus (EBOV) is a lethal pathogen that causes up to 90% mortality in humans, whereas H5N1 avian influenza has a 60% fatality rate. Both viruses are considered pandemic threats. The objective was to evaluate the protective efficacy of a bivalent, recombinant vesicular stomatitis virus vaccine expressing both the A/Hanoi/30408/2005 H5N1 hemagglutinin and the EBOV glycoprotein (VSVΔG-HA-ZGP) in a lethal mouse model of infection.

Methods. Mice were vaccinated 28 days before or 30 minutes after a lethal challenge with mouse-adapted EBOV or selected H5N1 influenza viruses from clades 0, 1, and 2. Animals were monitored for weight loss and survival, in addition to humoral and cell-mediated responses after immunization.

Results. A single VSVΔG-HA-ZGP injection was efficacious when administered 28 days before a homologous H5N1 and/or mouse-adapted EBOV challenge, as well as a heterologous H5N1 challenge. Postexposure protection was only observed in vaccinated animals challenged with homologous H5N1 and/or mouse-adapted EBOV. Analysis of the adaptive immune response postvaccination revealed robust specific T- and B-cell responses, including a potent hemagglutinin inhibition antibody response against all H5N1 strains tested.

Conclusions. The results highlight the ability of vesicular stomatitis virus–vectored vaccines to rapidly confer protection against 2 unrelated pathogens and stimulate cross-protection against H5N1 influenza viruses.

Keywords. Ebola virus; H5N1 influenza virus; mice; vaccine; vesicular stomatitis virus.
is estimated that up to 100 million people worldwide have perished [4]. Virus transmission from the avian reservoir to humans can occur through a porcine intermediate, because pigs are susceptible to both human and avian influenza strains [5] and thus are ideal hosts for influenza virus genome reassortment. The H5N1 subtype of influenza A has a mortality rate approaching 60% [6] and is currently a major threat for a worldwide pandemic. The 1997 outbreak of H5N1 among birds in Hong Kong was also accompanied by human cases, in which direct avian-to-human transmission of the virus was reported [7, 8]. H5N1 subsequently reappeared in Hong Kong in 2002, as well as most of Eastern and Southeastern Asia, including South Korea, Japan, China, Vietnam, Thailand, as well as Indonesia during 2003–2004 [9, 10]. The outbreak of H5N1 among migratory birds at Qinghai Lake in China during mid-2005 introduced these viruses into European and African countries, and to date, avian H5N1 influenza viruses have been spread to more than 60 countries in addition to becoming endemic among poultry in Southeast Asia [11]. The 2006 H5N1 outbreak in Indonesia demonstrated the first human-to-human transmission of influenza [12, 13]. In addition, H5N1 are becoming more genetically and antigenically diverse and are now separated into 10 different clades based on hemagglutinin (HA) sequence similarity with the majority of viruses classified in clades 0, 1, and 2 [14].

The main goal of this study was to evaluate the protective efficacy of a recombinant vesicular stomatitis virus (VSV)–based vaccine simultaneously expressing both the EBOV glycoprotein (ZGP) and the H5N1 A/Hanoi/30408/2005 (Hanoi 05) influenza HA antigens in a lethal mouse model of infection with mouse-adapted Ebola virus (MA-EBOV) and/or selected H5N1 influenza strains from clades 0, 1, and 2. A study with the dual H5N1 HA and ZGP vaccine demonstrated the versatility of VSV vectors as multivalent vaccine candidates able to confer protection to multiple unrelated and highly virulent pathogens, without significantly compromising the efficacy of each individual component. A second objective was to characterize the ability of this recombinant vaccine (VSVAG-HA-ZGP) to generate cross-protection against multiple H5N1 influenza subtypes.

**MATERIALS AND METHODS**

**Plasmid Construction and Recombinant Virus Rescue**

The codon-optimized EBOV glycoprotein sequence [15] and the Hanoi 05 H5N1 HA antigen sequences [16] were cloned into pATX VSVΔG [17]. The resulting construct, termed pATX VSVAG-HA-ZGP, contained the HA sequence in a multiple cloning site flanked by MluI and AvrII restriction sites, as well as the ZGP sequence flanked by XhoI and NheI. Both genes were downstream from the matrix gene (M) and upstream from the RNA-dependent RNA gene (L). The complementary DNA of the pATX VSVAG-HA-ZGP construct along with helper plasmids containing the T7, N, L, and phosphoprotein genes were transfected with Lipofectamine 2000 reagent (Invitrogen) using a 1:1 mix of 293T/Vero E6 cell mixture. The supernatant was blind passaged onto subconfluent Vero E6 cells and incubated until cytopathic effects (CPEs) were observed.

**Cells and Viruses**

VSVAG-HA-ZGP and MA-EBOV (Ebola virus USAMRIID/BALB/c-lab/COD/1976/Mayinga-MA-p3) [18] were grown on Vero E6 cells and titers calculated using the Reed-Muench method for endpoint dilutions [19]. Stock VSVAG-HA-ZGP was prepared by resuspending the virus in Dulbecco’s Modified Eagle’s medium (DMEM) supplemented with 2% fetal bovine serum (FBS). Influenza H5N1 viruses A/Hanoi/30408/2005 (Hanoi 05, clade 1) (generously supplied by Q. Mai Le and T. Hien Nguyen, National Institute of Hygiene and Epidemiology, Hanoi), A/Hong Kong/483/97 (Hong Kong 97, clade 0), A/Vietnam/1203/04 (Vietnam 04, clade 1), and A/Indonesia/5/05 (Indonesia 05, clade 2.1.3) were maintained in Madin-Darby canine kidney (MDCK) cells using minimal essential medium (MEM) supplemented with 0.1% bovine serum albumin (BSA) and 1% penicillin/streptomycin (Gibco). The titers of these viruses were quantified using a standard plaque assay on MDCK cells.

**Immunoblotting**

Expression of HA and ZGP from VSVAG-HA-ZGP was confirmed by immunoblotting. Vero E6 cells infected with VSVAG-HA-ZGP was harvested at 48 hours postinfection along with cell supernatant, and centrifuged at 2500 rpm. After removal of excess supernatant, the virus lysate was combined with sodium deoxycholate–polyacrylamide gel electrophoresis (SDS-PAGE) buffer containing 250 mM Tris–HCl pH 6.8, 30% glycerol (volume/volume), 8% SDS, 0.02% bromophenol blue, and 10% 2-mercaptoethanol (volume/volume). Proteins were separated by electrophoresis on a 10% polyacrylamide gel and transferred to a polyvinylidene fluoride (PVDF) membrane (GE Healthcare) overnight. The HA and ZGP antigens were detected using mouse serum containing anti-H5N1 HA or a monoclonal mouse anti-ZGP antibody, respectively, at 1:1000. The control antigen was detected using a monoclonal mouse anti-β-actin at 1:5000 (Sigma). Goat antimouse antibody conjugated to horseradish peroxidase (Sigma) was used at 1:3000 as a secondary antibody. Bands were visualized using the enhanced chemiluminescence detection kit following manufacturer’s instructions (Amersham).

**Vaccination and Challenge**

Groups of 10 female BALB/c mice, 6–to 8-weeks old (Charles River Laboratories) were immunized with DMEM, 1 x 10^7 plaque-forming unit (PFU) recombinant VSV expressing enhanced green fluorescent protein (VSV-eGFP), or an equal dose of VSVAG-HA-ZGP via intramuscular (I.M.) injection,
Humoral Immune Responses

VSV-eGFP- or VSVΔG-HA-ZGP-vaccinated mouse serum samples were harvested on day 25 postvaccination and evaluated by hemagglutinin (HI) and neutralizing antibody (NAb) assays. A total of 14 mice (7 control, 7 vaccinated) were used for the experiments shown in all 4 panels, and thus the levels of NAbs, immunoglobulin G (IgG), and HI antibodies against EBOV and the various H5N1 influenza viruses originated from the same set of mice. Harvested serum samples were treated overnight with receptor-destroying enzyme for influenza virus (Denka Seiken) at 37°C followed by inactivation at 56°C for 45 minutes. HI assays were performed using a 96 V-bottom microtiter plate. Each sample was serially diluted 1:2 in PBS starting at a 1:10 dilution, added to 4 agglutinating doses of corresponding homologous or heterologous virus, and incubated with horse red blood cells. HI titers were recorded as the reciprocal of the highest dilution that did not block erythrocyte agglutination.

For NAb assays, the serum samples harvested from VSV-eGFP or VSVΔG-HA-ZGP mice were serially diluted 2-fold from an initial 1:10 dilution of each sample in MEM containing 0.1% BSA for H5N1 influenza virus or DMEM containing 2% FBS for EBOV-eGFP. The samples were then added to either 100 PFU of Hanoi 05, Hong Kong 97, Vietnam 04 and Indonesia 05, or 100 PFU of EBOV-eGFP (Ebola virus NML/H.sapiens-lab/COD/1976/Mayinga-eGFP-p3) and subsequently incubated at 37°C for 60 minutes. Following incubation, the influenza and EBOV virus mixtures were incubated for 10 minutes with subconfluent MDCK cells or Vero E6 cells, respectively, in 96-well flat bottom plates at room temperature. A total of 100 µL MEM with 0.1% BSA containing 2.0 µg/mL TPCK-trypsin or 100 µL DMEM containing 2% FBS was added to the influenza or EBOV virus sample wells, respectively, and plates were incubated for 48 hours at 37°C with 5% CO2. NAb titers were reported as the reciprocal of the highest dilution that did not show CPE for H5N1 influenza, or greater than 50% reduced eGFP expression for EBOV-eGFP.

Total IgG was measured by enzyme-linked immunosorbent assay (ELISA) using a recombinant His-tagged ZGP as a capture antigen [20]. The capture antigen was diluted in PBS and 50 µL/well of 1 µg/mL was used to coat Immulon 2 HB 96-well flat bottom Microtiter ELISA plates (Thermo Scientific). Inactivated mouse serum samples were serially diluted 1:2 in blocking buffer starting at 1:100, and 50 µL was applied to each well then allowed to incubate for 60 minutes at 37°C. After washing plates 3 times, horseradish peroxidase (HRP)–conjugated rat antimouse IgG (Jackson Laboratories) was added to each well and incubated at 37°C for 60 minutes. HRP substrate was added to each well and developed at room temperature for 30 minutes. The VMax Kinetic ELISA Microplate Reader ( Molecular Devices) was used to analyze the optical density at 405 nm in conjunction with CellMaxPro software. The resulting data are...
expressed as endpoint dilutions, and a sample was deemed positive when the absorbance was greater than 5 standard deviations from the prevaccination control sample for each animal.

**Cell-Mediated Immunity**

VSV-eGFP- or VSVΔG-HA-ZGP-vaccinated mouse splenocytes were harvested and pooled 7 days postvaccination, and then stimulated ex vivo with either an HA or ZGP peptide library spanning the entire antigen and consisting of 10 overlapping 15mers per peptide pool. A total of 6 mice (3 control, 3 vaccinated) were used for these experiments, and thus the results against HA or ZGP originated from the same set of mice. One day prior to harvest, 96-well microtiter plates with a PVDF membrane (BD Sciences) were coated with purified antimouse interferon (IFN)–γ antibody and incubated overnight at 4°C. The following day, the plates were blocked with Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin for 4 hours. Spleens were pooled, broken up against a fine mesh filter, and resuspended in L-15 media (Gibco). Peptide pools consisting of 167 or 112 total 15mer peptides with 10-amino-acid overlaps were used for splenocyte stimulation, corresponding to the entire ZGP (Genscript) or HA (Mimitopes) antigens, respectively. Peptides were diluted in dimethyl sulfoxide and pooled with 10 peptides per group. Each pool was diluted with RPMI 1640 and added to the microtiter plates at 2.5 µg/mL per well. The splenocytes were centrifuged and resuspended in RPMI supplemented with 10% FBS, 1% penicillin/streptomycin, 1% L-glutamine, 1% nonessential amino acids, 1% sodium pyruvate, 1% HEPES buffer, and 5 × 10⁻³ M 2-β-mercaptoethanol, and 5 × 10⁵ cells were added to each well. Plates were incubated at 37°C at 5% CO₂ overnight. The plates were then washed with PBS-0.1% TWEEN and incubated with antimouse IFN-γ antibody for 2 hours at room temperature. Following incubation, streptavidin-conjugated HRP antibody was added for 1 hour and IFN-γ-positive cells were detected with the BD AEC Substrate Reagent Set (BD Biosciences). Spots formed by IFN-γ-secreting splenocytes were quantified using an enzyme-linked immunospot (ELISPOT) Plate Reader (Cell Technology).

**RESULTS**

**Generation of VSV Encoding Both HA and ZGP**

The recombinant, bivalent VSVΔG-HA-ZGP vaccine was constructed to express both the Hanoi 05 HA and ZGP (Figure 1A),

| Group | Challenge Virus | No. Surviving Animals/No. Total Animals | Percent Survival | Percent Weight Loss | Mean Time to Death | P Valuea | P Valuеб |
|-------|----------------|----------------------------------------|-----------------|--------------------|--------------------|----------|----------|
| Vaccination | DMEM Hanoi 05 | 0/10 | 0 | 24 | 10.2 ± 0.9 | . . . | . . . |
| | VSV-eGFP | 0/10 | 0 | 26 | 9.3 ± 1.2 | .1146 (ns) | . . . | . . . |
| | VSVΔG-HA-ZGP | 10/10 | 100 | <5 | N/A | <.0001 (*** | <.0001 (***|
| | DMEM Hong Kong 97 | 0/10 | 0 | 13 | 7.8 ± 0.8 | . . . | . . . |
| | VSV-eGFP | 0/10 | 0 | 17 | 7.9 ± 0.7 | .8757 (ns) | . . . | . . . |
| | VSVΔG-HA-ZGP | 10/10 | 100 | <5 | N/A | <.0001 (*** | <.0001 (***|
| | DMEM Vietnam 04 | 0/10 | 0 | 24 | 8.0 ± 0.5 | . . . | . . . |
| | VSV-eGFP | 0/10 | 0 | 17 | 8.5 ± 1.1 | .1470 (ns) | . . . | . . . |
| | VSVΔG-HA-ZGP | 10/10 | 100 | <5 | N/A | <.0001 (*** | <.0001 (***|
| | DMEM Indonesia 05 | 0/10 | 0 | 27 | 8.9 ± 0.9 | . . . | . . . |
| | VSV-eGFP | 0/10 | 0 | 16 | 8.8 ± 1.0 | .9019 (ns) | . . . | . . . |
| | VSVΔG-HA-ZGP | 10/10 | 100 | <5 | N/A | <.0001 (*** | <.0001 (***|
| | DMEM MA-EBOV | 0/10 | 0 | 18 | 6.3 ± 0.8 | . . . | . . . |
| | VSV-eGFP | 0/10 | 0 | 17 | 6.5 ± 1.3 | .5999 (ns) | . . . | . . . |
| | VSVΔG-HA-ZGP | 10/10 | 100 | <5 | N/A | <.0001 (*** | <.0001 (***|
| | DMEM MA-EBOV and Hanoi 05 | 0/10 | 0 | 16 | 6.2 ± 1.3 | . . . | . . . |
| | VSV-eGFP | 0/10 | 0 | 18 | 7.9 ± 0.7 | .1078 (ns) | . . . | . . . |
| | VSVΔG-HA-ZGP | 10/10 | 100 | <5 | N/A | <.0001 (*** | <.0001 (***|

Abbreviations: DMEM, Dulbecco’s Modified Eagle’s medium; EBOV, Ebola virus; eGFP, enhanced green fluorescent protein; HA, hemagglutinin; MA-EBOV, mouse-adapted EBOV; N/A, not applicable; ns, not significant; VSV, vesicular stomatitis virus; ZGP, EBOV glycoprotein.

a Compared with the DMEM group.
b Compared with the VSV-eGFP group.

* * *P value < .001.
which was confirmed by immunoblotting (Figure 1B). The desired antigens were expressed at the correct molecular size, with HA1 at 50–55 kDa and HA2 at 25 kDa for the H5N1-positive control and the VSVG-HA-ZGP sample, ZGP at 75 kDa in the EBOV-positive control and the VSVG-HA-ZGP sample. A control β-actin antigen was also probed for all samples with the correct molecular size appearing at 42 kDa.

Survival and Weight Loss After MA-EBOV Challenge
Control mice given either DMEM or 1 × 10⁷ PFU VSV-eGFP challenged I.P. with 1000 × LD₅₀ of MA-EBOV resulted in rapid weight loss, and 100% mortality between days 4 and 9 postinfection (Tables 1 and 2). In contrast, complete protection was observed in mice vaccinated 28 days previously with negligible weight loss (Table 1), whereas 9 of 10 mice survived when given the vaccine 30 minutes postexposure with approximately 14% weight loss (Table 2).

Survival and Weight Loss After H5N1 Challenge
Infection of DMEM- or VSV-eGFP-treated control mice with the homologous Hanoi 05 resulted in rapid weight loss and 100% mortality between days 7 and 12 postinfection (Tables 1 and 2). Complete protection was observed in mice given VSVΔG-HA-ZGP 28 days before homologous challenge with negligible weight loss (Table 1). Mice given VSVΔG-HA-ZGP 30 minutes postexposure failed to protect against a heterologous H5N1 challenge (Table 2), but complete protection against all tested heterologous H5N1 strains was achieved with negligible weight loss if VSVΔG-HA-ZGP was administered 28 days before the challenge (Table 1).

Survival and Weight Loss After a Simultaneous H5N1 and MA-EBOV Challenge
Infection of DMEM or VSV-eGFP control mice under these challenge conditions resulted in rapid weight loss and 100% mortality between days 7 and 9 postinfection (Tables 1 and 2). Complete protection with negligible weight loss was observed in mice vaccinated 28 days prior to challenge (Table 1). However, only 4 of 10 mice survived when given the vaccine postexposure with approximately 29% weight loss (Table 2).

Cellular Immunity
An IFN-γ ELISPOT assay was performed with stimulation from either a HA or ZGP peptide library on pooled mouse splenocytes, to confirm whether the VSVG-HA-ZGP vaccine can elicit immune responses against both EBOV and H5N1 influenza.

Table 2. Postexposure Therapy With VSVΔG-HA-ZGP in Mice Against a Challenge With MA-EBOV, Homologous, and/or Heterologous H5N1 Influenza Virus

| Group                   | Challenge Virus          | No. Surviving Animals/No. Total Animals | Percent Survival | Percent Weight Loss | Mean Time to Death | P Valueᵃ | P Valueᵇ |
|-------------------------|--------------------------|----------------------------------------|------------------|---------------------|--------------------|----------|----------|
| Postexposure            |                          |                                        |                  |                     |                    |          |          |
| DMEM                    | Hanoi 05                 | 0/10                                   | 0                | 31                  | 9.3 ± 1.3          | .        | .        |
| VSV-eGFP                |                          | 0/10                                   | 0                | 29                  | 9.5 ± 1.3          | .0281 (ns)| .        |
| VSVG-HA-ZGP             |                          | 8/10                                   | 80               | 23                  | 8.5 ± 0.7          | .0007 (***)| .0012 (**)|
| DMEM                    | Hong Kong 97             | 0/10                                   | 0                | 13                  | 9.0 ± 1.9          | .        | .        |
| VSV-eGFP                |                          | 0/10                                   | 0                | 31                  | 8.6 ± 0.5          | .5264 (ns)| .        |
| VSVG-HA-ZGP             |                          | 0/10                                   | 0                | 25                  | 7.9 ± 0.7          | .1514 (ns)| .1195 (ns)|
| DMEM                    | Vietnam 04               | 0/10                                   | 0                | 31                  | 8.2 ± 1.2          | .        | .        |
| VSV-eGFP                |                          | 0/10                                   | 0                | 33                  | 9.4 ± 0.7          | .1412 (ns)| .        |
| VSVG-HA-ZGP             |                          | 0/10                                   | 0                | 28                  | 9.1 ± 1.0          | .3692 (ns)| .6035 (ns)|
| DMEM                    | Indonesia 05             | 0/10                                   | 0                | 28                  | 8.7 ± 0.8          | .        | .        |
| VSV-eGFP                |                          | 0/10                                   | 0                | 29                  | 8.4 ± 0.8          | .4925 (ns)| .        |
| VSVG-HA-ZGP             |                          | 0/10                                   | 0                | 30                  | 9.2 ± 0.8          | .3760 (ns)| .0987 (ns)|
| DMEM                    | MA-EBOV                  | 0/10                                   | 0                | 16                  | 6.6 ± 1.3          | .        | .        |
| VSV-eGFP                |                          | 0/10                                   | 0                | 24                  | 9.3 ± 1.5          | .0193 (*) | .        |
| VSVG-HA-ZGP             |                          | 9/10                                   | 90               | 14                  | 8.0 <.0001 (***)   | .0001 (***)| .0114 (**)|
| DMEM                    | MA-EBOV and Hanoi 05     | 0/10                                   | 0                | 23                  | 9.1 ± 1.2          | .        | .        |
| VSV-eGFP                |                          | 0/10                                   | 0                | 27                  | 10.9 ± 1.1         | .0225 (*) | .        |
| VSVG-HA-ZGP             |                          | 4/10                                   | 40               | 29                  | 11.5 ± 1.4         | .0004 (***)| .0114 (**)|

Abbreviations: DMEM, Dulbecco’s Modified Eagle’s medium; EBOV, Ebola virus; eGFP, enhanced green fluorescent protein; HA, hemagglutinin; MA-EBOV, mouse-adapted EBOV; ns, not significant; VSV, vesicular stomatitis virus; ZGP, EBOV glycoprotein.

ᵃ Compared with the DMEM group.
ᵇ Compared with the VSV-eGFP group.

* P value < .05; ** P value < .01; *** P value < .001.
postvaccination. In VSV-eGFP-treated mice, background levels of 310 and 448 spot-forming cells (SFCs) per million splenocytes were detected for HA or ZGP peptide stimulation, respectively. Significant levels of antigen-specific cellular immune responses were observed for the VSVΔG-HA-ZGP group with 3442 and 6662 SFCs per million splenocytes when stimulated with HA or ZGP peptides, respectively (Figure 2), corresponding to activated, IFN-γ-secreting immune cells.

**Humoral Immunity**

Levels of antibodies induced by VSVΔG-HA-ZGP immunization were assessed by assays quantifying NAb, HI antibodies, and ZGP-specific IgG antibodies. Significant levels of neutralizing antibodies against both EBOV and Hanoi 05 were induced by VSVΔG-HA-ZGP vaccination at 23 ± 16 (P = .0056) and 36 ± 23 (P = .0036), respectively, compared to VSV-eGFP-vaccinated mice, which were below the limit of detection set at 10 reciprocal dilutions (Figure 3A and 3B). Neutralizing antibodies were not detected against the tested heterologous H5N1 influenza strains for both the VSV-eGFP and the VSVΔG-HA-ZGP groups (Figure 3B). Significant levels of total ZGP-specific IgG were detected in the VSVΔG-HA-ZGP group at 128 000 ± 64 000 reciprocal dilutions, whereas specific IgG antibodies were not detected for the VSV-eGFP control group (P = .0009) (Figure 3C). HI assays revealed the presence of high levels of broad-spectrum H5N1 antibodies for mice immunized with VSVΔG-HA-ZGP, at 320 ± 160 (P = .0019) for Hanoi 05, 320 ± 160 (P = .0019) for Hong Kong 97, 389 ± 181 (P = .0013) for Vietnam 04, and 503 ± 363 (P = .0106) for Indonesia 05, compared to the control VSV-eGFP mice, which were below the limit of detection set at 10 reciprocal dilutions (Figure 3D).

**DISCUSSION**

VSV is a well-characterized vaccine platform protective against several highly virulent pathogens in addition to EBOV [21] and influenza viruses [22], including severe acute respiratory syndrome, Marburg, and Andes viruses [4, 23, 24]. VSV-based vaccines are attractive vaccine candidates because they induce strong humoral and cell-mediated immune responses in vivo, and have been shown to be efficacious postexposure [25] as well as conferring long-term immunity in animal studies [26, 27]. While a past study documents in immunocompromised NHPs the safety of recombinant, live-attenuated VSV [28], concerns still persisted with its use for mass immunization in humans. However, the 2014 EBOV outbreak in West Africa has accelerated VSV-vectored vaccines toward clinical development, and VSVΔG/EBOV-GP is 1 of 2 candidates under consideration to be used in a clinical setting to combat current and future outbreaks [29]. Furthermore, a broad-spectrum VSVΔG-HA-ZGP can also be applied to vaccinations in susceptible animal populations, such as NHPs for both H5N1 and EBOV. This study aims to highlight the versatility of VSV vectors as viable multivalent vaccines with the ability to confer protection against multiple unrelated and highly virulent pathogens.

A single injection of VSVΔG-HA-ZGP at 1 × 10⁷ PFU was shown to be fully protective 28 days postvaccination and partially protective postexposure against an otherwise lethal challenge with MA-EBOV and/or homologous H5N1 influenza virus. Cross-protection against 3 other heterologous H5N1 viruses was observed when exposure occurred at 28 days after vaccination, but was not observed when the vaccine was given at 30 minutes postexposure. Interestingly, broadly cross-neutralizing antibodies were not detected postvaccination, which implies the mechanism for cross-protection against H5N1 influenza may be based on other antibody mechanisms, or cellular immunity. The results show, for the first time, rapid postexposure protection against a highly pathogenic H5N1 virus with pandemic potential as well as a simultaneous challenge with 2 unrelated pathogens.

While the cellular immune response has been shown to play a role generating a broad-spectrum immune response resulting in protection from heterologous H5N1 infection [30], the most promising candidates with cross-protection ability have come from treatments that elicit antibodies aimed at the conserved stem portion of HA [31, 32]. The role of humoral immunity against HA in protection against influenza has been clearly established, in which postimmunization HI antibody titers of greater than 1:40 considered protective in at least 50% of individuals [33]. The VSV vaccine tested elicited titers significantly above this protective limit against all tested H5N1 strains, resulting in...
full cross-protection across clades 0, 1, and the highly divergent clade 2 of H5N1 viruses with a single administration.

A dose of $2 \times 10^7$ PFU per mouse of VSV expressing ZGP (VSVΔG-ZGP) has previously been shown to be sufficient to elicit complete postexposure protection against MA-EBOV [25]. However, in this study, an increased dose of $1 \times 10^7$ PFU VSVΔG-HA-ZGP did not result in complete protection (Table 2). This suggests that there may be competition between the HA and ZGP antigens, or that the VSVΔG-HA-ZGP vaccine may be attenuated due to the addition of 2 distinct antigens into the genome. Because the VSV genome would be elongated with the addition of 2 antigens instead of 1, the replication kinetics of VSVΔG-HA-ZGP may be slower than that of VSVΔG-ZGP, thus impairing the ability of the bivalent vaccine to mount a quick and robust immune response leading to postexposure protection. Another possible explanation may be that the placement of the ZGP gene downstream of HA in the VSVΔG-HA-ZGP genome resulted in lowered expression levels of ZGP compared to that of VSVΔG-ZGP, as was shown previously for the genes in the wild-type VSV genome [34]. However, with the development of promising antibody-based therapeutics against EBOV [35, 36, 37] and the availability of anti-influenza drugs such as oseltamivir and zanamivir, VSV-vectored vaccines are expected to play a bigger role as a prophylactic candidate rather than as a therapeutic. Future experiments should include the testing of the bivalent VSVΔG-HA-ZGP vaccine in higher animal models to confirm the observed protective effects, such as against EBOV in NHPs, and against both homologous and heterologous H5N1 strains in ferrets. While VSVΔG-HA-ZGP was well tolerated in mice, there is a need to fully elucidate the safety profile of this bivalent vaccine in order to facilitate clinical acceptance.

The efficacy of VSVΔG-HA-ZGP against a single or dual virus challenge increases its value as a vaccine against multiple
pathogens as well as highlighting the vaccine’s utility and flexibility, which may eventually result in an efficacious broad-spectrum H5N1 influenza vaccine for use in humans. Furthermore, these observations could help establish the foundation and immunologic requirements to support the future design and production of a clinical broad-spectrum vaccine against multiple pathogens with pandemic potential, without significant concerns of interference.

Notes

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