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A chimpanzee adenoviral vector-based rabies vaccine protects beagle dogs from lethal rabies virus challenge

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ABSTRACT

Rabies continues to poses serious threats to the public health in many countries. The development of novel inexpensive, safe and effective vaccines has become a high priority for rabies control worldwide. We previously generated a novel recombinant rabies vaccine by cloning rabies virus glycoprotein into a chimpanzee adenoviral vector, termed ChAd68-Gp. The present study evaluated the immune responses and protection afforded by this vaccine in beagle dogs. The results demonstrated that intramuscular immunization with both low-dose and high-dose of ChAd68-Gp induced strong immune responses and provided complete protection in beagles even at low-dose. However, when administered orally, high-dose vaccination was protective while low-dose vaccination was ineffective. Further investigation indicated that the low-pH value of gastric juice in the stomach of beagles might decompose the adenovirus. Therefore, suitable formulation for adenovirus-based oral vaccine should be considered and developed. The chimpanzee adenovirus-vectored rabies vaccine ChAd68-Gp warrants extensive test for clinical application.

1. Introduction

Rabies is a major fatal zoonosis caused by the rabies virus, which infects wild animals, livestock, and humans. Rabies virus infects the peripheral nerves and then migrates into the central nervous system of the brain through the spinal cord, causing fatal encephalitis and myelitis, resulting the death of the infected subjects. As rabies virus infects the central nervous system, which is difficult for anti-viral drugs to reach, and the time interval from rabies onset to death is very short (Zhou et al., 2016). Once symptomatic, this disease is virtually 100% fatal (Brunker and Mollentze, 2018). Near 59,000 individuals worldwide die from rabies every year, mainly in Africa and Asia, and around 40% of deaths are children <14 years of age (Fooks et al., 2018). Rabies is also considered as a neglected tropical disease that primarily affects poor and vulnerable individuals in remote areas. Presently, approximately 80% of those exposed to rabies are from poor areas of Africa and Asia who cannot receive treatment timely (https://www.who.int/news-room/detail/10-12-2015-new-global-framework-to-eliminate-rabies). Vaccination is the best way to prevent the rabies infection. Rabies vaccine is highly recommended for the individuals at high risk of exposure, and for those post-exposure. The commonly used rabies vaccine is a type of inactivated viral vaccine that is propagated in cell culture, and normally applied for pre-exposure or post-exposure when combined with rabies immunoglobulin (RIG) to prevent or avoid most rabid dog bite tragedies (McGettigan, 2010). However, high cost and repeated vaccination seriously hinder the use and acceptance of the current rabies vaccine in developing countries (Fooks et al., 2017). Thus, the development of novel inexpensive, safe and effective vaccines becomes a top priority for rabies control in developing countries.

Novel rabies vaccines with durable antigen expression and highly immunogenicity, which are easy to use and inexpensive are under active development. Among these, a viral vector-based rabies vaccine is more promising for both veterinary and human use (Ertl, 2009; Fooks et al., 2018; Giel-Moloney et al., 2017). A recombinant vaccinia virus expressing the glycoprotein of the rabies virus was tested as an oral rabies vaccine (Weyer et al., 2009), which is formulated as a “bait” and can induce protective immunity in several wild animals. Unfortunately, it has lower efficacy in skunks and other animals, and the worst

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consequence is that it may cause infection and/or allergic in humans (Centers for Disease Control Prevention, 2013). Adenovirus is one of the most popular vaccine vectors that elicit potent cellular, humoral, and mucosal immunities in innoculated animals and individuals. Therefore, it has been widely applied in vaccine development for various diseases (Guo et al., 2018; Zhang and Zhou, 2016). Replication-competent human adenovirus type 5 (AdHu5) expressing rabies glycoprotein, AdRG1.3 (trade name ONRAB), was developed as an oral vaccine baits distributed in the wild by Artemis Technologies Inc (Guelph, Canada) (Knowles et al., 2009). The distribution of several million doses AdRG1.3 in Canada since 2006 has achieved encouraging results in the assessment of field efficacy in skunks and raccoons, without causing any serious human contact or public safety issues.

Compared with replication-competent adenoviral vector, replication-defective adenoviral vectors are more efficious and safe (Ertl, 2009). Thus, rabies vaccine based on E1-deleted AdHu5 have been developed and yielded promising results in both rodents and canines (Tims et al., 2000; Wang et al., 1997; Xiang et al., 1996, 2002). However, 45–90% of the human population has detectable titers of virus neutralizing antibodies to AdHu5, which dampen the immune responses elicited by the AdHu5 vector (Ertl, 2009). To circumvent the problem of pre-existing immunity to the vaccine carrier, rare serotypes of human adenovirus or those originating from other species, such as chimpanzee, were generated and applied in novel vaccine development. Chimpanzee adenoviruses including ChAd68, ChAd7, ChAd3, ChAd63, etc. rarely circulate in humans, and have recently been engineered to express various antigens and some have demonstrated impressive safety and immunogenicity in clinical studies (Guo et al., 2018). We choose ChAd68 for vaccine development due to its low pre-existing immunity and overall safety profile.

We previously generated a novel recombinant rabies vaccine by cloning rabies virus glycoprotein into ChAd68 and tested it in mice (Zhang et al., 2017). In the present study, we evaluated the immune responses and protection afforded by this vaccine in beagles in an attempt to accelerate its advancement to clinical applications.

2. Materials and methods

2.1. Vaccines, cells and viruses

A codon-optimized glycoprotein of rabies Evelyn Rokitniki Abelseth (ERA) strain virus was cloned into an E1/E3-deleted chimpanzee adenoviral vector ChAd68, termed “ChAd68-Gp”, as described previously (Zhang et al., 2017). ChAd68-Gp was propagated in HEK293 cells and purified by cesium chloride density-gradient centrifugation methods in a single centrifugation step. ChAd68-empty virus was used as a control virus. HEK293 cells were maintained in complete Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (HyClone, Utah, USA) and 2% penicillin and streptomycin (HyClone, Utah, USA) and cultured at 37 °C and 5% CO_2. Rabies street virus strain BD06 (GenBank: EU549783.1) was propagated and maintained in mouse brains. BD06 was originally isolated from a rabid dog in Baoding district of Hebei province, China, in 2006. Phylogenetically, BD06 belongs to the China clade 1, which is responsible for most rabies cases in humans and dogs in China (Wang et al., 2015). Rabies Challenge Virus Standard 11(CVS-11) was kindly provided by Wuxi Xinlianxin Biotech co., LTD. (Wuxi, China) and was propagated on BHK-21 cells and titrated in BALB/c mice. The CVS-11 strain has been approved as a challenge virus in RABV neutralizing antibody tests (Yu et al., 2013).

2.2. Beagles

Sixteen 3- to 4-month-old beagles (8 female and 8 male) (Table 1) were purchased from Sichuan Institute of Musk Dear Breeding, Sichuan, China, and housed at Institute of Laboratory Animal, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital. All the dogs were chimpanzee adenovirus antibody negative according to screening before immunization, and left unvaccinated with rabies vaccines. The dogs were separately fed in cages and were randomly divided into 6 groups. Group1-3 were immunized intramuscularly (i.m.), and group 4–6 were immunized orally (oral) with different doses of ChAd68-Gp or ChAd68-empty. All animal experiment procedures were approved by the Institutional Animal Care and Use Committee of Institute of Laboratory Animal, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, and all methods were performed in accordance with the relevant guidelines and regulations.

2.3. Immunization and challenge

In the i.m. injection groups, the dogs were primed with different doses of the ChAd68-Gp (5×10^{10} virus particle(vp) and 1.7×10^{12}vp) and ChAd68-empty (5×10^{10}vp). While in the oral groups, dogs were orally instilled with ChAd68-Gp (5×10^{10}vp and 1.7×10^{13}vp) and ChAd68-empty (5×10^{10}vp). Eight weeks after the prime, all dogs were boosted with the same doses of ChAd68-Gp or ChAd68-empty viruses as the prime.

Rabies street virus strain BD06 was used as challenge virus and diluted with sterile phosphate-buffered saline. Four weeks after boosting, some of beagle dogs were randomly selected to be sedated and inoculated in the unilateral hind limb muscles with 1.0 ml of BD06 virus at 6×10^4 50% mouse lethal dose (MLD_{50}). The dogs were checked daily and recorded individually for 3 months after the challenge.

2.4. Blood samples collection

Blood samples were harvested from all dogs every two weeks until 12 weeks after the prime. Blood samples were stored at room temperature for 2 h and then transferred to 4 °C overnight and then centrifuged at 3000 rpm for 15 min at 4 °C. Serum (1–2 mL) was collected and frozen at −20 °C until use. Before testing, serum was thawed and then heat-inactivated at 56 °Cfor 30 min.

2.5. Virus neutralization assay

Rabies virus neutralizing antibodies (RVNA) were measured using the fluorescent antibody virus neutralization (FAVN) test as previously described (Cliquet et al., 1998). Briefly, 3-fold serial dilutions of standard serum (0.5 IU/ml) and test serum samples were prepared in quadruplicate in a multi-well plate, and mixed with 100TCID50 (50 μl) of CVS-11. After incubation at 37 °C in a humidified 5% CO_2 incubator for 1 h, a 50 μl suspension containing 2×10^4 BHK-21 cells was added, and the incubation was continued for 48 h. The cells were fixed at 4 °C by treatment with 80% acetone for 30 min and stained with FITC-labeled anti-RABV-N monoclonal antibodies (Veterinary Research Institute, Changchun, China). Fluorescence was observed using ultraviolet microscopy, and the RVNA titers were calculated using the Spearman-Karber formula. According to guidelines from the World Health Organization, a RVNA titer ≥0.5 IU is adequate to provide full protection (Xiang et al., 2014).

2.6. Enzyme-linked immunosorbent assay

Anti-rabies IgG antibody was detected by rabies virus IgG-antibody assay kit for animal (Ningbo Tianrun Bio-pharmaceutical Co., Ltd., Ningbo, China) following the manufacturer's instructions.

2.7. Adenovirus neutralization assays

An adenovirus neutralization assay was performed based on previously described methods (Wang et al., 2014). Before testing, 10-fold serial dilutions of the recombinant adenoviruses were prepared, and 50 μl of each dilution was added to 96-well plates, followed by 50 μl of

Table 1
Beagles in different groups.

Group	Immunization routes	Vaccines	Animal no.	Animal gender	Doses(VP/dose)	Challenge or not
1	i.m.	ChAd68-Gp	3	Female	5.00E + 10	None
			4	Female	5.00E + 10	None
			7	Female	5.00E + 10	Challenge
			8	Female	5.00E + 10	Challenge
2	i.m.	ChAd68-Gp	12	Male	1.70E + 12	None
			17	Female	1.70E + 12	None
3	i.m.	ChAd68-empty (control)	18	Male	5.00E + 10	Challenge
			19	Female	5.00E + 10	Challenge
4	oral	ChAd68-Gp	1	Male	5.00E + 10	None
			2	Male	5.00E + 10	None
			5	Male	5.00E + 10	Challenge
			6	Female	5.00E + 10	Challenge
5	oral	ChAd68-Gp	10	Male	1.70E + 13	Challenge
			11	Male	1.70E + 13	Challenge
6	oral	ChAd68-empty (control)	15	Female	5.00E + 10	Challenge
			16	Male	5.00E + 10	Challenge

Dulbecco's modified Eagle's medium with 5% fetal bovine serum. One hundred microliters of HEK293 cell suspension (2.5×10^5 cells/ml) was added to wells in the same 96-well plate. Twenty-four hours later, green fluorescent protein levels were examined by fluorescence microscopy to determine a suitable virus concentration to use in the neutralization test. Viruses were then diluted to this concentration, mixed with two-fold serially diluted (1:10–1:1280) dog serum in 96-well plates and incubated at 37 °C for 1 h. After incubation, 100 μl of an HEK293 cell suspension (2.5×10^5 cells/ml) was added to each well and the plates were then incubated at 37 °C in a 5% CO_2 atmosphere, and 24 h later, the 96-well plates were examined by fluorescence microscopy. Dulbecco's modified Eagle's medium without serum was used as the negative control. The neutralizing antibody titer was expressed as the reciprocal of dilutions for which the proportion of green fluorescent protein-expressing cells was reduced to approximately 50% of that for the negative control. A titer ≥20 was regarded as positive for the serotype-specific neutralizing antibodies (Zhang et al., 2013).

2.8. Passive protection

Thirty 8-week-old recipient BALB/c mice were divided into 6 groups, with 5 mice per group. Each mouse was passively transferred 500 μl antisera collected from the corresponding group of immunized beagle dogs (low-dose i.m. ChAd68-Gp group; high-dose i.m. ChAd68-Gp group; i.m. ChAd68-empty group; low-dose oral ChAd68-Gp group; high-dose oral ChAd68-Gp group; oral ChAd68-empty group), respectively, by intraperitoneal injection. Twenty-four hours later, the mice were challenged with rabies virus CVS-11 administered intranasally at 10 MLD_{50}. Body weights and survival rates were monitored daily post-challenge for 21 days. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of Institut Pasteur of Shanghai (Shanghai, China), and all methods were performed in accordance with relevant guidelines and regulations.

2.9. Plaque assay for low-pH value treated adenoviruses

Six-well plates containing 2×10^6 HEK293 cells per well were prepared. Twenty-four hours later, the plated cells were approximately 100% confluent. A batch of 4×10^3 plaque-forming unit (pfu) adenovirus ChAd68-Gp were incubated with simulated gastric fluid (SGF) and Tris-HCl solutions with different pH values of 1.5, 2.1, 6.2, 6.6, and 7.4, respectively. The treated adenoviruses were maintained at 37 °C for 30min, 60min, and 90min, respectively. After treatment, the solutions were adjusted to pH values of 7.0 by adding different pH buffers and then transferred to the six-well plates and incubated in the incubator for 2 h. The supernatants were removed from the six-well plates and 0.8% low-melting agar was added to each well. When the agar overlay turns solid, the plates were gently transferred to the incubator. Seven days later, the cells were fixed with formaldehyde and stained with crystal violet. The number of plaques in each well were counted.

2.10. Statistical analysis

Plaque numbers were compared among different pH-value groups with one-way analysis of variance; P-values < 0.05 were considered statistically significant. GraphPad Prism software v6.0 (GraphPad, CA, USA) was used for statistical analysis.

3. Results

3.1. Antibody responses in the i.m. groups

In the i.m. immunization group, dog sera were collected every 2 weeks to measure the total IgG against rabies virus, and the neutralizing antibodies to rabies virus and adenovirus ChAd68, respectively. As shown in Fig. 1A, a high titer of total IgG against rabies virus can be detected 2 weeks after the prime, and continued to increase at 4 weeks in both vaccine groups. At 8 weeks after the prime, IgG titer continued to rise in the high-dose (1.7×10^{12}vp) group, while it decreased slightly in the low-dose (5×10^{10}vp) group. After boosting, total IgG in beagles in both vaccine groups increased dramatically.

RVNA is the most important indicator in evaluating whether a rabies vaccine is effective. Two weeks after i.m. immunization with ChAd68-Gp, both the low- and high-dose groups induced strong neutralizing antibodies against rabies (Fig. 1B), the RVNA titers in both vaccine groups reached 20 IU. Rabies neutralization antibody responses continue to maintain at a high level until 8 weeks, and climbed to a peak (300 IU) 2 weeks after the boost immunization.

The neutralizing antibodies against the adenoviral vector ChAd68 were also measured, as shown in Fig. 1C. Both ChAd68-Gp and ChAd68-empty induced strong neutralizing antibodies against ChAd68 2 weeks after the prime. After boosting, adenoviral vector neutralizing antibodies continued to rise significantly.

3.2. Antibody responses in the oral groups

In the oral groups, there were no difference in antibody responses between the low-dose vaccine group and the control group, rabies virus total IgG, neutralizing antibodies to rabies virus and adenoviral vector were all negative (Fig. 2A.,2B.,2C). However, high-dose (1.7×10^{13}vp) immunization group exhibited a specific immune response. As shown in Fig. 2A, the total IgG response against rabies virus was detected in the high-dose group 2 weeks after the prime and peaked at 4 weeks, with a slight decrease at 8 weeks after priming but a rapid increase 2 weeks

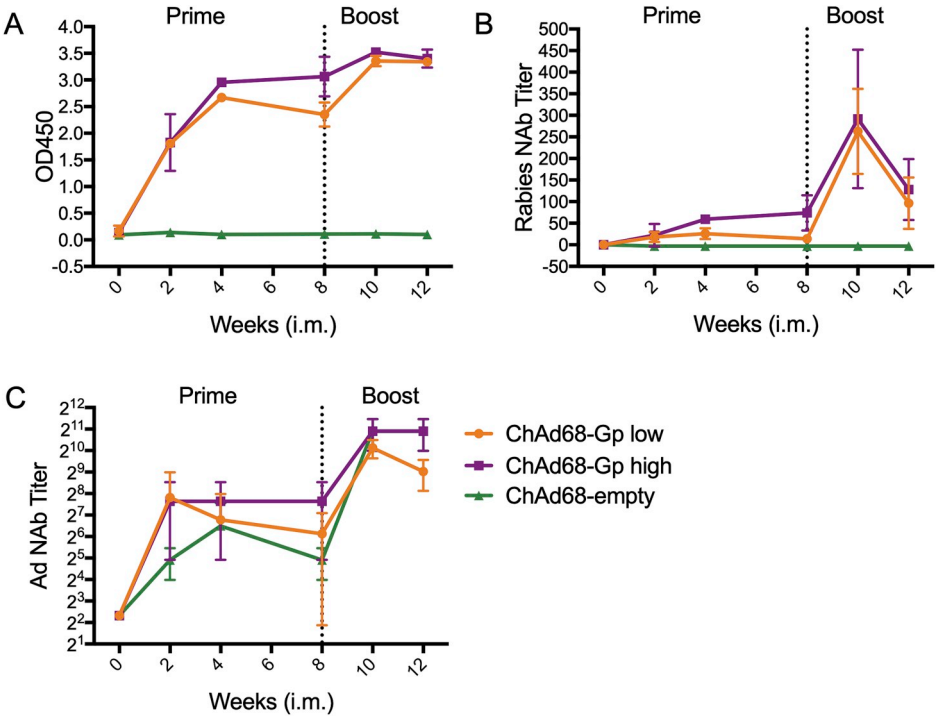

Fig. 1. Antibody responses after immunization in the intramuscularly immunized groups. Three groups of beagle dogs were primed at week 0 and boosted at week 8. Serum samples were obtained at week 2, 4, 8, 10 and 12 to detect the total immunoglobulin (Ig)G against rabies virus, rabies-neutralizing antibodies and adenovirus-neutralizing antibodies in the sera of beagle dogs, respectively. (A) Total IgG against rabies virus. (B) Neutralizing antibodies against rabies virus. (C) Neutralizing antibodies against ChAd68 vector.

after boosting. As shown in Fig. 2B, neutralizing antibody responses against rabies virus were detected in the high-dose immunization group 2 weeks after the prime, and antibody titers continued to rise 4–8 weeks after priming. RVNA titer increased significantly 2 weeks after oral boosting.

Immune responses against the adenoviral vector were assessed (Fig. 2C). The primary immunization could not effectively induce the adenoviral neutralizing antibody response, while high-dose vaccination elicited strong vector neutralization titer after boosting.

3.3. Survival after challenge

Four weeks after the boost immunization, some of the dogs were challenged with lethal rabies virus. Survival status was monitored every day until 3 months after challenge. In the i.m. groups, all the dogs in vaccine group remained disease-free, while all the dogs in control group appeared symptomatic and were euthanized on the 11th day after rabies virus challenge (Fig. 3A). Regarding the oral groups, dogs in control group appeared symptomatic and were euthanized on the 11th days after challenge. One of the two dogs in the low-dose group appeared

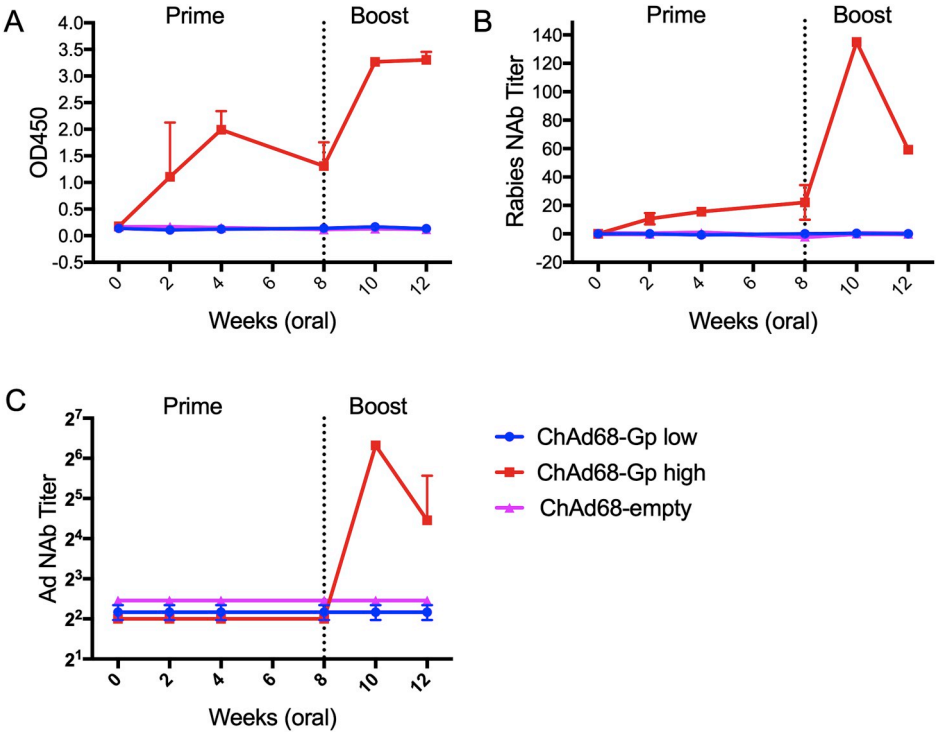

Fig. 2. Antibody responses of beagle dogs after oral immunization. Three groups of beagle dogs were primed at week 0 and boosted at week 8. Serum samples were obtained at week 2, 4, 8, 10 and 12 to detect the total immunoglobulin (Ig)G against rabies virus, rabies-neutralizing antibodies and adenovirus-neutralizing antibodies in the sera of beagle dogs, respectively. (A) Total IgG against rabies virus. (B) Neutralizing antibodies against rabies virus. (C) Neutralizing antibodies against ChAd68 vector.

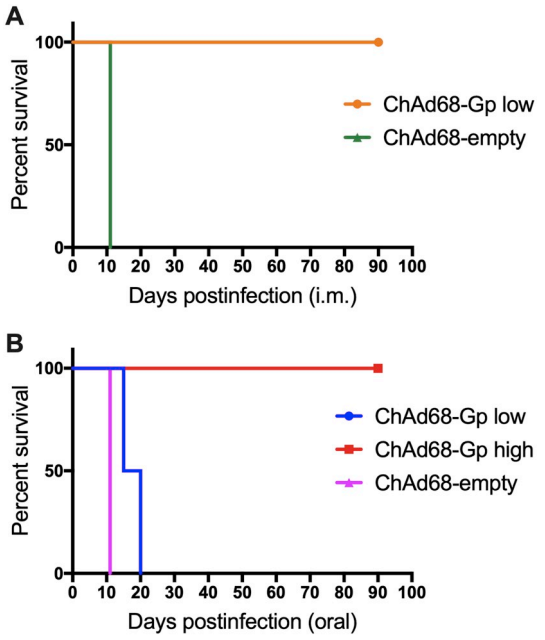

Fig. 3. Survival of the beagle dogs after lethal rabies virus challenge. Four weeks after the boost immunization, the dogs were challenged with 6×10^4 MLD_{50} BD06 rabies virus. Survival status was monitored every day until 3 months after challenge. (A). Survival of the beagles in the intramuscularly injected groups. (B). Survival of beagles in the oral groups.

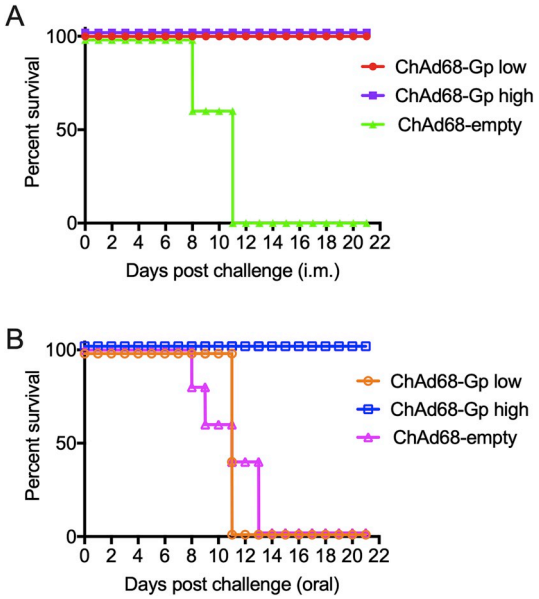

Fig. 4. Survival of rabies virus-challenged mice that received antisera from vaccine-immunized beagle dogs. The mice in each group were passively received 500 μl serum derived from immunized beagles. Twenty-four hours later, all mice were challenged with 10 MLD_{50} CVS-11 rabies virus administered intranasally. (A). Survival of the mice received antisera from beagles immunized intramuscularly. (B). Survival of the mice received antisera from beagles immunized orally.

symptomatic and then was euthanized at day 15, the other one showed symptoms and was euthanized at day 20. While all the beagles in high dose immunization group survived healthily three months after challenge (Fig. 3B).

3.4. Passive protection

Antisera from beagles in each group were harvested and pooled after the dogs received boosting, and then passively transferred to naïve mice by intraperitoneal injection to explore whether the antibodies induced in beagles could protect other animals from rabies virus. The recipient mice were challenged with lethal rabies virus 24 h after receiving antisera from the beagles. As shown in Fig. 4, mice that received antisera from the low-dose (RNAb titer 262) and high-dose i.m. ChAd68-Gp groups (RNAb titer 291) and the high-dose oral ChAd68-Gp group (RNAb titer 135) remained alive. While the mice in the control groups (RNAb titer 0) and the low-dose oral ChAd68-Gp group (RNAb titer 0) died on the 8th day post-infection.

3.5. The effect of gastric acid on adenovirus

To explore why the low-dose oral immunization could not provide protection in the beagles, different pH solutions were prepared to mimic gastric acids to investigate the effect of gastric acid on the stability of adenovirus. As shown in Fig. 5, adenoviruses were treated with artificial gastric juice and buffers with different pH values for 30 min, 60 min, and 90 min, and then added to the HEK293 cells to test the infectivity of these viruses. The results revealed that the SGF and the pH1.5 solution completely inactivated the adenoviruses. Under condition at pH 2.1, if the treatment time exceeded 30 min, the infectivity of the adenoviruses was partially impaired, although the damage was more serious with time. The solutions with pH values of 6.2–7.4 had little effect on the adenovirus within 30–90 min of treatment (Fig. 5A.,C.,5E). In fact, the pH values in stomach of beagle are 1.5 and 2.1 in the fast and fed situations, and are 6.2, 6.2, 6.6 in the duodenum, upper jejunum and lower jejunum, respectively (Willmann et al., 2010). Therefore, the reason that the low-dose oral immunization with naked adenovirus could not provide protection was that the adenovirus was essentially inactivated by the low pH of gastric juices and, thus, could not provide immune protection.

4. Discussion

ChAd68 is one serotype of adenoviruses isolated from chimpanzee, retaining enough similarity to subgroup C adenoviruses to allow high-level replication of an E1-deleted ChAd68 vector in HEK 293 cells containing the E1 region of AdHu5. In addition, it can induce similar immune responses as AdHu5, but are not impaired by pre-existing immunities in humans (Xiang et al., 2002). An E1-deleted replication-deficient ChAd68 vector expressing rabies virus glycoprotein was initially developed as rabies vaccine by the Ertl HC lab in the Wistar Institute, Philadelphia (Pennsylvania, USA), which has been tested in mice administered i.m., intranasally or orally, and could provide fully protection even at low dose against lethal challenge of rabies virus (Xiang et al., 2002, 2003a, 2003b; Zhou et al., 2006). It has also been tested in a non-human primate model, a single immunization with a moderate dose of this vaccine resulted in sustained titers of rabies virus neutralizing antibodies and completely protection against a lethal rabies virus challenge in monkeys (Xiang et al., 2014).

We generated an E1/E3-deleted ChAd68 vector expressing codon-optimized glycoprotein of rabies virus and tested in mice previously, it induced long-lasting immune responses and 100% protection against lethal infection of rabies virus (Zhang et al., 2017). In the present study, we tested the efficacy of ChAd68-Gp in beagles and compared the protective effects between the oral and i.m. immunizations with different dosages. After i.m. immunization, antibody responses to both vector virus and rabies virus were induced and boosted significantly after the second immunization. Although the neutralizing antibody to vector itself was developed after the prime, it could not neutralize all viruses in the boost. The remaining viruses activated the immune memory and triggered strong immune responses toward both the vector virus and rabies virus. The boosting effects could retain about two

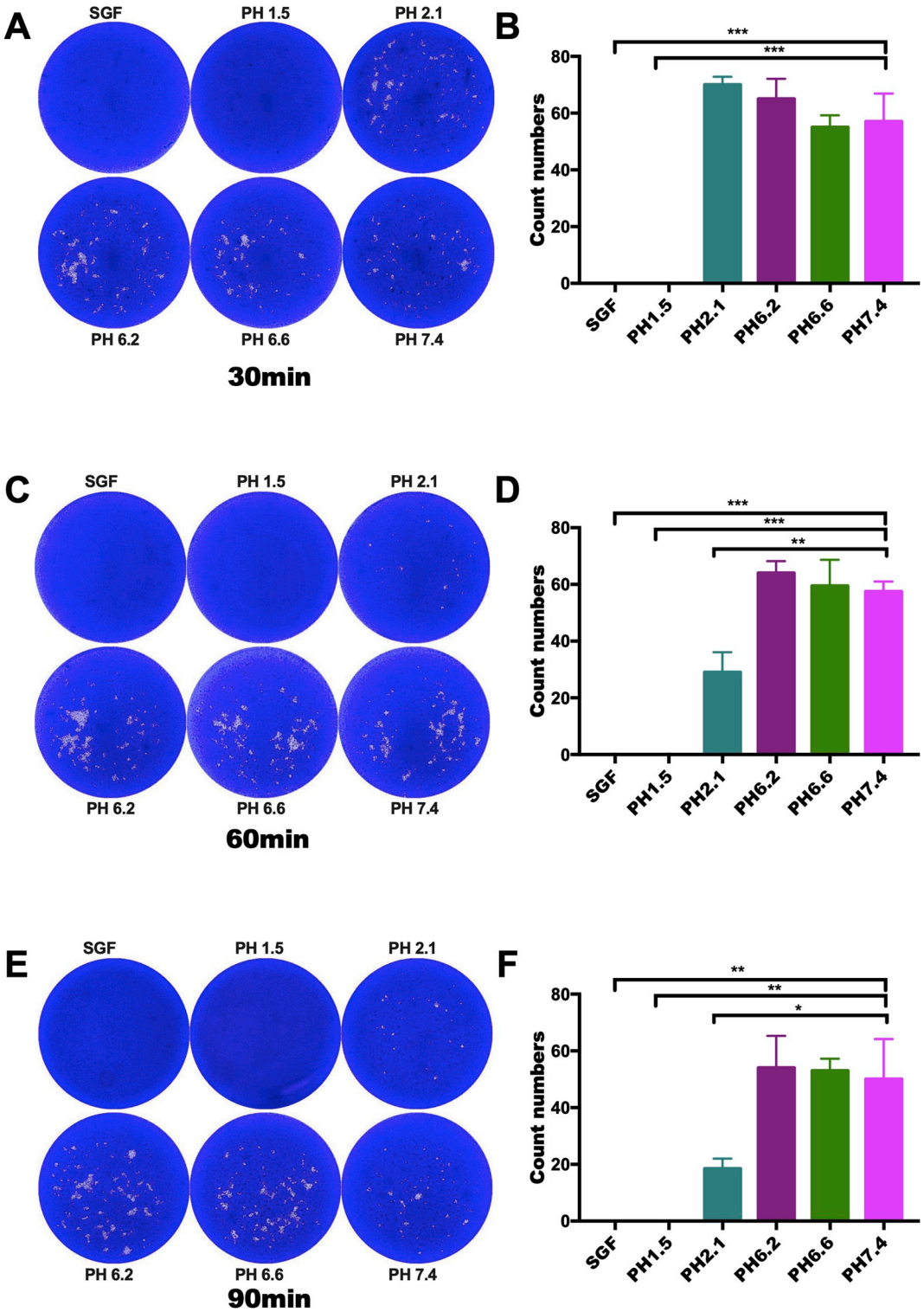

Fig. 5. The effect of simulated gastric acid (SGF) on adenovirus. Plague assays were performed and quantified for ChAd68-Gp treated with SGF and low-pH buffer for 30min (A, B), 60min (C, D), and 90min (E, F), respectively.

weeks and then declined but still remained at a high level compared with those after the prime. Unexpectedly, low-dose oral immunization group failed to activate any immune responses against rabies virus or adenoviral vector, and thus failed to provide protection against rabies virus challenge. Oral vaccination with high-dose vaccine could induce rabies-specific antibody responses, but no antibody response against adenoviral vector after the prime. After boosting, high-dose oral vaccine group exhibited a high level of immune response against both rabies virus and adenoviral vector, and protected the dogs against the lethal challenge. Our results are partially consistent with a previous study (Vos et al., 2001), in which dogs received replication-defective AdHu5 expressing rabies virus glycoprotein by direct oral instillation could not develop detectable levels of RVNA even after oral boosting, however, dogs that received i.m. boosting produced high levels of rabies virus specific immune responses. The authors in that study suspected the reason for the poor efficiency of oral immunization was the

inefficiency of mucosal delivery for adenovirus because adenovirus naturally infecting mucosal tissues requires frequent infections (Vos et al., 2001), but they only used relatively low dose of adenovirus for vaccination.

Oral vaccination is a cost-effective, socially acceptable technique often used to control rabies in terrestrial wildlife, and is easy to manipulate. However, we speculated that gastric juice of beagle dogs is harmful to adenovirus. When a small amount of adenovirus directly reaches the stomach of the beagle (i.e., through swallowing), the adenoviral particles including the proteins and DNAs which consist of the virus maybe completely destroyed by the low pH of the stomach environment. When a large quantity of adenoviruses reach the stomach, a certain percentage of adenovirus maybe fully destroyed, while the coat proteins on the remaining proportion of adenoviruses might be partially damaged, so that it is not sufficient to induce detectable neutralizing antibodies against adenoviral vector after the prime. However, the internal parts of the virus, including the inserted foreign gene of interest, the glycoprotein of rabies virus which existed as an internal protein in the viral particle, may not be seriously affected and, thus can induce the rabies-specific immune responses. After the boost, the partially impaired adenovirus coat proteins activate the immunological memory and lead to enhanced adenovirus-specific neutralizing antibody responses. Therefore, in some conditions, for example, gastric juice with low pH only destroys the surface of virus particle, and the internal proteins including the expressed foreign gene are released and then trigger the host immune responses. Compared with AdRG1.3 wrapped in a plastic blister pack, which can be chewed by animals and thus the adenovirus can be released and lead to a mucosal infection in the oral cavity, we in this study delivered the vaccine directly to the stomach of the beagle dogs, there is no chance of oral mucosal immunity to be elicited. To confirm our hypothesis, we prepared different pH solutions to mimic gastric acids to explore the effect of gastric acid on the stability of adenovirus. The results in this study indicated that the gastric acid with low pH values seriously impairs adenovirus. Therefore, suitable formulation of the vaccine is very important for efficient induction of immune response, especially to the oral vaccination.

In conclusion, chimpanzee adenoviral-based rabies vaccine ChAd68-Gp can effectively activate immune response and completely protect beagle dogs from lethal rabies virus challenge through i.m. administration. In addition, we found out the reason for the poor efficiency of oral immunization of the ChAd68-Gp is that the gastric juice in the stomach of beagles might decompose the adenovirus due to the low-pH value. Thus it is necessary to further investigate the formulation of adenoviral vector-based oral vaccine in order to improve the protective immunity.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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