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Communication

Human Adenovirus Type 5 as a Delivery Vector is Not Neutralized in Field Serum Samples of Cattle, Pig, and Goat of Republic of Korea

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Human adenovirus type 5 (hAd5) vectors have been demonstrated to be useful vehicles for gene expressions in animals. However, it has not been reported whether hAd5 transduction might be hampered in the sera of livestock animals in Republic of Korea. We collected 205 samples of livestock animals, such as pig (n=84), cattle (n=84), and goat (n=37) in Korea. The neutralizing antibody (NAb) titers to hAd5 virus were less than 15 in most of samples. Only 8% of goat samples had a NAb titer of 15 or 30. Thus, we showed that hAd5 virus was not neutralized in sera from cattle, pig, and goat, and suggest that the hAd5 vector could be used for the effective delivery of vaccines or proteins in livestock animals in the field.

Key Words: Human adenovirus type 5, Antibody, Serum, Korea

INTRODUCTION

Human adenovirus (hAd) based vectors have been demonstrated to act as excellent vehicles for gene expressions, including vaccines (1). Among these, human adenovirus type 5 (hAd5) is most widely used as a vector. The effectiveness of recombinant human adenovirus-delivered vaccines against human infectious diseases such as human immunodeficiency virus (HIV) and human hepatitis B has been demonstrated in animal models and in clinical trials on humans (2, 3). However, the practical usefulness of the hAd5 vector is hampered by the wide prevalence of preexisting adenoviral immunity in mice, primates, and humans (4~6). For this reason, several alternatives, such as genetic engineering of the Ad vector, nasal delivery, and use of non-human adenoviruses have been suggested (7~9). A study on non-human adenoviral vectors showed that bovine adenovirus and porcine adenovirus was not significantly neutralized in human serum samples (10), which indicated that porcine and bovine adenovirus - based vectors could be alternatives employed to prevent immunity in humans.

The previous studies have reported that hAd5 vector could be promising in animals. Human Adenovirus type 5 vector mediated delivery attributed to its broad host range has been applied on vaccines or antiviral agents against animal diseases, such as foot-and-mouth disease (FMD), influenza, vesicular stomatitis virus (VSV) and classical swine fever (CSF) (11~16). In addition, porcine adenovirus or bovine adenovirus vector-specific rabbit antibodies were not cross-

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neutralized with hAd5 (8) and that Ad antibody prevalence was very low in Belgian cattle (17). However, it has not been reported whether hAd5 transduction was hampered in sera of livestock animals in Republic of Korea because preexisting adenovirus antibodies has not been determined. As this could interrupt the effective delivery of hAd vector for field animal trials, we performed a virus neutralization test in serum samples of farm animals such as pig, cattle, and goat. The 205 serum samples of pig, cattle, and goat were collected from seven nationwide provinces from the Republic of Korea. Our study showed that hAd5 virus was not neutralized in the sera of cattle, pig, and goat, and on the basis of our finding. We propose that the hAd5 vector could be used as an effective delivery method for vaccines or proteins for livestock animals in the field.

MATERIALS AND METHODS

Human embryonic kidney cells, including human adenovirus type 5 E1 DNA (293A cells) were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; pH 7.4) at 37℃ with 5% CO_2. Recombinant adenovirus type 5 expressing the lacZ was produced as previously described (18). The titer of adenovirus was determined in 293A cells and the 50% tissue culture infective dose ($TCID_{50}$) was calculated using the formula of Reed and Muench (19). A total of 205 serum samples [cattle (n = 84), pig (n = 84) and goat (n = 37)] were collected randomly from 47 farms [cattle (n = 21), pig (n = 17) and goat (n = 9)] from seven provinces of the Republic of Korea (Table 1). Adenovirus neutralization assays were performed as described previously (17). The serum was incubated at 56℃ for 30 minutes and then diluted in DMEM in two-fold increments starting from 1:15 dilution in 96 well plates. One hundred microliters of each dilution was mixed with 200 $TCID_{50}$ of recombinant adenovirus type 5 expressing lacZ and incubated for 1 hour at 37℃. The rabbit polyclonal antibody to the adenovirus type 5 (Abcam ab6982, USA) was used as a positive serum control, after which 10^4 of 293A cells were added into each well and incubated at 37℃ for 7 days. The virus neutralizing antibody titer was estimated from the reciprocal of the highest serum dilution that completely prevented the development of a cytopathic effect (CPE).

Table 1. Serum samples of cattle, pig, and goat used in this study

Province	The number of samples for species		
	Cattle	Pig	Goat
Chungbuk	8	8	12
Gyeongnam	12	12	8
Jeju	8	8	
Gyeonggi	12	12	
Gyeongbuk	28	16	9
Jeonbuk	16	16	8
Gangwon		12	
Total	84	84	37

RESULTS AND DISCUSSION

In this study, we examined whether preexisting neutralizing antibodies (NAb) would cross-neutralize hAd5 in 205 field serum samples of cattle, pig, and goat using a virus-neutralization test. We enhanced representativeness of the samples by random sampling in a nationwide collection from Korea (Table 1). In the virus neutralization assay, hAd5 virus was neutralized by NAb titer > 100 of the positive serum control and had completes CPE in no treatment wells. And the titers higher than 15 were scored as positive for the presence of hAd5-specific NAb in this study (20). The hAd5-specific NAb titers were less than 15 in 100% of serum samples from cattle (n = 84) and pig (n = 84) (Fig. 1). Even though most of goat samples also had the NAb titer of less than 15, 8% of the goat serum samples showed NAb titer of more than 15, two samples had a titer of 15 in Gyeongbuk and Jeonbuk province, and one sample in Jeonbuk province had a titer of 30. We suggested that the level of NAb titers of cattle, pig, and goat in the fields might not affect transduction of human adenoviral vector and only some of the goat samples could have low levels of titer for cross-neutralization.

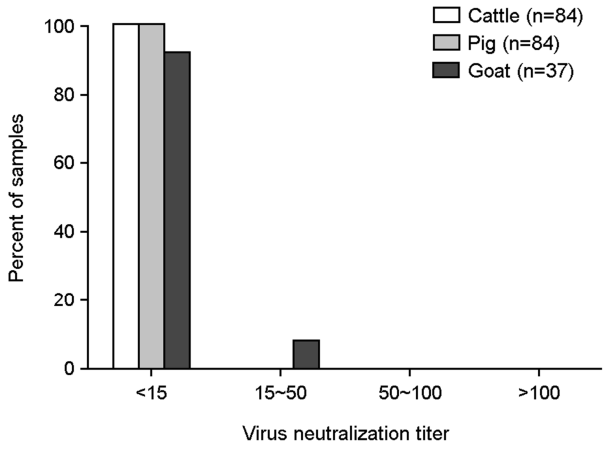

Figure 1. Distribution of serum samples from cattle, pig and goat according to neutralizing antibody to human adenovirus type 5 virus (hAd5). The virus neutralizing antibody titer was estimated from the reciprocal of the highest serum dilution that completely prevented the development of a cytopathic effect (CPE). The percent of serum samples that display hAd5-specific neutralization titers of <15, 15~50, 50~100, and >100 are shown. The rabbit polyclonal antibody to adenovirus type 5 virus (Abcam ab6982, USA) was used as a positive serum control.

Cattle, pig, and goat are major livestock animals in many countries, including the Republic of Korea. The adenoviral vectors could be promising delivery systems for vaccines and antiviral agents against infectious diseases such as foot-and-mouth disease (FMD), classical swine fever (CSF), and porcine productive and respiratory syndrome (PRRS) in cattle, pig, and goat (12, 21, 22). However, in more than 90% of instances of ovine adenovirus and bovine adenovirus, prevalence of NAb has been reported in sheep and cattle in the US (23). Occurrences of 89.5% and 92.3% of bovine adenovirus type 1 and 3 antibodies have been reported in Turkey (24) and an 18.3% prevalence of swine adenovirus antibody has been reported in 1983, in the US (25). The prevalence of antibodies for bovine, porcine, or ovine adenovirus was expected to be high in Korea as well, and the possibility that preexisting adenovirus antibodies might act as blocking factors in the effective delivery of the hAd vector for field animal trials could not be ruled out. Therefore, the virus-neutralization test for hAd5 was necessary for the study of a large set of animal serum samples from several farms.

Adenoviruses, including human adenovirus, bovine adenovirus, porcine adenovirus, and ovine adenovirus are hypothesized to have evolved with their hosts (26). Natural infection is known to be restricted to single or related host species, although some adenoviruses used as delivery vectors could be used to infect a broad range of hosts (27). Previously, it was reported that bovine adenovirus and porcine adenovirus were not significantly cross-neutralized by NAb to human adenovirus (NAb<4) (10). Another study suggested that virus-neutralizing epitopes differ significantly in human adenovirus 5, bovine adenovirus 3, and porcine adenovirus 3 (8). The study also showed that porcine adenovirus- or bovine adenovirus- specific rabbit antibodies significantly neutralized porcine and bovine adenovirus but did not cross-neutralize hAd5. Thus, our study was based on the findings that the efficiency of porcine and bovine adenovirus - based vectors could be significantly decreased on neutralization by preexisting immunity in cattle and pigs. Furthermore, we anticipated that the human adenovirus - based vectors could serve as more effective delivery systems for animals owing to circumvention of preexisting host-specific adenovirus immunity.

In conclusion, we confirmed that there was no hindrance in the use of hAd5-based virus in the sera of cattle, pig, and goat, and suggest that the hAd5-based vector could be used effectively for the field trials of livestock animals in many countries, including the Republic of Korea. Moreover, we also suggest that the human adenovirus-based vectors are more effective than the bovine or porcine adenovirus-based vectors in the study of livestock animals.

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