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Short note

Bovine herpesvirus 1 glycoprotein D expression in bovine upper respiratory tract mediated by a human adenovirus type 5

Sacha GOGEV[a], Jean-Pierre GEORGIN[b], Frédéric SCHYNTS[a], Alain VANDERPLASSCHEN[b], Etienne THIRY[a]*

[a] Virology, Department of Infectious and Parasitic diseases, Faculty of Veterinary Medicine, University of Liège, boulevard de Colonster 20, B43b, 4000 Liège, Belgium
[b] Immunology-Vaccinology, Department of Infectious and Parasitic diseases, Faculty of Veterinary Medicine, University of Liège, boulevard de Colonster 20, B43b, 4000 Liège, Belgium

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Abstract – Bovine herpesvirus 1 glycoprotein D (gD) gene expression by recombinant replication defective human adenovirus type 5 (HAdV-5) was investigated in calves using indirect immunofluorescence microscopy (IIFM), confocal laser scanning microscopy (CLSM) and RT-PCR. One fold intranasal instillation of HAdV-5-expressing gD in the cattle upper respiratory tract showed a short term expression of at least 5 days, but not 10 days, limited only to epithelial cells localised in the epithelium of the nasal mucosa in one out of six calves. Observed limited gene transfer into well differentiated cattle airway epithelial cells must be taken into consideration in order to enhance transfection efficiency, and consequently the vaccine potential of this vector.

bovine herpesvirus 1 / glycoprotein D / recombinant human adenovirus type 5 / cattle

1. INTRODUCTION

Recombinant replication defective human adenovirus type 5 (HAdV-5) was largely used as a transfer vector and has proven useful for achieving high-level expression of a variety of foreign genes as well as effective in inducing an immune response following either systemic or mucosal immunisation [4, 5, 13, 17, 19]. This virus is unable to replicate in cells which do not complement the defective gene but is still able to express the gene of interest inserted in the E1 region when the cells are infected at a high multiplicity of infection [3, 22]. It has been reported that this vector, belonging to the first generation of adenovirus vectors, efficiently transduces intrapulmonary airway epithelial cells; however, transgene expression is no longer detectable 2–3 weeks post infection [11, 16, 25, 26, 28]. In vivo testing of second generation vectors (deletion of the viral E, E3 and E2A regions or of the E1, E3 and E4 regions) showed no reduction of the anti-vector immune response and no significant improvement of transgene persistence compared to those obtained with the first generation vectors [14]. Furthermore, it has also been shown that the efficiency of human adenovirus vector-mediated gene transfer to poorly differentiated human or rodent airway epithelial cells in vitro is high whereas

* Corresponding author: etienne.thiry@ulg.ac.be

the efficiency of gene transfer to well differentiated human or rodent ciliated airway epithelial cells in vivo is low [6, 8, 20, 21].

In this study, the efficacy of recombinant HAdV-5 mediated bovine herpesvirus 1 (BoHV-1) glycoprotein D (gD) gene transfer in vivo was investigated. We previously tested the capacity of replication defective HAdV-5 expressing the BoHV-1 glycoprotein C or D, administered intranasally alone or in combination with chitosan based intranasal adjuvants, to elicit an immune response and to protect cattle against virulent BoHV-1 challenge [4, 5]. The aim in the current study was to investigate the localisation, and the temporal pattern of gD gene expression in the airway epithelial cells of the upper respiratory tract of cattle upon one fold intranasal inoculation of calves with the defective HAdV-5 expressing BoHV-1 gD when used as a vaccine vector.

2. MATERIALS AND METHODS

Eight calves were randomly allocated into four groups of two calves. All the calves were seronegative to BoHV-1 and HAdV-5. The bovine viral diarrhea antigen test performed on these calves was also negative. Each calf in three of the groups was inoculated once intranasally by an aerosol of 2 mL per nostril of a solution containing 10^{10} $CCID_{50}$ (Cell Culture Infectious Dose) of recombinant replication defective HAdV-5 expressing gD of BoHV-1, whereas the non inoculated group served as a negative control group. The pair of calves of the negative control group was euthanised on day 0, while the other pairs of calves were euthanised every five days. Calf care and experimental procedures were carried out in accordance with the Belgian law (AR 14/11/93) implementing the European Council directive number 86/609/ECC of November 24, 1986.

Whole nasal, pharyngeal and tracheal mucosa were skillfully dissected. The mucosa samples from the vestibular, respiratory and olfactory regions of the nasal cavity, from the nasopharynx and laryngopharynx and from the cranial, middle and caudal parts of the trachea of each calf were prepared and then frozen at –80 °C. The expression of the delivered gD gene in the respiratory tract was sought on randomly selected frozen mucosa sections fixed on microscope slides using indirect immunofluorescence microscopy (IIFM) and confocal laser scanning microscopy (CLSM).

The slides were dried at room temperature and entirely immersed in 95% acetone at –20 °C for 30 min. The slides were held frozen at –20 °C for later examination. Acetone–treated slides were immersed completely for 30 min in PBS supplemented with 10% HS (horse serum) and were washed for a 10 min period with agitation. After saturation of the slides an anti BoHV-1 gD MAb, kindly received from Dr G.L. Letchworth (Department of Veterinary Science, Madison, Wisconsin, USA), was applied on each section and then incubated for one hour at room temperature. After washes with PBS, the slides were further incubated with fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG (Dako, Glostrup, Denmark) for 45 min at room temperature and then stained with PBS-Evans Blue and mounted with Mowiol 4–88 (Hoechst, Frankfurt, Germany).

Expression of BoHV-1 gD gene mRNA was also tested by RT-PCR. Total RNA was extracted from the frozen mucosa samples by RNA NOW (Biogentex, Seabrook, TX, USA) in accordance with the manufacturer's protocol. To ensure that eventual residues of gD gene DNA were eliminated, prior to conversion of mRNA to cDNA, all total RNA samples were treated with DNase I (Amersham Pharmacia Biotech, Uppsala, Sweden) in an assay buffer: 40 mM Tris-HCL (pH 7.5), 6 mM $MgCl_2$, 1 mM DTT and 40 U RNasin (Promega, Madison, WI, USA) and incubated for two hours at 37 °C. This was followed by RNA re-purification in a High Pure RNA Isolation Kit (Roche, Mannheim, Germany) and then the RNA

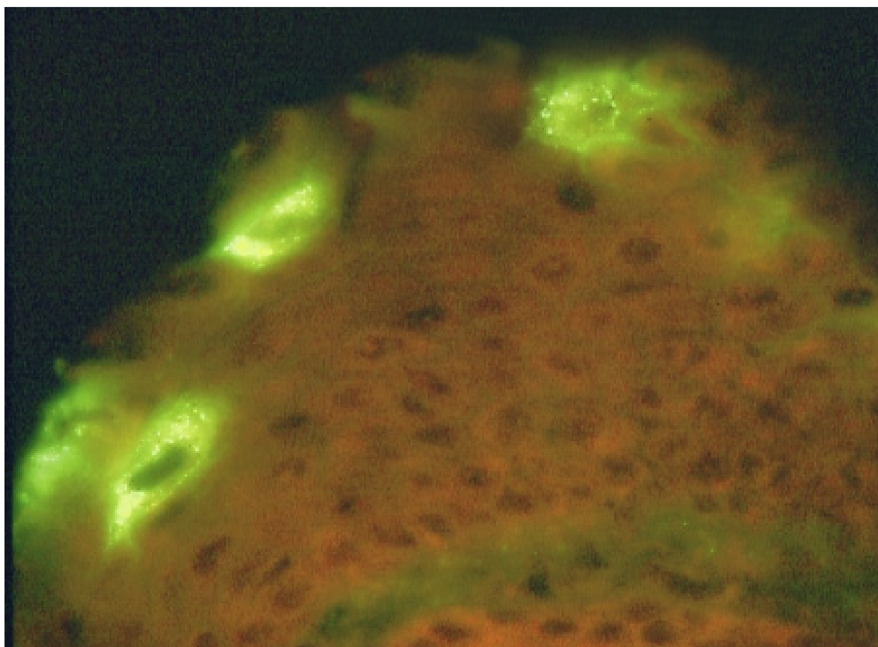

Figure 1. gD gene expression detected in the epithelium of the cranial third of the nasal cavity on day 5 PI with recombinant replication defective HAdV-5gD (magnification, × 500; Leitz fluorescence microscope).

samples were tested for gD gene DNA presence by PCR described hereinafter. Ten microlitres of each RNA sample (gD gene DNA negative by PCR) were incubated for a 10 min period at 65 °C with 1 μL (80 pmoles) of Oligo $(dT)_{15}$ primer (Roche, Mannheim, Germany). A cDNA synthesis reaction was carried out in 1 × reverse transcriptase buffer: 40 mM KCl, 50 mM TrisHCl, 5 mM $MgCl_2$, 250 M each dNTP, 10 mM DTT and 50 U Expand reverse transcriptase (Roche, Mannheim, Germany), in a final volume of 20 μL. The reaction was incubated at 42 °C for one hour, 95 °C for 5 min and cooled to 4 °C.

For PCR amplification of gD gene cDNA, the following primers (in the 5' 3' orientation) and conditions were used: AACATGCAAGGGCCGACATTGG and GACCGTGCCGTCGATGTACAGC; 95 °C for 30 s, 56 °C for 1 min, and 72 °C for 1 min and 20 s for 35 cycles. The PCR reaction was carried out in 1 × buffer 50 mM KCl, 10 mM TrisHCl, 1.5 mM $MgCl_2$, 200 μM each dNTP, 50 ng each primer, 6% DMSO and 2.5 U Red Goldstar DNA Polymerase (Eurogentec, Seraing, Belgium) in a final volume of 25 μL. The expected fragment length was 552 bp. Amplicons were visualised using 2% agarose gel electrophoresis-ethidium bromide staining.

3. RESULTS

Histological examination of frozen nasal tissue sections by IIFM showed that the expression of the gD gene delivered by recombinant HAdV-5 was detected in groups, pairs and individual epithelial cells localised in the epithelium of the nasal cavity (Fig. 1) in one calf and on day 5 PI. Although a small number of epithelial cells were detected to be positive for gD gene expression, they abundantly expressed glycoprotein D. On the contrary, gD gene expression

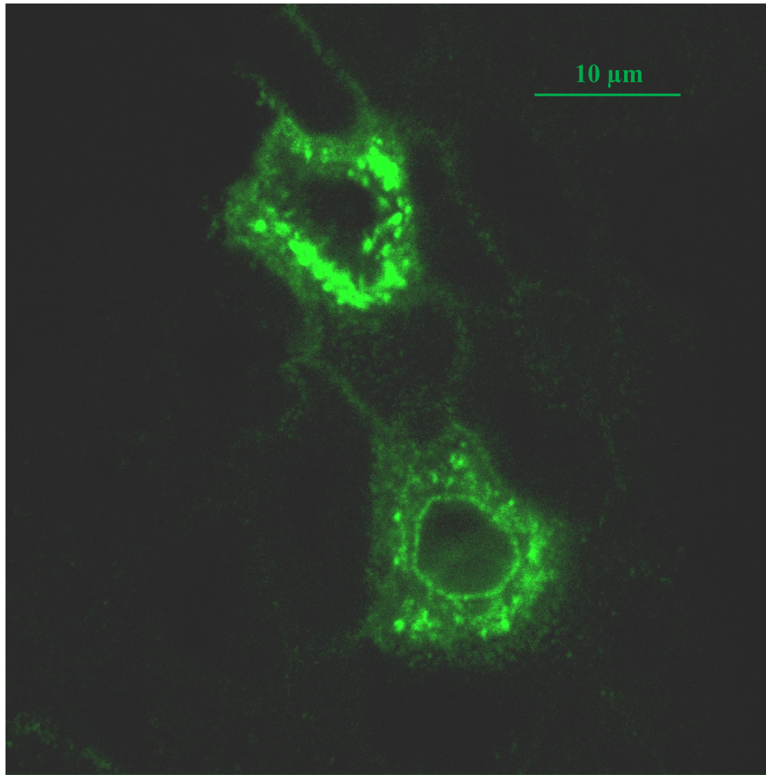

Figure 2. High amounts of glycoprotein D expressed in the epithelial cells of the nasal cavity 5 days PI with recombinant replication defective HAdV-5gD (Leica confocal laser scanning microscope).

was not observed on pharyngeal and tracheal tissue sections of this calf nor on those from the other calves. CLSM was also used to examine the tissue sections, to confirm the previous positive results detected by IIFM. As depicted in Figure 2, the epithelial cells localised in the fore part of the nasal cavity 5 days PI with HAdV-5gD were confirmed positive. Confocal microscopy-generated XZ sections revealed high amounts of glycoprotein D accumulated in the cytoplasm and on the cell membrane of positive epithelial cells.

The BoHV-1 gD gene mRNA was sought by RT-PCR in a randomly selected tissue sample. Samples from the non inoculated group, which served as a negative control group, did not show any signal. gD gene mRNA was also not detected in any frozen tissue samples from the calves evaluated 10 and 15 days after administration of recombinant replication defective HAdV-5 harbouring the BoHV-1 gD gene, except in those from the cranial third of the nasal mucosa in one of two calves from the group inoculated on day 5 (Fig. 3). These results confirmed previous data obtained in the same calf by IIFM and CLSM.

4. DISCUSSION

The current experiment was designed to study for the first time in vivo adenovirus-mediated transgene expression in cattle. By using recombinant replication defective

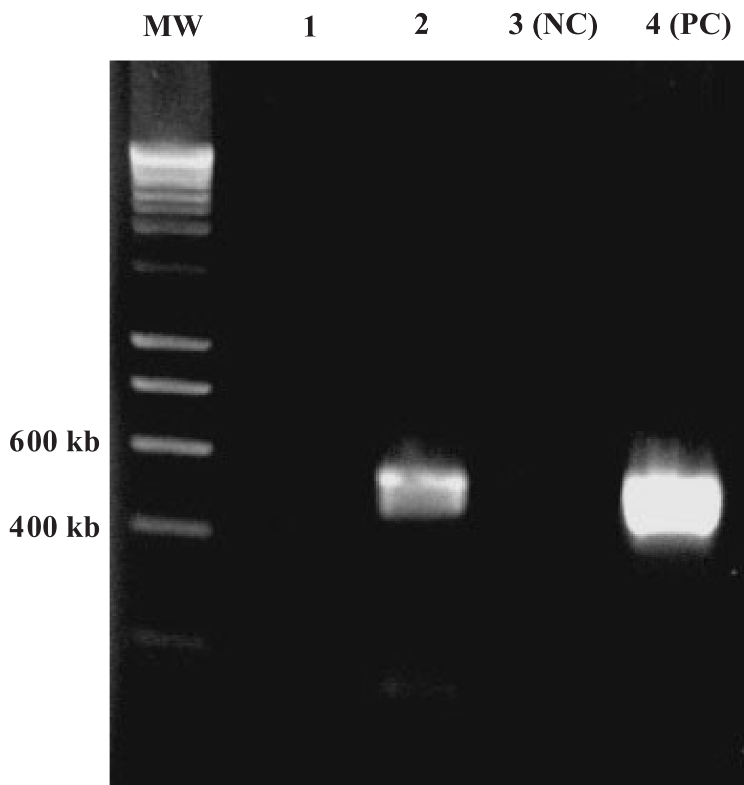

Figure 3. Detection of mRNA expression encoding for glycoprotein D in the epithelial cells of the cranial third of the nasal cavity of one calf 5 days PI using the RT-PCR technique. Lane 1: absence of residual gD gene DNA after DNase I treatment; lane 2: detection of BoHV-1 gD gene amplified fragment (552 bp) after mRNA conversion to cDNA; lane 3: negative control (NC) DNAse and RNAse free water; lane 4: positive control (PC) BoHV-1 Iowa strain DNA.

HAdV-5 as a vector carrying the gD gene of BoHV-1, we demonstrated a gD gene overexpression only in the epithelial cells localised in the epithelium of the cranial third of the nasal cavity of one calf and for at least 5 days after intranasal administration with HAdV-5gD. The BoHV-1 gD gene mRNA was also detected using RT-PCR, but only in the vestibular part of the nasal mucosa in the same calf 5 days PI.

Detected short gD gene expression, however, restricted only to the nasal cavity after a single inoculation observed in one cattle, was enough to induce an immune response and to protect cattle against virulent BoHV-1 challenge, upon twofold intranasal administration of the replication defective HAdV-5 expressing the BoHV-1 glycoprotein gC or gD [4, 5]. After inoculation, once vector gene expression is turned on in infected cells, expression of the glycoprotein D encoded by the first-generation adenoviral vector backbone can trigger destructive cellular immune responses mediated by cytotoxic T lymphocytes (CTLs) that rapidly eliminate the transduced cells [27–29]. A similar situation is expected, particularly if one takes into account that the BoHV-1 glycoprotein D is immunodominant because it contains neutralising antibodies and CTL epitopes [2,

23]. Thus the duration of its expression is likely to be short, as shown in the current experiment (between 5–9 days), particularly since the peak activity of cellular response in cattle occurs at 7-10 days post infection with BoHV-1 [1]. In addition, this HAdV-5gD vector was not intended for use in gene therapy, but in vaccination where the expression of immunogenic transgene products on the cell surfaces is aimed at eliciting an immune response, and consequently, gene expression is transient because the infected cells are rapidly eliminated.

Nevertheless, some further work could be done to improve the duration and the transduction efficiency of HAdV-5 mediated transgene expression eventually by the means of nongenetic retargeting [10] and construction of chimeric HAdV-5 genetically retargeted with broadened or narrowed tropism [7, 12, 15, 30]. Since it has been shown that human adenovirus mediated gene transfer is inefficient in cells lacking sufficient coxsackievirus and adenovirus receptor expression, such as a differentiated airway epithelium in humans and mice [21, 24], then the construction of HAdV-5 containing the HAd5/35 chimeric fiber protein could overcome this limitation [9, 18]. The vaccine potential of HAdV-5 could also be improved by association with an intranasal adjuvant as already described in our study using chitosan based adjuvants [5].

In conclusion, to enhance transfection efficiency, vector design should take into account observed limited and short gD gene expression when an adenovirus is devoted to be developed for intranasal vaccination. In addition, a large-animal model, such as the cattle model, could also be useful for the assessment of the efficacy of improved adenovirus vector-mediated gene delivery in man.

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REFERENCES

[1] Babiuk L.A., van Drunen Littel-van den Hurk S., Tikoo S.K., Immunology of bovine herpesvirus 1 infection, Vet. Microbiol. 53 (1996) 31–42.

[2] Denis M., Slaoui M., Keil G., Babiuk L.A., Ernst E., Pastoret P.P., Thiry E., Identification of different target glycoproteins for bovine herpes virus type 1-specific cytotoxic T lymphocytes depending on the method of in vitro stimulation, Immunology 78 (1993) 7–13.

[3] Eloit M., Bouzghaia H., Toma B., Identification of antigenic sites on pseudorabies virus glycoprotein gp50 implicated in virus penetration of the host cell, J. Gen. Virol. 71 (1990) 2179–2183.

[4] Gogev S., Vanderheijden N., Lemaire M., Schynts F., D'Offay J., Deprez I., Adam M., Eloit M., Thiry E., Induction of protective immunity to bovine herpesvirus type 1 in cattle by intranasal administration of replication-defective human adenovirus type 5 expressing glycoprotein gC or gD, Vaccine 20 (2002) 1451–1465.

[5] Gogev S., Versali M.F., Gautier S., Thiry E., Glycol chitosan improves the efficacy of intranasally administered replication-defective human adenovirus type 5 expressing glycoprotein D of bovine herpesvirus 1, Vaccine 22 (2004) 1946–1953.

[6] Goldman M., Su Q., Wilson J.M., Gradient of RGD-dependent entry of adenoviral vector in nasal and intrapulmonary epithelia: implications for gene therapy of cystic fibrosis, Gene Ther. 3 (1996) 811–818.

[7] Goossens P.H., Havenga M.J., Pieterman E., Lemckert A.A., Breedveld F.C., Bout A., Huizinga T.W., Infection efficiency of type 5 adenoviral vectors in synovial tissue can be enhanced with a type 16 fiber, Arthritis Rheum. 44 (2001) 570–577.

[8] Grubb B.R., Pickles R.J., Ye H., Yankaskas J.R., Vick R.N., Engelhardt J.F., Wilson J.M., Johnson L.G., Boucher R.C., Inefficient gene transfer by adenovirus vector to cystic fibrosis airway epithelia of mice and humans, Nature 371 (1994) 802–806.

[9] Havenga M.J., Lemckert A.A., Ophorst O.J., van Meijer M., Germeraad W.T., Grimbergen J., van Den Doel M.A., Vogels R., van Deutekom J., Janson A.A., de Bruijn J.D., Uytdehaag F., Quax P.H., Logtenberg T., Mehtali M., Bout A., Exploiting the natural diversity in adenovirus tropism for therapy and prevention of disease, J. Virol. 76 (2002) 4612–4620.

[10] Hong S.S., Galaup A., Peytavi R., Chazal N., Boulanger P., Enhancement of adenovirus-mediated gene delivery by use of an oligopeptide

with dual binding specificity, Hum. Gene Ther. 10 (1999) 2577–2586.

[11] Jooss K., Yang Y., Wilson J.M., Cyclophosphamide diminishes inflammation and prolongs transgene expression following delivery of adenoviral vectors to mouse liver and lung, Hum. Gene Ther. 7 (1996) 1555–1566.

[12] Kirby I., Davison E., Beavil A.J., Soh C.P., Wickham T.J., Roelvink P.W., Kovesdi I., Sutton B.J., Santis G., Mutations in the DG loop of adenovirus type 5 fiber knob protein abolish high-affinity binding to its cellular receptor CAR, J. Virol. 73 (1999) 9508–9514.

[13] Klonjkowski B., Denesvre C., Eloit M., Adenoviral vectors for vaccines, in: Seth P. (Ed.), Adenoviruses: basic biology to gene therapy, RG Landes Company, Austin, 1999, pp. 163–173.

[14] Lusky M., Christ M., Rittner K., Dieterle A., Dreyer D., Mourot B., Schultz H., Stoeckel F., Pavirani A., Mehtali M., In vitro and in vivo biology of recombinant adenovirus vectors with E1, E1/E2A, or E1/E4 deleted, J. Virol. 72 (1998) 2022–2032.

[15] Magnusson M.K., Hong S.S., Boulanger P., Lindholm L., Genetic retargeting of adenovirus: novel strategy employing "deknobbing" of the fiber, J. Virol. 75 (2001) 7280–7289.

[16] Minter R.M., Rectenwald J.E., Fukuzuka K., Tannahill C.L., La Face D., Tsai V., Ahmed I., Hutchins E., Moyer R., Copeland E.M. 3rd, Moldawer L.L., TNF-alpha receptor signaling and IL-10 gene therapy regulate the innate and humoral immune responses to recombinant adenovirus in the lung, J. Immunol. 164 (2000) 443–451.

[17] Mittal S.K., Papp Z., Tikoo S.K., Baca-Estrada M.E., Yoo D., Benko M., Babiuk L.A., Induction of systemic mucosal immune responses in cotton rats immunized with human adenovirus type 5 recombinants expressing the full truncated forms of bovine herpesvirus type 1 glycoprotein gD, Virology 222 (1996) 299–309.

[18] Mizuguchi H., Hayakawa T., Adenovirus vectors containing chimeric type 5 and type 35 fiber proteins exhibit altered and expanded tropism and increase the size limit of foreign genes, Gene 285 (2002) 69–77.

[19] Papp Z., Babiuk L.A., Baca-Estrada M.E., Induction of immunity in the respiratory tract protection from bovine herpesvirus type 1 infection by different routes of immunization with recombinant adenovirus, Viral Immunol. 11 (1998) 79–91.

[20] Pickles R.J., Barker P.M., Ye H., Boucher R.C., Efficient adenovirus-mediated gene transfer to basal but not columnar cells of cartilaginous airway epithelia, Hum. Gene. Ther. 7 (1996) 921–931.

[21] Pickles R.J., McCarty D., Matsui H., Hart P.J., Randell S.H., Boucher R.C., Limited entry of adenovirus vectors into well-differentiated airway epithelium is responsible for inefficient gene transfer, J. Virol. 72 (1998) 6014–6023.

[22] Ragot T., Eloit M., Perricaudet M., Recombinant E1A-defective adenoviruses expressing pseudorabies and Epstein-Barr virus glycoproteins induce immunological responses as live vaccines in rabbits and mice, in: Cohen-Haguenauer O., Boiron M. (Eds.), Human Gene Transfer, John Libbey, London, 1991, pp. 260–249.

[23] Van Drunen Littel-Van Den Hurk S., Parker M.D., Massie B., van den Hurk J.V., Harland R., Babiuk L.A., Zamb T.J., Protection of cattle from BHV-1 infection by immunization with recombinant glycoprotein gIV, Vaccine 11 (1993) 25–35.

[24] Walters R.W., Grunst T., Bergelson J.M., Finberg R.W., Welsh M.J., Zabner J., Basolateral localization of fiber receptors limits adenovirus infection from the apical surface of airway epithelia, J. Biol. Chem. 274 (1999) 10219–1026.

[25] Wilmott R.W., Amin R.S., Perez C.R., Wert S.E., Keller G., Boivin G.P., Hirsch R., De Inocencio J., Lu P., Reising S.F., Yei S., Whitsett J.A., Trapnell B.C., Safety of adenovirus-mediated transfer of the human cystic fibrosis transmembrane conductance regulator cDNA to the lungs of nonhuman primates, Hum. Gene Ther. 7 (1996) 301–318.

[26] Wilson C.B., Embree L.J., Schowalter D., Albert R., Aruffo A., Hollenbaugh D., Linsley P., Kay M.A., Transient inhibition of CD28 and CD40 ligand interactions prolongs adenovirus-mediated transgene expression in the lung and facilitates expression after secondary vector administration, J. Virol. 72 (1998) 7542–7550.

[27] Yang Y., Nunes F.A., Berencsi K., Furth E.E., Gonczol E., Wilson J.M., Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy, Proc. Natl. Acad. Sci. USA 91 (1994) 4407–4411.

[28] Yang Y., Li Q., Ertl H.C., Wilson J.M., Cellular and humoral immune responses to viral antigens create barriers to lung-directed gene therapy with recombinant adenoviruses, J. Virol. 69 (1995) 2004–2015.

[29] Yang Y., Su Q., Wilson J.M., Role of viral antigens in destructive cellular immune responses to adenovirus vector-transduced cells in mouse lungs, J. Virol. 70 (1996) 7209–7212.

[30] Yotnda P., Onishi H., Heslop H.E., Shayakhmetov D., Lieber A., Brenner M., Davis A., Efficient infection of primitive hematopoietic stem cells by modified adenovirus, Gene Ther. 8 (2001) 930–937.