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Research paper

Time-dependent biodistribution and transgene expression of a recombinant human adenovirus serotype 5-luciferase vector as a surrogate for rAd5-FMDV vaccines in cattle

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ABSTRACT

Replication-defective recombinant adenovirus 5 (rAd5) vectors carrying foot-and-mouth disease virus (FMDV) transgenes elicit a robust immune response to FMDV challenge in cattle; however mechanistic functions of vaccine function are incompletely understood. Recent efforts addressing critical interactions of rAd5 vectors with components of the bovine immune system have elucidated important aspects of induction of protective immunity against FMDV. In the current study, a rAd5-Luciferase (rAd5-Luc) surrogate vector was utilized for indirect assessment of rAd5-FMDV distribution during the first 48 hours post inoculation (hpi). To compare vector distribution dynamics and time-dependent transgene expression, bovine cells were inoculated in vitro with rAd5-FMDV and rAd5-Luc vectors. Superior transgene expression was detected in cells infected with rAd5-Luc compared to rAd5-FMDV. However, both vectors behaved remarkably similar in demonstrating elevated mRNA transcription at 24 and 48 hpi with peak occurrence of transgene expression at 48 hpi. Injection sites of cattle inoculated with rAd5-Luc contained mononuclear inflammatory infiltrates with hexon and transgene proteins associated with antigen-presenting cells. Luciferase activity, as well as microscopic detection of luciferase antigens, peaked at 24 hpi. Presence of viral mRNA also peaked at 24 hpi but unlike luciferase, remained strongly detected at 48 hpi. Cell-associated luciferase antigens were detected as early as 6 hpi at the cortical interfolicullar areas of local LN, indicating rapid trafficking of antigen-presenting cells to lymphoid tissues. This work provides mechanistic insights on rAd5-mediated immunity in cattle and will contribute to ongoing efforts to enhance rAd5-FMDV vaccine efficacy.

1. Introduction

Foot-and-mouth disease virus (FMDV), an Aphtovirus of the Picornaviridae family (Fauquet et al., 2005), can cause severe disease in susceptible livestock (Grubman and Baxt, 2004). In enzootic regions, FMD-associated morbidity causes food insecurity and limits trade. In FMDV-free regions, considerable resources are directed towards preventing introduction of the virus. Control strategies are of upmost importance and have historically relied in the use of an inactivated whole virus vaccine that can effectively control clinical disease (Doel, 2003; Rodriguez and Gay, 2011). However, because of a number of limitations of the current vaccine preparations, new vaccination approaches have been envisioned and explored in recent

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years (Grubman and Baxt, 2004; Rodriguez and Gay, 2011; Rodriguez and Grubman, 2009). One novel approach utilizes a replication-defective recombinant human adenovirus type 5 (rAd5) vaccine containing FMDV coding sequences (rAd5-FMDV) (Grubman et al., 2010; Mayr et al., 1999; Moraes et al., 2002). This platform has been demonstrated to be a highly efficient system for delivering antigens to the host and eliciting protective immune responses against FMDV challenge (Grubman et al., 2010; Grubman and Baxt, 2004; Montiel et al., 2012; Pacheco et al., 2005). Vaccination trials using rAd5-FMDV have demonstrated complete protection against virulent challenge at 21 days post vaccination (dpv) and partial protection before 7 dpv in presence of low levels of anti-FMDV antibodies (Grubman et al., 2010; Sanz-Parra et al., 1999a,b). Furthermore, recent studies on rAd5 vector biodistribution in cattle indicated that during the first 48 hours after inoculation with rAd5-FMDV, adenoviral and transgene proteins were found highly associated with cells phenotypically consistent with antigen presenting cells (APC) primarily at the injection site and secondarily at various local lymph nodes (Montiel et al., 2012). These results implicate the innate immune system in early events associated with generation of protective immunity, which are of critical importance in driving a protective response against FMDV.

Several groups have explored novel approaches using sensitive reporter systems based on expression of the luciferase gene, both in vitro and in vivo. Porcine cell lines infected with a rAd5-Luciferase (rAd5-Luc) vector (Torres et al., 1996), and plasmid DNA containing the firefly luciferase gene directly inoculated into the muscle of pigs (Everett et al., 2000) and mice (Manthorpe et al., 1993; Wolff et al., 1990), have shown detectable and stable levels of biologically active recombinant proteins, demonstrating the feasibility of using such systems in gene transfer-related studies. To establish validity as a surrogate vector for rAd5-FMDV vaccine biodistribution studies, our lab recently demonstrated transgene expression in human and bovine cells lines infected with a rAd5-Luc construct, and correlated these data with PCR and microscopy (Montiel et al., 2012). We also demonstrated that upon intramuscular inoculation of cattle with rAd5-Luc, luciferase was strongly detected at the injection site and, to a lesser extent, at the local lymph nodes. The luciferase transgene was also detected by PCR and luciferase antigens by immunomicroscopy in bovine tissues (Montiel et al., 2012).

The current study provides a comprehensive investigation of rAd5 biodistribution and transgene expression using recently validated detection techniques in tissues of cattle (Montiel et al., 2012), and should contribute to enhancing our current understanding of host–vector interactions for the improvement of vaccine effectiveness against FMD.

2. Materials and methods

2.1. Cells and viruses

The replication-defective recombinant (r) Ad5 vectors used in this study have deletions in the E1, E3, and E4 regions that render them incapable of generating progeny virus in non-complementary cell lines. The first vector, rAd5-FMDV strain A24 Cruzeiro (Ad5-A24; Adt.A24.11D), contains the FMDV P1-2A and $3C^{pro}$-coding regions (Grubman et al., 2010). The second (i.e. surrogate) vector, rAd5-Luciferase (rAd5-Luc; AdL.11D), contains the firefly (*Photinus pyralis*) luciferase gene. Both vectors' transgene coding regions are under the control of a cytomegalovirus promoter. In vitro infections were carried out as previously described with minor variation (Pacheco et al., 2010). Briefly, bovine LFBK (Swaney, 1988) and human 293 (Graham et al., 1977) cells were seeded onto 6-well plates and 1-well tissue culture slides (cat# 154453, Lab-Tek®II Chamber Slide™ System; Nalgene Nunc, International; Naperville, IL), inoculated with rAd5 vectors at m.o.i. 100 and 300, and incubated at 37 °C for varying times. Following incubation of chamber slides, the monolayers were rinsed twice with media and the upper structure removed. The slides were then fixed in acetone at room temperature for 10 min and stored at −70° C until assayed by microscopy.

2.2. Experimental animals and inoculation system

Six 300 kg Holstein steers were obtained from an experimental-livestock provider accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (Thomas-Morris Inc., Reistertown, MD). All studies were conducted under an approved Institutional Animal Care and Use Committee (IACUC) protocol and animals were individually housed in a biosafety level-3 (BSL-3) animal facility from time of inoculation until time of euthanasia. Animals were examined prior to inoculation to ensure absence of pre-existing medical conditions. On Day 0 each animal received three simultaneous 3-ml intramuscular injections with a total dose of 1.5×10^{11} PU of the rAd5-Luc vector resuspended in Final Formulation Buffer (cat# B5003, FFB; Lonza, Walkersville, MD, USA) containing adjuvant D. Adjuvant D is a proprietary lipid containing emulsion designed for producing defined particle size formulas for delivery of live virus or virus-vector vaccines and immunotherapeutics and was provided by Benchmark Biolabs (Lincoln, NE) through an agreement with DHS S&T PIADC.

2.3. Animal sample collection

In vivo experiments were terminated by euthanasia via intravenous barbiturate overdose at predetermined end points at 6, 24, and 48 hours post-inoculation (hpi). Necropsies were performed immediately thereafter. Sample collection and processing was performed as previously described (Montiel et al., 2012). In order to maximize retrieval of the injection depot from the injection site (IS) area, 8 specimens of the regional muscle were collected based on anatomic location and visualization of focal hemorrhage and/or edema associated with deposit of the inoculum. Non-IS specimens collected from each animal were spleen, thymus, liver, lung, pancreas, adrenal, kidney, and local and distant lymph nodes.

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Table 1
Antibodies used in the study.

Antibody	Clone	Specificity	Isotype	Manufacturer
Anti-Adenovirus	2Q1850; 8C4	Hexon protein; hexon protein	IgG_1; IgG_{2a}	cat# A0977; USBiological, Swampscott, MA; cat# ab8249; Abcam, Cambridge, MA
Anti-FMDV	6HC4	VP1 capsid protein	IgG_{2b}	Hybridoma-A12 VP1
Anti-Luciferase	LUC-1	Firefly luciferase	IgG_1	cat# L2164; Sigma–Aldrich, St. Louis, MO
CD11c	BAQ153A	Mono[a], MΦ[b], sub T, NK, sub B, DC[c]	IgM	cat# BAQ153A; VMRD, Inc., Pullman, WA
MHCII (HLA-DR α)	TH14B	MHC class II	IgG_{2a}	cat# TH14B; VMRD
CD14 (M-M9)	CAM36A	Mono, MΦ, DC subset	IgG_1	cat# CAM36A; VMRD
CD172a	CC149	Mono, granulo	IgG_{2b}	cat# MCA2041G; AbD Serotec
MAC387	MAC387	Granulo[d], mono, histiocytes	IgG_1	cat# M0747; Dako, Carpinteria, CA
Von Willebrand Factor (vWf)	Polyclonal	Endothelial cells	Rabbit IgG	cat# A0082; Dako
Anti-Collagen I	COL-1	Extracellular matrix (ECM)	IgG_1	cat# ab90395; Abcam
ACTA-1	Polyclonal	Actin (N-terminus)	Rabbit IgG	cat# LS-B3339; LSBio, Seattle, WA
CAR	3C100	Human CAR receptor	IgG_1	cat# sc-70493; Santa Cruz Biotechnology, Inc., Santa Cruz, CA

[a] Mono = monocytes.
[b] MΦ = macrophages.
[c] DC = dendritic cells.
[d] Granulo = granulocytes.

2.4. Extraction of viral nucleic acids and transgenic product from cell lines and tissues

Nucleic acids (DNA and RNA) and total protein were extracted from cell cultures and tissues as previously described (Montiel et al., 2012), following the manufacturer's recommendations. The nucleic acid and luciferase-containing supernatants were frozen and stored at −70 °C until assayed.

2.5. Real-time PCR (rPCR and rRT-PCR) and luciferase assays

The strategy to characterize rAd5 vector biodistribution and infection distinctly utilizing two separate nucleic acid detection systems has been recently described in vitro and in vivo (Montiel et al., 2012). Systems used for detection of rAd5 DNA (PIX) and rAd5 mature mRNA transcripts (splice, representing transgene expression in infected cells) including probe and primer sequences were utilized as described previously (Montiel et al., 2012). For the in vitro assessment, values correspond to the mean Ct values of 3 separate experiments performed independently in triplicate. For the ex vivo assessment, for each time-point, PCR results were expressed as the Ct values of 39 distinct tissues, each of which was collected and analyzed independently in duplicate. Results are expressed as cycle threshold (Ct) mean values with specific detection defined for positive samples at Ct < 40. Luciferase activity was also determined based on a recently published methodology (Montiel et al., 2012). Briefly, luciferase content of each sample was recorded as relative light units (RLU) and normalized by the respective total protein concentration of the samples (RLU/mg total protein) obtained by the bicinchoninic acid assay (BCA) (Smith et al., 1985).

2.6. Immunomicroscopy

Localization of rAd5 and transgene products (FMDV-A24 capsid proteins and luciferase) in cell lines and bovine tissues was similarly determined by immunohistochemistry (IHC) and multichannel immunofluorescence (mIF) techniques as previously described (Arzt et al., 2009; Montiel et al., 2012) with minor variation. Primary antibodies (Table 1) were used individually for IHC or in combination for mIF assays. For mIF, detection was performed using goat-anti-rabbit and isotype-specific, anti-mouse secondary antibodies labeled with Alexa Fluor dyes (Invitrogen) diluted in Fluorescence Antibody Diluent (Biocare Medical, Concord, CA). For IHC, immunoreactivity was detected with a micropolymer alkaline phosphatase kit (Biocare Medical). Isotype controls were included with each experiment. Slides were evaluated with a wide-field epifluorescence microscope and images captured with a Nikon DS-Qi1 digital camera. Images of individual detection channels were adjusted for contrast and brightness and merged in commercially available software (Adobe Photoshop CS2). Differential interference contrast (DIC) microscopy was utilized on selected images to enhance cellular and extracellular matrix structural detail.

2.7. Statistical analysis

Data analyses and graphic representations were performed by using Microsoft Office Excel 2007. Statistical differences were determined by using a Student's *t* test when applicable. A cutoff value of $p < 0.05$ was considered significant.

3. Results

3.1. Time-dependent detection and quantitation of rAd5 nucleic acids and luciferase in LFBK cells

Luminometry, rPCR, and rRT-PCR were utilized to assess vector dynamics that occurred in LFBK cells during the first 72 hpi after inoculation with rAd5-FMDV or rAd5-Luc vectors at fixed m.o.i. (Fig. 1). Vector DNA of both constructs was detected at similar levels throughout the time-course 3–72 hpi (Ct: 27.64–26.16 for rAd5-FMDV and 26.93–25.08 for rAd5-Luc). In rAd5-FMDV infected cells,

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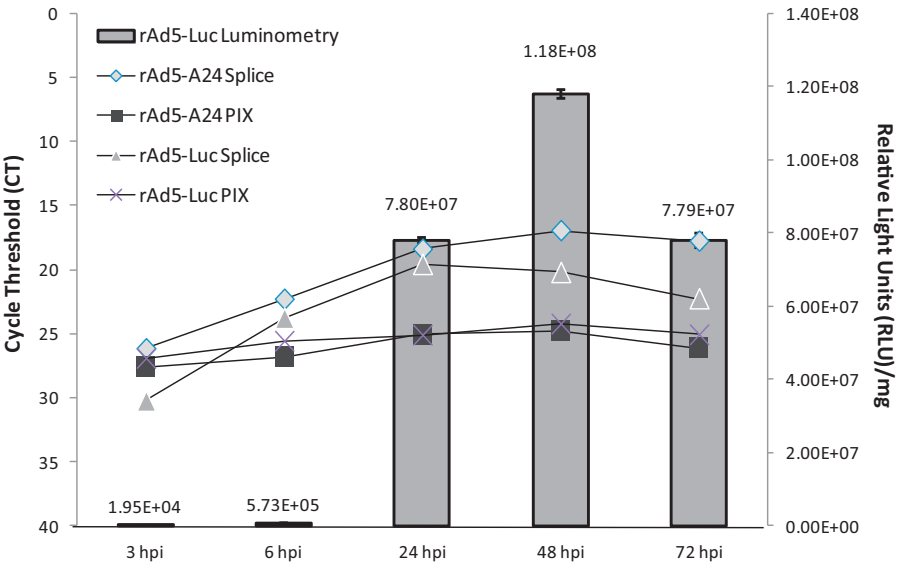

Fig. 1. Detection of nucleic acids (mRNA and DNA) and luciferase activity in cells infected with rAd5-Luc or rAd5-A24 vectors at different time-points. Each column represents mean luciferase activity (relative light units (RLU) per mg of total protein) ± SD of 3 replicates assayed simultaneously. Lines correspond to levels of rAd5-A24 mRNA (diamonds), rAd5-A24 DNA (squares), rAd5-Luc mRNA (triangles), and rAd5-Luc DNA (crossing lines) as determined by rRT-PCR (splice) and rPCR (PIX) in LFBK cells infected at fixed m.o.i. = 300 (rAd5-A24) or 100 (rAd5-Luc). Ct values are reported as mean Ct values of 3 different experiments assayed in triplicate.

mRNA signal increased with time post-inoculation with a peak observed at 48 hpi (Ct 16.94). Relative to rAd5-Luc-infected cultures, mRNA content in rAd5-FMDV-infected cultures was consistently higher (lower Ct values) at all time-points. Nonetheless, detection of mRNA in rAd5-Luc cultures followed a remarkable similar pattern than the observed in rAd5-FMDV positive cells during the first 24 hpi (Ct 30.27 at 3 hpi, Ct 23.80 at 6 hpi, and Ct 19.58 at 24 hpi). However, mRNA signal peaked at 24 hpi (Fig. 1).

In rAd5-Luc-infected cultures, luciferase activity increased with time post-exposure between 3 and 48 hpi followed by a decrease between 48 and 72 hpi (Fig. 1). The most significant difference regarding luciferase activity levels across consecutive time-points was observed between 6 and 24 hpi (136 ± 24-fold; $p < 0.05$). Moreover, the peak of luciferase activity was detected at 48 hpi ($1.18 \times 10^8 \pm 1.25 \times 10^6$ RLU/mg protein). It is noteworthy that luciferase activity levels at 24 ($7.80 \times 10^7 \pm 6.87 \times 10^5$) and 72 ($7.79 \times 10^7 \pm 1.88 \times 10^6$) hpi were approximately 50% lower than at the peak (1.18×10^8 RLU). These results demonstrate that bovine LFBK cells provide a suitable model for the study of time-dependent kinetics of rAd5 infection in vitro, which can be further applied for the study of rAd5 vaccine pathogenesis in vivo.

3.2. Immunomicroscopy in rAd5-infected LFBK cells

In order to further characterize suitability of rAd5-Luc infection as a surrogate model for rAd5-FMDV, LFBK cells were inoculated with either rAd5-FMDV or rAd5-Luc and analyzed at similar times post inoculation (Fig. 2). Adenoviral protein (hexon) and transgene product of both constructs (FMDV capsids and luciferase) were detected using specific antibodies (Table 1). Luciferase-expressing cells were found as early as 3 hpi with an average of one luc+ cell per field. A clear peak of detection occurred at 48 hpi with more than 70% of cells identified as luc+. At 24 and 72 hpi, LFBK cell cultures had a similar protein expression pattern and were visualized containing substantially less luc+ cells (50% or less) than at 48 hpi. Localization of the Coxsackievirus and Adenovirus Receptor (CAR) in rAd5-Luc+ LFBK cultures was consistently membrane-associated and unaltered by the presence of luciferase or hexons throughout the time-course.

By contrast, FMDV antigens (FMDV-A24 capsids) were not detected in rAd5-FMDV-infected LFBK cell cultures until 24 hpi, suggesting expression of transgene antigens was not substantial until after 6 hpi. At 24–48 hpi, around 30% of cells were immunoreactive to the anti-FMDV antibody, and at 72 hpi only few A24+ cells were found in the cultures. From 24 to 72 hpi in rAd5-FMDV cultures, there was progressive accumulation of clusters of degenerate, rounded cells and autofluorescent cell bodies (Fig. 2, arrows). Detection of CAR in rAd5-FMDV-inoculated cultures was similarly strong and uniform at 3, 6, and 24 hpi; however, altered patterns of CAR detection were observed at 48 and 72 hpi with significantly reduced expression intensity (Fig. 2).

3.3. Time-dependent detection and quantitation of rAd5-Luc nucleic acids and luciferase in bovine tissues

In order to characterize vector dynamics in vivo, tissue specimens from rAd5-Luc inoculated cattle were collected from animals euthanized at 6, 24, and 48 hpi and subsequently processed by rPCR, rRT-PCR, and luciferase bioassay (Fig. 3).

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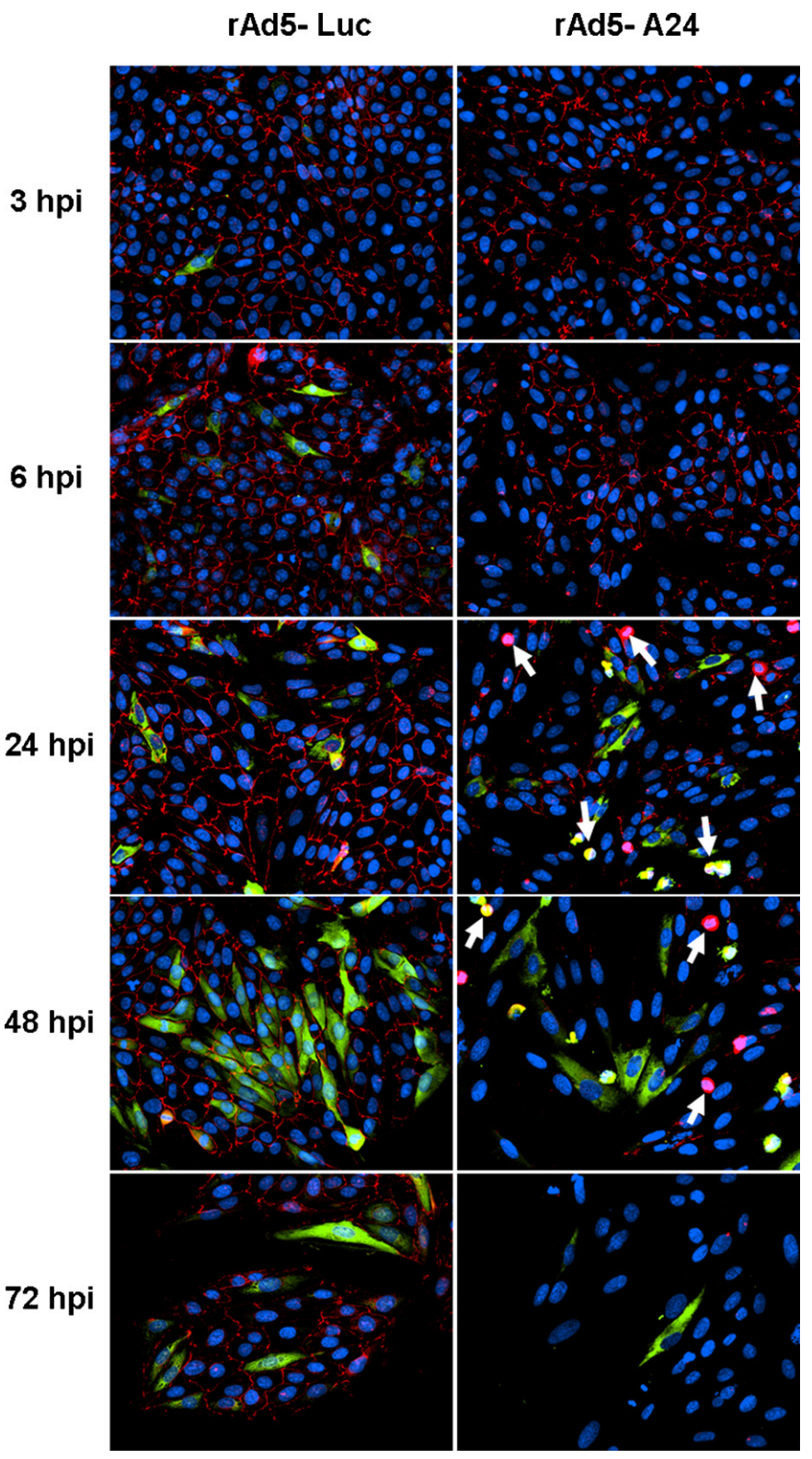

Fig. 2. Detection of transgene products in LFBK cells infected with rAd5 vectors during time-course experiments. Diffuse cytoplasmic distribution of FMDV capsids and luciferase (green) in the cytoplasm of rAd5-A24 or rAd5-Luc infected cells at m.o.i. 300 or 100, respectively. Membrane-associated expression of the coxsackie and adenovirus receptor (CAR) is shown in red. Multichannel immunofluorescence (mIF) microscopy. Degenerate autofluorescent cells (arrows) only seen in rAd5-A24 infected cells. Nuclei DAPI counterstain. 20× magnification.

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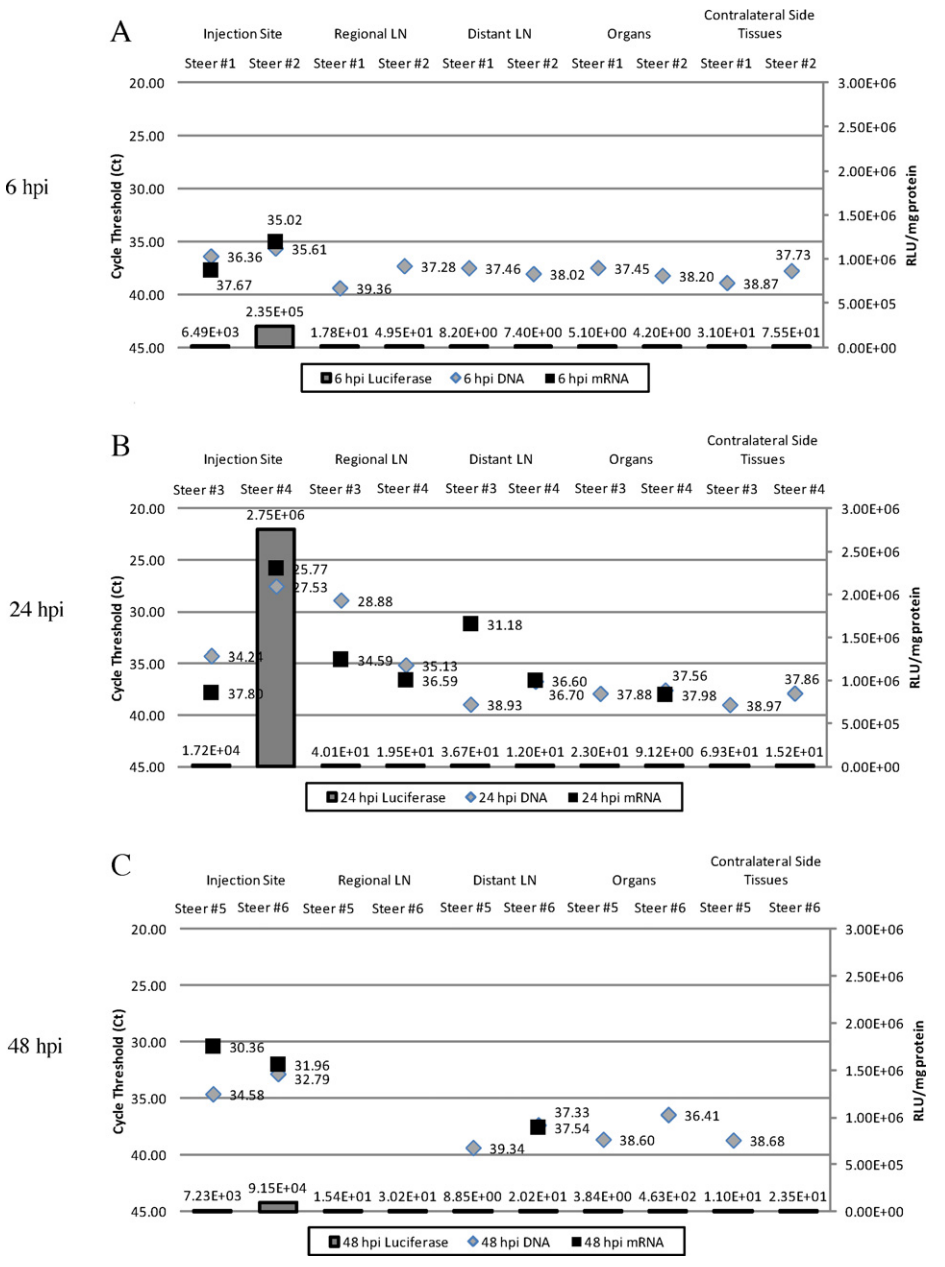

Fig. 3. Detection of rAd5-Luc nucleic acids (mRNA and DNA) and luciferase activity in tissues of inoculated animals euthanized at 6, 24, and 48 hpi. Luciferase detection (columns) was only substantial at injection sites (relative light units (RLU) per mg of total protein). Diamonds correspond to levels of rAd5-Luc DNA (PIX); squares represent detection of rAd5-Luc mRNA (splice). PCR results are expressed as mean Ct values of individual samples assayed in duplicate with minimum detection threshold of Ct < 40.

As early as 6 hpi (steers #1 and #2), rAd5 DNA was detected at the injection site (IS), local LN, and systemically disseminated to visceral organs and distant lymph nodes (Fig. 3A). Ct values ranged between 35 and 40 which were interpreted as low level detection. Mature mRNA transcripts were also detected at low levels at the IS, but not in LN or organs of 6 hpi steers (Ct > 40). Similarly, low level luciferase activity was present in the IS but not in other tissues of the 6 hpi steers (mean = $1.2 \times 10^5 \pm 1.6 \times 10^5$ RLU/mg protein) (Fig. 3A).

At 24 hpi (steers #3 and #4), rAd5 DNA was detected locally at IS and systemically distributed (Fig. 3B). Overall, greater quantities of DNA and RNA (Ct values between 25 and 35) were detected relative to the corresponding tissues of 6 hpi animals. Unlike at 6 hpi, mature (splice) mRNA transcripts were detected in several tissue specimens beyond the IS (local and distant LN and viscera). The lowest Ct value (strongest PCR signal) (Ct = 25.77) was detected at the IS. Moderate quantities of mRNA were also detected in regional (Ct 34.59) and distant (Ct 31.18) LN. Luciferase activity (mean = $1.38 \times 10^6 \pm 1.9 \times 10^6$ RLU/mg

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protein) correlated with the strongest detection by PIX (DNA; Ct = 27.53) and splice (mRNA; Ct = 25.77) PCR (Fig. 3B). Interestingly, luciferase activity was detected at the IS, but not in LN or any other tissues.

Adenovirus biodistribution (DNA) and gene expression (mature mRNA transcripts and luciferase) at 48 hpi (steers # 5 and 6) was largely similar to that of the 6 hpi group (Fig. 3C). As with earlier time points DNA distributed to distant LN and organs. Beyond the IS, rAd5 mRNA transcripts were also found within distant LN but not in other organs. Interestingly, nucleic acids (DNA and mRNA) were undetectable at regional LN. Luciferase activity at 48 hpi was the lowest (mean = $4.8 \times 10^4 \pm 6 \times 10^4$ RLU/mg protein) of the time-course; similar to 6 and 24 hpi, luciferase was generally not detected in regional LN or systemically distributed (Fig. 3C). The only exception was in steer #6 (48 hpi) which had elevated luciferase activity (99-fold above background controls) in the adrenal glands (3×10^3 RLU/mg protein) (data not shown). Similar to 6 and 24 hpi, at 48 hpi luciferase activity correlated well with detection of mature mRNA transcripts at the IS of the two replicates (steer #5 and #6; 7.2×10^3 and 9.1×10^4 RLU/mg protein, and Ct 30.36 and 31.96, respectively) (Fig. 3C).

3.4. Gross pathology and microscopy of injection sites of rAd5-Luc inoculated cattle

Injection sites of all animals were identified postmortem as multifocal, 0.5–1.5 cm intramuscular hemorrhages with variable edema (Fig. 4A–C). One of the 48 hpi steers (#5) had a moderately demarcated, firm, edematous granuloma at the IS in the splenius muscle (Supplemental Figure). The mass was composed of multiple individual firm nodules of 3 cm × 3 cm surrounded by gelatinous edema.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.vetimm.2012.10.003.

Histologically, all IS had variable extents of regions in which normal muscle architecture was multifocally disrupted by extensive edema and cellular infiltrates with limited regional myocyte degeneration and necrosis. Inflammatory infiltrates composed predominantly of large mononuclear cells with fewer neutrophils and small mononuclear cells dissected myofibers following the interstitial connective tissue of the endomysium and perimysium (Fig. 4A–C). At 6 hpi, infiltrates were mild and predominantly perivascular (Fig. 4A). Small foci of granular basophilic material (suggestive of adjuvant) were identified at the IS of one 6 hpi steer (Fig. 4A, inset). At 24–48 hpi, inflammation was more extensive (Fig. 4B and C) with effacement and replacement of myocytes with regionally extensive aggregates of infiltrates and occasional intralymphatic myocyte emboli (Fig. 4B, inset). The intramuscular mass of steer #5 was comprised of numerous microgranulomas often centered upon pools of amphophilic liquid.

Hexon proteins were immunohistochemically localized within or in close proximity to inflammatory infiltrates in all the subjects included in the study. Throughout the time-course, rAd5 hexon signal intensity was low with an observable peak at 24 hpi. Hexon antigens were found inconsistently associated with cells expressing CD14, MHCII (Fig. 4D) and/or CD11c (Fig. 4E) antigens, presumably antigen-presenting cells (APC; dendritic cells and macrophages). There was a rapid influx and increase in signal intensity of cells consistent with the APC phenotype from 6 to 24 hpi (Fig. 4F and G). However, in contrast to hexon signal decrease after 24 hpi, expression of APC antigens reached a plateau by 48 hpi.

The transgene product luciferase was observed within IS tissues immunomicroscopically at all time-points (6, 24, and 48 hpi) studied. Distribution of luciferase immunoreactivity was consistently similar to hexon, associated with mononuclear cells within perimysial connective tissue. At 6 hpi, IHC, but not IF, demonstrated presence of scarce amounts of luciferase antigens at injection sites (Fig. 5A) which increased substantially by 24 hpi (Fig. 5B). As with hexon, signal intensity associated with luciferase antigen detection declined by 48 hpi (data not shown). Within infiltrates, luciferase antigen colocalized to hexon+ cells and in close association with cells of APC phenotype (Fig. 5C and D).

3.5. Microscopy of non-injection site tissues of rAd5-Luc inoculated cattle

Hexon antigens were found in several LN (lateral and medial retropharyngeal LN and infrequently at the axillary and prescapular LN) as early as 6 hpi in the lymphofollicular dark (marginal) zone (Fig. 6A). Luciferase antigens were also detected in lymph nodes at 6 hpi, although at lesser quantities and with distinct distribution relative to hexon. Small clusters of luc+ cells were present in subcapsular and trabecular sinuses and in interfollicular paracortex regions of the prescapular and lateral retropharyngeal LN. Hexon detection in LN was minimal at 24 hpi and completely absent at 48 hpi. However, at 24 hpi, abundant luc+ cells were found at the axillary LN paracortex and sinus regions (Fig. 6B). No luc+ cells were found within lymphoid follicles. Luciferase signal (IHC) was also present at 48 hpi and followed a similar pattern that at 24 hpi; however, at 48 hpi, detection was only limited to the axillary and the prescapular LN. Luciferase (and hexon) immunoreactivity was absent from all other non-injection site tissues analyzed.

4. Discussion

We have previously demonstrated that a replication-defective recombinant human adenovirus 5-Luciferase (rAd5-Luc) vector can be used as a surrogate agent for rAd5-FMDV, providing a convenient and effective screening method in host–vector interaction and vaccination studies in cattle (Montiel et al., 2012). Results of those studies indicated that molecular signatures and cellular events occurring after intramuscular inoculation with a rAd5-Luc vector were remarkably similar to those induced by vaccination with a rAd5-FMDV vector. Expression of the luciferase transgene in tissues of vaccinated animals facilitated localization of the adenovector that further allowed examination of key interactions with various cellular phenotypes of the immune system. In the current work, we hypothesized that rAd5-Luc could be implemented as

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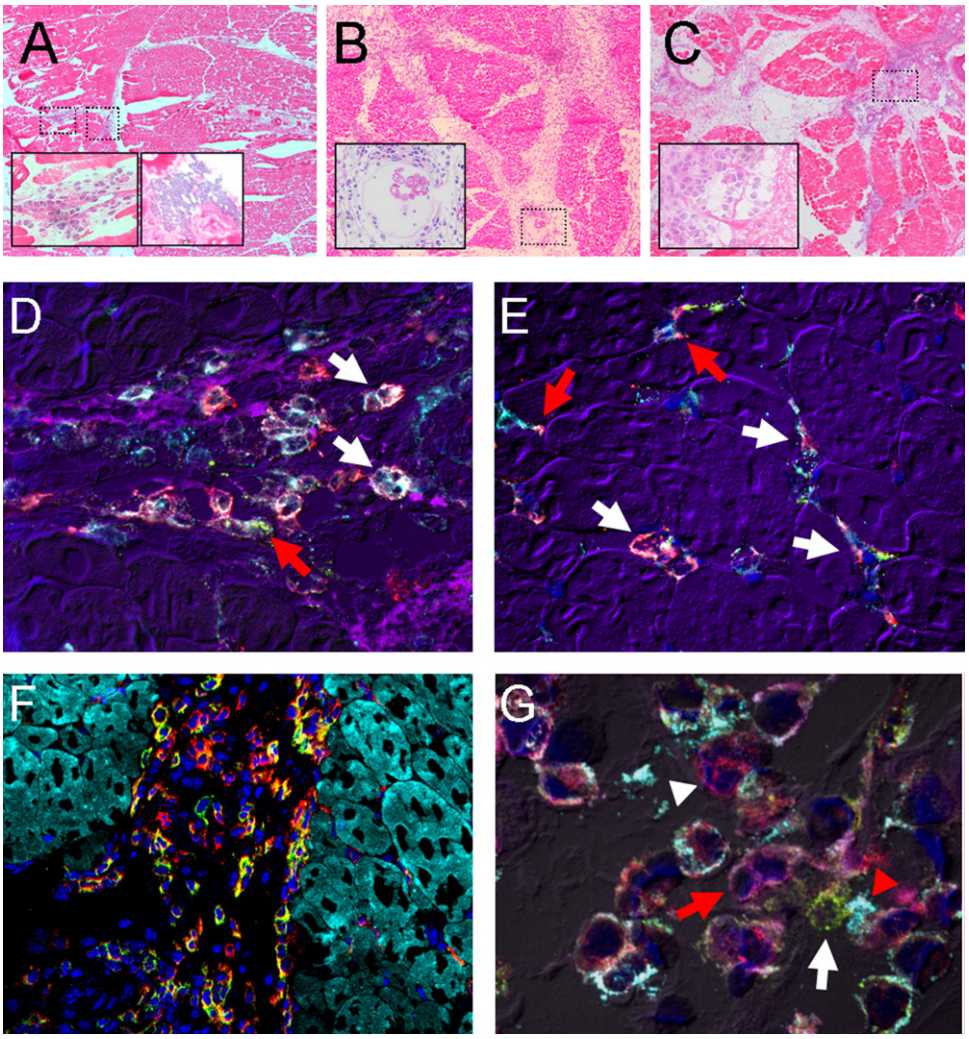

Fig. 4. Microscopy of injection sites (IS) of steers inoculated with rAd5-Luc at 24 hpi. (A–C) Progression of skeletal muscle lesions at IS of steers (#3 and 4) inoculated with adjuvanted rAd5-Luc. (A) 6 hpi, mild perivascular lymphoplasmacytic inflammation (box 1, inset 1) and rare aggregates of extracellular basophilic material (presumptive adjuvant; box 2, inset 2). (B) 24 hpi, severe endomysial–perimysial lymphohistiocytic myositis with intralymphatic myoemboli (inset). (C) 48 hpi, modestly less cellular myositis with edema, fibrin, and predominantly large mononuclear infiltrates (inset). H&E stain. (D) Steer #4, immunofluorescent localization of hexon (green, red arrow), MHCI (light blue), and MHCII (red) antigens to interstitial tissue of endomysium of the IS. Cells expressing MHCI or MHCII complexes, and colocalization of both markers (white arrows) are shown. Collagen (pink). (E) Steer #4, immunofluorescent visualization of hexon (green), MHCII (red), and CD11c (light blue) in between myocytes. Areas with varying degrees of double MHCII-CD11c (red arrows) or triple MHCII-CD11c-hexon (white arrows) colocalization can be seen at the endomysium. Myocyte architecture in purple using differential interference contrast (DIC) technique (in D and E). (F) Steer #4, infiltrated region of intermuscular connective tissue (perymisium) showing separation of myocytes (actin, light blue). Cells positive for CD14 (green) or CD11c (red) are shown. Double positive CD14-CD11c cells are seen as yellow (F). (G) Steer #4, expanded region of infiltrates of IS showing colocalization of cells expressing either CD14 (pink, red arrow), MHCII (green, white arrow), CD172a (red, white arrow head), and CD11c (light blue, red arrow head) or multiple combinations of these markers. Nuclei are shown in blue.

a surrogate agent for time-dependent studies on biodistribution and transgene expression of rAd5-based FMDV vaccines, in vivo. For this purpose, critical events related to rAd5-Luc vector dissemination and expression of transgene products were investigated during a time-course of 72 hours post-inoculation in vitro and during the first 48 h subsequent to intramuscular administration to cattle.

The need for an alternative vector for FMDV adenovirus-based vaccination studies (such as rAd5-Luc) is based upon observations of cytotoxicity (presumably induced by the 3C protease ($3C^{pro}$) in rAd5-FMDV-vectored vaccines) (Moraes et al., 2011). In the current study when rAd5-FMDV and rAd5-Luc were compared, cytopathogenic effects were notably absent in rAd5-Luc inoculated cell cultures but present in rAd5-FMDV-infected cultures. Moreover, both transgene expression levels and cell stability were superior in rAd5-Luc inoculated cells. Detection of CAR, was altered in rAd5-FMDV (all time-points post inoculation), but not in rAd5-Luc cultures, further demonstrating deleterious effects of rAd5-FMDV vectors on cell integrity.

With the use of rAd5-Luc, we demonstrated increased transgene expression (luciferase activity) in inoculated cultures at 24 hpi in relation to earlier time points, with a further peak of luciferase activity at 48 hpi. Microscopy

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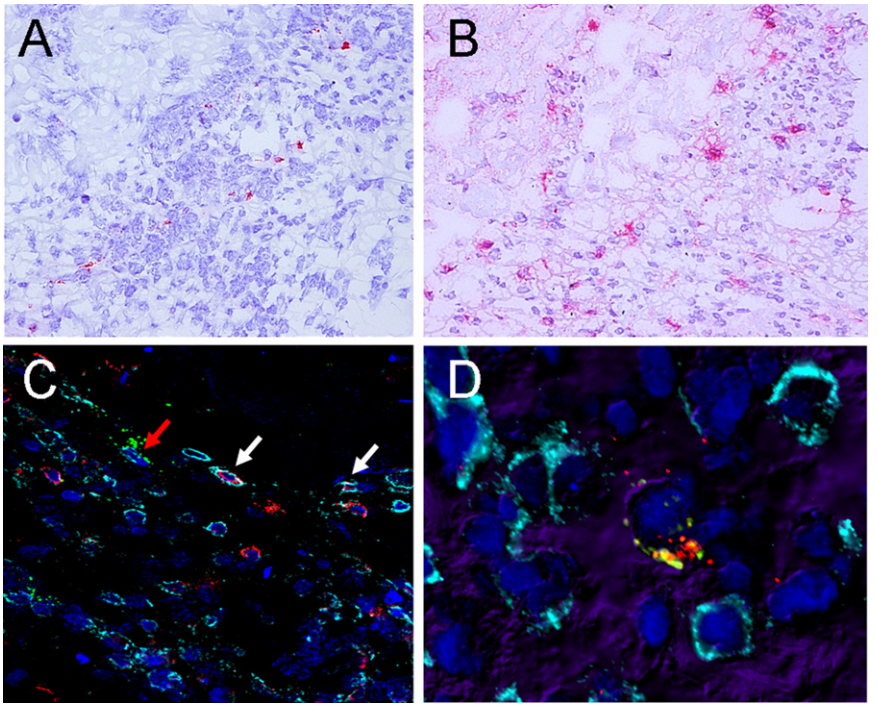

Fig. 5. (A and B) Presence of luciferase antigens in large mononuclear cells is shown by immunohistochemistry at 6 hpi (A) and at 24 hpi (B). (C) Immunofluorescent localization of luciferase (green), MHCII (red), and CD11c (light blue) antigens within infiltrates of the IS of a 24 hpi animal. Luc+CD11c+ colocalization (red arrows) in the proximity to single CD11c+ or MHC II+, or double CD11c+MHCII+ cells (white arrows), presumably corresponding to dendritic cells or macrophages. (D) Serial section of panel C, a double hexon+ (red) luc+ (green) positive cell can be observed surrounded by CD11c+ (light blue) cells. Nuclei are shown in blue.

data also followed the same detection pattern observed by luminometry throughout the time-course. By immunomicroscopy, detection of luciferase proteins was superior to detection of FMDV capsid products. Additionally, detection of increasing levels of luciferase (microscopy) lagged behind detection of rAd5 mRNA transcripts (representing transgene expression) by PCR; rAd5 DNA levels remained steady throughout the time-course for both vectors. As it was previously suggested (Montiel et al., 2012), it is possible that differences in efficiency of post-transcriptional expression of transgenes or reduced sensitivity of the anti-FMDV antibody compared to the anti-Luc antibody may account for differences in microscopic visualization of adenovirus transgene products in cells. However, in the current study, direct comparisons between luminometry results (integrated with PCR data) and microscopy, confirmed high sensitivity and a high degree of correlation of both protein detection-based techniques. This phase of the study demonstrated the utility of the rAd5-Luc vector as a suitable surrogate for study of biodistribution and gene expression in vitro and in vivo.

Consistent with our previously published results (Montiel et al., 2012), subsequent to inoculation with rAd5-Luc, luciferase activity was detected only at the injection site of all the experimental subjects with a statistically significantly higher peak of activity at 24 hpi followed by a rapid decrease by 48 hpi. Unlike luciferase activity, severity of the inflammatory infiltrates in response to inoculation also reached a plateau at 24 hpi and remained high at 48 hpi. Together, these observations confirmed (1) active production of transgene protein products (luciferase) and (2) rapid and efficient recruitment of leukocytes to the sites of rAd5 inoculum-induced inflammation. Steady levels of inflammatory infiltrates, including presence of macrophages, and a rapid rise and fall of luciferase activity during the first 48 hpi is consistent with previous studies that indicate that during the first 24 h after muscle injury phagocytic macrophages are attracted to inflamed areas to degrade damaged muscle (Schiaffino and Partridge, 2008). These phagocytic functions of macrophages could partially explain the short-lived presence of luciferase and the substantial differences in luciferase detection between 24 and 48 hpi in muscle. Furthermore, immunomicroscopy of injection sites demonstrated that luciferase followed a multifocal and heterogeneous distribution in muscle with varying degrees of association with cells of the infiltrates. Consistent with luciferase activity, visualization of luciferase proteins in muscle increased from 6 to 24 hpi followed by a rapid decrease in detection at 48 hpi. Therefore, it is likely that following inoculation with rAd5-Luc, specific target cells are efficiently infected, luciferase is effectively produced and detected at the highest levels at 24 hpi, and rapidly commences to be degraded within phagocytic macrophages.

Alternatively, it has been proposed that IFN-γ induced by innate anti-adenoviral immune responses inhibit adenovirus-mediated transgene expression (Sung et al., 2001), and that the actions of anti-IFN-γ antibodies can

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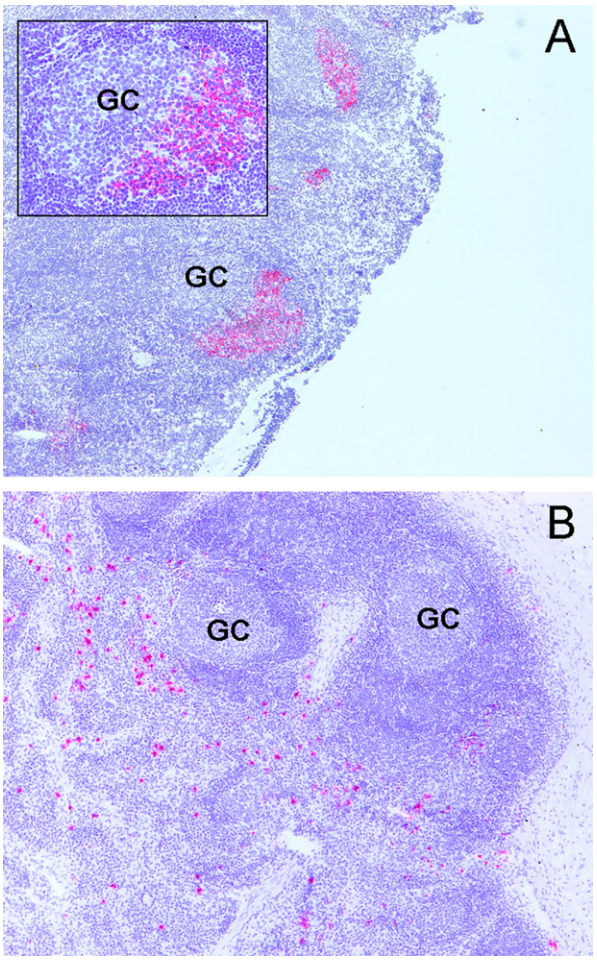

Fig. 6. Immunohistochemical detection of hexon and luciferase antigens in local LN of rAd5-Luc inoculated steers. (A) Steer #1, hexon signal is observed at the cortex of one of the medial retropharyngeal LN within primary lymphoid follicles, at 6 hpi. Note hexon-laden mantle zones of primary lymphoid follicle (A, inset). (B) Steer #3, luciferase positive cells are seen scattered throughout the perifollicular cortex, subcortex, and corticomedullary junction of the axillary LN of rAd5-Luc inoculated steer #3, at 24 hpi. GC, germinal center. Micropolymer alkaline phosphatase technique.

reverse these effects, enhancing immunogenicity of recombinant adenovirus vectors (Jackson et al., 2011). During the acute phase of inflammation and immune responses, IFN-γ is produced by NK cells and antigen-presenting cells (APCs), especially interleukin-12 -stimulated macrophages and dendritic cells (Frucht et al., 2001). Thus, it is plausible that our observations could also be attributed to a combination of both phagocytic effects of macrophages and actions of IFN-γ during and after the first 24 hpi. While it is possible that discrepancies observed on levels of luciferase detected by luminometry and/or microscopy may be due to intrinsic variability of the techniques and or to inter-animal variation factors, our data strongly suggest that the majority of the effects observed are attributable to true biological processes in response to rAd5 infection.

We previously reported that detection of rAd5 DNA and rAd5 mRNA transcripts in tissues of rAd5-FMDV-vaccinated cattle elucidated important aspects of adenovirus distribution and transgene expression (Montiel et al., 2012). In the current study, patterns of biodistribution and expression of rAd5-Luc-derived nucleic acids were remarkably similar to those of a rAd5-FMDV (reported in our previous study). A cross-analysis of luminometry, PCR, and immunomicroscopy results clearly demonstrates correlation across detection techniques. In muscle, the highest levels of mRNA transgene expression corresponded to strong detection of luciferase (by luminometry) at 24 hpi. However, at 48 hpi, despite abundant rAd5 mRNA (Ct values between 30 and 32) at the IS, luciferase activity was found at surprisingly lower levels. This may be explained by selective phagocytic elimination of the subset of infected cells with active translation of luciferase; thus PCR signal may persist from infected (translationally inactive) cells that remain in tissues. Alternatively, luciferase may be degraded within the phagolysosomal compartment, whereas nucleic acids (particularly viral DNA) persist in a distinct cellular compartment.

While detection of luciferase in muscle was consistently demonstrated by microscopy and luminometry, luciferase activity was only marginally present in all other tissue specimens examined, including local and regional LN. This finding is consistent with observations for our previous report where luciferase was only present at the injection site of a single rAd5-Luc inoculated steer. It is likely that lack of detection is due to the dilution effect created by inoculation of a replication-incompetent vector into a 300 kg steer. Alternatively this may represent suboptimal sensitivity of the luciferase bioassay in non-injection sites as compared to immunomicroscopy. It has been shown that both liver and adrenal are major targets for both wild-type adenoviruses and recombinant vectors (Bernt et al., 2003; Duncan et al., 1978; Wang et al., 2003; Ying et al., 2009). While hepatic tissue has been demonstrated to be the principal organ for adenovirus sequestration (Shayakhmetov et al., 2004), persistent transgene expression and functional gene activity in adrenal tissue of animals undergoing gene transfer experiments using rAd has been reported by several groups (Alesci et al., 2007; Senoo et al., 2000; Ying et al., 2009). Our observations of low level luciferase activity detected in adrenal tissue are supported by the published studies. Complete lack of detection in liver remains confounding. However, non-canonical tropism may be the result of atypical tropism of a human rAd5 vector interacting with bovine tissues and cells.

In agreement with previous reports (Banchereau and Steinman, 1998; Guidotti and Chisari, 2001; Montiel et al., 2012; Steinman et al., 1999), the rapid recruitment of dendritic cells and macrophages to injection sites in areas where hexon proteins and transgene product (luciferase) localize, is highly suggestive of critical early immunological events triggered by inoculation with the rAd5 vector. Importantly, subsequent to inoculation with rAd5-Luc, a peak of hexon and transgene protein expression and detection occurs at 24 hpi followed by a rapid decrease by 48 hpi, while expression of various combinations of CD14+, CD11c+, MHCI+, MHCII+ phenotypes within cellular infiltrates reach steady levels after 24 hpi. In addition, during

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the first 48 hpi, luciferase proteins were detected in cortical and interfollicular regions of some of the local LN. Overall, these findings suggest that at the site of inoculation, adenovirus and transgene products are first captured and processed for antigen presentation by APCs through MHC I and/or II -dependent mechanisms, followed by rapid trafficking of transgene product (free or cell-associated) to lymphoid tissues (lymph nodes) where induction of protective immune responses is presumably initiated. The extent to which this trafficking was modulated by the adjuvant used in this study remains undetermined; however microscopic localization of adjuvant at 6 hpi and adjuvant-associated inflammation at 48 hpi suggest a contributory role in the immunopathogenesis.

5. Conclusions

Recent work (Montiel et al., 2012) has documented the biodistribution in cattle of a recombinant Ad5 construct encoding FMDV capsid antigens (rAd5-FMDV) which is the first recombinant vaccine granted conditional license for use in cattle by the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service's Center for Veterinary Biologics (CVB) (Press release[1,2]). In the present study, we assessed biodistribution and transgene expression of a rAd5 containing the luciferase gene and demonstrated that it behaves remarkably similar to rAd5-FMDV. Thus, these baseline descriptions obtained by the former and the current work characterizing rAd5 inoculation in cattle will contribute to a better understanding of rAd5-FMD vaccine immunopathogenesis and toward efforts to enhance adenoviral-based FMDV vaccine potency and efficacy.

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[1] http://dairybusiness.com/dairyline_headline.php?item=New+Foot-And-Mouth+Disease+Vaccine+Gets+Licensed.

[2] http://benchmarkbiolabs.com/2012/06/a-world-free-one-most-virulent-animal-diseases-planet/.

References

Alesci, S., Perera, S.M., Lai, E.W., Kukura, C., Abu-Asab, M., Tsokos, M., Morris, J.C., Pacak, K., 2007. Adenoviral gene transfer in bovine adrenomedullary and murine pheochromocytoma cells: potential clinical and therapeutic relevance. Endocrinology 148, 3900–3907.

Arzt, J., Gregg, D.A., Clavijo, A., Rodriguez, L.L., 2009. Optimization of immunohistochemical and fluorescent antibody techniques for localization of foot-and-mouth disease virus in animal tissues. J. Vet. Diagn. Invest. 21, 779–792.

Banchereau, J., Steinman, R.M., 1998. Dendritic cells and the control of immunity. Nature 392, 245–252.

Bernt, K.M., Ni, S., Gaggar, A., Li, Z.Y., Shayakhmetov, D.M., Lieber, A., 2003. The effect of sequestration by nontarget tissues on anti-tumor efficacy of systemically applied, conditionally replicating adenovirus vectors. Mol. Ther. 8, 746–755.

Doel, T.R., 2003. FMD vaccines. Virus Res. 91, 81–99.

Duncan, S.J., Gordon, F.C., Gregory, D.W., McPhie, J.L., Postlethwaite, R., White, R., Willcox, H.N., 1978. Infection of mouse liver by human adenovirus type 5. J. Gen. Virol. 40, 45–61.

Everett, R.S., Gerrard, D.E., Grant, A.L., 2000. Factors affecting production of luciferase and epitope-tagged IGF-I in porcine muscle after DNA injection. J. Endocrinol. 166, 255–263.

Fauquet, C., Mayo, M., Maniloff, J., Desselberger, U., Ball, L., 2005. Virus Taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press, San Diego, CA.

Frucht, D.M., Fukao, T., Bogdan, C., Schindler, H., O'Shea, J.J., Koyasu, S., 2001. IFN-gamma production by antigen-presenting cells: mechanisms emerge. Trends Immunol. 22, 556–560.

Graham, F.L., Smiley, J., Russell, W.C., Nairn, R., 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. 36, 59–74.

Grubman, M., Moraes, M., Schutta, C., Barrera, J., Neilan, J., Ettyreddy, D., Butman, B., Brough, D., Brake, D., 2010. Adenovirus serotype 5-vectored foot-and-mouth disease subunit vaccines: the first decade. Future Virol. 5, 51–64.

Grubman, M.J., Baxt, B., 2004. Foot-and-mouth disease. Clin. Microbiol. Rev. 17, 465–493.

Guidotti, L.G., Chisari, F.V., 2001. Noncytolytic control of viral infections by the innate and adaptive immune response. Annu. Rev. Immunol. 19, 65–91.

Jackson, S.S., Schmitz, J.E., Letvin, N.L., 2011. Anti-gamma interferon antibodies enhance the immunogenicity of recombinant adenovirus vectors. Clin. Vaccine Immunol. 18.

Manthorpe, M., Cornefert-Jensen, F., Hartikka, J., Felgner, J., Rundell, A., Margalith, M., Dwarki, V., 1993. Gene therapy by intramuscular injection of plasmid DNA: studies on firefly luciferase gene expression in mice. Hum. Gene Ther. 4, 419–431.

Mayr, G.A., Chinsangaram, J., Grubman, M.J., 1999. Development of replication-defective adenovirus serotype 5 containing the capsid and 3C protease coding regions of foot-and-mouth disease virus as a vaccine candidate. Virology 263, 496–506.

Montiel, N., Smoliga, G., Arzt, J., 2012. Early detection and visualization of human adenovirus serotype 5-viral vectors carrying foot-and-mouth disease virus or luciferase transgenes in cell lines and bovine tissues. Vaccine.

Moraes, M.P., Mayr, G.A., Mason, P.W., Grubman, M.J., 2002. Early protection against homologous challenge after a single dose of replication-defective human adenovirus type 5 expressing capsid proteins of foot-and-mouth disease virus (FMDV) strain A24. Vaccine 20, 1631–1639.

Moraes, M.P., Segundo, F.D., Dias, C.C., Pena, L., Grubman, M.J., 2011. Increased efficacy of an adenovirus-vectored foot-and-mouth disease capsid subunit vaccine expressing nonstructural protein 2B is associated with a specific T cell response. Vaccine 29, 9431–9440.

Pacheco, J.M., Brum, M.C., Moraes, M.P., Golde, W.T., Grubman, M.J., 2005. Rapid protection of cattle from direct challenge with foot-and-mouth disease virus (FMDV) by a single inoculation with an adenovirus-vectored FMDV subunit vaccine. Virology 337, 205–209.

Pacheco, J.M., Piccone, M.E., Rieder, E., Pauszek, S.J., Borca, M.V., Rodriguez, L.L., 2010. Domain disruptions of individual 3B proteins of foot-and-mouth disease virus do not alter growth in cell culture or virulence in cattle. Virology 405, 149–156.

Please cite this article in press as: Montiel, N.A., et al., Time-dependent biodistribution and transgene expression of a recombinant human adenovirus serotype 5-luciferase vector as a surrogate for rAd5-FMDV vaccines in cattle. Vet. Immunol. Immunopathol. (2012), http://dx.doi.org/10.1016/j.vetimm.2012.10.003

Rodriguez, L.L., Gay, C.G., 2011. Development of vaccines toward the global control and eradication of foot-and-mouth disease. Expert Rev. Vaccines 10, 377–387.

Rodriguez, L.L., Grubman, M.J., 2009. Foot and mouth disease virus vaccines. Vaccine 27 (Suppl. 4), D90–D94.

Sanz-Parra, A., Jimenez-Clavero, M.A., Garcia-Briones, M.M., Blanco, E., Sobrino, F., Ley, V., 1999a. Recombinant viruses expressing the foot-and-mouth disease virus capsid precursor polypeptide (P1) induce cellular but not humoral antiviral immunity and partial protection in pigs. Virology 259, 129–134.

Sanz-Parra, A., Vazquez, B., Sobrino, F., Cox, S.J., Ley, V., Salt, J.S., 1999b. Evidence of partial protection against foot-and-mouth disease in cattle immunized with a recombinant adenovirus vector expressing the precursor polypeptide (P1) of foot-and-mouth disease virus capsid proteins. J. Gen. Virol. 80 (Pt 3), 671–679.

Schiaffino, S., Partridge, T., 2008. Skeletal muscle repair and regeneration. Adv. Muscle Res. 3, 380.

Senoo, M., Matsubara, Y., Fujii, K., Nagasaki, Y., Hiratsuka, M., Kure, S., Uehara, S., Okamura, K., Yajima, A., Narisawa, K., 2000. Adenovirus-mediated in utero gene transfer in mice and guinea pigs: tissue distribution of recombinant adenovirus determined by quantitative TaqMan-polymerase chain reaction assay. Mol. Genet. Metab. 69, 269–276.

Shayakhmetov, D.M., Li, Z.Y., Ni, S., Lieber, A., 2004. Analysis of adenovirus sequestration in the liver, transduction of hepatic cells, and innate toxicity after injection of fiber-modified vectors. J. Virol. 78, 5368–5381.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76–85.

Steinman, R.M., Inaba, K., Turley, S., Pierre, P., Mellman, I., 1999. Antigen capture, processing, and presentation by dendritic cells: recent cell biological studies. Hum. Immunol. 60, 562–567.

Sung, R.S., Qin, L., Bromberg, J.S., 2001. TNFalpha and IFNgamma induced by innate anti-adenoviral immune responses inhibit adenovirus-mediated transgene expression. Mol. Ther. 3, 757–767.

Swaney, L.M., 1988. A continuous bovine kidney cell line for routine assays of foot-and-mouth disease virus. Vet. Microbiol. 18, 1–14.

Torres, J.M., Alonso, C., Ortega, A., Mittal, S., Graham, F., Enjuanes, L., 1996. Tropism of human adenovirus type 5-based vectors in swine and their ability to protect against transmissible gastroenteritis coronavirus. J. Virol. 70, 3770–3780.

Wang, Y., Groot-Wassink, T., Lemoine, N.R., Vassaux, G., 2003. Cellular characterization of the tropism of recombinant adenovirus for the adrenal glands. Eur. J. Clin. Invest. 33, 794–798.

Wolff, J.A., Malone, R.W., Williams, P., Chong, W., Acsadi, G., Jani, A., Felgner, P.L., 1990. Direct gene transfer into mouse muscle in vivo. Science 247, 1465–1468.

Ying, B., Toth, K., Spencer, J.F., Meyer, J., Tollefson, A.E., Patra, D., Dhar, D., Shashkova, E.V., Kuppuswamy, M., Doronin, K., Thomas, M.A., Zumstein, L.A., Wold, W.S., Lichtenstein, D.L., 2009. INGN 007, an oncolytic adenovirus vector, replicates in Syrian hamsters but not mice: comparison of biodistribution studies. Cancer Gene Ther. 16, 625–637.

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