Efficient dengue virus (DENV) infection of human muscle satellite cells upregulates type I interferon response genes and differentially modulates MHC I expression on bystander and DENV-infected cells

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Dengue virus (DENV) is a mosquito-borne flavivirus that causes an acute febrile disease in humans, characterized by musculoskeletal pain, headache, rash and leukopenia. The cause of myalgia during DENV infection is still unknown. To determine whether DENV can infect primary muscle cells, human muscle satellite cells were exposed to DENV in vitro. The results demonstrated for the first time high-efficiency infection and replication of DENV in human primary muscle satellite cells. Changes in global gene expression were also examined in these cells following DENV infection using Affymetrix GeneChip analysis. The differentially regulated genes belonged to two main functional categories: cell growth and development, and antiviral type I interferon (IFN) response genes. Increased expression of the type I IFN response genes for tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), melanoma-derived antigen 5 (MDA-5), IFN-α-inducible protein 10 (IP-10), galectin 3 soluble binding protein (LGals3BP) and IFN response factor 7 (IRF7) was confirmed by quantitative RT-PCR. Furthermore, higher levels of cell-surface-bound intracellular adhesion molecule-1 (ICAM-1) and soluble ICAM-1 in the cell-culture medium were detected following DENV infection. However, DENV infection impaired the ability of the infected cells in the culture medium to upregulate cell-surface expression of MHC I molecules, suggesting a possible mechanism of immune evasion by DENV. The findings of this study warrant further clinical research to identify whether muscle cells are targets for DENV infection during the acute stage of the disease in vivo.

INTRODUCTION

Dengue is an increasingly troublesome, mosquito-borne viral disease that is highly prevalent in both tropical and subtropical regions of the world. Annually, 50–100 million cases of dengue fever (DF) and 250,000 cases of dengue haemorrhagic fever (DHF) occur worldwide, as determined by the World Health Organization (Nathan & Dayal-Drager, 2006; Petersen & Marfin, 2005). Infection with any one of the four serotypes of DENV (DENV1–4) can cause DF or DHF. The general mechanisms that control virus replication and are involved in disease pathogenesis are not well understood in this complex, acute illness.

Monocytes, dendritic cells and B cells are known to be susceptible to DENV in vivo (Halstead, 1989; King et al., 1999; Lin et al., 2002; Marovich et al., 2001; Wu et al., 2000), whilst DENV antigen has been detected in sinusoidal endothelial cells (Jessie et al., 2004) and hepatocytes (de Macedo et al., 2006; Rosen et al., 1999) in autopsy studies. In addition, a variety of primary human cells, including mast cells (King et al., 2002), endothelial
cells (Warke et al., 2003), epithelial cells (Lee et al., 2007) and hepatocytes (Suksanpaisan et al., 2007), can serve as hosts for DENV in vitro.

Symptomatically, patients with dengue often present with general muscle affection as well as severe, persisting myalgia, headache and rash (Chaturvedi et al., 1970; Halstead, 1966). Higher serum levels of creatine phosphokinase (CPK), which is specifically produced by muscle cells (Kalita et al., 2005), have been found in DENV patients (Kalita et al., 2005; Malheiro et al., 1993; Rajajee et al., 2005). Similar to DENV infection, individuals affected with Chikungunya virus present with persisting myalgia (Chaturvedi et al., 1970; Halstead, 1966; Weaver, 2005) and a recent study involving muscle biopsies of acutely infected individuals demonstrated that Chikungunya virus infects muscle satellite cells but not differentiated multinucleated fibres (myotubes) (Ozden et al., 2007). These findings suggest that myalgia, muscle weakness and elevated serum CPK levels in dengue might be a result of direct virus infection of muscle satellite cells.

No clinical report published to date has looked at the presence of DENV in muscle cells. However, two clinical studies have reported myositis during acute DENV infection (Kalita et al., 2005; Rajajee et al., 2005). Previous studies of DENV infection in mice have reported biochemical and ultrastructural changes in skeletal muscle (Nath et al., 1982; Rajajee et al., 2005), as well as the detection of infectious DENV progeny in the muscles (Agrawal et al., 1978). Fatal myocarditis has also been observed during acute DENV infection (I. Villalobos & J. Rodriguez, personal communication).

Muscle satellite cells are resident, proliferative cells found in the skeletal muscle (Allen et al., 1997; Campion, 1984; Ozden et al., 2007). These cells express muscle-specific markers such as neural adhesion molecule (NCAM or CD56) and desmin, a type III intermediate filament protein (Belles-Isles et al., 1993; Brady et al., 2005; Illa et al., 1992) and will spontaneously fuse to form mature, contractile, skeletal muscle (Campion, 1984). Skeletal muscle satellite cells can be cultured in vitro from human muscle biopsy tissues as undifferentiated, mononucleated cells, and their proliferative capacity makes them an ideal model for in vitro infection studies of skeletal muscle. Desmin and CD56 are both considered reliable markers for muscle satellite cells among cells cultured from skeletal muscle (Ozden et al., 2007; Stewart et al., 2003; Zheng et al., 2006). Fibroblasts, which co-propagate in cell culture, do not express CD56 (Allen et al., 1997).

Previous in vitro studies have shown that various immunological and/or inflammatory stimuli can induce or increase expression of human MHC I, intracellular adhesion molecule 1 (ICAM-1), co-stimulatory/inhibitory molecules (CD40 and PD-L1) and cytokines (interleukin-6 and transforming growth factor-β) in muscle satellite cells (Marino et al., 2001, 2003; Wiendl et al., 2005). Although these proteins are expressed below detectable levels in vivo, their expression levels increase in various inflammatory myopathies (Marino et al., 2001, 2003; Wiendl et al., 2005).

To determine whether these cells could serve as a host for DENV infection, we exposed them to DENV in vitro and determined their susceptibility to a productive DENV infection. We demonstrated in vitro infection and replication of DENV in primary human muscle satellite cells using several independent methods. We also determined the activation level of these DENV-infected cells and confirmed changes in expression levels of several type I interferon (IFN)-inducible genes. In light of these findings, we suggest further studies to determine whether these in vitro findings hold true for muscle cells in vivo during acute DENV infection.

**METHODS**

**Preparation of muscle satellite cells.** Cryopreserved muscle satellite cells were derived from muscle biopsies taken from the thigh muscle of healthy human donors and were donated by Mytogen. The donor biopsies were obtained under an approved clinical study protocol (Dib et al., 2005). The donor identification protocol number was erased after cell preparation and culture of the satellite cells. Connective tissue was removed by placing the biopsy samples in Ringer’s solution with heparin followed by three cycles of enzymic digestion (0.5 mg trypsin ml⁻¹, 0.5 mg collagenase ml⁻¹). The first cycle of enzymic digestion was carried out for 60 min, followed by two 40 min cycles. The temperature was maintained at 37 °C and a total volume of 5 ml fresh digestion mixture was used for each cycle. After each cycle of digestion, the cells were trypsinized and centrifuged in complete growth medium at 1000 r.p.m. for 5 min for 4 °C. Cells were seeded in one T175 flask per digestion cycle. All cells were expanded for up to 10–12 cell doublings in order to optimize yield and viability and were then cryopreserved. The muscle satellite cells were thawed and grown in Dulbecco’s minimal essential medium containing 15% fetal calf serum (FCS), recombinant human epidermal growth factor (10 ng ml⁻¹) and dexamethasone (0.39 μg ml⁻¹). Muscle satellite cells at approximately 15 cell doublings were used for experiments in this study. No myotubes were evident by microscopic inspection of the culture at the time of infection. The conditions described above are considered to be optimal for muscle satellite cell phenotype and survival for transplantation purposes (Dib et al., 2005).

**Infection of cells.** Monolayers of muscle satellite cells maintained in fully supplemented medium were washed with fresh cell-culture medium with no FCS. DENV2 strain New Guinea C (NGC), previously grown in Aedes albopictus C6/36 cell monolayers and titrated in Vero cells, was added to confluent monolayers of muscle satellite cells at an m.o.i. of 1–2. The cell-culture supernatant was removed after 2 h, the monolayers were carefully washed three times and fresh growth medium containing 10% FCS was added to each well. After 48 h of infection, the culture supernatant was collected and stored at –70 °C for plaque assays and ELISA. The cells were trypsinized and centrifuged twice at 700 g for 5 min each and kept on ice for antibody staining or stored at –70 °C for RNA extraction. Cells were infected with DENV2 strain NGC, DENV1 strain Hawaii or DENV4 strain 814669. The DENV1 Hawaii, DENV2 NGC and DENV4 814669 serotypes of DENV used to infect the muscle satellite cells were only passaged three, three and five times, respectively, in C6/36 cells from Aedes albopictus larvae (ATTC CRL-1660).
Detection of dengue virus

Transmission electron microscopy (TEM). Muscle satellite cells were infected with DENV2. At 48 h post-infection (p.i.), both uninfected and DENV-infected muscle satellite cells were fixed overnight at 4 °C with freshly prepared 2.5 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) and then embedded in epoxy resin. Processed samples were cut into ultrathin sections (70 nm) using a diamond knife, stained with uranyl acetate and lead citrate, and examined under a Philips CM 10 transmission electron microscope. Images were obtained using a digital photographic device and scales were digitally drawn using the Gatan Digital Micrograph imaging software.

Flow cytometry. Uninfected and DENV-infected muscle satellite cells were stained at 24 and/or 48 h p.i. Antibodies used in this study were FITC-conjugated anti-DENV-complex monoclonal antibody (clone D3-2H2-219; Chemicon), phycoerythrin (PE)-conjugated anti-CD56 (clone B159; Becton Dickinson), allophycocyanin (APC)-conjugated anti-MHC I (clone G46-2.6; Becton Dickinson) and PE-conjugated anti-ICAM-1 (clone HA58; Becton Dickinson). Permeabilization and fixation of cells was done using a Cytofix/Cytoperm Plus kit (Pharmingen). The percentage of DENV-infected muscle satellite cells was assessed using a FACSArray Flow Cell Sorter (Becton Dickinson). FlowJo version 6.3.1 software (Tree Star) was used to analyse the data. Live cells were gated on forward scatter (FS) and side scatter (SS) plots. CD56-positive cells were selected and further analysis was restricted to this population. Cell doubles and aggregates were eliminated from the analysis by electronic doublet discrimination to decrease the number of false positives.

Immunofluorescence. Cells plated on 22 mm glass coverslips were infected with DENV2 (m.o.i. of 2) for 2 h at 37 °C. At 48 h p.i., cells were washed and fixed with 4 % paraformaldehyde for 15 min at room temperature. Cells were rinsed with PBS, permeabilized with 0.5 % Triton X-100 for 5 min on ice, washed and incubated for 1 h with rabbit anti-desmin polyclonal antibody (clone H76; Santa Cruz Biotechnology) at 1:150 dilution, and anti-DENV–FITC monoclonal antibody at 1:200 dilution. Cells were washed and then incubated for 1 h with PE-conjugated goat anti-rabbit IgG (Sigma-Aldrich). Coverslips were mounted on slides using polyvinyl alcohol mounting medium with Dabco anti-fading agent (Sigma-Aldrich). Images of stained cells were obtained using a Nikon Eclipse E800 microscope fitted with an RT Slider Spot camera (Diagnost Instruments) and processed using Adobe Photoshop 8.0 professional.

TaqMan quantitative (q)RT-PCR. TaqMan quantification of DENV RNA was performed as described previously (Warke et al., 2003). TaqMan primers and probes for tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), melanoma-derived antigen 5 (MDA-5), IFN-γ-inducible protein 10 (IP-10), galectin 3 soluble binding protein (LGals3BP) and IFN response factor 7 (IRF7) were obtained from Applied Biosystems. Results were calculated using a relative quantification method using qPCR software (Applied Biosystems). β-Actin was used as an endogenous control to equalize loading of total RNA among samples. Each data point was measured in triplicate.

Plaque assay. Muscle satellite cells were infected with DENV as described above. Culture supernatants were collected at 24, 48 h p.i. and at 0 and 48 h p.i. for DENV2 and DENV4, respectively. DENV titres were determined by plaque assay using LLCMK2 cells (a rhesus monkey kidney cell line). Serial 10-fold dilutions of cell-culture supernatants were adsorbed for 2 h onto duplicate wells. Cells were washed with PBS and covered with an overlay containing 1 % medium-viscosity carboxymethyl cellulose (Sigma-Aldrich) in 2 x minimal essential medium (Gibco) supplemented with 10 % FCS. On day 6, cells were fixed and stained with crystal violet. Plaques were counted and titres expressed as p.f.u. ml⁻¹.

Affymetrix GeneChip analysis. The GeneChip hybridization and analysis protocol published by Warke et al. (2008) was followed in this study. We assessed the expression levels of genes known to be transcriptionally induced by type I IFN in DENV-infected muscle satellite cells at 48 h p.i. For comparison, gene expression levels were also measured in primary human monocytes, which are known to support DENV infection and are a primary site of DENV replication in vivo (Halstead, 1989). Type I IFN-inducible gene expression responses have been characterized previously using DNA microarrays in HUVEC (primary endothelial) cells by Indraccolo et al. (2007). The gene expression levels of the top 50 genes induced by type I IFN treatment (1000 IU ml⁻¹ for 5 h) in that study were compared with DENV-infected muscle satellite cells and monocytes. In our experiments, DENV infection of muscle satellite cells and monocytes induced 88 and 94 % of these genes, respectively, more than twofold.

RESULTS

DENV infection of muscle satellite cells

Primary human muscle satellite cells (CD56-positive cells) were infected with low-passage DENV2 strain NGC, DENV1 strain Hawaii or DENV4 strain 814669 at an m.o.i. of 2 for 24 or 48 h and stained for the presence of DENV antigens using flow cytometry. CD56 surface antigen (Stewart et al., 2003) was used for gating muscle satellite cells. Our gating strategy consisted of initially gating live cells based on SS and FS plots. The live cells were further gated on CD56 antigen and SS to eliminate CD56-negative cells from the analysis in order to identify cells that were positive for both DENV and CD56. Approximately 15–30 % of the live cells did not stain positively for CD56 (data not shown). Intracellular staining with a DENV-specific antibody was used to determine the percentage of infected cells. High proportions of muscle satellite cells stained positively for DENV2 antigen at the 24 h (16.9 ± 4.2 %, n = 3) and 48 h (9.5 ± 3.4 %, n = 3) time points (Fig. 1a and b). As DENV NGC is a prototype strain of DENV, we investigated whether DENV1 and DENV4 strains could also productively infect muscle satellite cells.

Muscle satellite cells also stained positively for DENV1 (6.6 ± 2.7 %, n = 2) and DENV4 (21.5 ± 12.3 %, n = 2) antigens (Fig. 1b) as determined by flow cytometry. A small subset (0.1–1.5 %) of the CD56-negative cells, which are mostly fibroblasts (Allen et al., 1997), stained positively for DENV antigen (data not shown). These results demonstrated that muscle satellite cells are susceptible to infection with multiple DENV serotypes in vitro.

As an additional means of demonstrating DENV infection and to determine the subcellular localization of DENV, we examined DENV-infected muscle satellite cells by TEM. Clusters of virus-like particles of the expected 40–60 nm diameter (Lee et al., 2007; Zhang et al., 2003) were detected inside cytoplasmic vacuoles in DENV-infected cells (Fig. 2a). Perinuclear actinomysin fibre bundles were detected in the cells containing virus-like particles (Fig. 2a). We also demonstrated the direct presence of DENV...
antigen in muscle satellite cells using immunofluorescence microscopy. Cells were grown on microscope coverslips overnight and then infected with DENV2 at an m.o.i. of 2. After 48 h, cells were fixed and stained for DENV and desmin. Desmin and DAPI double-positive cells clearly stained positive for DENV antigen in the cytoplasm (Fig. 2b). DENV staining was spotty and localized around the nucleus (perinuclear) (Fig. 2b).

The presence of intracellular, positive-strand DENV RNA was confirmed by qRT-PCR (Fig. 3a). We performed a multistep growth curve for DENV2 to determine the production and release of infectious virus at 0, 24 and 48 h p.i. Very few infectious virus particles were detected in the supernatant at the 0 h time point. The multistep growth curve showed an increase in infectious DENV produced by muscle satellite cells at both 24 (10^2–10^5 p.f.u. ml^-1) and 48 (10^5–10^6 p.f.u. ml^-1) h p.i. (Fig. 3b). This result confirmed that muscle satellite cells are susceptible to DENV infection (Fig. 1a and b) and support replication of DENV (Fig. 3b). High levels of infectious DENV progeny (10^5–10^6 p.f.u. ml^-1) were also detected in the supernatant of DENV4-infected muscle satellite cells using plaque assays (Fig. 3b). DENV titres in culture supernatants correlated positively with the levels of DENV antigen staining by flow cytometry analysis (Figs 1b and 3b). Taken together, these results demonstrate that DENV can both enter and replicate in muscle satellite cells, producing infectious progeny in vitro.

MHC I and ICAM-1 cell-surface expression following DENV infection

Primary endothelial cells and monocyte-derived dendritic cells, which are host cells for DENV infection, become activated and upregulate MHC molecules and ICAM-1
after DENV infection (Libraty et al., 2001; Peyrefitte et al., 2006). Hence, we assessed whether DENV infection increased the cell-surface levels of these proteins on muscle satellite cells. MHC I expression was found to be upregulated following DENV infection in muscle satellite cell cultures (Fig. 4a). However, we found that, in DENV-infected cell cultures, only bystander (uninfected) cells upregulated (>1 log) the surface expression of MHC I (Fig. 4b). DENV antigen-positive cells did not show upregulation of MHC I expression. MHC II expression was not detected on the surface of either uninfected or DENV-infected muscle satellite cells (data not shown).

Fig. 2. Localization of DENV in muscle satellite cells. (a) Muscle satellite cells were infected at an m.o.i. of 2 for 48 h. Uninfected and DENV-infected muscle satellite cells were examined by TEM as described in Methods. Panel (i) shows perinuclear actinomyosin fibre bundles detected in cells containing virus-like particles. Panels (ii) and (iii) demonstrate the clusters of virus-like particles (DV) found inside the cytoplasmic vacuoles of DENV-infected cells. N, Nucleus; C, cytoplasm; V, vacuole; M, perinuclear actinmyosin fibre bundles. Arrows indicate clusters of virus-like particles. (b) Immunofluorescence microscopy of muscle satellite cells. Panels (i) and (ii) represent uninfected cells and (iii) and (iv) cells infected with DENV at an m.o.i. of 2. Panels (i) and (iii) are stained for the presence of desmin (red), and panels (ii) and (iv) for DENV (green). All four muscle satellite cells were positively stained with anti-DENV antibody indicating DENV infection. Nuclei were visualized using DAPI staining (blue). Magnification 20.
Next, we looked at both cell-surface-bound and soluble ICAM-1 (sICAM-1) levels following DENV infection. The percentage of cell-surface ICAM-1-positive cells significantly increased on DENV-infected cells compared with uninfected cells (mean ± SD of 44.08 ± 8.39 % versus 25.93 ± 6.04 %; P<0.001, paired t-test; Fig. 5a). Cell-surface ICAM-1 levels were preferentially upregulated (~50 % of the upregulated ICAM-1 levels) on DENV-infected cells in culture (Fig. 5b). In addition, increased levels of sICAM-1 protein were detected in DENV2-infected cell-culture supernatants by ELISA (Fig. 5c). sICAM-1 protein levels in supernatants differed among donors; however, sICAM-1 levels were consistently higher in DENV-infected muscle satellite cell supernatants compared with uninfected cell cultures. These results indicated that DENV infection induces activation but inhibits antigen presentation by DENV-infected cells.

**Gene expression analysis**

To identify DENV infection-induced changes in gene expression in muscle satellite cells, we performed Affymetrix GeneChip (HG-U133A) analysis. Data were analysed using GeneSpring software. Among the differentially regulated DENV infection-inducible common-response genes, we selected the IP-10, TRAIL, IRF7, MDA-5 and LGals3BP genes with known antiviral and/or immune functions. qRT-PCR analysis confirmed the increased expression of these five selected genes (Table 1), further supporting the hypothesis that muscle satellite cells are productively infected by DENV.

**DISCUSSION**

Muscle satellite cells are proliferative muscle cell precursors that are present in muscle fibres and are responsible for muscle repair and regeneration. They have the potential to fuse and form muscle fibre de novo. In addition, muscle satellite cells are an accepted model for studying changes in skeletal muscle cells in vitro (Goebels et al., 1992). Therefore, we used human muscle satellite cells as a model for DENV infection of muscle cells.

Our data showed that DENV can productively infect muscle satellite cells, produce high levels of infectious viral progeny, regulate activation of the infected cells and induce changes in expression of immune and antiviral genes. Previous studies of DENV infection of muscle cells have used mouse models to identify and document biochemical and structural changes in skeletal muscle during acute DENV infection (Agrawal et al., 1978; Nath et al., 1982). However, mice are not a natural host for DENV (Gubler, 1994). Muscle involvement has been studied infrequently during acute DENV infections in humans. A recent clinical study found that seven of 16 patients (44 %) positive for acute DENV infection clinically and serologically (by IgM ELISA) presented with acute flaccid weakness (Kalita et al., 2005). These seven DENV-positive patients had an incidence of acute motor quadriplegia along with elevated serum levels of CPK and glutamic pyruvic transaminase, muscle weakness, pain and tenderness, which are findings consistent with myositis (Kalita et al., 2005). In addition, Rajajee et al. (2005), in a study conducted in Chennai, India, found that 50 % of children presenting with clinical and laboratory features of benign acute childhood myositis were positive for DENV by serological tests (PanBio Dengue Duo IgM and IgG capture ELISA). Furthermore, Malheiro et al. (1993) detected moderate perivascular mononuclear infiltrates in 12/15 muscle biopsies performed in serologically positive DENV-infected individuals. These studies indicate that human skeletal muscle cells are indirectly or directly damaged during the acute stage of DENV infection.
In the present study, we used flow cytometry, immunofluorescence, TEM and qRT-PCR to demonstrate for the first time that primary human muscle satellite cells are susceptible to infection by multiple DENV serotypes. We showed that DENV replicates in muscle cells, based on plaque assays showing high levels of DENV progeny in culture supernatants. These data demonstrate that DENV infects and replicates in human muscle satellite cells. Interestingly, only dendritic cells infected in vitro show a higher rate of infection, as demonstrated by flow cytometry analysis (Lozach et al., 2005; Navarro-Sanchez et al., 2005; Wu et al., 2000). Furthermore, DENV infection impaired the ability of DENV-infected muscle satellite cells to upregulate MHC I protein levels, suggesting a mechanism of immune evasion by DENV.

Previous studies have shown that both type I and type II IFNs are critical in controlling different stages of DENV infection in mice (Shresta et al., 2005). Furthermore, type I and type II IFNs inhibit DENV in primary human cells in culture (Ho et al., 2005). Fink et al. (2007), Sariol et al. (2007), Simmons et al. (2007) and Warke et al. (2008) have reported changes in type I IFN genes as an important part of the cellular response to DENV infection, both in vitro (monocytes, dendritic cells and HepG2 cells) and in vivo (PBMCs). Hence, global gene expression analysis was performed in muscle satellite cells to identify IFN-responsive genes differentially regulated following DENV infection. We confirmed upregulation of five of the type I IFN-inducible antiviral and immune regulation genes (IRF7, MDA-5, TRAIL, IP-10 and LGals3BP) by qRT-PCR. These five genes were also found to be differentially regulated in response to in vitro DENV infection (Fink et al., 2007; Simmons et al., 2007; Warke et al., 2008).

Among the genes analysed, IRF7, an essential transcriptional regulator of type I IFN and MDA-5, a member of the helicase family that induces the type I IFN response pathway, might be involved in the antiviral response
against DENV (Berghall et al., 2006; Honda et al., 2005; Ramirez-Ortiz et al., 2006). In addition, TRAIL, a member of the tumour necrosis factor family, was recently found to be a potent antiviral against DENV (Warke et al., 2008), and IP-10, a CXC chemokine, has been shown to inhibit binding of DENV to cells (Chen et al., 2006).

LGals3BP, also known as Mac-2BP, has been detected at higher levels in the serum of human immunodeficiency virus- and hepatitis C virus-infected individuals and has been implicated in immune defence and immune regulation (Kittl et al., 2000; Natoli et al., 1993). Future studies should investigate the functional significance of the increased expression of these genes during DENV infection.

Table 1. qRT-PCR validation of five DENV response genes

In vitro DENV infections in muscle satellite cells were analysed by qRT-PCR. Relative fold induction was calculated with qRT-PCR software (Applied Biosystems). qRT-PCR analysis of uninfected and DENV-infected muscle satellite cells confirmed the induction of the five selected type I IFN-inducible genes following Affymetrix GeneChip HG-U133A analysis. The fold induction values for the gene chip and qRT-PCR analyses are the means of two and four independent experiments, respectively.

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Fig. 5. ICAM-1 expression levels on a muscle satellite cell population infected with DENV. Muscle satellite cells infected with DENV2 were stained for ICAM-1 (PE) (a) or ICAM-1 (PE) and DENV complex (FITC) (b) at 48 h (n=3) after infection at an m.o.i. of 2. An isotype control for IgG1a (PE) was used to set the gates. (c) Fold changes in sICAM-1 levels in cell-culture supernatants. Cells were infected with DENV2 for 48 h at an m.o.i. of 2. Data are represented as the ratios of sICAM-1 levels in supernatants of DENV-infected muscle satellite cell cultures compared with uninfected muscle satellite cell cultures for each donor. The SD for six experiments is shown.
inhibition of MHC I expression on the surface of DENV-infected cells has been shown previously in DENV-infected DCs in vitro (Librarty et al., 2001; Palmer et al., 2005). In addition, a study by Mathew et al. (1999) found that T-cell activation is suppressed during the early days of infection in PBMCs from DENV-infected individuals, which might be a result of lower MHC I expression on DENV-infected antigen-presenting cells, which are critical in the initiation of the immune response. Overall, lower MHC I expression on DENV-infected antigen-presenting cells represents a virus mechanism to evade recognition by the cytotoxic effector cells of the immune system.

ICAM-1 cell-surface expression was upregulated on both DENV-infected and bystander cells. Pervascular mononuclear infiltrate has previously been found in muscle biopsies of DENV-infected individuals (Malheiros et al., 1993). The higher cell-surface ICAM-1 levels detected in this study could lead to adhesion of DENV-infected muscle satellite cells to activated mononuclear cells expressing LFA-1 (Makgoba et al., 1988). Thus, inhibition of MHC I expression might help DENV to evade recognition and lysis by effector mononuclear cells possibly recruited by the ICAM-1–LFA-1 interaction. siCAM-1 protein levels were also elevated in DENV-infected cell supernatants: secreted siCAM-1 may regulate binding of activated immune cells to muscle cells expressing cell-surface ICAM-1 (Marino et al., 2003). It will be informative in the future to know whether siCAM-1 is secreted by DENV-infected or bystander cells. Differential induction of ICAM-1 and MHC I expression on muscle satellite cells following DENV infection may play a role in determining the outcome of the interaction of DENV-infected cells with inflammatory or effector immune cells.

A recent clinical study demonstrated that Chikungunya virus infects muscle satellite cells but not differentiated multinucleated fibres (myotubes) (Ozden et al., 2007). However, the role of muscle cells as reservoirs presenting viral antigen during acute dengue disease has not been studied. Ours is the first study to show that DENV infects and replicates in human muscle cells. Future clinical studies should determine whether skeletal/ cardiac muscle cells are targets for DENV in vivo. These studies may provide insight into the pathogenesis of DENV infection.

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