Journal of Immunological Methods 368 (2011) 24–35

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Validation of assays to monitor immune responses in the Syrian golden hamster (*Mesocricetus auratus*)

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ARTICLE INFO

Article history:
Received 28 October 2010
Received in revised form 7 February 2011
Accepted 8 February 2011
Available online 17 February 2011

Keywords:
Syrian Golden hamster
qRT-PCR
Host immune response
Reference genes

ABSTRACT

The Syrian golden hamster (*Mesocricetus auratus*) is a valuable but under-utilized animal model for studies of human viral pathogens such as bunyaviruses, arenaviruses, flaviviruses, henipaviruses, and SARS-coronavirus. A lack of suitable reagents and specific assays for monitoring host responses has limited the use of this animal model to clinical observations, pathology and humoral immune responses. The objective of this study was to establish and validate assays to monitor host immune responses in the hamster including important pro-inflammatory, anti-inflammatory and innate immune responses, as well as markers of apoptosis, cell proliferation, cell junction integrity and coagulation. Commercially available mouse and rat ELISA and luminex panels were screened for potential cross-reactivity, but were found to be of limited value for studying host responses in hamsters. Subsequently, quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assays for the detection of 51 immune-related and four internal reference genes were developed. To validate the immune-related assays, hamsters were infected with vesicular stomatitis virus (VSV), Indiana species, or treated with lipopolysaccharide (LPS) and host immune responses were monitored in selected organs. Ribosomal protein L18 was identified as the most stable internal reference gene. In conclusion, these new assays will greatly improve the use of the hamster as an important small animal model in infectious disease research.

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1. Introduction

Syrian golden hamsters (*Mesocricetus auratus*; hereafter referred to as hamster) have been used in numerous studies as infection and disease models (Table 1) due to their ease of handling and disease development, which often mimics the natural course of human diseases. In particular, hamsters have been recognized as valuable animal models for studying emerging and high consequence acute human viral diseases caused by bunyaviruses (Niklasson et al., 1984; Hooper et al., 2001; Milazzo et al., 2002; Fisher et al., 2003) arenaviruses (Smee et al., 1993; Sbrana et al., 2006), henipaviruses (Wong et al., 2003), flaviviruses (Tesh et al., 2001; Xiao et al., 2001; Siirin et al., 2007) and SARS-coronavirus (Roberts et al., 2005). For some of these pathogens, such as Andes virus, a New World hantavirus and a causative agent of hantavirus pulmonary syndrome, hamsters are the only lethal disease model (Hooper et al., 2001). However, due to a lack of available reagents and specific assays to monitor host responses in hamsters, including disease-decisive acute and early innate immune responses, investigators are currently limited to studies on disease progression (clinical symptoms),

Abbreviations: β2M, Beta-2-microglobulin; BBQ, Blackberry quencher; HPRT, Hypoxanthine phosphoribosyltransferase; LPS, Lipopolysaccharide; RPL18, Ribosomal protein L18; VSV, vesicular stomatitis Indiana virus; Yak, Yakima yellow.

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0022-1759/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.jim.2011.02.004

Table 1
Selected biological agent disease models in Syrian Golden hamsters.

Agent	Disease modeled	Reference
Amebic liver abscess	Entamoeba histolytica	Ghadirian et al., 1980
Andes virus	Hantavirus pulmonary syndrome	Hooper et al., 2001
Babesia	Babesiosis	Braga et al., 2006
Eastern equine encephalitis virus	Eastern equine encephalitis	Paessler et al., 2004
Gabek Forest virus	Rift Valley fever-like [a]	Fisher et al., 2003
Japanese encephalitis virus	Japanese encephalitis	Larson et al., 1980
Leishmania	Visceral leishmania	Melby et al., 2001
Leptospira	Leptospirosis	Haake, 2006
Maporal virus	Hantavirus pulmonary syndrome	Milazzo et al., 2002
Nipah virus	Nipah virus encephalitis	Wong et al., 2003
Oncolytic adenoviruses	Pancreatic carcinoma	Spencer et al., 2009
Pichinde virus	Lassa fever-like	Smee et al., 1993
Pirital virus	Lassa fever-like	Sbrana et al., 2006
Prions	Scrapie, Creutzfeldt–Jakob disease	Beekes et al., 1996; Chabry et al., 1999
Punta Tora virus	Rift Valley fever-like	Fisher et al., 2003
Rift Valley virus	Rift Valley fever	Niklasson et al., 1984
St. Louis encephalitis virus	Chronic St. Louis encephalitis [a]	Siirin et al., 2007
SARS coronavirus	Severe acute respiratory syndrome [a]	Roberts et al., 2005
Venezuelan equine encephalitis virus	Venezuelan equine encephalitis**	Jackson et al., 1991
West Nile virus	West Nile neurological syndrome	Xiao et al., 2001
Western equine encephalitis virus	Western equine encephalitis	Zlotnik et al., 1972
Yellow fever virus [b]	Yellow fever	Tesh et al., 2001

[a] Infection model, not disease model.
[b] Adapted viruses used in model.

humoral immune responses (antibodies) and pathology. For other disease models using rodents, nonhuman primates and ferrets, expression microarray proteome and luminex technology-based quantification assays have been developed and utilized (Kayo et al., 2001; Datson et al., 2007; Bruder et al., 2010; Ierna et al., 2010). However, for Syrian (Golden) hamsters similar specific assays are not available due to the lack of a complete genome sequence.

Here, we describe the testing of a large panel of ELISA and luminex based assays for cross-reactivity against hamster proteins and the development of an extended panel of real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assays which were based on hamster mRNA sequences currently registered in Genbank. Due to limited cross-reactivity in ELISA and luminex based assays, the development of qRT-PCR assays seemed the most practical and useful strategy to quantify specific host responses in the hamster model. A few qRT-PCR assays for the quantification of a limited number of immunological responses have been previously reported (Melby et al., 1998; Muller et al., 2001; Vernel-Pauillac and Merien, 2006). We developed a unique extended panel of TaqMan® primer/probe assays for 51 different genes targeting specific markers in the pro-inflammatory, anti-inflammatory, innate immunity, apoptosis, cell junction and coagulation responses of the hamster. With ribosomal protein L18 we identified a more suitable and stable housekeeping gene. The assays were validated by monitoring host responses to treatment with lipopolysaccharide (LPS) and infection with vesicular stomatitis Indiana virus (VSV), both of which are known to strongly activate host responses in the hamster (Fultz et al., 1981b; Muller et al., 2001). The new assays will allow for a broader monitoring of host responses against infection and an advanced utilization of the hamster model.

2. Materials and methods

2.1. Inoculations and sample preparation

Recombinant VSV was rescued from cloned cDNA and infectivity was titered by conventional plaque forming assay, as described previously (Lawson et al., 1995; Garbutt et al., 2004). Three groups of six hamsters (female, 4–6 weeks old Harlan Laboratories Inc, Indianapolis, IN, USA) were injected intraperitoneally (ip) with either 50 μg LPS (Sigma, St. Louis, MO USA), 10^6 plaque forming units (pfu) of VSV (both diluted in Dulbecco's modified Eagle's medium (DMEM)), or DMEM only (control) in a total volume of 400 μL. Three of the controls and VSV infected, and six of the LPS treated hamsters were euthanized by exsanguination while under deep anesthesia (inhalational isoflurane) one day post inoculation. The remaining control and VSV inoculated hamsters were euthanized at 3 days post inoculation. Whole blood was collected by cardiac puncture into EDTA vacutainers, after which plasma was separated by centrifugation and frozen at −80 °C. Lung, liver and spleen samples were harvested from euthanized hamsters, cut into approximately 50–100 mg pieces and submerged in 1 mL of RNAlater (Qiagen, Valencia, CA, USA) overnight at 4 °C. The following day RNAlater was removed and the tissues were frozen at −80 °C. Approximately 30 mg of the tissue samples were mechanically homogenized in RLT buffer and the RNA was extracted from the homogenate using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. The concentration of the extracted RNA was determined using a NanoDrop 8000 instrument (Thermo Scientific, Waltham, MA, USA) and adjusted to a final concentration of 10 ng/μL. All experiments were conducted under BSL2 conditions, and approval for animal experiments was obtained from the Institutional Animal Care and Use Committee. Animal work was performed by certified staff in a facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care.

2.2. ELISA and Milliplex panel preparation

Rat TNFα, IL-2, IL-6 and IL-10, mouse CXCL10/IP-10, IL-13 and IL-1β/IL-1F2, and mouse/rat/porcine/canine TGFβ1 ELISA kits were purchased from R&D Systems (Minneapolis, MN, USA) and tested according to manufacturer's instructions with individual hamster plasma (Table 2). Individual control and VSV infected and pooled LPS hamster plasma were sent to Millipore (Billerica, MA, USA) for analysis by Milliplex® MAP Mouse (32 plex) and Rat (23 plex) Cytokine panels (Table 2).