Expression and evaluation of a 24-kDa recombinant protein of the N-terminal E2 glycoprotein of chikungunya virus

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ABSTRACT

Chikungunya is an acute febrile illness caused by chikungunya virus (CHIKV). In this study, a short 24-kDa N-terminal of E2 glycoprotein of chikungunya virus was cloned and expressed in E.coli expression system. The E2 recombinant protein was expressed as a fusion protein to 6-Histidine for ease of purification. The expression of the 24-kDa recombinant protein was detected by SDS-PAGE and the protein reactivity was evaluated by western blot analysis. The immunogenicity of the 24 kDa protein was further tested against human positive and negative sera for chikungunya and dengue. The results showed that the recombinant antigen was able to detect CHIKV positive sera and no cross reactivity was observed with dengue virus positive serum. © 2015 Trade Science Inc. - INDIA

KEYWORDS

Chikungunya virus; Glycoprotein; Recombinant antigen; Immunoblot assay; E.coli expression system.

INTRODUCTION

Chikungunya fever is an acute illness caused by chikungunya virus (CHIKV), an alphavirus of the family Togaviridae. CHIKV is transmitted to humans by mosquitoes of the genus Aedes, particularly Aedes aegypti and Aedes albopictus. The hallmark of CHIKV infection is a long lasting polyarthralgia, which may persist for months or even years[1]. Though generally a non-fatal condition, CHIKV infections may rarely be associated with complications such as encephalopathy and hepatic failure[2] and occasional deaths have been reported over the last decade[3]. The clinical illness is often associated with prolonged morbidity, which can impose enormous social and economic disadvantages on affected communities[4]. The first formal description of the disease was during an outbreak of chikungunya fever in 1952 in Tanzania[5] and the subsequent isolation of CHIKV[6]. The first outbreak in Asia was documented in Bangkok, Thailand in 1958 and since then, outbreaks have been reported in Cambodia, Vietnam, Laos, Myanmar, Malaysia, Singapore, the Philippines, and Indonesia[7]. Malaysia reported its first outbreak between December 1998 and February 1999[8]. There are three distinct lineages of CHIKV, a West African, an East Central and South African (ECSA) and an Asian lineage[9]. In 2004-2005, CHIKV of the ECSA lineage caused massive outbreaks in the
Indian Ocean island of La Reunion\textsuperscript{19}. The single muta-
tion in the E1 (A226V) of the CHIKV during the In-
dian Ocean outbreaks enhanced the ability of the virus
to replicate in Aedes albopictus mosquitoes that greatly
facilitated the transmission of the disease\textsuperscript{11}. Currently
there are no specific treatments for CHIKV infections
and no licensed vaccine for any alphavirus is available
for human use.

The CHIKV genome consists of a linear, positive-
sense, single-stranded RNA of approximately 11.8kb,
and encodes four non-structural proteins (nsP1, nsP2,
nsP3 and nsP4) at the 5’ end and five structural pro-
teins (C, E3, E2, 6k and E1) at the 3’ end. The non-
structural proteins are required for viral replication
whereas the structural proteins are produced by trans-
lation of an mRNA that is generated from an internal,
sub-genomic promoter immediately downstream of the
non-structural open reading frame\textsuperscript{12}. The 5’ end of
the genome has a 7-methylguanosine cap, while the 3’ end
is polyadenylated. Two important viral glycoproteins
E1 and E2 are conserved among alphaviruses\textsuperscript{13}. The
E1 glycoprotein of CHIKV mediates fusion of the viral
and host cell membranes during virus entry and the E2
glycoprotein is responsible for receptor binding to host
cells\textsuperscript{14}. Therefore the glycoproteins E1 and E2 serve
as the major targets for diagnostic and vaccine de-
velopment.

Along with the clinical diagnosis based on
symptoms, laboratory confirmation of CHIKV infec-
tion is critical, especially in dengue endemic areas, as
clinical symptoms of the two diseases are similar. The
ability to distinguish CHIKV infection from dengue vi-
rus infection is important to launch different control stra-
egies. Enzyme-linked immunosorbent assays (ELISAs) and reverse transcriptase-polymerase chain
reactions (RT-PCR) are among the recognized sero-
logical and molecular tools for the specific detection
of CHIKV in patient samples\textsuperscript{15}. RT-PCR is an ex-
cellent tool for the early phase confirmation of CHIKV
infections, and many protocols have been established
for this purpose\textsuperscript{16}. Unfortunately, this viral detection
method is limited to the vireamic phase, which is usually
one to five days after fever onset\textsuperscript{17}. Thereafter, confir-
mation of CHIKV infection requires serological tests.
The serological tests include hemagglutination inhibition
(HI) and ELISAs detecting IgM antibodies of CHIKV.

**EXPERIMENTAL**

**Propagation of CHIKV in cell culture**

The virus, named BS5 was isolated from a local
outbreak of CHIKV in Sarawak in 2009. The virus
was propagated in Vero cells, which was maintained in
Dulbecco’s Modified Eagle’s Medium, DMEM (Gibco,
South America), supplemented with 5% Fetal Calf Se-
rum (FCS), 100 U/ml Penicillin G and 100 µg/ml Strep-
tomycin Sulfate. Vero cells were maintained in a hu-
midified incubator at 37°C supplemented with 5% CO\(_2\).

**RNA isolation and RT-PCR**

The N-terminal of E2 gene was amplified using primer pairs as listed in TABLE 1. Prior to the amplifi-
cation, RNA was extracted using High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Germany) ac-
cording to the manufacturer’s manual. The RNA was
subjected to reverse transcription PCR (RT-PCR). For
this purpose 6 µl of the extracted RNA was mixed with
1 µl of downstream primer for 10 minutes at 70°C and
immediately chilled on ice afterwards. A master mix
containing 0.5 µl of 10 mM dNTPS, 2.0 µl l of 5X RT