Available online at www.sciencedirect.com
SCIENCE DIRECT
Vaccine 24 (2006) 2803–2809
Vaccine
www.elsevier.com/locate/vaccine

Characterization of a viscerotropic yellow fever vaccine variant from a patient in Brazil

Amber R. Engel [a], Pedro F. C Vasconcelos [e], Monica A. McArthur [b], Alan D. T. Barrett [a,b,c,d,*]

[a] *Department of Pathology, The University of Texas Medical Branch, Galveston, TX 77555-0609, USA*
[b] *Department of Microbiology and Immunology, The University of Texas Medical Branch, Galveston, TX 77555-0609, USA*
[c] *Sealy Center for Vaccine Development, The University of Texas Medical Branch, Galveston, TX 77555-0609, USA*
[d] *Center for Biodefense and Emerging Infectious Diseases, The University of Texas Medical Branch, Galveston, TX 77555-0609, USA*
[e] *Department of Arbovirology and Hemorrhagic Fevers, Instituto Evandro Chagas, Ministry of Health, Belém, Pará, Brazil*

Received 7 August 2005; received in revised form 28 December 2005; accepted 2 January 2006
Available online 18 January 2006

Abstract

Although the live attenuated yellow fever (YF) 17D vaccine is considered to be one of the safest vaccines in the world today, several cases of disease associated with administration of the vaccine have been reported, including YF vaccine-associated viscerotropic disease (YF-VAVD), which was first described in 1996. All YF-VAVD isolates sequenced to date have shown very little genomic change when compared to their parental vaccine strains. In this study, we report the characterization of an isolate, BeH291597 (Brazil75), from a 1975 fatal case of YF-VAVD in Brazil. Comparison of Brazil75 with the genomic sequence of the parental 17DD vaccine strain revealed two amino acid substitutions (at positions M-49 and NS4B-240) that were unique to Brazil75. Although still a rare occurrence, this isolate suggests that YF-VAVD has been present much longer than previously recognized.

Keywords: Yellow fever; YF-VAVD; 17DD vaccine; Vaccine-associated adverse events

1. Introduction

Yellow fever virus (YFV) is a mosquito-borne member of the *Flavivirus* genus, family *Flaviviridae*. Symptoms resulting from yellow fever (YF) can range from a mild, non-specific illness to a hemorrhagic fever involving disease of the liver and other organs (i.e., viscerotropic disease). Approximately 20–50% of severe YF cases result in death due to liver failure and hemorrhage [1].

A very successful live attenuated vaccine has been developed to control YF. The wild-type Asibi strain (isolated in Ghana, 1927) was given 176 serial passages in mouse embryo and chicken tissue cultures to derive the attenuated 17D strain [2]. Two substrains were independently derived from 17D, termed 17D-204 and 17DD. 17D-204 was derived at passage 204, while 17DD was independently derived from 17D after passage 195 by further subculturing 17D in embryonated chicken eggs until passage 284 [3]. The 17DD substrain is now currently in use only in South America, while the 17D-204 substrain is used throughout the rest of the world.

Over 400 million doses of the 17D vaccine have been administered since the introduction of the seed lot system in 1945 [3]. However, despite being one of the safest and most efficacious vaccines developed to date, several instances of YF-vaccine-associated neurotropic disease (YF-VAND) and YF-vaccine-associated viscerotropic disease (YF-VAVD) have been reported [4–9]. While YF-VAND has been recognized for 60 years, YF-VAVD is a recently recognized phenomenon. Eighteen cases of YF-VAVD have been reported since 1996; approximately half of these cases resulted in death [10]. Most patients with YF-VAVD have presented with symptoms that are similar to wild-type YF such as fever, myalgia, athralgia, liver and renal failure, and

* Corresponding author. Tel.: +1 409 772 6662; fax: +1 409 772 2500.
E-mail address: abarrett@utmb.edu (A.D. T. Barrett).

0264-410X/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2006.01.009

hemorrhage, beginning from 2 to 5 days post-vaccination [10].

In 1975, a yellow fever virus strain (BeH291597) was isolated from a fatal case of YF in Brazil. As reported by WHO [11], there was only one fatal YF case reported in Brazil in 1975. This patient became ill and displayed symptoms 5 days post-vaccination and died 9 days post-vaccination. Due to the viscerotropic nature of the disease in this case, it was originally thought that Brazil75 was wild-type YF. However, partial genomic sequence of this isolate showed that Brazil75 was not a wild-type South American YFV isolate, and was, in fact, derived from the 17D vaccine [12]. Therefore, although YF-VAVD is still an uncommon occurrence, the isolation of Brazil75 suggests that this phenomenon has been present for at least 20 years prior to its initial description in 1996.

2. Materials and methods

2.1. Viruses

BeH291597 was isolated from the blood of a human in Aripuaña, Mato Grosso, Brazil on November 14, 1975 by the Instituto Evandro Chagas, Belém, Brazil and passaged twice in suckling mouse brain and once in mosquito C6/36 cells. There was one further passage in Vero monkey kidney cells to amplify the virus for sequencing and to generate viral working stocks. The genomes of three 17DD substrains [17DD (GenBank accession number U17066), 17DD-Brazil batch 182A (GenBank accession number DQ100292), and 17DD-Senegal (GenBank accession number L06480)] were used for comparison with Brazil75 [13–15]. The published sequence of 17DD-Senegal contained only the structural region of the vaccine, while full-length sequences of 17DD and 17DD-Brazil batch 182A were compared. 17DD-Brazil batch 182A virus was used as a comparison to Brazil75 in cell culture and hamster studies. Wild-type Asibi virus (GenBank accession number AY640589), wild-type FVV (GenBank accession number U21056), vaccine substrain 17D-204 (GenBank accession number X03700), and FNV (GenBank accession number U21055) were also used for comparison with Brazil75 [16–18].

2.2. Nucleotide sequence analysis

Viral RNA RT-PCR and sequencing of Brazil75 were undertaken as previously reported [19,20]. All sequence data was generated by direct sequencing of PCR products or by sequencing at least three clones of each PCR product. Sequence analysis was performed using Vector NTI (InforMax).

2.3. Growth curve assays

Hepatocellular carcinoma cells, HepG2 (ATCC) and Huh7 (a gift from Dr. R. Rijnbrand, UTMB), and monkey kidney Vero cells (ATCC) were infected in triplicate at an moi of 0.1 with either the Brazil75 strain or 17DD-Brazil batch 182A vaccine strain of YFV. Samples were assayed for infectivity using the 50% tissue culture infectious dose ($TCID_{50}$) assay in Vero cells.

2.4. Virulence studies

Hamsters were obtained from Harlan Sprague–Dawley. All animals were handled according to federal and institutional guidelines.

Groups of four three- to four-week-old female Golden Syrian hamsters (*Mesocricetus auratus*) were inoculated intraperitoneally (i.p.) with 4.4 $\log_{10}$ $TCID_{50}$ units of Brazil75, 17DD-Brazil batch 182A, Asibi, or Asibi hamster p7 strains. Asibi hamster p7 virus was derived by passaging wild-type Asibi seven times in hamster livers and displays a highly viscerotropic phenotype in hamsters [21]. Animals were checked daily and monitored for mortality and signs of morbidity for 14 days. All animals were bled via saphenous vein on day 3 post-infection to isolate serum and determine peak titers of the virus in the serum for each virus through $TCID_{50}$ titrations.

3. Results

3.1. Nucleotide sequence analysis

The consensus sequence of Brazil75 was compared to sequences of two wild-type South American YFV strains, Panama74B and Bolivia99D (M.A. McArthur, unpublished data), wild-type West African strains Asibi and FVV, published vaccine substrains 17D-204, FNV, 17DD, 17DD-Senegal, and the 17DD-Brazil batch 182A stock used in these studies [13–18]. Brazil75 shared the least identity with two South American strains, Bolivia99D (South American geno-

Table 1
Sequence identity between Brazil75 and other strains of YF virus

Virus	Nucleotide identity with Brazil75 (%)	Amino acid identity with Brazil75 (%)
17DD-Brazil batch 182A	99.8	99.8
17DD	99.7	99.7
17D-204	99.7	99.7
Asibi	99.4	99.0
FVV	99.3	98.9
FNV	98.9	98.3
Bolivia99D	84.8	96.0
Panama74B	85.1	95.7

Nucleotide identity was compared between the entire genomes of Brazil75, vaccine strains 17DD, 17D-204, and FNV, wild-type African strains Asibi and French viscerotropic virus (FVV), and wild-type South American strains Bolivia99D (South American genotype II) and Panama74B (South American genotype I). Amino acid identity was compared between the open reading frames of Brazil75, 17DD, 17D-204, Asibi, FVV, FNV, Bolivia99D, and Panama74B.