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Journal of Clinical Virology 27 (2003) 205–209

JOURNAL OF CLINICAL VIROLOGY

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Short communication

The genomic analysis of rubella virus detected from outbreak and sporadic cases in Rio de Janeiro state, Brazil

Flávia F. Donadio [a], Marilda M. Siqueira [a,*], Andrew Vyse [b], Li Jin [b], Solange A. Oliveira [c]

[a] *Department of Virology, Instituto Oswaldo Cruz, FIOCRUZ, Avenida Brasil, 4365, Rio de Janeiro 21045-900, Brazil*
[b] *Enteric, Respiratory and Neurological Virus Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK*
[c] *Department of Infectious Diseases, Hospital Universitário Antônio Pedro, Universidade Federal Fluminense, UFF, Niterói, Brazil*

Received 30 May 2002; received in revised form 21 October 2002; accepted 7 November 2002

Abstract

Background: The molecular epidemiology of rubella virus (RV) based on the analysis of the viral E1 gene sequences indicated the existence of two genotypes that differ from each other by 8 to 10% in their nucleotide sequences: genotype I is present in Europe, North America and Asia; and genotype II is present only in Asia. *Objectives:* The purpose of the study was to identify the RV genotypes circulating in Brazil. *Study design:* In this study, we analysed 86 clinical samples collected between 1996 and 1999 during a rubella outbreak and from sporadic cases of rubella in Rio de Janeiro State. For the molecular characterisation of RV strains we have used PCR/nested amplification and direct sequencing of a 513-nucleotide region of the E1 gene. *Results:* The E1 gene sequences of 14 RVs were obtained and were assigned to two lineages, both within genotype I. The percentage divergence of nucleotide sequence ranged from 3.4 to 5.1% between these two lineages. These results were in agreement with the pattern of variation observed among the sequences obtained from other lineages of RV. *Conclusions:* This work demonstrated that two new lineages of RV circulated simultaneously between the years 1996 and 1999 in the state of Rio de Janeiro. These results provided new approaches for monitoring the progress of vaccination efforts in Brazil.

Keywords: Rubella; Genotype; Molecular epidemiology

1. Introduction

Rubella virus (RV) belongs to the Togaviridae family and is the only member of the genus *Rubivirus*. Humans are the only known host and infection caused by RV in adults and children is usually mild or asymptomatic. However, infection during pregnancy often leads to severe birth defects known as congenital rubella syndrome (CRS). RV is an enveloped virus with a positive-sense, single stranded RNA genome. This is approximately 10 000 nucleotides in length, con-

* Corresponding author. Tel.: +55-21-2598-4360; fax: +55-21-2573-9591
E-mail address: mmsiq@ioc.fiocruz.br (M.M. Siqueira).

1386-6532/02/$ - see front matter
PII: S1386-6532(02)00270-6

tains an unusually high proportion of guanosine (G) and cytidine (C) residues, and consists of two nonoverlapping open reading frames (ORFs). One is 5′ proximal encompassing two thirds of the genome and encodes non-structural proteins. The other is 3′ proximal encompassing the remaining third of the genome and encodes three structural proteins, consisting of the capsid (C) and two glycoproteins, E1 and E2. The glycoproteins are embedded in the lipid envelope and form a heterodimer in which E1 is the more exposed and against which the majority of the host's humoral response is directed. Studies using murine monoclonal antibodies suggest antigenically important epitopes are located between amino acid residues 210 and 286 within E1 (Frey et al., 1998).

The information currently available regarding the molecular epidemiology of RV is limited. The most comprehensive studies to date (Katow et al., 1997; Frey et al., 1998) have included samples from Europe, the US and Asia and suggest the RV may be classified into two genotypes, designated I and II, with the majority of samples sequenced being categorised as genotype I.

In Brazil, seroprevalence studies conducted during the pre vaccination era demonstrated that rubella was endemic all year round with epidemics occurring every 6 or 7 years (Schatzmayr, 1985). The measles–mumps-rubella vaccine (MMR) was first introduced in Sao Paulo state in 1992. Subsequently it has been gradually introduced in the other regions of Brazil and, in 2000, the vaccination program reached all Brazilian states. However, despite these significant efforts by the Ministry of Health to control rubella and CRS, a number of rubella outbreaks and sporadic cases are still reported (Anonymous, 2001).

In this study we describe the phylogeny of RV using clinical samples from both sporadic cases and from an outbreak, collected in Rio de Janeiro state during 1996 and 1999. These data contribute towards a more complete understanding of the molecular epidemiology of RV strains that have recently been circulating in Brazil and represent the first such study in a South American population.

2. Materials and methods

2.1. Specimens

A total of 86 specimens were used in this study, collected in the city of Tres Rios, Rio de Janeiro state, during a rubella outbreak that occurred in 1996 and from cases of rubella that occurred in Rio de Janeiro state in 1997 that were identified during a measles epidemic. The specimen panel comprised 43 urines, 40 bloods and two nasopharyngeal aspirates (NPA) and were from patients with a serologically confirmed rubella infection. Also included was a saliva sample collected in 1999 from a confirmed case of rubella in Rio de Janeiro state.

2.2. Laboratory methods

All clinical samples were stored at −70 °C until required for laboratory testing. They were processed in accordance with the protocol described in the Manual for Laboratory Procedures in Measles/Rubella, Measles National Reference Centre/FIOCRUZ.

RV RNA was extracted directly from 100 μl of each specimen using the silica–guanidinium isothiocyanate method (Boom et al., 1990) and reverse transcribed into cDNA using random priming (dN6) as described previously for measles virus (Jin et al., 1996). Samples were then screened for rubella genome by nested PCR (Vyse and Jin, 2002) that amplifies a 513-nucleotide region of the E1 gene. The Judith strain (JUD), Liverpool, England (GenBank accession no: L9420) was used as a positive control. Nested PCR products were visualised by staining with ethidium bromide after electrophoresis on a 2% agarose gel.

2.3. Sequencing and data analysis

All samples that were RT-PCR positive for rubella genome were sequenced. The specific PCR products were gel purified using Geneclean (BIO 101 Inc., USA) and purified DNA fragments were directly sequenced using the primers RE1.3 (3.2 pmol/μl) and RE1.4R (3.2 pmol/μl) (Vyse and Jin 2002). Nucleotide sequences were determined

using the Perkin–Elmer dye-deoxy terminator sequencing kit in an ABI automatic 373 DNA sequencer (Applied Biosystems Ltd., UK). Sequence data was analysed and a phylogenetic tree constructed using the ClustelV method of Megalign programme (DNAStar, USA).

3. Results

Of a total of 86 samples screened in this study, 14 (16.27%) tested positive by RT-PCR. These consisted of 11/43 urines, 1/40 bloods, 1/2 NPAs, and 1/1 saliva sample.

Six RT-PCR positive samples (five urines and one NPA) were collected during the 1996 outbreak. All were collected <6 days after the onset of rash. Seven RT-PCR positive samples were from cases of rubella in 1997. These consisted of six urines (no information on timing of collection) and one blood collected 1 day after the onset of rash. The sole saliva sample collected in 1999 was RT-PCR positive (Table 1).

A phylogenetic tree showing the results of this study is shown in Fig. 1. Every sequence obtained from samples used in this study was compared with 24 other strains of RV contained in the GenBank that represented both genotypes I and II (Frey et al., 1998).

The rubella sequences obtained in this study fell into two distinct lineages on the phylogenetic tree. Both contained samples collected in 1996, 1997 and 1999. The nucleotide sequence divergence between these two groups ranged from 3.4 to 5.1% based on the 513 nt of E1 gene generated. One lineage consisted of ten strains that showed a high degree of similarity with nucleotide variation ranging from 0 to 0.7% and formed a new branch in comparison to previously published strains of rubella. The second lineage consisted of four strains with a nucleotide difference ranging from 0.8 to 2.4% and were most closely related to strains of rubella that were circulating in Europe in the late 1980s and early 1990s. An analysis of the nucleotide sequences used in this study showed that no more than two amino acid changes resulted in any one sequence from the nucleotide changes observed.

4. Discussion

This study has shown that RV genome can be amplified from urine, blood, NPA and saliva specimens collected from cases of rubella. However, rubella genome was amplified from only 16% specimens used, though all had been collected <1 week after rash onset. These data are similar to

Table 1
Specimens testing PCR positive

ID	Year collected/Location of collection	Age (years)	Specimen	Onset symptoms to collection (days)	Lineage
B102BR96	1996/TR	7	NPA	6	First
B52BR96	1996/TR	6	Urine	4	First
B54BR96	1996/TR	8	Urine	4	First
B63BR96	1996/TR	3	Urine	3	First
B48BR96	1996/TR	11	Urine	3	Second
B64BR96	1996/TR	10	Urine	5	Second
B32BR97	1997/RJ	NK	Urine	1	First
B33BR97	1997/RJ	NK	Urine	1	First
B38BR97	1997/RJ	NK	Urine	NK	First
B40BR97	1997/RJ	17	Urine	1	First
B41BR97	1997/RJ	NK	Urine	1	First
B24BR97	1997/RJ	17	Blood	1	First
B68BR97	1997/RJ	NK	Urine	2	Second
BR99	1999/RJ	3	Saliva	3	Second

TR, city of Três Rios, Rio de Janeiro State; RJ, city of Rio de Janeiro, Rio de Janeiro State; NK, not known.

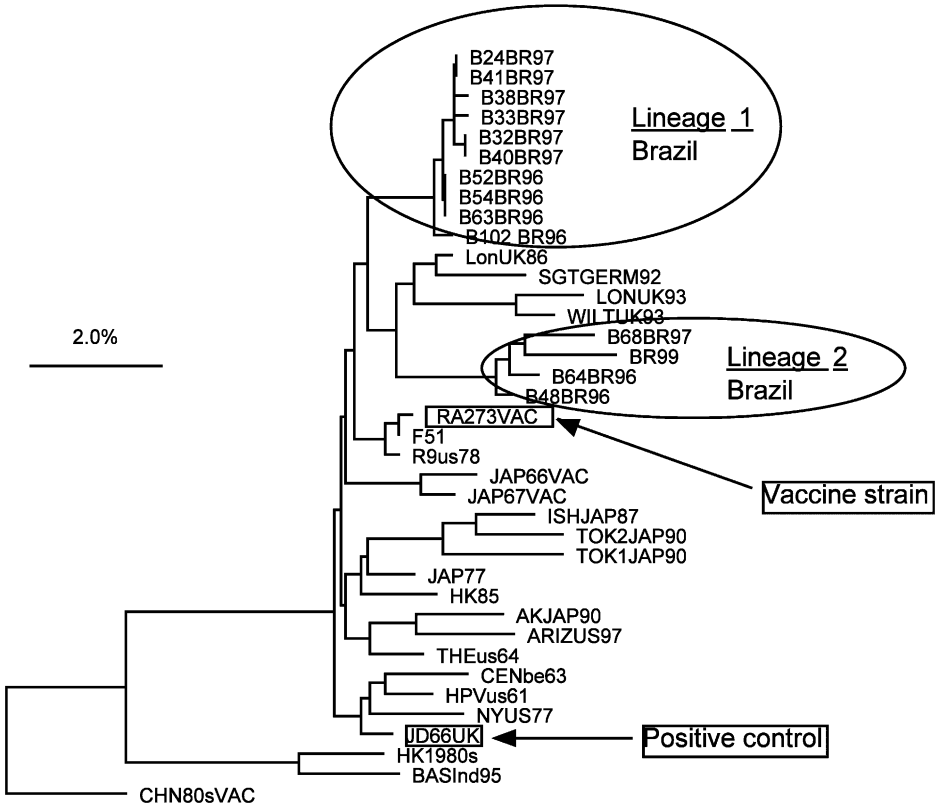

Fig. 1. Genetic relationship between rubella strains detected in Brazil during 1996–1999. Unrooted tree diagram was constructed on the basis of the 513 nt of E1 gene sequence using the ClustalV method of Megalign (DNAStar, USA). Strains reported in this study were indicated as well as the vaccine strain and the strain used as positive control through out the study.

previous results of RV detection in urine, blood, NPA and saliva specimens (Bosma et al., 1996). They also confirm previous findings that early collection is critical if samples are to be successfully used for molecular studies (Jin et al., 2002). Delay in collecting a specimen may result in clearance of the virus by the hosts' immune system and degradation of any viral genome by nucleases.

Phylogenetic analysis shows that two strains of RV were co-circulating in Rio de Janeiro state during 1996, 1997 and 1999. Both may be classified as genotype I and show approximately 9% difference in nucleotide sequence from those classified as Genotype II (Katow et al., 1997; Frey et al., 1998). Whilst one strain showed some similarity to those that were circulating in Europe during the late 1980s and early 1990s, the other strain formed a new branch on the tree and may represent a strain not previously described. Neither strain was similar to the strain, which was circulating in the UK and Greece in 1999, (Vyse and Jin, 2002) showing a 4% (20 nucleotide) and 5% (25 nucleotide) difference in nucleotide sequence for the amplicon generated. The lack of nucleotide sequence changes leading to amino acid substitutions suggests that little antigenic drift occurs for RV and is consistent with the findings of other studies. The divergence between these strains and the vaccine strain, RV273 (Fig. 1), which is currently used in Brazil, was 2.2–5.5%. However, there is no evidence that the vaccine strain would not protect against all wild type RV infections.

The work carried out in this study, therefore, identifies for the first time strains of rubella that have been circulating recently in Brazil. It also

reinforces the value of genomic sequencing of rubella for epidemiological related cases, contributing to the global view of RV divergence and providing supplementary information for surveillance purposes.

Acknowledgements

To Dr Maureen Oliveira Fiuza in memoriam. We are also grateful to Dr David Brown/CPHL for encouragement and Dr Bernard Cohen/CPHL for helpful advice. This project was supported by a grant from FIOCRUZ/British Council.

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