

BRIEF COMMUNICATION

FIRST ISOLATION OF DENGUE 3 IN BRAZIL FROM AN IMPORTED CASE

Iray Maria ROCCO(1), Berenice Bustamanti KAVAKAMA(2) & Cecília Luiza Simões SANTOS(3)

SUMMARY

The authors report the isolation of dengue 3 virus for the first time in Brazil. The patient, resident in Limeira-SP, traveled to Nicaragua on May 16th, 1998, where he stayed for two months. Starting on August 14th he had fever, headache, myalgia, arthralgia, retro-orbital pain and diarrhea. He returned to Brazil on August 16th and was hospitalized in the next day. The patient had full recovery and was discharged on August 20th. The virus was isolated in C6/36 cell culture inoculated with serum collected on the 6th day after the onset of the symptoms. The serotype 3 was identified by indirect immunofluorescence assays performed with type-specific monoclonal antibodies. This serotype was further confirmed by polymerase chain reaction analysis. The introduction of a new dengue serotype in a susceptible population is a real threat for the occurrence of severe forms of the disease. The isolation and identification of dengue virus are important in order to monitoring the serotypes circulating in Brazil and to take the measures necessary to prevent and control an epidemic.

KEYWORDS: Dengue 3; Virus isolation; PCR

Dengue, a mosquito-borne virus infection, is considered one of the most important and widespread reemerging infectious diseases in developing countries. Clinically, the infection can be asymptomatic or it can manifest as an undifferentiated febrile illness, known as dengue fever (DF) characterized by "flu-like" symptoms including fever, headaches and myalgia. Major complications are known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), with occurrence of plasma leakage and thrombocytopenia, leading to death in a significant number of cases³.

Dengue is caused by dengue virus, a positive-stranded RNA virus of the family *Flaviviridae*. It is transmitted to humans by *Aedes* sp mosquitoes and are divided into four closely related but antigenically distinct serotypes from 1 to 4. Infection by one serotype does not protect the individual against infection by a second serotype of dengue virus⁶. Instead, the appearance of clinically severe infections such as DHF and DSS seems to be mostly related to subsequent infections by a virus of different serotype from that of former infection. The mechanisms involved in the pathogenesis of dengue infections remain unresolved. Epidemiological studies have shown a higher incidence of DHF/DSS in secondary infection compared with the primary one, supporting the antibody-dependent enhancement hypothesis⁵. On the other hand, variation in virulence among viral strains may account for severe dengue disease in confirmed cases of primary infection¹⁰. At present, in spite of

many studies all over the world, no dengue vaccine is available and the prevention of infection depends mainly on mosquito vector control.

In Brazil, millions of dengue infections have been notified all over the country since 1986. In the State of São Paulo outbreaks of DF have been notified since 1987, when the first autochthonous cases by serotype 1 virus were detected. Dengue 2 virus was introduced in 1996 and nowadays both serotypes are present in many regions of the State, as in other States of the country^{2,9}.

In 1998, dengue serotype 3 was isolated in our laboratory from an imported case. The patient, a 46-year-old male, living in Limeira, traveled on May 16th to Nicaragua, where he stayed for three months. Starting on August 14th he had fever, headaches, myalgia, arthralgia, retro-orbital pains and diarrhea. He arrived in Brazil on August 16th and was hospitalized in the next day. He had full recovery and was discharged on August 20th.

The virus isolation was accomplished in C6/36 cell culture inoculated with serum collected on the 6th day after the onset of disease symptoms. Indirect immunofluorescence assays performed with type-specific monoclonal antibodies, showed positive reaction for dengue 3 virus⁴. This serotype identification was further confirmed by polymerase chain reaction (PCR) analysis. Virus RNA isolated from infected cell culture

Financial support: Secretaria de Estado da Saúde, São Paulo; Fapesp, Fundação de Amparo à Pesquisa.

(1) Seção de Vírus Transmitidos por Artrópodos, Instituto Adolfo Lutz, São Paulo, SP, Brasil.

(2) Divisão de Zoonoses, Centro de Vigilância Epidemiológica, São Paulo, SP, Brasil.

(3) Laboratório de Biologia Molecular, Instituto Adolfo Lutz, São Paulo, SP, Brasil.

Correspondence to: Iray Maria Rocco; Instituto Adolfo Lutz; Av. Dr. Arnaldo 355, 01246-902 São Paulo, SP, Brasil, Fone (11) 3068-2902, E-mail: irayrocco@hotmail.com

supernatant by silica method¹, was reverse-transcribed and amplified with oligonucleotide consensus primers developed by LANCIOTTI *et al.*⁷ The second-round of PCR, performed with type-specific primers, detected DNA products of 290 bp, characteristic of dengue virus serotype 3 (Fig. 1).



Fig. 1 - Identification of dengue 3 serotype by reverse-transcriptase-polymerase chain reaction. Ethidium bromide stained agarose gel showing: molecular markers (A), the amplified product corresponding to 290bp obtained with dengue 3 specific primers set (Lanciotti, 1992) (B) and negative control (C).

Dengue 3 was introduced in Nicaragua in 1994 causing a large epidemic by DF/DSS and in the following year it spread to other Central America countries and Mexico. Molecular analysis showed that this virus was genetically distinct from that one which circulated 16 years before in the Americas, but it presented the same genotype as the one isolated in the epidemic that has caused major epidemics of DHF in Sri Lanka and India⁸.

The introduction of a new dengue serotype in a susceptible population is a real threat for the occurrence of severe forms of the disease, whatever the mechanism of pathogenesis involved. That is the case of our country, where there is a great number of people who have already contracted dengue infection by serotype 1 or 2.

The isolation and characterization of dengue 3 virus in our laboratory show the necessity of constant monitoring of dengue virus serotypes circulating in our country in order to alert the health authorities of any relevant changes observed so that measures to prevent and control a new epidemic are taken.

RESUMO

Primeiro isolamento de dengue 3 no Brasil de um caso importado

Os autores relatam o isolamento do vírus dengue 3 pela primeira vez no Brasil. O paciente, residente em Limeira-SP, viajou em 16 de maio de 1998 para a Nicarágua, onde permaneceu por três meses. A partir de 14 de agosto, ele apresentou febre, dor de cabeça, mialgia, artralgia, dor retro-orbital e diarreia. Ele retornou ao Brasil em 16 de agosto e foi hospitalizado no dia seguinte. O paciente teve completa recuperação, recebendo alta em 20 de agosto. O vírus foi isolado em cultura de células C6/36 inoculadas com soro colhido no 6^o dia após o início dos sintomas. O sorotipo 3 foi identificado por imunofluorescência indireta com anticorpos monoclonais tipo-específico. O sorotipo foi posteriormente confirmado por PCR. A introdução de um novo sorotipo de dengue em uma população susceptível é uma ameaça para a ocorrência de formas severas da doença. O isolamento e a identificação do vírus dengue são de grande importância a fim de monitorar a introdução de novos sorotipos e para que medidas sejam tomadas para prevenir e controlar novas epidemias.

ACKNOWLEDGEMENTS

We are grateful to Dr. Tiyo Sakurai for a critical reading of the manuscript.

REFERENCES

1. BOOM, R.; SOL, C.J.A.; SALIMANS, M.M.M. *et al.* - Rapid and simple method for purification of nucleic acids. *J. clin. Microbiol.*, **28**: 495-503, 1990.
2. FIGUEIREDO, L.T.M. - Dengue in Brazil. I: history, epidemiology and research. *Virus Res. & Res.*, **1**: 9-16, 1996.
3. GUBLER, D.J. - Dengue. In: MONATH, T.P. *The arboviruses: epidemiology and ecology*. Boca Raton, CRC Press, 1988. v. 2, p. 223-260.
4. GUBLER, D.J.; KUNO, G.; SATHER, G.E.; VELEZ, M. & OLIVER, A. - Mosquito cell cultures and specific monoclonal antibodies in surveillance for dengue viruses. *Amer. J. trop. Med. Hyg.*, **33**: 158-165, 1984.
5. HALSTEAD, S.B. - Challenges to molecular biology. *Science*, **239**: 476-481, 1988.
6. HENCHAL, E.A. & PUTNAK, J.R. - The dengue virus. *Clin. Microbiol. Rev.*, **3**: 376-396, 1990.
7. LANCIOTTI, R.S.; CALISHER, C.H.; GUBLER, D.J.; CHANG, G.J. & VORNDAM, A.V. - A rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J. clin. Microbiol.*, **30**: 545-551, 1992.

8. PINHEIRO, F. & NELSON, M. - Re-emergence of dengue and emergence of dengue haemorrhagic fever in the Americas. **Dengue Bull.**, 21: 16-24, 1997.

9. ROCCO, I.M.; FERREIRA, I.B.; KATZ, G. *et al.* - Ocorrência de dengue no Estado de São Paulo, Brasil, de 1986 a 1996. **Rev. Inst. Adolfo Lutz**, 57: 7-12, 1998.

10. ROTHMAN, A.L. - Viral pathogenesis of infection. In: GUBLER, D.J. & KUNO, G., ed. **Dengue and dengue hemorrhagic fever**. Wallingford, CABI, 1997. p.245-271.

Received: 15 September 2000

Accepted: 16 October 2000