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Studies on antigenic and genomic properties of Brazilian rabies virus isolates

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Abstract

Despite the recognized stability of rabies virus, differences among isolates from different species have been found. This work was carried out with the aim to identify antigenic and genomic differences in Brazilian rabies virus isolates and to verify whether such alterations would bear any relationship with the different hosts for the virus in nature. For that, 79 Brazilian rabies viruses isolated from different host species and from distinct regions within Brazil were submitted to antigenic characterization with a panel of 11 monoclonal antibodies (Mabs) directed to lyssavirus antigens and to genomic analyses by the reverse transcriptase-polymerase chain reaction (RT-PCR) amplification of the N gene followed by restriction endonuclease analysis (REA). In addition, the nucleotide sequences of part of the N gene (225 bp) of seven isolates, taken as representative of the majority of the viruses under study, were determined. The analyses with the Mabs and RT-PCR/REA allowed the identification of two major groups of variants, the first formed by most isolates of cattle and bats and the second formed by viruses of dog origin. Partial sequencing of the N gene confirmed the similarity among isolates from cattle origin and those of vampire bats. However, viruses from non-haematophagous bats exhibited consistent differences from those of vampire bat isolates. Such findings suggest that the variants have evolved fairly stable modifications, which are not altered after passage in a dead-end host of a distinct species. No association could be established between antigenic or genomic alterations and geographic distribution of the isolates, which suggests that evolution of the virus has been directed to adaptation to the host species.

Keywords: Rabies; RT-PCR; REA; Monoclonal antibodies; Sequencing

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1. Introduction

Rabies virus is a member of the *Lyssavirus* genus within the *Rhabdoviridae* family (Tordo, 1996). The *Lyssavirus* genus is presently divided into seven genotypes based on nucleotide and amino acid sequence analyses (Bourhy et al., 1993; Amengual et al., 1997; Gould et al., 1998). Genotype 1 (GT 1; classical rabies virus) has worldwide distribution and is so far the only genotype isolated in the Americas. The virus causes rabies in humans and other mammals, being transmitted predominantly by the bite of an infected vector species (King and Turner, 1993). In Brazil, the infection is maintained in two main cycles the urban rabies, where dogs are the main reservoirs, and the sylvatic cycle, where the main reservoirs are haematophagous bats (Wiktor and Koprowski, 1982; Dietzschold et al., 1988), particularly the species *Desmodus rotundus* (Baer, 1975), which is the main source of infection for cattle. In addition, vampire bats are second only to dogs as cause of contamination of humans in Brazil (Anonymous, 1996). Nevertheless, other bats may occasionally be infected, although their role in the transmission and perpetuation of the infection disease remains uncertain (Germano, 1994). Despite the recognized antigenic stability of rabies virus, differences among isolates from different species have been found (Roehe et al., 1997; Loza-Rubio et al., 1999; Ito et al., 2001, 2003; Páez et al., 2003; Romijn et al., 2003). Such studies have usually relied on antigenic analyses with monoclonal antibodies (Mabs; Wiktor and Koprowski, 1978; Wiktor et al., 1980; Flamand et al., 1980; Dietzschold et al., 1988). The application of genomic methods of analysis revealed that apart from such antigenic differences, rabies virus isolates adapted to different species have conserved gene alterations which can also be used as epidemiological markers (Arai et al., 1997; Ito et al., 2001, 2003; Páez et al., 2003). In Brazil, the two main ''natural'' cycles of infection involving either haematophagous bats or domestic dogs as hosts had already been identified in previous studies by both antigenic and genomic methods (Roehe et al., 1997; Ito et al., 2001, 2003; Schaefer et al., 2002; Romijn et al., 2003). Here, we report the identification of another possible natural cycle involving non-haematophagous bats and isolates with genomic adaptations to these hosts. Such modifications were made evident by nucleotide sequencing of a fragment of the N gene.

2. Material and methods

2.1. Viruses

Seventy-nine rabies virus isolates from calves (47), dogs (13), 9 from non-haematophagous bats (4 from *Tadarida brasiliensis*, 1 from *Molossus molossus* and 4 from bats with no species identification), wild-dogs (*Cerdocyon thous*; 3), 2 from cats, 2 from horses, 2 from unidentified hosts and 1 from a human case were obtained from different Brazilian states (Rondônia, RO; Paraíba, PB; Alagoas, AL; Pernambuco, PE; Mato Grosso, MT; Mato Grosso do Sul, MS; Minas Gerais, MG; Bahia, BA; São Paulo, SP; Rio de Janeiro, RJ and Rio Grande do Sul, RS; Fig. 1) and analysed in the present study. Standard strains Challenge Virus Standard (CVS) and Pasteur Virus (PV) were included as controls (Table 1).

2.2. Rabies virus identification and multiplication

Initial rabies virus detection was carried out by a direct fluorescence antibody test (DFAT; Dean et al., 1996). Viruses were then multiplied by intracerebral inoculation of 3–4-week-old mice with infected brain suspensions (10% in PBS, pH 7.4), as described by Koprowski (1996). Mice were observed daily and brains collected *in extremis*.

2.3. Monoclonal antibodies

A panel of eight monoclonal antibodies, produced by the immunization of BALB/C mice with rabies-related members of the *Lyssavirus* genus (Mabs L3 and L18 from Lagos Bat virus; DB1, DB3, DB4 and DB9 from European Bat Lyssaviruses, previously denominated ''Denmark bat''; D3 from Duvenhage virus and M11 from Mokola virus), was prepared elsewhere (King, 1991). Three additional Mabs, one prepared with the CVS strain (Mab CVS-2; Roehe et al., 1997) and two other prepared with a Brazilian rabies virus isolate of bovine origin (Mabs 5A3 and 5G2; Schaefer, 1999), were also included in the panel.