Genomic sequence analysis identifies Jembrana disease virus as a new bovine lentivirus

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Jembrana disease virus, the cause of an acute, severe disease in Bali (Bos javanicus) cattle in Indonesia was recently identified as a retrovirus, and possibly a lentivirus. We have produced sequence data representing 598 bp of the pol gene, amplified by PCR from viral cDNA using broadly reactive universal primers for retroviruses and more specific genus-reactive primers for lentiviruses. When the sequence data were compared with that of known lentiviruses and other bovine retroviruses, the closest alignment was with bovine immunodeficiency-like lentivirus (BIV), showing 74% nucleotide sequence identity. This confirmed that JDV is a lentivirus and that it is distinguishable from BIV.

Jembrana disease in Bali cattle (Bos javanicus) was first recognized in 1964 in Bali and is now endemic throughout parts of Indonesia (Hartaningsih et al., 1993). It is an acute, severe disease with a mortality of about 17% and a short incubation period of 5-12 days (Soeharsono et al., 1990). Fever, lymphadenopathy and lymphopenia are the major clinical changes (Soesanto et al., 1990). Pathological changes reflect an intense lymphoproliferative disorder. There is marked enlargement of lymph nodes and spleen, which feature proliferating lymphoblastoid cells in parafollicular areas and follicular atrophy (Teuscher et al., 1981; Dharma et al., 1991; Budiarso & Rikihisa, 1992). Proliferative lymphoid infiltrate is also found in many other tissues, but the central nervous system is spared. High titres of infectious virus of about 10⁸/ml are found in the plasma during the febrile stage of the disease and a persistent viraemia can be demonstrated for at least 25 months in animals that survive the acute disease (Soeharsono et al., 1990). Surviving animals show regression of lesions commencing about 5 weeks post-infection (Dharma et al., 1991).

The causative agent, designated Jembrana disease virus (JDV), was recently identified as a retrovirus on the basis of virus morphology, C-type budding from cell membranes and reverse transcriptase activity (Kertayadnya et al., 1993). Further evidence in the same report, including strong serological cross-reactivity between the capsid proteins of JDV and bovine immunodeficiency-like virus (BIV), and morphological characteristics of the nucleocapsid, suggested that JDV may be a lentivirus.

To further characterize JDV by sequence analysis, cDNA was produced from viral genomic RNA using Superscript (BRL). Virus was purified by sucrose gradient centrifugation from the plasma of Bali cattle experimentally infected with the Tabanan/87 isolate of JDV, as previously described (Wilcox et al., 1992). Amplification by PCR from this cDNA using a nested set of primers specific for the BIV gag gene did not generate any specific products. However, amplification using universal degenerate primers from a conserved region of the pol gene of retroviruses (Donehower et al., 1990) yielded a product of the expected size, 135 bp. The primer sequences were: RVf, 5' ctcggatccGTNYTNC-NCARGG 3', RVr, 5' ctcgtcgacRTCRTCCATRTA 3' (lower-case letters represent non-homologous 5' extensions). This product was recovered from a 1% agarose gel (Gene-Clean II; Bio 101) and sequenced.

Further amplification was carried out using another set of six degenerate primers demonstrated by us to detect lentiviruses, but not other retroviruses. These primers were designed to amplify various fragments from
Fig. 1. Alignment of 598 bases and the predicted amino acid sequence of a conserved reverse transcriptase domain in the JDV genome with the BIV pol gene (bases 2117–2714). Bases and amino acids of JDV that do not match those of the BIV gene (BIV127; GenBank M32690) are highlighted with an asterisk.
within a 640 bp region of the pol gene, also within the highly conserved amino-terminal portion of the reverse transcriptase-coding region. This region entirely encompasses the region flanked by the retrovirus primers described above. Primer sequences (in order of their position in the pol gene) were: LV1Bf, 5' ggtgatcc-CARTGGGCCHTT 3'; LV1Af, 5' CAATGCGCATT-ACAAAWGARAA 3'; LV3f, 5' GATTYYAGAGAA-YTAAAAAY 3'; LV3r, 5' TTRTTTARTTCYCTRA-ACAAAWGARAA 3'; LV5r, 5' AARTATGCATCYCCTAYR-TC 3'; LV8r, 5' GGATGNARTTCRWAANCNACACCA 3'. The strongest amplifications were achieved with the combinations LV1Bf/LV5r (290 bp) and LV3f/LV8r (473 bp). These two overlapping fragments were also recovered from agarose and sequenced.

The three overlapping fragments purified were sequenced directly (373A automated DNA sequencing system; Applied Biosystems). The DNA fragments amplified by the lentivirus primers gave 598 bp of contiguous nucleotide sequence data for JDV; 91 bp of this sequence data was duplicated and confirmed by the short fragment amplified by the retrovirus primers. Comparison of the JDV sequence data with known viral sequences (BLAST at NCBI) revealed an alignment with the BIV pol gene (bases 2117–2714 of BIV 127; GenBank M32690), which showed 74 % identity at the nucleotide level and 76 % at the amino acid level, with no deletions or insertions (Fig. 1). A phylogenetic tree was constructed which shows that, based on the pol gene data, JDV is most closely related to, but distinct from BIV (Fig. 2). The genetic distance observed between JDV and BIV indicates that JDV is a new bovine lentivirus.

The acute, severe nature of Jembrana disease, with no recurrence of disease in those animals that survive the initial disease, is not typical of most lentivirus infections. There are, however, striking similarities between Jembrana disease and an acute, severe disease syndrome recently described in pig-tailed macaques infected with a lethal variant of simian immunodeficiency virus (SIV), SIVsMMPBj14 (Fultz et al., 1989; Dewhurst et al., 1990; Lewis et al., 1992; Israel et al., 1993). Both feature a severe lymphopenia initially, followed by a rapid, intense lymphoproliferative disorder that leads to accumulations of large numbers of blastic lymphocytes in parafollicular regions of lymph nodes, spleen and lymphoid tissues of other organs. Very high levels of infectious virus occur in the plasma in each case and there is no recurrence of clinical signs in recovered animals.

It is remarkable that from among a group of viruses generally known for their ability to cause chronic infections of a relentlessly progressive nature two viruses such as JDV and SIVsMMPBj14 should emerge that induce an acute lymphoproliferative disease. The implications of SIVsMMPBj14 infection as a unique model of acute lentiviral pathogenesis, and the parallels between this syndrome and the acute transient syndromes associated with other lymphotropic lentivirus infections such as HIV, have been previously discussed (Martin, 1990). The identification of a natural disease-causing agent such as JDV as a new lentivirus, with similar unusual biological characteristics to SIVsMMPBj14, adds significantly to the emerging pattern of the evolution and biology of lentiviruses as a group.
References


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