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# Isolation and Characterization of Simian Retrovirus Type D from *Macaca fascicularis* and *M. nemestrina* in Indonesia

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Simian type D retroviruses (SRVs) are one of the causative agents of simian acquired immunodeficiency syndrome (AIDS) in Asian macaques. In the past, SRV isolates from macaques had only been identified at the US primate centers, outside the country of origin and after the animals had been introduced into a new environment. In this study, we report the first isolation, cultivation and molecular characterization of the type D simian retrovirus naturally infecting wild caught macaques in their natural habitats in the country of origin, in this case, Indonesia. When peripheral blood mononuclear cells (PBMC) from *Macaca fascicularis* (Mf) and *M. nemestrina* (Mn) were co-cultured with Raji human B-cell line, syncytia were observed microscopically and confirmed by immunofluoresence assay using antibody to SRV-2. Immunoblot analysis of purified Mf-ET1006 from cell culture supernatants demonstrated that the viral core and envelope proteins reacted with rabbit anti-SRV. Sequence analysis of Mf isolates in the viral envelope region revealed high homology to SRV-2 (94-96%). On the other hand, the homologies in the envelope region of Mn isolates were less than 80% to SRV-1, SRV-2, SRV-3 and Mf isolates. This study suggests that the isolate from Mn may be different from any other published SRV isolates..

Key words: type D simian retrovirus, *Macaca fascicularis*, *Macaca nemestrina*

For many years, the Retroviridae family has been studied primarily because of the ability of these viruses to transform mammalian cells and cause naturally occurring tumors in many animal species. It was recognized that this virus family could also cause non-oncogenic disease, including immunosuppresive disorders such as AIDS (Gardner *et al.* 1988). Among family Retroviridae, the lentivirus (simian immunodeficiency virus, SIV) and exogenous type D retroviruses (simian retrovirus, SRVs) have been identified as the etiologic agents of infectious immunodeficiency diseases in several macaque species that have some clinical similarities to human AIDS (Lerche *et al.* 1995).

SRV infection is endemic and for the first time was reported in *Macaca* sp. populations at some Primate Research Centers in the United States in the early 1980s (Daniel *et al.* 1984; Gardner *et al.* 1988; Pamungkas *et al.* 1991). Until 1988, five serotypes of SRV had been isolated and identified from macaques (SRV-1 to SRV-5). However, from these five serotypes, only four (SRV-1, SRV-2, SRV-3 and SRV-4) had been completely sequenced and reported to GenBank (Daniel *et al.* 1984; Marx *et al.* 1984; Gardner *et al.* 1988; Li *et al.* 2000). SRVs have emerged as significant pathogens in captive macaques, although infection appears to be sporadically prevalent in Asian macaques at breeding facilities and these species are probably the natural hosts of SRV, but prevalence of infection in feral macaques remains undetermined (Daniel *et al.* 1984; Gardner *et al.* 1988; Pamungkas *et al.* 1991). Serological studies of *M. fascicularis*, *M. nemestrina*, and *Pongo pygmeus* in Indonesia show variable prevalence of SRV-2 leading to the assumption that the animals have been infected with SRV (Iskandriati *et al.* 1998a; Iskandriati *et al.* 1998b; Warren *et al.* 1998).

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SRV isolates, found to date from macaques at primate centers out of the animals' original country, have been obtained after the animals were introduced to the new environment. According to Grant (1995), the isolates obtained over many years from the Washington Primate Center have a very high homology level, which shows that these SRV isolates probably came from the same source or a small number of source animals. However, the characteristics of virus growth and genetic sequence variability from wild type isolates have not been described yet. Until today there has been no report on findings of wild isolate in its natural population. Since SRV infection has negative impact on the management of macaques breeding facility, it is very important that we be able to identify and characterize new SRV isolates in the animals' original population.

The objective of this research was to identify SRV from *M. fascicularis* and *M. nemestrina* in Indonesia using virus isolation technique, and to identify and characterize provirus DNA from those isolates. The resulting amino acid sequences obtained were then compared with those from other type D retroviruses to describe their genetic relationship.

## MATERIALS AND METHODS

**Sample Collection and Processing.** Fifty-three blood samples from Lampung and Palembang, Indonesia and 76 blood samples from *M. nemestrina* in Palembang were obtained from several breeding facilities in Indonesia. *M. fascicularis* and *M. nemestrina* with a total number of 129 were placed in individual cages during the screening process. Blood samples were taken within the first week of the animals' arrival at the breeding facilities. This was carried out to get accurate data about the animals' condition. If the animals were infected by SRV, it can confirm that the