SURVEY OF IMPORTANT PRIMATE VIRAL ANTIBODIES IN *Macaca fascicularis* AND *M. nemestrina* IN INDONESIA

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Introduction

The benefits of non-human primates particularly macaques as animal model in the AIDS-related and other infectious diseases research have been recognized for a long time. A number of retroviruses that infect macaques has similar characteristics as human immunodeficiency virus (HIV). In an experimental setting, certain retroviruses infection in rhesus monkeys are widely spread and produce many similarities with HIV infection in human beings.

Among the Retroviridae family members, simian immunodeficiency virus (SIV) and type D simian retrovirus (SRV) have been identified as the causal agents of immune system disorders in macaque species which caused clinical symptoms similar to human AIDS, and referred to as simian AIDS (SAIDS). Clinical symptoms induced by SRV include fever, chronic diarrhea, wasting syndrome, and anemia. Sometimes retroperitoneal fibromatosis (RF) was found in animals infected by SRV. In addition to the above clinical symptoms, opportunistic infections were often found in SIV-infected animals, however, no RF was noted. Many macaques colonies were naturally infected with simian retroviruses that would confound experimental data relevant to **AIDS-related** research and other infectious disease.

Simian T-lymphotrophic virus type-1 (STLV-1) is a **member** of Retroviridae family, which was reported to cause immunosuppression in macaques.

Herpesvirus simiae or *Cercopithecine **herpesvirus** 1* (CHV-I), also known as herpes B virus. is widely spread in macaques populations and continues to represent **a significant health** threat **to** personnel who works closely with monkeys or **handle** their tissue specimens. The B virus is the most challenging infection to eliminate from an SPF population.

Here we report the results of retrospective serological survey from 2004 to 2007 **for the** presence of antibodies against SRV, SIV, HTLV-1 and HSV-1 in macaques population in Indonesia.

Materials and Methods

Viral antigens

HTLV-1, SIV, HSV-1 were purchased from Advanced Biotechnologies Inc (Columbia, MD). Purified preparations contained a total protein concentration of 1 mg/ml. SRV-2 was **purified using ultra-centrifugation** and sepharose method. HTLV-1 and HSV-1 are serologically cross-reactive to STLV-1 and herpes B virus (B virus), respectively.

Antigen preparation for ELISA and Western blot

Viral antigens for coating in ELISA were prepared by 2:1 mixing of the purified virus preparation with 0.3% Sodium Dodecyl Sulphate (SDS). **While virus** preparation for Western blot were diluted in sample **buffer** containing SDS, β-mercaptoethanol, **glycerol** and bromphenol blue.

Monkey serum/ plasma samples

Thousands of serum or plasma samples from *Macaca fascicularis* and *M. nemestrina* were tested by the Microbiology and Immunology Laboratory at the Primate Research Center, Bogor Agricultural University, as part of the **laboratory's** diagnostic **services** from 2004 through 2007. The majority af samples source were *M. fascicularis*.

ELISA

Several different ELISA systems were **used,** depending on the need and availability. One system consisted of commercial HTLV-1/2 ELISA kit (Genelabs® Diagnostics, Singapore) was used for detection of STLV in 2004 and 2005. The **kits were** used according to the manufacturer's instruction. For HTLV-1 (after 2005) and HSV-1 antibodies detection in routine serology diagnostics performed at our center, purified whole viral antigens obtained from ABI (Columbia, MD) were used. **Briefly,** ELISA plates were coated with viral lysates of HTLV and HSV at optimal protein concentrations of 400 ng/ml and 220 ng/ml,

respectively. Viral antigens were diluted in 0.1M carbonate- bicarbonate buffer (pH 9.6). Diluted antigens were added to ELISA plate wells and incubated overnight at 4°C and blocked with 5% blotto (containing 5% skim milk in p8S with 0.1% Tween-20). For antibody detection, serum samples were diluted at 1:33 in 5% blotto added to the plates. Bound antibodies were detected by horseradish peroxidase-labeled goat anti-monkey IgG antibodies **(Sigma,** USA) with TMB (tetra methyl benzidine) chromogen substrate (Sigma, USA). The reaction was stopped by adding 50 ul 2 N sulfate acid (H_2SO_4). Color development was read at **450** nm filter measured **as** optical density (OD) BioRad microplate reader model 3550).

Western blot

Immunoblotting technique was carried out by using a modified techniques of Harlow and Lane (1988) for SRV and SIV antibodies. The purified viral protein was run in a sodium-dodecyl-sulfate poly-acrylamide gel electrophoresis (SDS-PAGE) system using gradient gel with the concentration from 7.5% to 17.5% at optimal protein concentrations at 500 ng/strip (SRV) and 150 ng/strip (SIV). Sequentially, the proteins in the gel was transferred to nitrocellulose **membranes** and blocked with 5% blotto. For antibody detection, serum samples were diluted at 1.50 in 5% blotto, then **added** to each test strip. Bound antibodies were detected by alkaline phosphatase-labeled goat anti-human IgG antibodies (Sigma, USA) with BCIP-NBT (5-bromo-4-chloro-3-indolylphosphate toluidinium-nitroblue tetra-zolium) chromogen substrate (Sigma, USA). Commercial HTLV-1/2 Western blot kits (MP Diagnostic, Singapore) was used for confirmatory test of STLV. This kits were used according to the manufacturer's instructions.

Results

Serum or plasma samples tested in *M. fascicularis* and *M. nemestrina* obtained from 2004 to 2007 were summarized in Table 1, presented according to the viruses of interest.

Discussion

In the **case** of STLV, the significant increase of its antibody prevalence in 2006 compared with the data from 2005 might **be** caused by different assays that were used in those years. The commercial HTLV antibody ELISA kit that was previously used in 2005 and before was not as sensitive as the assay used in 2006. Some samples from 2005 showed false negative results when confirmed with Western blot **assay.** There was notable relatively constant positive detection of antibodies against SRV, SIV and HSV. Antibodies to SRV was detected in the range of around 20 to 30% of all tested samples. while SIV antibodies were detected constantly in low percentage below 1%, except for the samples from 2004, and HSV antibodies were detected in quite high percentage of the samples tested, reaching almost 70%, **except** for the samples from 2007. The low percentage of HSV antibodies detected in 2007 was probably due to the majority of samples obtained from young animals under 2 years of **age.**

Conclusion

The prevalence of SRV, SIV and HSV-11 **CHV-1** antibodies in *M. fascicularis* from several locations in Indonesia was around 20-30%, 1% and 70%, respectively;

Further optimization of the STLV-11 HTLV-1 antibody detection assay **was** done to better reveal the data of its antibody detection. The in-house kit for STLV-1/ HTLV-1 antibody detection proved to have a higher sensitivity with better specificity.

Tabe 1. Summary of antibodies testing In **2004-2007** in **both *M. fascicularis*** and ***M. nemestrina***

Agents	2004		2005		2006		2007	
of nterest	No of samples	% Positive	No of samples	% Positive	No of samples	% Positive	No of samples	% Positive
SRV	2'74	23 09%	2270	**24 58%**	2907	20 88%	**2858**	30.79%
SIV	**343**	1.17%	1043	0 96%	1422	0 98%	2573	0 47%
STLV	503	**9** 74%	673	7 28%	1500	41 33%	1064	18.52%
HSV	*1771*	67 64%	376	70 74%	490	69 **59%**	655	**21** 98%

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