大韓바이러스學會誌 : 第 27 卷 第 1 號 1997
J. Korean. Soc. Virology, Vol. 27, No. 1, 1997

One-Stage Polymerase Chain Reaction for the Comprehensive Detection of Type D Retrovirus Provial DNA

Yong Seok Jeong

Department of Biology, College of Liberal Arts and Sciences, Kyung Hee University, Seoul 130-701, Korea

=국문초록=

Type D Retrovirus 감염의 포괄적 검색을 위한 One-Stage 중합효소 연쇄반응법의 개발

경희대학교 문리과대학 생물학과

정 용 석

본 연구에서는 영장류에서 감염성 면역결핍 증상을 일으키는 type D simian retrovirus (SRV)를 검출하기 위해 SRV *env* 유전자의 특정 지역을 선정하여 증폭, 검색하는 중합효소 연쇄반응 (PCR)법을 개발하였다. 증폭반응의 대상부위인 SRV *env* 유전자의 3' 후반부는 서로 다른 3 종류의 SRV subtype 1, 2, 그리고 subtype 3에 걸쳐 높은 보존율을 보이고 있다. 반응 결과, 1차 PCR 만으로 3 종류의 SRV subtypes를 동시에 검출, 증폭하였으며 SRV와 더불어 영장류에서 감염성 면역결핍을 유도하는 주요 바이러스 simian immunodeficiency virus 또는 simian T-lymphotropic virus type 1에 감염된 영장류의 peripheral blood mononuclear cells (PBMCs)을 비교, 완전한 증폭 특이성이 확인되었고 교차반응으로 인한 위양성반응은 발견되지 않았다. 한편, 위 증폭반응의 검출 민감도 측정을 위해 준정량적 적정 PCR을 수행하였으며 SRV 게놈과 증폭 대상 부위의 분자량을 기준으로 한 본 PCR법의 검출 한계는 단 한 번의 증폭과 간단한 ethidium bromide 염색만으로 적어도 5-7$\times 10^4$ 개의 PBMCs 중 한 개의 감염세포를 검출할 수 있는 것으로 확인 되었다. 본 연구에서 확인된 신속성과 반응 특이성, 그리고 높은 민감도의 본 PCR법은 현재 유일한 AIDS 연구모델인 영장류의 SIV 감염연구 전후에 반드시 필요한 효과적인 SRV 감염검색에서 ELISA법의 위양성에 의한 약점을 보완하고 보다 높은 검출 민감도를 확보함으로써 연구모델 실험의 오류를 최소화하는 데 중요한 역할을 하게 될 것이다.

Key Words: Type D simian retrovirus (SRV), Polymerse chain reaction (PCR), Animal models of AIDS

INTRODUCTION

The development and exploitation of animal models of acquired immunodeficiency syndrome (AIDS) in human is an absolute necessity when we are to better understand the pathogenesis induced by the retrovirus, develop and test vaccine candidates [1], and test possible therapies involving variety of antiviral drugs or modifiers of immune activity as cytokines, especially in combination. There are animals manifesting immunosuppressive disease symptoms comparable to human AIDS with various degree through infection with their species-specific retroviruses. Whilst those retroviral infections and disease progress with their natural hosts have provided only limited information in studying human AIDS [2], experimental infections of rhesus macaques by

molecularly cloned simian immunodeficiency virus (SIVmac239) turned out to be highly promising which most closely approximates infection of humans with human immunodeficiency virus (HIV) [3, 4]. Desrosiers and his colleagues [5] established persistent viremia in experimentally infected macaques by the virus from the SIV molecular clone, SIVmac239, at the New England Regional Primate Research Center (NERPRC), and demonstrated possible attenuation of the genetically engineered SIV, at least in adult macaques, by the removal of 'nef' sequences [6, 7, 8].

Currently, most of rhesus macaques used for human AIDS research have been bred as captive colonies in regional research centers from the wild Asian macaques. Several type D simian retroviruses (SRV) are indigenous in feral macaques in India and Indonesia and so in captive macaques of various U.S. primate center and other research facilities [9]. SRV transmission, under natural conditions, is mainly mediated by the parenteral inoculation of saliva during habitual contact with their offspring but vertical or sexual transmission rarely occurs [10]. Experimental infections by intravenous (i. v.) inoculation of blood, plasma, spinal fluid, cell-free tissue extracts, or culture fluids are readily reproducible. Broad cell tropism of the SRV including macaque T and B lymphocytes, fibroblasts, and epithelial cells in salivary glands, pancreas, choroid plexus, the skin, oral mucosa, and upper and lower gastrointestinal tract has been demonstrated *in vitro* as well as *in vivo* [11]. SRV infection, *in vivo,* causes a severe depletion of both T and B lymphocytes followed by profound immunosuppression which may be fatal with opportunistic infections [12, 13]. Asymptomatic infection of the central nervous system at the end-stage of the SRV serotype 1 (SRV-1) and the characteristic fibromatosis formation by the SRV serotype 2 (SRV-2) infections has been described in macaques. Indistinguishable clinical end-results and its pathobiology by the SRV infection from simian model study of human AIDS by the SIV infection in macaques produce serious complications in interpretations of experimental data. Therefore, application of serologic and virologic tests to identify SRV-infected animals has been carried out prior to animal usage. The tests include enzyme-linked immunosorbent assay (ELISA), immunoblot, indirect immunofluorescent assay, and radioimmunoprecipitation assay (RIPA) for serum antibody screening, and infectious virus isolation from blood cell culture which should be the most confirmative [14, 15]. Serum-based tests, however, are frequently complicated by the fact that animals may be antibody positive while virus culture is negative, or culture positive without detectable antibody, thus necessitating multiple testing procedures [14, 16, 17]. Furthermore, a 2.5-year epidemiolgical study of a breeding group of rhesus macaques demonstrated that seroconversion was a poor indicator of infection rate, as approximately 50% of virus-positive juvenile monkeys had no antibody detectable by ELISA [17]. Selected regional primate research centers and other laboratories developed a polymerase chain reaction (PCR)-based test for detection of SRV infection of experimental animals for each serotypes recently [18, 19]. Although the present PCR test by specific amplification of integrated proviral DNA has been proven to be very confirmative without compromising its characteristic sensitivity, experimenters have to perform multiple PCR in order to identify each serotypes of SRV. Thus far, five distinct neutralization serotypes of this virus (SRV-1 to SRV-5) have been isolated from various species of macaques [2]. Three of these (SRV-1, -2, and -3) have been molecularly cloned and sequenced, and show envelop variation up to 40% [20]. Simian AIDS (SAIDS) has been induced by a molecular clone of SRV-1 [21]. The SRV-2 at the Oregon and Washington re-

gional primate research centers appears associated with retroperitoneal and subcutaneous fibromatosis in addition to SAIDS [22]. The prototype virus in this subfamily is the Mason-Pfizer monkey virus (MPMV), classified as SRV serotype 3, which was shown to be immunosuppressive and not oncogenic [2]. Serotype 4 and 5 have not been problematic in captive macaques at the present.

In this report, a novel PCR method using only a pair of primers is described in order to identify all three type D simian retroviruses after single round of reaction by amplification of proviral DNA integrated in host chromosome. Based on preliminary studies performed on samples from animals of known infection status [6], this PCR was proved to be confirmative and more sensitive than virus isolation for the detection of SRVs, while requiring only one-sixth the time. This technique will greatly enhance the efficiency of SRV diagnostics, and this technology into testing algorithms will be discussed.

MATERIALS AND METHODS

Cell lines

The cell lines used to evaluate the SRV PCR primer pair included three Rajis stably infected with each serotypes of SRV (R20216 for SRV-1, R10876 for SRV-2, and R23200 for SRV-3) and an uninfected Raji. Established human T cell line CEMx174 free from any known retrovirus infection was used to provide cellular DNA in order to adjust substrate DNA amount in the plasmid titration PCR for sensitivity tests.

Peripheral blood mononuclear cells (PBMC)

Chromosomal DNA of PBMCs from animals infected with simian retroviruses other than SRV were used to ensure the specificity of the primer pair and amplification reaction. Heparinized peripheral blood samples of SIV seropositive female rhesus macaque E968 infected with SIVmac239 through i.v. injection, and of seropositive rhesus macaque neonate 93-7 infected with SIVmac239 through oral mucosa were obtained from TSI Mason Laboratory (Worcester, MA). Simian T lymphotropic virus type 1 (STLV-1)-positive and STLV-I-negative baboon PBMCs were generously gifted by Dr. Jonathan Allan at the Southwest Foundation for Animal Research (San Antonio, TX). Approximately 10 ml (E 968), or 2 ml (93-7) of heparinized peripheral blood were separated through a Ficoll-Hypaque gradient. PBMCs were resuspended in RPMI-1640 supplemented with 15% FBS, and stored at -20℃ until DNA isolation for PCR analysis.

Plasmid DNA

Viral genome-cloned plasmids used to determine sensitivity and specificity of the PCR included pSRV-1 (SRV-1), D2C/Oregon (SRV-2), pSHRM15 (SRV-3), generously donated by Drs. P. Luciw (University of California at Davis) and E. Hunter (University of Alabama at Birmingham), p239SpSp5' (SIVmac239 5'half), p239SpE3' (SIVmac239 3' half), and pMT-2 (HTLV-1) which were kindly provided by Drs. R. Desrosiers (New England Regional Primate Research Center, MA) and J. Sodrosky (Dana-Farber Cancer Institute, Havard Medical School).

Polymerase chain reaction for proviral DNA and cloned viral DNA

For cellular DNA preparation, all cell samples used for PCR were lysed using a lysis buffer containing 10 mM Tris-HCl (pH 8.3), 30 mM NaCl, 20 mM EDTA (pH 8.0), 0.5% SDS, and 60 μg/ml proteinase K. The samples were extracted with phenol/chloroform after 10 hours incubation at 37℃ in the lysis buffer followed by 10 min at 95℃, and then stored

at -20℃ in TE buffer prior to PCR analysis. After multiple conserved sequence analysis, a region conserved among SRV-1, -2, and -3 within envelop glycoprotein gene was chosen for PCR amplification. Locations and sequences of the primer pair, and expected fragment size after reaction were summarized in Table 1. For detective PCR amplification, approximately 1.0 μg of the sample cellular DNA or 0.1 μg of plasmid DNA containing cloned viral genome mixed with 1.0 μg of uninfected CEMx174 DNA was added to the reaction cocktail (2.5 mM $MgCl_2$, 200 μM dNTPs for final concentration) making 100 μl final reaction volume. For semiquantitative titration PCR using plasmid DNA containing viral genome, approximately 1.0 μg of uninfected CEMx174 cellular DNA was mixed with serially diluted pSRV-1, D2C/Oregon, and pSHRM15. The samples were overlaid with 100 μl of mineral oil and amplified in Ericomp thermocycler (San Diego, CA) using the following cycle conditions: 1 min denaturation at 94℃, 1 min annealing at 55℃, and 1 min extension at 72℃. This cycle was repeated 34 times followed by a 10 min final extension at 72℃. The reaction conditions were optimized for specificity and sensitivity. One-tenth, about 10 μl, of the each reaction products was separated by electrophoresis through an 1.5% agarose gel, and then stained with ethidium bromide. Throughout all PCR amplification, 5U of Taq polymerase (Perkin-Elmer) and 0.3 μM of each primer per reaction were used.

RESULTS

PCR amplification of cloned viral DNA

Viral genome-containing plasmids of three different serotypes of SRV and three other retroviruses were subjected to PCR amplification in order to evaluate the specificity of primers SRVenv65 and SRVenv72R for amplifying and detecting SRV-1, -2, and -3 all together. The primers were designed to amplify the target region which was rather conserved within *env* gene sequence. One round of reaction with the primer pair amplified and detected

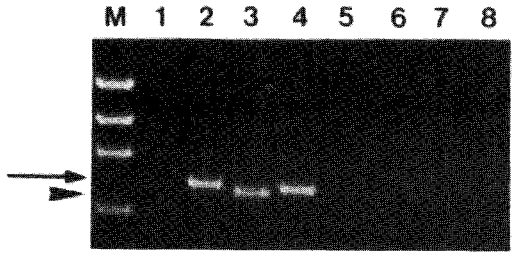


Fig. 1. PCR amplification of the SRV *env* region in SRV-1, SRV-2, SRV-3, genome-cloned plasmid DNA using cross-detective oligonucleotide primers. Each plasmid DNA, 1.0 × 10^{-2} μg per reaction, was mixed with 1.0 μg CEMx174 chromosomal DNA and subjected to one round of PCR as described in Materials and Methods. Lane M, ϕX174/ Hae III restriction fragment molecular size marker; lane 1, reaction mixture control, no target DNA; lane 2, pSRV-1 (SRV-1); lane 3, D2C/Oregon (SRV-2); lane 4, pSHRM15 (SRV-3); lane 5, p 239SpSp5′ (SIVmac239 5'-half); lane 6, p239SpE3 (SIVmac239 3'-half); lane 7, pMT-2 (HTLV-1); lane 8, 1.0 μg CEMx174 chromosomal DNA only. Arrow and arrowhead indicated the 725 or 728, and 701bp PCR products, respectively. One-tenth of the each reaction product was separated by electrophoresis through 1.5% agarose gel, and then stained with ethidium bromide.

Table 1. Sequence and position of the primers used, and expected fragment size from the PCR

Primer designation	Sequence (5'-3')	Position/ Region/ Subtype	Fragment size (bp)
SRV1 env65	GCCCCTGGTAAAGAAAAAATTG	6500-6521/ env/ SRV-1	728
		6468-7168/ env/ SRV-2	701
		6884-7608/ env/ SRV-3	725
SRV1 env72R	ACTTCTGCTAGAGAGTCTAC	7208-7227/ env/ SRV-1	
		7149-7168/ env/ SRV-2	
		7589-7608/ env/ SRV-3	

the corresponding regions in all three types of SRV genome-cloned plasmids (Fig. 1). Electrophoretic analyses of the reaction products on ethidium bromide-stained 1.5% agarose gel revealed expected fragment sizes for each serotype. SRV-1 and SRV-3, known to share very high sequence homology up to 92%, produced similar fragment sizes in the reaction. Estimated product sizes were 728 nts for SRV-1 and 725 nts for SRV-3 generated from published sequences (Fig. 1, lanes 2 and 4, respectively). The amplified product of SRV-2 migrated slightly faster than the other two as expected of which should be about 701 nt-long (Fig. 1, lane 3). Homology of SRV-2 to SRV-1 was about 80% overall but the sequences for the primer pair to anneal were selected for complete match in all three serotypes. Plasmid clones of SIVmac239 and HTLV-1 were not amplifiable at all by the SRV PCR primer pair used in this reaction. Therewith, the PCR using a primer pair hybridizing two conserved sequences of SRV *env* gene is not only able to detect all three subtypes of SRV in one round of reaction, but it does so with very high specificity.

PCR amplification of SRV-infected cell lines

To further evaluate the utility of the SRV PCR primer pair, chromosomal DNA extracted from three SRV-infected Rajis and an uninfected Raji cell line were tested whether SRV proviral DNA are detectable and amplifiable as shown in the PCR amplifying cloned viral genome. Three Rajis R20216, R10867, and R23200 persistently infected with subtype 1, 2, and 3 respectively, produced specifically amplified PCR fragments of expected size (Fig. 2, lanes 3, 5, and 7). PBMCs obtained from rhesus macaques either fatally infected with cloned SIVmac239 or of infection-free, and from STLV-1-infected or uninfected baboons were subjected to the PCR amplification.

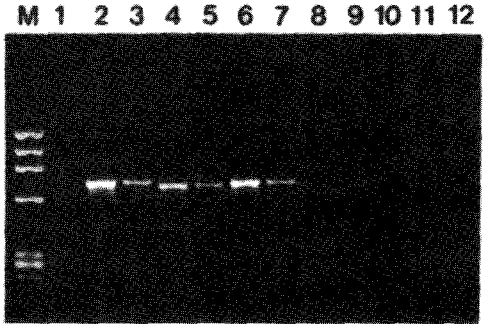
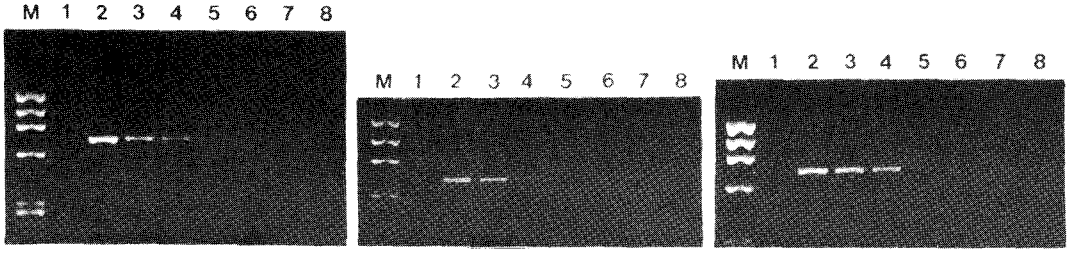


Fig. 2. Detection and amplification of the SRV *env* region by the PCR in Raji cells persistently infected with each subtype of SRV. Chromosomal DNA was prepared directly from cultured SRV-infected control cells and from uncultured rhesus macaque or baboon PBMC. One microgram of each cellular DNA was subjected to one round of PCR amplification. For the size comparison, 1.0×10^{-2} μg of viral genome-cloned plasmid DNA for each SRV subtype mixed with 1.0 μg CEMx174 chromosomal DNA, was amplified at the same reaction condition. PCR was performed as described in Materials and Methods. Lane M, ϕX174/Hae III restriction fragment molecular size marker; lane 1, reaction mixture control, no target DNA, lane 2, R20216 (SRV-1); lane 3, pSRV-1; lane 4, R10867 (SRV-2), lane 5, D2C/Oregon; lane 6, R23200 (SRV-3), lane 7, pSHRM15; lane 8, uninfected Raji; lane 9 to 12, PBMCs of E968, 93-7, PAPx4513, and PAP4446, respectively, and then stained with ethidium bromide.

Neither any other cell lines nor PBMCs involved in specific retrovirus infection other than SRV produced detectable PCR products at the given condition (Fig. 2, lanes 8-12). Repeated trials for amplifying SRV provirus from the various concentrations of infected cellular DNA confirmed that the PCR was highly reproducible without compromising its excellent specificity (data not shown).

Sensitivity evaluation of the PCR using viral genome-cloned plasmid DNA titration

To demonstrate the sensitivity of the PCR using primers SRVenv65 and SRVenv72R, a semiquantitative titration PCR was performed in which each type of SRV genome-cloned plasmid DNA was serially diluted, mixed with approximtely 1.0 μg of uninfected CEMx174 chromosomal DNA, and then subjected to one



(A) (B) (C)

Fig. 3. Sensitivity evaluation of the SRV PCR using the cross-detective primer pair. Each plasmid DNA for three subtypes of SRV was serially diluted and mixed with 1.0 μg CEMx174 chromosomal DNA, and then subjected to one round of amplification. PCR was performed as described in Marerials and Methods. **(A)** Plasmid pSRV-1 titration PCR for SRV-1 detection. Lane M, ϕX174/Hae III restriction fragment molecular size marker; lane 1, reaction mixture control with CEMx174 DNA, but no target DNA; lane 2, 1×10^{-2} μg; lane 3, 1×10^{-4} μg; lane 4, 1×10^{-6} μg; lane 5, 1×10^{-7} μg; land 6, 1×10^{-8} μg lane 7, 1×10^{-9} μg ; lane 8, 1×10^{-10} μg. **(B)** Plasmid D2C/Oregon titration PCR for SRV-2 detection. Lane M, ϕX174/Hae III retriction fragment molecular size marker; lane 1, reaction mixture control with CEMx174 DNA, but no target DNA; lane 2, 1×10^{-2} μg; lane 3, 1×10^{-4} μg; lane 4, 1×10^{-6} μg; lane 5, 1×10^{-7} μg; land 6, 1×10^{-8} μg pSRV-1; lane 7, 1×10^{-9} μg ; lane 8, 1×10^{-1} μg. **(C)** Plasmid pSHRM15 titration PCR for SRV-3 detection. Lane M, ϕX174/Hae III retriction fragment molecular size marker; lane 1, reaction mixture control with CEMx174 DNA, but no target DNA; lane 2, 1×10^{-2} μg; lane 3, 1×10^{-4} μg; lane 4, 1×10^{-6} μg; lane 5, 1×10^{-7} μg; lane 6, 1×10^{-8} μg pSRV-1; lane 7, 1×10^{-9} μg ; lane 8, 1×10^{-10} μg. One-tenth of the each reaction product was separated by electrophoresis through 1.5% agarose gel, and then stained with ethidium bromide.

round of amplification. One-tenth of the total end-product was electrophoresed for the analyses of each reaction. Titration PCR for SRV-1, revealed clearly visible bands on lane 6 which required only 1.0×10^{-9} μg of plasmid pSRV-1 (Fig. 3A). For SRV-2, amplified fragment from 1.0×10^{-9} μg of plasmid D2C/Oregon was shown with somewhat lower intensity but it was identifiable and reproducible (Fig. 3B; lane 6). For SRV-3 titration PCR, sharing very high sequence homology with SRV-1 throughout the genome, it was obvious that the reaction for pSHRM15 was as, if not more, sensitive as in the reaction for pSRV-1 (Fig. 3C). Based on molecular weight of each cloned SRV genome, the PCR of which resultants were simply stained with ethidium bromide should be sensitive enough to detect one SRV-infected cell per more than $5\text{-}7\times10^{4}$ uninfected cells.

DISCUSSION

In the absense of any official report regarding human infection by SRV and its pathogenesis, the readiness of transmission through habitual contact and the induction of profound immunosuppression accompanied with several indistinguishable symptoms from SIV infection among captive macaque colonies by SRV infections have been a major concern in the animal model research of human AIDS [9]. Attempts to preclude SRV-infected animals from the model study by type-specific PCR in selected laboratories has been successful [18, 19] though multiple PCR had to be done for the detection of each serotype. While any PCR methodology to amplify proviral DNA of STLV has not been developed yet, it had been demonstrated that the detection of STLV-1 by PCR using HTLV-1-derived primers targeting *tax* and *pol* gene with excellent specificity [23].

The novel PCR method described in this study was developed to detect proviruses of SRV subtypes after single round of amplification. The reaction required only one pair of primers to detect all three subtypes of SRV. The primers and the PCR demonstrated their excellent specificity and reproducibility as well. Sensitivity of the PCR, approximated by sem-

iquantitative titration analysis of ethidium bromide-stained band products, should be no less than virologic tests and certain serological tests like RIPA or immunoblot [14, 17]. Although ethidium bromide staining was employed to identify resulting products in order to simplify provirus detecting process as possible as it can be, it should not be unreasonable to assume that Southern hybridiztion of the amplified products with radioactive-labeled probe shall increase the reaction sensitivity about several fold at least. Furthermore, the second round of amplification using 'nested' primers, targeting the first round PCR resultants, has been known to increase PCR sensitivity roughly 100-fold. Attempts to develop the 'nested' PCR for SRV provirus detection is in progress in this laboratory. The one-stage PCR will render us to perform a large scale screening within a limited time and cost, even with higher sensisivity and dependability. Integration of this technology into currently available testing algorithms will greatly enhance the efficiency of SRV diagnostics.

SUMMARY

To develop the polymerase chain reaction (PCR) for the detection of type D simian retrovirus (SRV) infection, an oligonucleotide primer pair was designed to hybridize to the sequences within *env* gene of SRV subtype 1 (SRV-1). The 3' proximal *env* sequences annealing to the primers had been rather conserved among three different subtypes of SRV, SRV-1, SRV-2, and SRV-3 (Mason-Pfizer Monkey Virus: MPMV). The PCR using the primer pair targeting an *env* region successfully detected and amplified all three subtypes of SRV with excellent specificity after single round of reaction. The tests with peripheral blood mononuclear cells infected either with simian immunodeficiency virus or simian T-lymphotropic virus type 1, major immunosuppressive viral agents together with SRV in simian, verified the specificity of the PCR by excluding any cross reactivity. Semiquantitative titration PCR, amplifying serially diluted plasmid DNA of each subtype, was performed to evaluate sensitivity limits of the reaction. Based on molecular weight of each cloned SRV genome, the PCR should be able to detect one SRV-infected cell per more than $5\text{-}7 \times 10^4$ uninfected cells after simple ethidium bromide staining of resulting products. The PCR must be very efficient screening system with its quickness, certainty, and sensitivity for SRV-infected animals used in human AIDS research model. Second round amplification of the reaction products from the first PCR, or Southern hybridization by radiolabeled probes shall render to compete its efficacy to ELISA which has been the most sensitive technique to screen SRV infection but with frequent ambiguity problem.

Acknowledgment

This work was supported by a research grant from Kyung Hee University.

REFERENCES

1. Dezzutti CS, Frazier DE, Huff LY, Stromberg PC, Olsen RG: Subunit vaccine protects *Macaca nemestrina* (pig-tailed macaque) against simian T-cell lymphotropic-virus type-1 challenge. Cancer Research 50: 5687-5691, 1990.
2. Gardner MB, Luciw PA: Animal models of AIDS. FASEB 3: 2593-2606, 1989.
3. Desrosires RC, Letvin NL: Animal models for acquired immunodeficiency syndrome. Rev Infect Dis 9: 438-446, 1987.
4. Schneider J, Hunsmann G: Simian lentiviruses-the SIV group. AIDS 2: 1-9, 1988.
5. Naidu YM, Kestler HW III, Li Y, Butler CV, Silva DP, Schmidt DK, Troup CD, Sehgal PK, Sonigo P, Daniel MD, Desrosiers RC: Characterization of infectious

molecular clones of simian immunodeficiency virus (SIVmac) and human immunodeficiency virus type 2: persistent infection of rhesus monkeys with molecular cloned SIVmac. J Virol 62: 4691-4696.

6. Baba TW, Jeong YS, Penninck D, Bronson R, Green MF, Ruprecht RM: Pathogenicity of live, attenuated SIV after mucosal infection of neonatal macaques. Science 267: 1820-1825, 1995.
7. Daniel MD, Kirchhoff F, Czsjak SC, Desrosiers RC: Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene. Science 21: 377-380, 1992.
8. Kestler HW III, Ringer DJ, Mori K, Panicali DL, Sehgal PK, Daniel MD, Desrosiers RC: Importance of the nef gene for maintenance of high virus loads and for development of AIDS. Cell 65: 651-662, 1991.
9. Hu S-L, Zarling JM, Chinn J, Travis BM, Moran PA, Sias J, Kuller L, Morton WR, Heidecker G, and Benveniste RE: Protection of macaques against simian AIDS by immunization with recombinant vaccinia virus expressing the envelop glycoproteins of simian type D retrovirus. Proc Natl Acad USA 86: 7213-7217, 1989.
10. Lerche NW, Osborn KG, Marx PA, Prahalada S, Maul DH, Henrickson RV, Arther LO, Gilden RV, Munn RJ, Bryant ML, Heidecker-Fanning G, Gardner MG: Inapparent carriers of simian AIDS type D retrovirus and disease transmission with saliva. J Natl Cancer Inst 77: 489-496, 1986.
11. Maul DH, Zaiss CP, Mackenzie MR, Shigi SM, Marx PA, Gardner MG: Simian retrovirus D serogroup has a broad cellular tropism for lymphoid and non-lymphoid cells. J Virology 62: 1768-1773, 1988.
12. Benveniste RE, Todaro GJ: Evolution of primate oncoviruses: an endogenous virus from langurs (*presbystis spp.*) with related virogene sequences in other Old World monkeys. Proc Natl Acad USA 74: 4556-4561, 1977.
13. Lackner AA, Marx PA, Lerche NW, Gardner MB, Kluge JD, Spinner A, Kwang H-S, Lowenstine LJ: Asymptomatic infection of the central nervous system by the macaque immunosupressive type D retrovirus. J Gen Virol 70: 1641-1651, 1989.
14. Lerche NW, Yee JL: Establishing specific retrovirus-free breeding colonies of macaques: an approach to primary screening and surveillance. Lab Anim Sci 44: 217-221, 1994.
15. Spinner A, Sutjipto S: Simian AIDS ELISA: sensitivity, specificity and predictive values based on a comparison with Western blot technique. Lab Anim Sci 38: 568-572, 1988.
16 Chen Z, Ben K, Tian B, Zheng Y: Serological survey of a captive macaque colony in china for antibodies to simian type D retroviruses. J Med Primatol 21: 377-380, 1992.
17. Lerche NW, Marx PA, Osborn KG, Maul DH, Lowenstine LJ, Bleviss ML, Moody P, Henrickson RV, Gardner MB: Natural history of endemic type D retrovirus infection and acquired immune deficiency syndrome in group-housed rhesus monkeys. J Natl Cancer Inst 79: 847-854, 1987.
18. Grant RF, Windsor SK, Malinak CJ, Barts CR., Sabo A, Benveniste RE, Tsai CC: Characterization of infectious type D retrovirus from baboons. Virology 207: 292-296, 1995.
19. Heneine W, Lerche NW, Woods T, Spira T, Liff J, Eley W, Yee JL, Kaplan JE, Khabbaz, RF: The search for human infection with simian type D retroviruses. J Acquir Immune Defic Syndr 6: 1062-1066, 1993.
20. Thayer RM, Power MD, Bryant MB, Barr PS, Luciw PA: Sequence relationship of type D retroviruses which cause simian im-

munodeficiency syndrome. Virology 157: 317-329, 1987.
21. Heideker G, Lerche NW, Lowenstine LJ, Lackner A, Osborn KG, Gardner MB, Marx P: Induction of simian acquired immune deficiency syndrome (SAIDS) with a molecular clone of a type D SAIDS retrovirus. J Virol 61: 3066-3071, 1987.
22. Bryant ML, Marx PA, Shiigi SN, Wilson BJ, McNulty WP, Gardner MB: Distribution of type D retrovirus sequences in tissue of macaques with simian acquired immune deficiency and retroperitoneal fibromatosis. Virology 150: 149-160, 1986.
23. Dezzutti CS, Lazo A, Yee JY, Blakeslee JR, Mathes LE, Brown BG, Lairmore MD: Detection of simian T-lymphotropic virus type 1 using the polymerase chain reaction. Int J Cancer 50: 805-810, 1992.