

図1 精液中のHIV存在形式

表1 各方法による女性の2次感染確率と課題

性交渉	毎年約4.8% ずつ感染 血中 HIV 量と感染確率は相関 精子の数が少なく、運動率が低下す妊娠率が低い
従来の Swim up 法での人工授精	1/2000以下だが0ではない 回収精子数が少ないと妊娠確率が低い
われわれの改良法による体外受精	理論上は100%安全

でも、精液の単核球中の HIV proviral DNA は残し、精液から二次感染するリスクは残るのでコンドームの使用は必要になる²⁾。

受精法によるリスク (表1)

1. 性交渉による二次感染の危険性

異性間性交渉による HIV の二次感染率は1回あたり約0.15% (1~2/1000) といわれるが、AIDS が進行している場合は5/1000、安定している場合は0.7/1000程度である³⁾。しかしクラミジア感染症などの性感染症があると HIV に3~5倍感染しやすくなり、HIV に感染するとほかの性感染症に感染しやすくなる。

Quinn らは血中 VL が多いほど性交渉による二次感染の確立は高くなり、VL が400以下では二次感染が少ないと報告している⁴⁾ が、調査人数が少なく VL をどれだけ下げると安全かは不明である。花房らの検討では血中 VL と精液中 VL には相関が認められ⁵⁾、抗 HIV 療法で血中 VL が少なくなると、精液中の VL も少なくなるが、どれだけ減らすと二次感染率が低くなるかは正確には検討されていないし、調査は倫理面からも実施困難である。

2. 排卵日のみの性交渉による二次感染の危険性

HIV 陽性の夫が排卵日だけコンドームを使用し

ない場合では毎年約4.8%ずつ妻に感染する⁸⁾。

**不妊症の Swim up 法による
精子除去とその問題点**

HIV 陽性者の無処理の精液を用いた人工授精では約3.5%が二次感染した⁷⁾。それに対し、1992年、Semprini らは不妊症治療の swim up 法を応用して精子処理法を行った。精液中の HIV は精漿中の RNA や単核球中の proviral DNA の形で存在する。精液を遠心分離し、swim up によって HIV が検出感度以下の精子を得た。彼らは1997年までに2,000回以上人工授精を行い、二次感染がないと報告している⁸⁾。しかし、彼らの方法は回収精子で HIV RNA 量が800copies/mL以下としか確認しておらず、ウィルス除去が不十分で安全性に疑問がもたれてきた。

改良 Swim up 法 (不妊症 Swim up 法との相違点) (図2)

その後、より高いウィルス除去率を得るために Swim up 法も研究者によって改良が加えられた。花房らや Pasquier らは精液中の HIV を検出限界以下 (50copies/mL) にできたと報告した^{9) 9)}が、その後 nested PCR を用いた検出感度の高い方法を用いた検査では完全除去はできないとの報告が多い¹⁰⁾。不妊症で行われている従来の方法は HIV 除去が不十分であるうえ、単に検出限界以下というだけでは完全に HIV が除去されたかどうかを確認できない。筆者らは共同研究者の兼子が開発した改良 swim up 法により精液処理を行い、慶應微生物の加藤が開発した1 copy の HIV を検出する超高感度 PCR を用いて回収精子に HIV が1 copy もないことを確認した¹⁾。

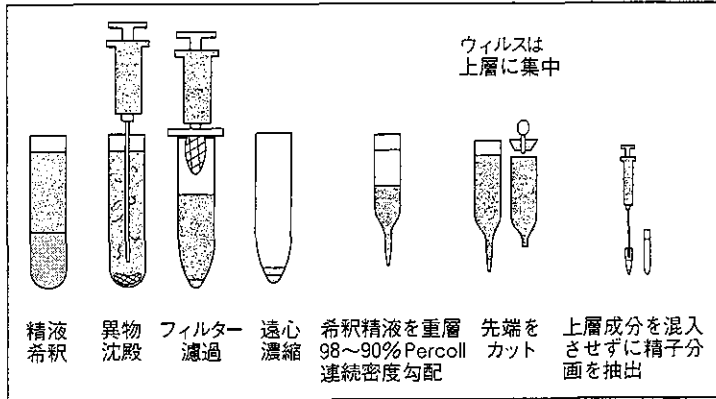


図2-a 精液から HIV 除去を目的とした方法の開発

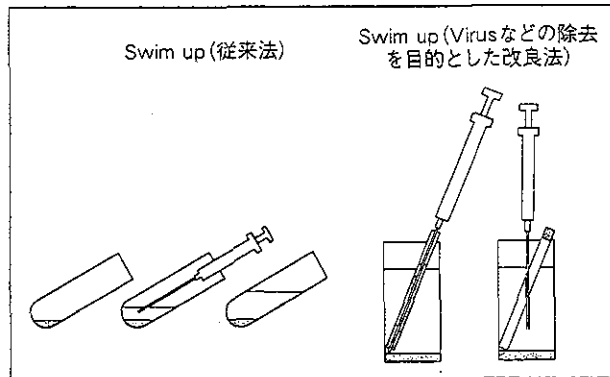


図2-b Swim up の相違

1. 精液希釈と夾雑物除去

精液には前立腺や精嚢腺の分泌物・金属結晶のほかに、尿道通過時の雑菌や下着の繊維などが混入している。そのまま分離液に重層して遠心すると夾雑物が底の精子分画に HIV を巻き込む危険性がある。筆者らは遠心前に精液を培養液で2倍に希釈して室温で20分静置後に上層の精子浮遊液を回収し、フィルターを使つてろ過して遠心分離した。

2. ウィルス分離液と濃度設定の選択

HIV 分離液として、Semprini らは1層のみのリンパ球分離液、ほかのグループらは45%と90%の2層の Pure Ception を用いて、400g、30分間遠心分離した。層を増やすほどウィルス除去率は高まるが精子の回収率が悪くなる。パコールにはエンドトキシン残存問題があり、最近では Pure Ception が一般的だが、Pure Ception による HIV 除去効率はパコールより低い¹¹⁾。筆者らは2000年以後、98%パコールを用いた連続密度勾配により、ウィルス除去率を $1/10^6$ 以上に高めた¹⁾。

3. 遠心分離後の精子分画の回収法 (図2a)

遠心分離後に、一番底の洗浄精子をどのように回収するかも問題である。遠心分離後、HIV は上層に分布し、単核球は中間層に分布している。上から順に吸引して精子を回収すると管壁を伝わり HIV が混入する危険性がある。筆者らは兼子らが開発した特殊試験管を用いて、最下層の精子分画部分をカットすることで上層成分を完全に遮断して底の精子を回収している。

4. Swim up 法の相違 (図2b)

遠心後の精子を培養液で洗浄した後に培養液を重層して Swim up を行うと、比重差がなくウィルスを攪拌する危険がある。われわれは精子液を底にガラス管を通じて静置し、運動精子を回収した。

5. 精子や卵への HIV の感染と付着の可能性

HIV が精子や卵に感染するか否かも議論がある。精子や卵は未分化細胞で、CD4、CCR5や CXCR4 が発現していない。HIV の GP120 が精子表面の糖脂質に付着し、容量依存性であるとの報告があり¹²⁾、Swim up 法で回収した精子に HIV が付着しているか否かが重大な問題だった。

HIV 感染成立に必要な CD4 蛋白は受精後10週以後にリンパ組織が分化して発現する。体外受精の培養液にウィルスが混入しても卵細胞にウィルスが感染することはない¹³⁾。

6. HIV 自体の感染性

HIV は毎日100億個産生されるが、感染力を持っているウィルスは全体の $1/10000$ 以下といわれている¹⁵⁾¹⁶⁾。われわれは、HIV を培養液の中で培養すると、感染性が $1/10$ 以下に低下することを示した¹⁶⁾。

体外受精と人工授精の比較

体外受精は人工授精に比して二次感染防止の面で優れているが、卵巣過剰刺激症候群など女性への負担が大きい。人工授精で HIV が混入した精子浮遊液を子宮に注入した場合、HIV 除去が完全でないと子宮内の細胞に感染する危険性がある。一方、体外受精では精子浮遊液に HIV が混入していたとしても卵には感染せず、HIV の感染性は培養後 $1/10$ 以下に低下する。さらに培養液を2日目に交換し、受精卵を洗浄すると、たとえ精子液に HIV が混入していても相当減少できる。われわれは図3に示すプロトコールに従って、胚移植前に培養液中の HIV がまったくないことを超高感度 PCR で確認して胚移植を実施した。

1. HIV 感染者の精子の問題

HIV 感染者は精子の数が減少し、奇形率も高く、運動率も低下している場合が多い。Swim up 法を用いると精子の回収率が低下し、HIV 除去率を高めた方法では人工授精では妊娠しなかったことが

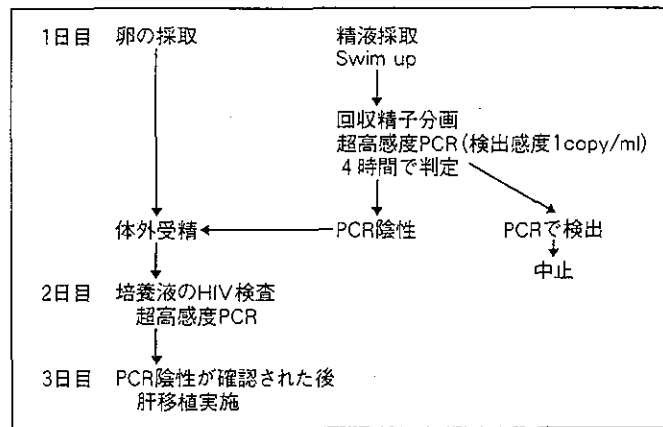


図3 今回実施した体外受精のプロトコール

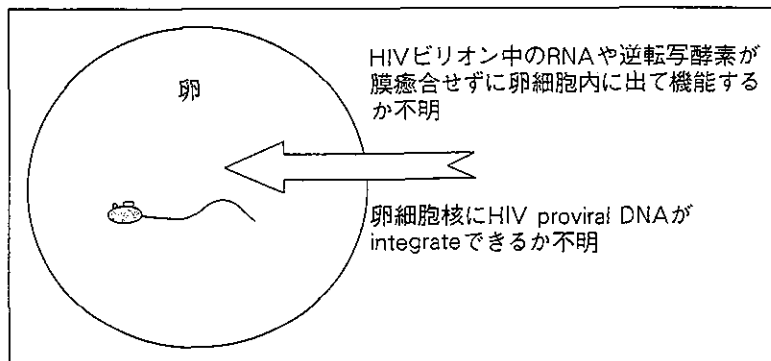


図 4

顕微授精で HIV が付着した精子を卵に強制注入した場合に感染が成立するかどうか不明

報告されている¹⁸⁾。しかし、今後、精子の数が多く、運動率も良い場合には人工授精の適応も必要と思われる。その場合も精子浮遊液の HIV の完全除去の確認が必須と考える。兼子が新たに開発した方法により、精子回収率を高め、ウィルス除去も十分で有れば人工授精の可能性も期待できる。

2. 顕微授精

筆者らの方法により、回収精子が少ない場合は顕微授精 (intracytoplasmic sperm injection: ICSI) でも二次感染なく妊娠出産できた。図4に示すように注入精子に HIV が付着していたり、注入液に HIV が混入していた場合に卵に HIV 感染が成立する可能性は相当低いが、絶対安全とは断言できず、完全除去の確認は必要である。ICSI 自体を問題

視する意見もあり今後慎重に対応したい。

3. C 型肝炎の問題

一般に HCV は HIV よりも性交渉による感染リスクは低いとされるが、体外受精による HCV の2次感染が報告されており¹⁹⁾、フランスなどはガイドラインにのっとり夫のウィルス感染検査を必要としている。Pasquier らは Swim up 法で精液中の HCV も除去されることを報告した²⁾。

今後の対応

今後、わが国の HIV 感染者の増加にともない、HIV 陽性男性が陰性女性と結婚して子どもを望む機会が増えると予測される。専門機関で精液を処

理し、HIV陰性精子を回収して冷凍保存し、地元
の協力産婦人科施設に送って人工授精や体外受精
を行うことができれば女性の負担は大幅に減少で
きる。わが国ではHIV感染者は先進国の中では
唯一急増しており、2003年で約15,000人が感染し、
2010年には5万人を越えると予測されている。今
後、多くの感染者が一般医療機関を受診するケー
スが増えるので、感染対策は必須である。

おわりに

EUではHIV感染者の人工授精を積極的に行う
ようになりつつあるが、アメリカCDCはいまだに

禁止勧告を解除していない。われわれは不十分な
HIV除去法による人工授精で二次感染した女性の
相談を受けた。精子浮遊液のHIV RNA, HIV pro-
viral DNAをどれだけ減らせば二次感染が生じな
いか不明である以上、完全なHIV除去確認が
されていない人工授精は危険である。日本産婦人
科学会は2003年からHIV感染者の人工授精は施
設の倫理委員会で承認された場合に限定するとの
勧告を出した。EUではHIVだけでなくHCV感染
症にもガイドラインを作成している国もあり、わ
が国でも生殖補助医療におけるウィルス感染対策
のガイドラインを導入することが必要と考えられ
る。

文 献

- 1) 花房秀次ほか：改良 swim up 法による HIV陽性男性の
精液からの HIV除去の評価と体外受精の臨床実施成績
について。第15回日本エイズ学会（東京）、11/29-
12/1 2001, p142, 2001.
- 2) Haase AT, Schacker TW: Potential for the transmis-
sion of HIV-1 despite highly active antiretroviral
therapy. *N Engl J Med* 339: 1846, 1998.
- 3) European Study Group on Heterosexual Transmission
of HIV: A longitudinal study of human immu-
nodeficiency virus transmission by heterosexual
partners. *N Engl J Med* 331: 341, 1994.
- 4) Quinn TC, et al: Viral load and heterosexual trans-
mission of human immunodeficiency virus type 1.
Rakai Project Study Group. *N Engl J Med* 342:
921, 2000.
- 5) Hanabusa H, et al: An evaluation of semen process-
ing methods for eliminating HIV-1. *AIDS* 14: 1611,
2000.
- 6) European Study Group on Heterosexual Transmission
of HIV: A longitudinal study of human immu-
nodeficiency virus transmission by heterosexual
partners. *N Engl J Med* 331: 341, 1994.
- 7) Araneta MR, et al: HIV transmission through do-
nor artificial insemination. *JAMA* 273: 854, 1995.
- 8) Semprini AE, et al: Reproductive counseling for
HIV-discordant couples. *Lancet* 349: 1401, 1997.
- 9) Pasquier C, et al: Sperm washing and virus nucleic
acid detection to reduce HIV and hepatitis C virus
transmission in serodiscordant couples wishing to
have children. *AIDS* 14: 2093-2099, 2000.
- 10) 五味淵秀人ほか：HIV感染者の精液を用いた夫婦間人
工授精についての検討—ウィルス除去法について—。
日本エイズ学会誌 3: 6, 2001.
- 11) Naoaki Kuji, et al: Sedimentation Kinetics of HIV-1 in
two gradient media. 58th Annual Meeting of American
Society for Reproductive Medicine. (Seattle) 2002,
10: 12-17, 2002.
- 12) Brogi A, et al: Interaction of human immunodeficiency
virus type 1 envelope glycoprotein gp120
with a galactoglycerolipid associated with human
sperm. *AIDS Res Hum Retroviruses* 12: 483, 1996.
- 13) Baccetti B, et al: Failure of HIV-1 to infect human
oocytes directly. *J Acquir Immune Defic Syndr* 21:
355, 1999.
- 14) Chan AW, et al: Transgenic monkeys produced by
retroviral gene transfer into mature oocytes. *Sci-
ence* 291: 309, 2001.
- 15) McKeating JA, et al: Differential loss of envelope
glycoprotein gp120 from virions of human immu-
nodeficiency virus type 1 isolates: effect on infectivity
and neutralization. *J Virol* 65: 8542, 1991.
- 16) Kato S, et al: A plaque hybridization assay for quan-
tifying and cloning infectious human immunodeficiency
virus type 1 virions. *J Virol Methods* 72: 1,
1998.
- 17) Seyaert SR, et al: Infections in IVF: review and
guidelines. *Hum Reprod Update* 6: 432, 2000.
- 18) Ohl J, et al: Assisted reproduction techniques for
HIV serodiscordant couples: 18 months of experi-
ence *Hum Reprod* 18: 1244-9, 2003.

**The quantity and diversity of infectious viruses
in various tissues of SHIV-infected monkeys
at the early and AIDS stages**

A. Miyake¹, Y. Enose¹, S. Ohkura¹, H. Suzuki¹, T. Kuwata¹, T. Shimada²,
S. Kato³, O. Narayan⁴, and M. Hayami¹

¹Institute for Virus Research, Kyoto University, Kyoto, Japan

²Department of Pathology and Tumor Biology, Graduate School of Medicine,
Kyoto University, Kyoto, Japan

³Keio University School of Medicine, Tokyo, Japan

⁴University of Kansas, Medical Center, Kansas City, Kansas, U.S.A.

Received December 2, 2002; accepted October 2, 2003

Published online December 23, 2003 © Springer-Verlag 2003

Summary. To detect the major sites of viral replication in immunodeficiency virus-infected individuals, we quantified proviral DNA and infectious viruses using quantitative PCR and a plaque assay, respectively, in various tissues of SHIV_{KU-2}-infected monkeys in the early and AIDS stages of infection. Compared the quantity of infectious virus among PBMC and the lymphoid tissues, the mesenteric lymph node had the largest number of infectious viruses at the AIDS stage more than at the early stage of infection. These results suggested that the gastrointestinal tract was a major site of viral replication. In the brain, proviral DNA was detected at the early and AIDS stage of infection, but infectious viruses were detected at only the AIDS stage. Moreover, we analyzed the nucleotide sequences of the *env* V3 region in infectious virus clones isolated from each plaque. The viruses in the lymphoid tissues of the monkey that developed AIDS diverged from the inoculated virus and had the same three amino acid substitutions. However, the viruses in the brain were almost identical to the inoculated virus, suggesting that the virus entered the brain early after infection and persisted without replication and genetic diversion until the AIDS stage.

Introduction

HIV-1-related mortality has significantly declined in the United States and other developed countries as a result of the use of effective antiretroviral drugs. Highly active antiretroviral therapy (HAART) has dramatically suppressed the virus load

in the plasma of patients to undetectable levels and retarded the progression of AIDS. However, it is difficult to completely eliminate the virus from the body because of inadequate drug concentration in certain tissues, drug resistance, and persistence of a latently infected pool of cells. Previous studies showed that the frequency of HIV-1-infected cells in lymphoid organs was higher than the frequency in peripheral blood [27, 31, 36]. Moreover, HIV-1-infected cells were detected in various tissues including brain, gut-associated lymphoid tissue and the genital tract [4, 33, 38]. The existence of persistent HIV-1-infected cells or organs could prompt the reappearance of the virus in the infected person even though the virus was once suppressed. Therefore, it is important to identify the reservoir tissues of the virus and the virus characteristics in these sites.

Simian immunodeficiency virus (SIV), which is closely related to HIV, infects monkeys by various routes and induces an AIDS-like disease. Therefore, the SIV-macaque model can provide information about HIV infection in humans [1, 8, 39]. Moreover, some laboratories have constructed chimeric simian-human immunodeficiency viruses (SHIVs), which contain the HIV-1 envelope gene (*env*) within a genetic background of SIVmac239. These SHIVs have been useful in clarifying the role of Env in viral infection, pathogenesis, and the relation between the viruses and the host immune response [11, 14, 21, 28]. Some SHIV strains were found to be pathogenic and cause disease in macaque monkeys. One of them is SHIV_{KU-2}, a polyclonal chimeric virus derived from the in vivo passage of SHIV-4, containing the *env* of HIV-1 HXBc2 [15, 24]. SHIV_{KU-2} was reported to cause an AIDS-like disease with encephalitis in macaque monkeys [16, 25].

In this study, to determine the quantity and diversity of viruses in various tissues, we quantified not only proviral DNA but also infectious viruses by quantitative PCR and plaque assay, respectively, and analyzed the hypervariable region (V3) sequences of infectious viruses in peripheral blood mononuclear cells (PBMC), brain, and lymphoid tissues of the SHIV_{KU-2}-infected monkeys in the early and AIDS stage of infection. The viral load in the infected individuals has usually been quantified either by the copy number of virus RNA or DNA using PCR [5, 31] or by the end-point-dilution of cell cultures [13, 36]. However, the former method did not differentiate between infectious and non-infectious viruses. Although the latter method is useful for quantification of infectious viruses, it is difficult to isolate single infectious virions from polyclonal viruses by this assay. Therefore, in addition to quantitative PCR, we used the plaque assay to quantify the infectious viruses and isolate the viruses as single clone. The plaque assay could quantify only the infectious viruses that were released from infected cells and could provide an infectious virus clone directly from a plaque because each plaque was derived from one infected cell [17]. One of the three monkeys used in this study developed AIDS. Our results showed that the viruses actively replicated not only in the lymphoid tissues but also brain of the monkey that developed AIDS and that the quasispecies of infectious viruses in the brain were different from those in the lymphoid tissues.

Materials and methods

Virus, monkeys, and inoculation

SHIV_{KU-2} was provided by one of the authors (Dr. Opendra Narayan). It is a polyclonal chimeric virus derived by the *in vivo* passage of SHIV-4 [15, 24]. The virus stock used in this study was grown in rhesus macaque PBMC, and stored at -80°C until use. Three adult rhesus macaques (*Macaca mulatta*) were used in this study. The monkeys were anesthetized by intramuscular injection of ketamine chloride and intravenously inoculated with 1×10^5 TCID₅₀ of the virus stock. Two monkeys, MM254 and MM255, were euthanized at 6 weeks post inoculation (p.i.) and one monkey, MM201, which developed an AIDS-like disease was euthanized at 26 weeks p.i. Throughout the experimental period, the monkeys were housed in accordance with regulations approved by the Institutional Animal Care and Use Committee of the Institute for Virus Research, Kyoto University.

Sample collection

Blood was periodically collected from all monkeys. PBMC and plasma were separated from heparinized blood by Percoll (Nacalai Tesque, Kyoto, Japan) density gradient centrifugation. Plasma was frozen at -80°C until use. Brain and tissues from various lymphoid organs were obtained at the time of euthanasia. A brain tissue sample was obtained from cerebral cortex, and bone marrow was harvested from the femur. Parts of the samples were frozen directly at -80°C until use for quantification of proviral DNA. Residual samples of brain, spleen, thymus, bone marrow, mesenteric lymph node and a mixture of axillary and mandibular lymph nodes were minced and filtered through a $40\ \mu\text{m}$ nylon filter (Becton Dickinson, Franklin Lakes, New Jersey). The obtained cells were immediately used in the plaque assay.

Quantification of plasma viral RNA

The viral RNA loads in plasma were determined by quantitative RT-PCR [34]. Total RNAs were prepared from plasma with a QIAamp Viral RNA kit (QIAGEN, Hilden, Germany). RT-PCR was performed with a Taqman EZ RT-PCR kit (Perkin Elmer, Wellesley, MA) for the SIV gag region using the following primers: SIV2-696F (5'-GGA AAT TAC CCA GTA CAA CAA ATA GG-3') and SIV2-784R (5'-TCT ATC AAT TTT ACC CAG GCA TTT A-3'). A labeled probe, SIV2-731T (5'-Fam-TGT CCA CCT GCC ATT AAG CCC G-Tamra-3'; Perkin Elmer), was used for detection of the PCR products. These reactions were performed with a Prism 7700 Sequence Detector (Applied Biosystems, Foster City, CA) and analyzed using the manufacturer's software. For each run, a standard curve was generated from dilutions whose copy numbers were known, and the RNA in the plasma samples was quantified based on the standard curve.

Quantification of proviral DNA

The proviral DNA loads in tissues were determined by quantitative PCR. DNA samples were extracted directly from frozen tissues with a QIAGEN DNeasy Tissue kit (QIAGEN). PCR was performed with a Taqman PCR Reagent kit (Perkin Elmer) using the same primer set and probe which were used in RT-PCR. A standard curve was generated from a plasmid DNA sample containing the full genome of SHIV NM-3rN, which was quantified with a UV-spectrophotometer.

Plaque assay

The infectious viruses were quantified and isolated using the plaque assay [17]. An agarose gel bilayer containing RPMI 1640 medium was made in plastic culture dishes of 100 mm; the

lower layer consisted of 12 ml of 1.2% agarose (Agarose NA; Pharmacia, Uppsala, Sweden) and the upper layer consisted of 12 ml of 0.4% low-gelling-temperature agarose (SeaPlaque Agarose; FMC, Rockland, ME). The dishes were incubated at 37 °C in 5% CO₂ overnight. The following day, 2×10^6 cells of each sample and 8×10^6 cells of M8166 were suspended in 3 ml of a 0.4% low-gelling-temperature agarose solution containing the culture medium and the mixture was immediately overlaid on the agarose gel layer previously prepared. To detect infectious viruses in the brain, approximately 10 cm³ of brain tissue was homogenized and then mixed with M8166 cells. The mixture was suspended in agarose solution and overlaid on the plate. To assay the plasma, 8×10^6 M8166 cells were incubated with 500 µl of plasma in 1 ml of medium for 1 h. The cells were suspended in agarose solution, and this mixture was overlaid on the plate. After the gel was hardened, the plates were covered with 12 ml of culture medium and incubated at 37 °C in 5% CO₂ for 10 days. The medium over the plates was replaced with fresh medium every day. After removal of the medium in the plates at 10 days, the plates were stained with 1.8 ml of 0.7% MTT for 2 h to count the number of the plaques. Each plaque represented an infectious virus clone. A portion of a plaque on the agarose gel was picked up with a pipette tip and suspended in 500 µl of medium. After centrifugation, the cell pellet was stored at -80 °C.

Sequence analysis

Total DNA was extracted from the cell pellet with a DNeasy Tissue Kit (QIAGEN). The 365 bp of the *env* V3 region was amplified by PCR with one primer pair: V3BgF1 (5'-CAG GCC AGT AGT ATC AAC TCA ACT GC-3') and YT001 (5'-ACA ATT TCT GGG TCC CCT CTG AGG A-3'), in a mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 200 µM of each dNTPs, 0.2 µM of each primer and 2.5 units of AmpliTaq DNA polymerase. The PCR products were purified with QIAquick Spin (QIAGEN) and directly sequenced with the sense primer V3BgF1 by a Taq cycle sequencing system using the Dyedeoxy termination method and analyzed with an ABI373A automated DNA sequencer (Applied Biosystems). The nucleotide sequences of the analyzed clones were converted into amino acid sequences and aligned with SHIV_{KU-2} [25] using the Clustal W program [37]. The nucleotide sequence data reported in this paper will appear in the GenBank nucleotide sequence database with accession numbers AF364413 to AF364476.

Results

Infection of rhesus macaques with SHIV_{KU-2}

Three rhesus macaques, MM201, MM254 and MM255, were intravenously inoculated with SHIV_{KU-2}. In all monkeys, viral RNAs in the plasma increased rapidly after infection and viremia reached peak levels (10^6 to 10^7 copies/ml) at 2 weeks post inoculation (p.i.) (Fig. 1). Concurrent with the increase in viremia, CD4⁺ T cell counts declined to 5 to 40% of normal range after the inoculation (data not shown). MM254 and MM255 were then euthanized at 6 weeks p.i. In the remaining monkey, MM201, plasma viral RNA remained high (in the range of 10^5 to 10^6 copies/ml) and CD4⁺ T cell counts remained low (less than 100 cells/µl) throughout the infection. Finally, MM201 developed an AIDS-like disease with neurological defects at the time of euthanasia, at 26 weeks p.i. Neurobehavioral and histopathological abnormalities with encephalitis were observed in this monkey (data not shown).

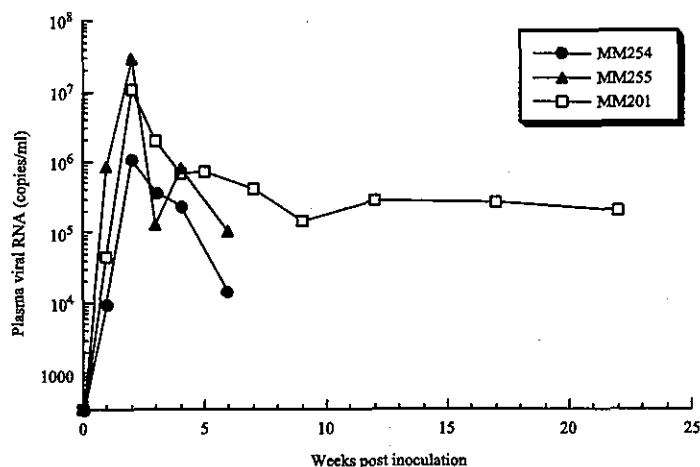


Fig. 1. Plasma viral RNA loads of three SHIV_{KU-2}-inoculated monkeys. MM255 and MM254 were euthanized at 6 weeks p.i. MM201, which developed an AIDS-like disease, was euthanized at 26 weeks p.i. The detection limit of this assay was 3×10^2 copies/ml

Quantification of proviral DNA

To examine the correlation between the amount of virus harbored in the various tissues of the individuals and the development of AIDS, the viral loads were quantified in the blood, brain and some lymphoid tissues of the monkeys at the early stage of infection (6 weeks p.i.) and at the AIDS stage (26 weeks p.i.). First, proviral DNA loads in the tissues were measured by quantitative PCR. In the early stage of infection, proviral DNA was detected in the PBMC, spleen, thymus and lymph nodes of the two monkeys (MM254 and MM255), ranging from 10^4 to 10^5 copies per microgram of total cellular DNA (Fig. 2). Proviral DNA was also detected in the brain tissues of both monkeys, although the copy numbers were lower than those in the other samples.

At the AIDS stage, the proviral DNA loads in all the tissue samples of the infected monkey (MM201) were higher (10 to 10^2 times) than those of the other two monkeys in the early stage of infection (Fig. 2). The proviral DNA load in the brain tissues was especially high, being 10^4 times higher than the loads in the other two monkeys. These results showed that the virus infected the brain tissues early after inoculation and increased at the late stage of infection.

Quantification of infectious viruses

Although the proviral DNA was present in each tissue of the infected monkeys, it was not clear which of the infected tissues released the infectious viruses that are considered to play a major role in virus spread in a body. Therefore, only the infectious viruses were quantified using the plaque assay in the various tissues of the infected monkeys. The infectious viruses were detected in PBMC, spleen,

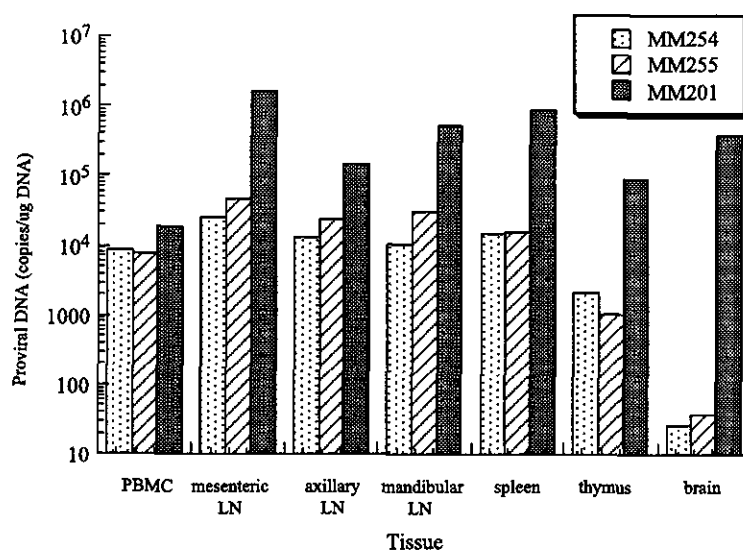


Fig. 2. Proviral DNA loads in various tissues of three SHIV_{KU-2}-inoculated monkeys. Viral loads were determined by quantitative PCR and are expressed as viral DNA copy numbers per microgram of total DNA extracted from the tissue homogenates. MM254 and MM255 were euthanized at 6 weeks p.i. MM201 was euthanized at 26 weeks p.i.

and mesenteric and axillary/mandibular lymph nodes of the two monkeys in the early stage of infection (Fig. 3). In MM254, the viral loads in PBMC, spleen, and lymph nodes were almost the same. In MM255, the viral loads in the spleen and axillary/mandibular lymph nodes were higher than those in PBMC and mesenteric lymph node. However, infectious virus was not detected in thymus, bone marrow and brain of both monkeys although proviral DNA was detected in these samples.

In the monkey that developed AIDS, MM201, infectious viruses were detected at various levels in all the tissues we examined, except for the thymus. The levels of infectious virus in the spleen, mesenteric and axillary/mandibular lymph nodes of MM201 were detected more than the level in PBMC, and the mesenteric lymph node of MM201 had an especially large number of infectious viruses (250 pfu per 10⁶ cells). Moreover, in MM201, the infected cells that produced infectious viruses were present in the brain and bone marrow, but not in these same tissues in the other two monkeys early after infection. These results showed that the productive viral infection occurs in the brain tissues at the AIDS stage of infection, not early after infection.

Quasispecies analysis

To characterize the genotype of the viruses that replicated in each tissue of the infected monkeys and caused AIDS, the 270-bp nucleotide sequence encompassing HIV-1 *env* V3 region, which is an immunologically important domain, was analyzed in the infectious viruses isolated from each plaque. Similarly, the viral

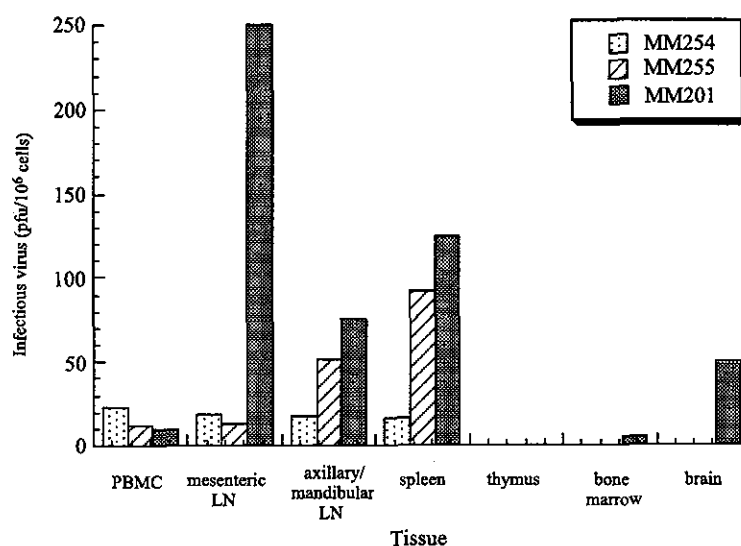


Fig. 3. Infectious virus loads in various tissues of three SHIV_{KU-2}-inoculated monkeys. Viral loads were determined by plaque assay and are expressed as the number of plaque forming units (pfu) per 10⁶ cells or 10 cm³ of brain tissues. MM254 and MM255 were euthanized at 6 weeks p.i. MM201 was euthanized at 26 weeks p.i.

genotypes in the virus stock were analyzed after isolation of the viral clones by plaque assay, resulting in a single genotype (Fig. 4).

Among the infectious viruses in different tissues of MM201, which developed AIDS, the nucleotide sequences encompassing the *env* V3 region varied greatly from the sequence of the inoculated virus. Moreover, three of the amino acid substitutions were commonly found in these sequences. One substitution [threonine (T) to lysine (K) or alanine (A)] was detected at a glycosylation site in the V3 region and the others [alanine (A) to threonine (T), phenylalanine (F) to tyrosine (Y)] were detected downstream of the V3 region, i.e., in the C3 region. Each viral genotype was named by three letters corresponding to these amino acid substitutions (Fig. 4): TAF, AAF, TAY, TTY, KAF, KAY, KTF, and KTY. In the PBMC, spleen, lymph nodes and bone marrow, all the viral genotypes, except for two clones, had both substitutions, i.e., the T-to-K substitution and the F-to-Y substitution. Moreover, the substitution from A to T was detected in 63% of all the genotypes in these tissue samples. Thus, at the AIDS stage of infection, the viral genotypes in most lymphoid tissues diverged from the virus stock. However, most of the viral genotypes in the brain were identical to that of the virus stock although two clones of the KAF and KAY types were detected. These results showed that the infectious viruses in the brain tissues were different from those in the lymphoid tissues, and that the infected cells in the brain tissues could produce both the original viruses and the diverged viruses at the AIDS stage of infection.

We next analyzed the nucleotide sequences of the infectious viruses from the other two monkeys at the early stage of infection, focusing on the viral genotypes

regardless of the infection period. However, the viral load in the lymphoid tissues was higher at the AIDS stage than at the early stage of infection. Especially, the mesenteric lymph nodes showed the greatest increases in the number of infectious viruses at the AIDS stage compared with the number of infectious viruses early after infection, although all three monkeys had almost the same plasma viral RNA loads. These results were confirmed by the quantification of both viral DNA and infectious virus. A previous study showed that mesenteric lymph nodes contain numerous SIV-infected cells at all stages of SIV-infection [12]. In the gastrointestinal tract, many lymphoid cells are constantly activated by antigenic stimuli, including food. Because HIV/SIV could replicate optimally in the activated T cells, the gastrointestinal tract might be a major site of viral replication at the time of development of AIDS.

Based on the sequences encompassing the *env* V3 region, the infectious viral populations in PBMC and mesenteric lymph nodes of the two monkeys at the early stage of infection hardly diverged from the inoculated virus. However, the sequences of infectious viruses in the monkey that developed AIDS revealed that the viral populations in PBMC and lymphoid tissues diverged from the inoculated virus and had three common amino acid substitutions in the predicted sequences of the infectious viruses. One substitution [threonine (T) to lysine (K)] was detected at the glycosylation site in the V3 region. A T-to-K substitution at this site could add a positive charge and abolish a potential N-linked glycosylation site. This substitution in HIV and SIV was reported to be correlated with a switch of the viral phenotype and a change in the interaction between the virus and the host immunity [9, 29]. Moreover, this site was also reported to be an epitope of cytotoxic T lymphocytes (CTLs) [30]. The other substitutions [alanine (A) to threonine (T) and phenylalanine (F) to tyrosine (Y)] were detected downstream of the V3 region, in the C3 region. Because this region was included in the outer domains of the envelope [22], it is possible that amino acids located on this region represent targets for CTLs or antibodies. Therefore, these infectious viruses with the amino acid substitutions might escape from the immune response in the infected monkeys and cause the development of AIDS.

The brain was reported as one of the tissues which the virus latently infected [10, 18, 20, 32, 35]. However, little is known about when the virus crosses the blood-brain barrier and infects the cell in the brain although various analyses about neuropathogenesis were performed in patients with HIV dementia. In this study, proviral DNA, but not infectious virus, was also detected in the brain of the monkeys in the early stage of infection. In contrast, not only proviral DNA but also infectious virus was detected in the brain of the monkey that developed AIDS with encephalitis. These results suggested that the brains of the infected monkeys were exposed to the virus during the acute infection but that the virus might hardly replicate in the brain. In SIV-infected macaques, both proviral DNA and viral RNA were detected in the brain during the acute infection [23, 26]. However, the detection of viral RNA was transient, whereas proviral DNA latently existed in the brain throughout infection [6]. Following persistent infection in the brain, viral RNA and viral protein, in addition to proviral DNA, were reproduced in the brain

at the late stage of infection [3, 7, 40]. In our study, with the progression of AIDS disease, the latent infection might be broken and the production of the infectious virus increased in the brain. Establishment of a productive infection in the brain might result in the neurological lesions in AIDS.

Previous studies showed genetic differences between the viral sequences derived from the blood and brain of HIV-1 infected patients [2, 19]. In our study, based on the sequence encompassing the *env* V3 region, the infectious viral population in the brain of the monkey that developed AIDS with encephalitis was similar to the inoculated virus, while that in the lymphoid tissues genetically diverged from the inoculated virus. The infectious viruses produced in the brain at the AIDS stage of infection resulted from the viruses which entered the brain early after infection and had persisted in the brain without genetic divergence. For individuals at the AIDS stage, immunological changes might trigger active viral replication in the brain and then result in neurological defects. Further studies of the evolution of the virus in different tissues may eventually lead to better methods for suppressing replication.

Acknowledgments

This work was supported by a Health Sciences Research Grant from the Ministry of Health, Labour and Welfare, Japan, a Grant-in-Aid for Scientific Research from the Ministry of Education and Science, Japan and a Research Grant on Health Sciences focusing on Drug Innovation from the Japan Health Sciences Foundation. Y. E. is recipient of a Research Fellowship of the Japan Society for the Promotion of Science for Young Scientists.

References

1. Anderson MG, Hauer D, Sharma DP, Joag SV, Narayan O, Zink MC, Clements JE (1993) Analysis of envelope changes acquired by SIVmac239 during neuroadaptation in rhesus macaques. *Virology* 195: 616–626
2. Ball JK, Holmes EC, Whitwell H, Desselberger U (1994) Genomic variation of human immunodeficiency virus type 1 (HIV-1): molecular analyses of HIV-1 in sequential blood samples and various organs obtained at autopsy. *J Gen Virol* 75: 67–79
3. Boche D, Khatissian E, Gray F, Falanga P, Montagnier L, Hurtrel B (1999) Viral load and neuropathology in the SIV model. *J Neurovirol* 5: 232–240
4. Cavert W, Haase AT (1998) A national tissue bank to track HIV eradication and immune reconstruction. *Science* 280: 1865–1866
5. Chun TW, Carruth L, Finzi D, Shen X, DiGiuseppe JA, Taylor H, Hermankova M, Chadwick K, Margolick J, Quinn TC, Kuo YH, Brookmeyer R, Zeiger MA, Barditch-Crovo P, Siliciano RF (1997) Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 387: 183–188
6. Clements JE, Babas T, Mankowski JL, Suryanarayana K, Piatak M Jr, Tarwater PM, Lifson JD, Zink MC (2002) The central nervous system as a reservoir for simian immunodeficiency virus (SIV): steady-state levels of SIV DNA in brain from acute through asymptomatic infection. *J Infect Dis* 186: 905–913
7. Demuth M, Czub S, Sauer U, Koutsilieris E, Haaf P, Heeney J, Stahl-Hennig C, ter Meulen V, Sopper S (2000) Relationship between viral load in blood, cerebrospinal fluid,

- brain tissue and isolated microglia with neurological disease in macaques infected with different strains of SIV. *J Neurovirol* 6: 187–201
8. Desrosiers RC (1988) Simian immunodeficiency viruses. *Annu Rev Microbiol* 42: 607–625
 9. Fouchier RA, Groenink M, Kootstra NA, Tersmette M, Huisman HG, Miedema F, Schuitemaker H (1992) Phenotype-associated sequence variation in the third variable domain of the human immunodeficiency virus type 1 gp120 molecule. *J Virol* 66: 3183–3187
 10. Gosztonyi G, Artigas J, Lamperth L, Webster HD (1994) Human immunodeficiency virus (HIV) distribution in HIV encephalitis: study of 19 cases with combined use of in situ hybridization and immunocytochemistry. *J Neuropathol Exp Neurol* 53: 521–534
 11. Harouse JM, Gettie A, Tan RC, Blanchard J, Cheng-Mayer C (1999) Distinct pathogenic sequela in rhesus macaques infected with CCR5 or CXCR4 utilizing SHIVs. *Science* 284: 816–819
 12. Heise C, Vogel P, Miller CJ, Lackner A, Dandekar S (1993) Distribution of SIV infection in the gastrointestinal tract of rhesus macaques at early and terminal stages of AIDS. *J Med Primatol* 22: 187–193
 13. Ho DD, Moudgil T, Alam M (1989) Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N Engl J Med* 321: 1621–1625
 14. Igarashi T, Kuwata T, Takehisa J, Ibuki K, Shibata R, Mukai R, Komatsu T, Adachi A, Ido E, Hayami M (1996) Genomic and biological alteration of a human immunodeficiency virus type 1 (HIV-1)-simian immunodeficiency virus strain mac chimera, with HIV-1 Env, recovered from a long-term carrier monkey. *J Gen Virol* 77: 1649–1658
 15. Joag SV, Li Z, Foresman L, Pinson DM, Raghavan R, Zhuge W, Adany I, Wang C, Jia F, Sheffer D, Ranchalis J, Watson A, Narayan O (1997) Characterization of the pathogenic KU-SHIV model of acquired immunodeficiency syndrome in macaques. *AIDS Res Hum Retrovir* 13: 635–645
 16. Joag SV, Li Z, Wang C, Jia F, Foresman L, Adany I, Pinson DM, Stephens EB, Narayan O (1998) Chimeric SHIV that causes CD4⁺ T cell loss and AIDS in rhesus macaques. *J Med Primatol* 27: 59–64
 17. Kato S, Hiraishi Y, Nishimura N, Sugita T, Tomihama M, Takano T (1998) A plaque hybridization assay for quantifying and cloning infectious human immunodeficiency virus type 1 virions. *J Virol Methods* 72: 1–7
 18. Koenig S, Gendelman HE, Orenstein JM, Dal Canto MC, Pezeshkpour GH, Yungbluth M, Janotta F, Aksamit A, Martin MA, Fauci AS (1986) Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science* 233: 1089–1093
 19. Korber BT, Kunstman KJ, Patterson BK, Furtado M, McEvilly MM, Levy R, Wolinsky SM (1994) Genetic differences between blood- and brain-derived viral sequences from human immunodeficiency virus type 1-infected patients: evidence of conserved elements in the V3 region of the envelope protein of brain-derived sequences. *J Virol* 68: 7467–7481
 20. Koyanagi Y, Miles S, Mitsuyasu RT, Merrill JE, Vinters HV, Chen IS (1987) Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. *Science* 236: 819–822
 21. Kuwata T, Shioda T, Igarashi T, Ido E, Ibuki K, Enose Y, Stahl-Hennig C, Hunsmann G, Miura T, Hayami M (1996) Chimeric viruses between SIVmac and various HIV-1 isolates have biological properties that are similar to those of the parental HIV-1. *Aids* 10: 1331–1337

22. Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA (1998) Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* 393: 648–659
23. Lackner AA, Vogel P, Ramos RA, Kluge JD, Marthas M (1994) Early events in tissues during infection with pathogenic (SIVmac239) and nonpathogenic (SIVmac1A11) molecular clones of simian immunodeficiency virus. *Am J Pathol* 145: 428–439
24. Li J, Lord CI, Haseltine W, Letvin NL, Sodroski J (1992) Infection of cynomolgus monkeys with a chimeric HIV-1/SIVmac virus that expresses the HIV-1 envelope glycoproteins. *J Acquir Immune Defic Syndr* 5: 639–646
25. Liu ZQ, Mukherjee S, Sahni M, McCormick-Davis C, Leung K, Li Z, Gattone VH, 2nd, Tian C, Doms RW, Hoffman TL, Raghavan R, Narayan O, Stephens EB (1999) Derivation and biological characterization of a molecular clone of SHIV(KU-2) that causes AIDS, neurological disease, and renal disease in rhesus macaques. *Virology* 260: 295–307
26. Marcondes MC, Burudi EM, Huitron-Resendiz S, Sanchez-Alavez M, Watry D, Zandonatti M, Henriksen SJ, Fox HS (2001) Highly activated CD8⁺ T cells in the brain correlate with early central nervous system dysfunction in simian immunodeficiency virus infection. *J Immunol* 167: 5429–5438
27. Pantaleo G, Graziosi C, Butini L, Pizzo PA, Schnittman SM, Kotler DP, Fauci AS (1991) Lymphoid organs function as major reservoirs for human immunodeficiency virus. *Proc Natl Acad Sci USA* 88: 9838–9842
28. Reimann KA, Li JT, Veazey R, Halloran M, Park IW, Karlsson GB, Sodroski J, Letvin NL (1996) A chimeric simian/human immunodeficiency virus expressing a primary patient human immunodeficiency virus type 1 isolate env causes an AIDS-like disease after in vivo passage in rhesus monkeys. *J Virol* 70: 6922–6928
29. Reitter JN, Means RE, Desrosiers RC (1998) A role for carbohydrates in immune evasion in AIDS. *Nat Med* 4: 679–684
30. Safrit JT, Lee AY, Andrews CA, Koup RA (1994) A region of the third variable loop of HIV-1 gp120 is recognized by HLA-B7-restricted CTLs from two acute seroconversion patients. *J Immunol* 153: 3822–3830
31. Sei S, Kleiner DE, Kopp JB, Chandra R, Klotman PE, Yarchoan R, Pizzo PA, Mitsuya H (1994) Quantitative analysis of viral burden in tissues from adults and children with symptomatic human immunodeficiency virus type 1 infection assessed by polymerase chain reaction. *J Infect Dis* 170: 325–333
32. Sinclair E, Gray F, Ciardi A, Scaravilli F (1994) Immunohistochemical changes and PCR detection of HIV provirus DNA in brains of asymptomatic HIV-positive patients. *J Neuropathol Exp Neurol* 53: 43–50
33. Smith PD, Meng G, Shaw GM, Li L (1997) Infection of gastrointestinal tract macrophages by HIV-1. *J Leukoc Biol* 62: 72–77
34. Suryanarayana K, Wiltout TA, Vasquez GM, Hirsch VM, Lifson JD (1998) Plasma SIV RNA viral load determination by real-time quantification of product generation in reverse transcriptase-polymerase chain reaction. *AIDS Res Hum Retroviruses* 14: 183–189
35. Takahashi K, Wesselingh SL, Griffin DE, McArthur JC, Johnson RT, Glass JD (1996) Localization of HIV-1 in human brain using polymerase chain reaction/in situ hybridization and immunocytochemistry. *Ann Neurol* 39: 705–711
36. Tamalet C, Lafeuillade A, Yahi N, Vignoli C, Tourres C, Pellegrino P, de Micco P (1994) Comparison of viral burden and phenotype of HIV-1 isolates from lymph nodes and blood. *Aids* 8: 1083–1088
37. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673–4680

38. Zhang H, Dornadula G, Beumont M, Livornese L Jr, Van Uiter B, Henning K, Pomerantz RJ (1998) Human immunodeficiency virus type 1 in the semen of men receiving highly active antiretroviral therapy. *N Engl J Med* 339: 1803–1809
39. Zink MC, Spelman JP, Robinson RB, Clements JE (1998) SIV infection of macaques—modeling the progression to AIDS dementia. *J Neurovirol* 4: 249–259
40. Zink MC, Suryanarayana K, Mankowski JL, Shen A, Piatak M Jr, Spelman JP, Carter DL, Adams RJ, Lifson JD, Clements JE (1999) High viral load in the cerebrospinal fluid and brain correlates with severity of simian immunodeficiency virus encephalitis. *J Virol* 73: 10480–10488

Author's address: Dr. Yoshimi Enose, Laboratory of Viral Pathogenesis, Research Center for AIDS, Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto, 606-8507, Japan; e-mail: yenose@virus.kyoto-u.ac.jp

原 著

妊娠中期胎児超音波スクリーニング検査による胎児異常検出率

(平成 15 年 5 月 26 日受付)

(平成 15 年 11 月 19 日受理)

国立成育医療センター周産期診療部
東京慈恵会医科大学産婦人科*

和田 誠司	左合 治彦	松本 隆万*	川口 里恵*
杉本 公平*	尾見 裕子	林 聡	小澤 伸晃
藤井絵里子	塚原 優己	久保 隆彦	北川 道弘
田中 忠夫*	名取 道也		

Key words

fetal ultrasonography
screening
second trimester

概要 対象は国立大蔵病院で妊娠初期から管理を行い、1996年1月から2002年2月までに分娩となった3416例中(胎児異常を疑われての紹介例を除く)、そのうち42例(1.2%)に先天異常を認めた。各臓器別の内訳は循環器系が13例(31%)と最も多く、ついで染色体異常の8例(19%)、消化器系7例(17%)、四肢骨格5例(12%)、泌尿器系3例(7%)の順であった。先天異常42例のうち21例は胎児超音波スクリーニングで出生前に診断された(診断率50%)。臓器別に診断率が高かったのは泌尿器(100%)、染色体異常(75%)、低かったのは消化器(14%)、四肢骨格の異常(20%)で、循環器系の診断率は46%であった。超音波機器などの進歩に伴い、胎児超音波診断技術は向上しているが、出生前に診断しやすい疾患としにくい疾患があり、当施設でのlow risk groupにおける先天異常の出生前診断率は50%であった。

緒 言

我が国では、妊娠中の超音波検査は胎児スクリーニングとして一般的に行われている。それにより多くの疾患が出生前に診断が可能となってきたが、いまだ出生前に診断がつかない症例も少なくない。

国立大蔵病院(国立小児病院と統廃合し、2002年3月より国立成育医療センターとなる)において出生した先天異常症例の出生前超音波検査を疾患、臓器別に後方視的に解析し、胎児診断の現況とその限界、及びその問題点について検討した。

方 法

対象は国立大蔵病院で1996年1月から2002年2月の閉院までに分娩となった症例のうち妊娠初期より当院に通院し、在胎22週以降に分娩となった3416例である(胎児異常を疑われて当院紹介となった18例は除いた)。胎児超音波スクリーニングは妊娠20週前後および30週前後の2回にわたり、産科医もしくは胎児超音波を専門としている超音波検査技師により施行した。使用した超

音波機器は東芝SSA-260Aであった。表1に示した各項目について検査し、胎児発育、羊水量、胎盤、臍帯、胎児形態異常をスクリーニングした。染色体異常については、羊水過多、IUGR、多発奇形などの所見がみられ疑われた症例に対し、羊水染色体検査を行った結果により染色体異常が見いだされた症例を診断例とした。1回の検査に要した時間は約15分間であった。今回我々は生後に先天異常が見いだされた例について臓器別に分類し、診断率ならびに診断に影響を及ぼす因子などについて検討した。

結 果

3416例の分娩中、出生後7日間の観察で形態異常がみられた新生児は42例であり、全体の1.2%であった。各臓器別の内訳は循環器系が13例(31%)と最も多く、ついで染色体異常の8例(19%)、消化器系7例(17%)、四肢骨格5例(12%)、泌尿器系3例(7%)の順であった(図1)。

先天異常全42例中、21例は胎児超音波スクリーニン

表1 超音波スクリーニングの項目

推定体重, 児頭横径, 大腿骨長
羊水量 (羊水ポケット, AFI)
胎盤, 臍帯
各臓器
頭部, 中枢神経系
顔面
心臓 (四腔断面, 大動脈, 肺動脈流出路, 下大静脈)
胸郭
腎臓, 膀胱
腹部 (胃胞, 臍帯など)
四肢骨格

図2 臓器別の診断率

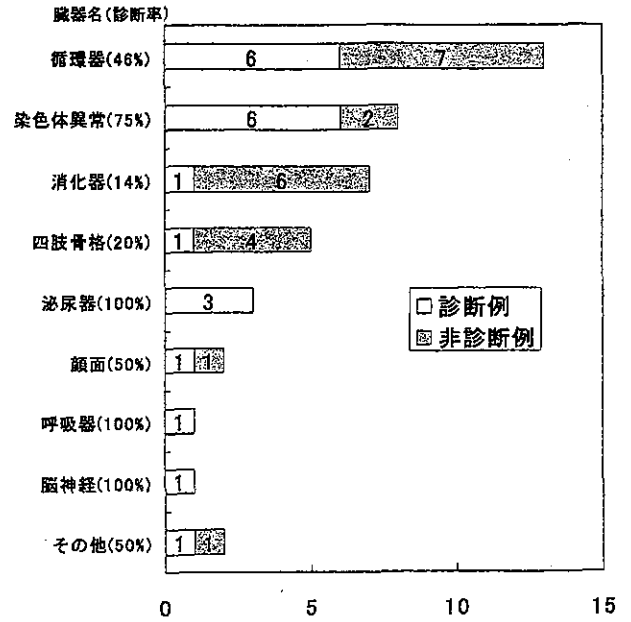
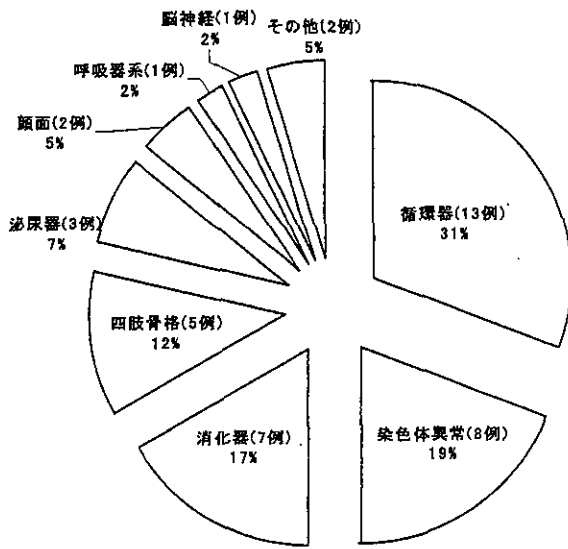


図1 全42例の臓器別内訳



で出生前に診断されたが、21例は出生前には診断できず、診断率は50%であった。各臓器別の診断率を図2に示す。臓器別分類(3例以上)で診断率が高かったのは泌尿器(100%)、染色体異常(75%)、低かった臓器は消化器(14%)、四肢骨格(20%)で、循環器系の診断率はその中間の46%であった。

診断できた疾患、できなかった疾患の内訳を循環器系とそれ以外の臓器で分けて検討した。循環器系以外で診断された疾患(表2)は18トリソミー、21トリソミー、13トリソミー、水腎症、嚢胞腎、肺無形成、髄膜瘤、肝血管腫、軟骨無形成症、口唇口蓋裂、筋緊張性ジストロフィー(羊水過多を契機に診断)で、診断されなかった疾患は21トリソミー、腸回転異常、鎖肛、食道閉鎖、指の異常、口唇口蓋裂、咽頭腫瘍であった。これらの疾患は現状のスクリーニング検査で検出するには困難と考えられる。

循環器系は表3のように中隔欠損、房室・弁の異常、大血管の異常、その他に分けて分類した。心室中隔欠損症4例、大血管転移、大動脈縮窄症、双胎の単心室が診

表2 循環器以外の診断例、非診断例

	診断例	非診断例
染色体異常	18トリソミー (3例) 21トリソミー 13トリソミー 4p-症候群	21トリソミー (2例)
泌尿器	水腎症 (2例) 嚢胞腎	
呼吸器	右肺無形成	
脳神経	髄膜瘤	
消化器	肝血管腫	腸回転異常 (2例) 鎖肛 (2例) 食道閉鎖 (2例)
四肢骨格	軟骨無形成症	合指症 手中指欠損症+合指症 両側第4指奇形 多指症
顔面	口唇口蓋裂	口唇口蓋裂
その他	筋緊張性筋ジストロフィー	咽頭腫瘍

断されなかった。この時点までのスクリーニング法においては心室中隔欠損と大血管系の異常は出生前に診断するのは難しいと考えられる。

胎児の構造異常のマーカーの一つである子宮内胎児発育遅延 (IUGR: intrauterine growth restriction, -1.5 SD以下)、羊水過多が診断に及ぼす影響について検討した(表4)。診断例ではIUGRは33%(7/21例)に、羊水過多は14%(3/21例)にみられたが、非診断例ではIUGRは9%(2/21例、腸回転異常、食道閉鎖)、羊水過多は5%(1/21例、咽頭腫瘍)しかみられなかった。IUGR、羊水過多ともに診断例に多くみられた。