特集 感染対策

産婦人科医に必要なエイズの知識

Guidance on HIV for obstetricians and gynecologists

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海外では圧倒的な数のHIV感染者，AIDS患者が報告されているが，幸いわが国では危機的な状況には至っていない．しかし，若者の性感染症の実態をみると今後の動向には注意が必要であり，積極的にHIV抗体検査を勧めるべきである．

わが国における妊婦のHIV感染症については，経腟分娩例に比べて，帝王切開分娩が施行された例では，母子感染率はわずかに1.9%であり，極めて良好な成績であるが，妊婦の抗HIV療法などには，なお未解決の問題も残されている．

Key Words　HIV，AZT，母子感染

はじめに

わが国においてHIV（human immunodeficiency virus）感染症は海外におけるoutbreakを尻目に感染者の報告数は幸いにも圧倒的に少ない．そのため性感染症（STD）としてのHIV感染症をスクリーニングする機会はあまり多くはない．一方，産科領域では妊婦にHIV抗体スクリーニングを行うことが多いためHIV感染の実体が明らかになり，母子感染防止対策も着実に成果を挙げほとんど母子感染を予防できるようになった．本稿ではHIV感染症の現状とHIV感染妊娠への対応を中心に述べたい．

HIV感染症の現状

当初，同性間性行為による感染症として注目されたHIV感染症は，その後異性間性行為によっても感染することが確認され，その他麻薬常習者による注射の回し打ちによる感染，母子感染などが感染の主要ルートであることが周知となった．不治の病とされたHIV感染症であるが1990年代後半からプロテアーゼ阻害剤を含む多剤併用療法（HAART）が推し進められ感染初期から強力な治療が行われた結果，死亡者数や日和見感染症の頻度は著明に減少した．しかし，感染者には長い期間の抗HIV薬の投与が必要であり，内服のアドヒアランス（服薬遵守），副作用，薬剤耐性，経済的負担などなお大きな問題が残されており，現在より長期的な計画に基づいた治療法も模索されている．

国内外の感染者の現況

UNAIDS（Joint United Nations Programme on HIV/AIDS）の2002年末の報告では，現在地球上には約4,200万人のHIV感染者AIDS患者が存在する（図1）．欧米先進国や東南アジアとくにタイでは，国の政策あるいは教育啓蒙の成果で

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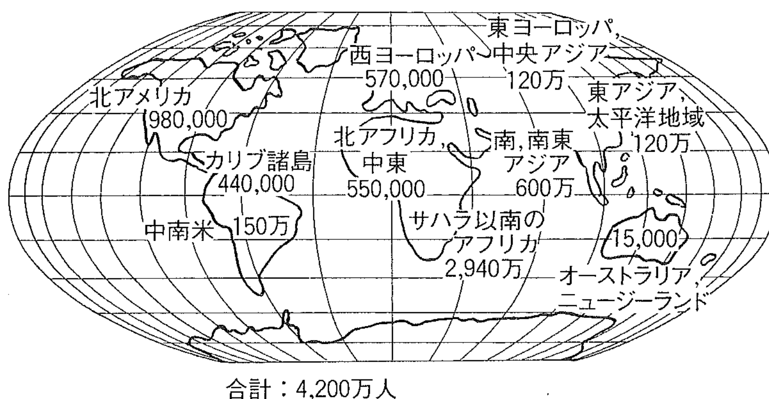

図1　世界における生存HIV感染者のエイズ患者の分布
(UNAIDS, 2002)

HIV感染症は明らかに減少してきており，また治療法の進歩によって死亡者の減少もみられている．

しかし，2002年には新規感染者数はなお500万人に上り，サハラ以南のアフリカ諸国，東欧諸国，中国などでは感染者は確実に増加している．とくにサハラ以南のアフリカのHIV感染者AIDS患者数は全世界の約2/3に相当する2,940万人に上り，この数は1996年の報告の2倍に達する．これらアフリカ諸国のなかには，妊婦のHIV感染者が40%を越す国も存在し，国の存亡の危機に直面している．このような地域では治療薬が十分に供給されず，また出生児の保育も母乳に頼らざるを得ないことから母子感染を防止することが困難となっており，感染者の増加に歯止めがかからないのが現状である．

一方，国内に目を向けると厚労省エイズ動向委員会2003年1月の報告では，わが国のHIV感染者は累積で5,121人，AIDS患者2,549人，凝固因子製剤による感染者が1,431人と報告されており，この累積数は海外における感染者数に比べればもちろん圧倒的に少数ではあるし，2002年には新規感染者は若干減少したとも報告されている．しかしながら，最近は20代，30代の感染者が増えており，その他潜伏した感染者の存在も懸念される．

性感染症とHIV感染症

近年，わが国においてはさらに若年の10代女性の性感染症と人口妊娠中絶の増加が問題となっており今後の動向から目が離せない．もっとも注目されている性感染症はクラミジアであるが，その他，淋菌，ヘルペス，梅毒などの性感染症に罹患していてもHIV感染，被感染のriskが2～5倍高くなるといわれている[1]．産婦人科外来診療の場においては性感染症を繰り返すhigh riskの患者に一度はHIV抗体検査を勧めるべきであろう．また，保健所では無料での検査も可能であることを教えてもよい．

妊娠とHIV感染症

1．母子感染防止への取り組み

母子感染は妊娠後期の経胎盤感染，出産時の産道感染，母乳による感染に分けられるが，1994年米国を中心としたPACTG（Pediatric AIDS Clinical Trial Group）はHIV感染妊婦に妊娠中期以降産褥期まで，出生した児に6週間抗HIV薬であるAZT（zidovudine）を投与することによって母子感染率が25.5%から8.3%と約1/3に低下したことを発表した[2]（表1）．その後，タイでは経済的な理由からAZTの投与を妊娠36週から行うshort programを実施し，母子感染率を1/2に低下させることに成功した[3]．さらにヨーロッパでは1999年に，妊婦へのAZT投与に加えて選択的帝切分娩を行うことによって母子感染率が2.0%まで低下したと発表[4]，一方米国では妊婦に強力な抗

表1 PACTG 076TRIAL

妊娠中	妊娠14週〜34週から全妊娠期間中AZT 100mgを1日5回内服.
分娩中	分娩開始からAZTを2 mg/kg/hで1時間, その後は1mg/kg/hで経静脈的に投与.
分娩後	出生後8〜12時間までに新生児に対しAZTシロップ2 mg/kg投与を開始し, 生後6週間継続する.
母子感染率	AZT投与なし群 25.5% AZT投与群 8.3%

HIV多剤併用療法を行い, ウイルス量を十分に低下させれば経腟分娩でも母子感染が防止できるとの見解も出された.

2. 妊婦HIV抗体スクリーニング

1) わが国における妊婦HIV抗体スクリーニングの実施状況

われわれ「厚生労働省HIV母子感染予防の臨床的研究」班が全国の産婦人科を標榜する病院に行ってきた調査[5]では, 妊婦のHIV抗体検査実施率は平成11年には73.2%だったが, 平成13年には82.6%に上った. これは諸外国に比べれば高率でHIV感染妊娠の啓蒙や感染妊婦の増加のほかに, 日本産婦人科学会が妊婦の抗体検査実施を推奨したことも一因と思われる. しかしなお検査の実施状況については地域格差が顕著で, HIV感染者の多い関東甲信越ブロックでは抗体検査の実施率が96.6%に上るのに, 感染者の少ない北海道・東北, 中国・四国, 九州ブロックでは実施率が低い. また, 抗体検査は現状では公費の補助を受けている地区はごく少数であり, 母子感染防止が確実に行い得るようになった現在, 全妊婦が公費で検査を受けられるようになることが望まれる.

2) 抗体検査と確認試験

抗体検査は妊娠初期に妊婦に検査の意義を十分に説明しインフォームドコンセントを文書で得て行う. 検査はPA法かELISA法で行われるが, この場合false positiveが問題となることがある. 妊婦では非特異的に抗体陽性となることが稀にみられるので抗体陽性や判定保留の結果が出てもウェスタンブロット法やPCR法による確認試験でHIV感染が確定するまで判断は待つ.

3) HIV陽性の告知, カウンセリング

確認試験で感染が確定したら, 日時を設定し十分時間をかけ妊婦個人に告知する. 妊婦が同意すればそのパートナーにも同席してもらう. 感染の告知, CD4やウイルス量に基づく病状の説明, 母子感染の経路と国内外の治療法の進歩や抗HIV療法の現状の説明, その他費用の問題, ほかの家族の検査の問題などを充分に説明する.

3. 妊娠中の基本的な対応

1) 妊婦健診

HIV感染が確認され妊娠の継続が確定したら, 健診ではHIV RNAコピー数や免疫機能の指標となるCD4, CD8を4週ごとに測定し病状の把握の目安とする. 妊娠中期以降はウイルス感染のriskとなる陣痛や破水を予防すべく, 切迫早産の管理, 絨毛羊膜炎の予防, その他腟炎, 頸管炎の有無をチェックする. また, 抗HIV薬の投与に際しては服薬のアドヒアランスに注意するとともに, 抗HIV薬のさまざまな副作用やほかの薬剤との併用で現れる相互作用などにも配慮する.

2) 妊婦に対する抗HIV療法

妊婦への抗HIV療法は催奇形性を考慮して妊娠14週頃から開始される. 最近は妊婦にも多剤併用療法が選択されることが多い. 多剤併用療法はAZTと3TCなどの核酸系逆転写酵素阻害剤 (NRTI) とNelfinavirなどのプロテアーゼ阻害剤 (PI) の3剤さらには4剤で行われる. しかし, 最近多剤併用療法でNRTIによる母児の乳酸アシドーシス, ミトコンドリア障害などの重篤な副作用が報告され, その投与には細心の注意が喚起されている. とくにd4TとddIを含む併用療法は禁忌とされている.

米国のHIV感染症治療ガイドライン[6] (2002年5月改定) では, 妊婦への投薬について血中ウイル

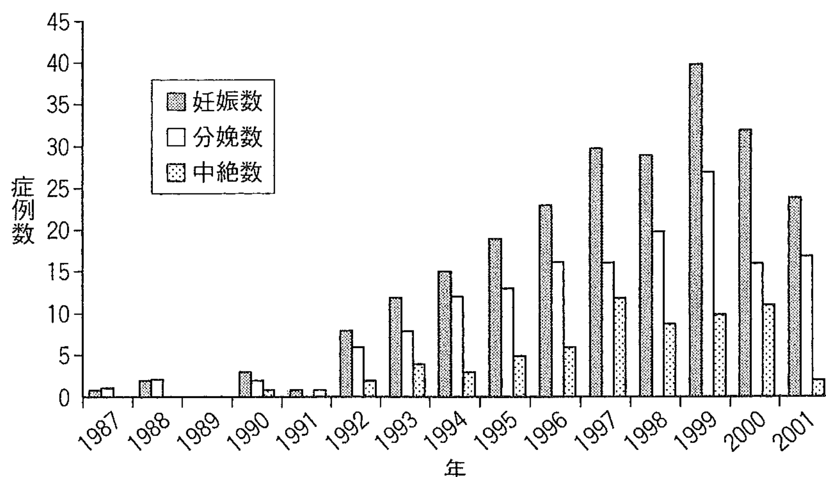

図２　わが国におけるHIV感染妊娠の年次推移
（厚生労働省，平成13年度研究報告書[5]より）

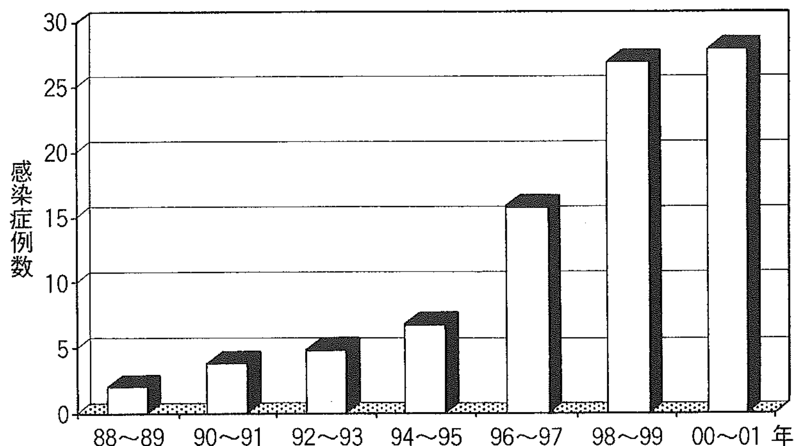

図３　HIV感染日本国籍妊婦の年次推移
（厚生労働省，平成13年度研究報告書[5]より）

ス量が1,000コピー/ml以上の場合は多剤併用療法を推薦しているが，1,000コピー/ml未満の場合にはAZT単剤投与選択の余地も示唆している．このように抗HIV薬の投与に関してはなお問題点も少くないので，十分に説明して妊婦の選択を尊重すべきであろう．以上のHIV感染妊婦への対応については平成13年度HIV母子感染予防対策マニュアル[7]（第２版：http：//api-net.jfap.or.jp/siryou/siryou_Frame.htm）を参考されたい．

４．わが国におけるHIV感染妊娠と母子感染の現況

１）HIV感染妊娠の年次推移

「HIV母子感染予防の臨床的研究」班によるアンケート調査[5]では，2002年３月までわが国におけるHIV感染妊娠数は248例である．図２に発生の年次別推移を示した．1992年から漸増していた感染妊娠数は1999年の40例をピークに若干の減少傾向を示している．しかし，必ずしも今後について安心できないのは日本国籍のHIV妊婦数をみると少しずつなお増加の傾向がみられていることである（図３）．先に述べたように日本の若年女性の性行動，性感染症の実態を考えると，今後の動向にはなお目が離せない状況と思われる．

２）分娩様式と母子感染

われわれの研究班で詳細のわかった母子感染例14例を分娩様式別にみると帝切分娩では106例中母子感染例はわずかに２例で感染率は1.9％と極

表2　わが国におけるHIV感染妊婦の分娩様式と母子感染

分娩様式	非感染	感 染	感染率 %	不 明	合 計
帝 切	104	2	1.9（2/106）	24	130
経 腟	12	12 5*	50.0（12/24） 29.4（5/17）**	5	29
合 計	116	14	10.8（14/130）	29	159

*児の異常による受診を機に母親の感染が確認された7例を除く
** P＜0.001，Fisher's exact test
（厚生労働省，平成13年度研究報告書[5)]）

めて良好な成績であった．一方，経腟分娩では24例中12例が感染している．感染の12例中7例は児の異常から後に母子感染が明らかにされたもので，その7例を除いても母子感染例は17例中5例に発生し感染率は29.4%にも上り，帝切分娩群に比べて有意に母子感染率が高かった（表2）．

妊娠中の抗HIV療法は帝切例では130例中80例（61.5%）に行われ，そのうちAZT単剤投与が48例，3剤併用療法が28例に行われていた．1998年以降は3剤併用療法が行われたケースが増えている．一方，経腟分娩の29例中妊娠中に抗HIV療法が行われたのはわずかに2例であった．これは飛び込み出産や産後にHIV感染が明らかになったケースが多いためと思われる．また，帝切分娩では母子感染率が極めて低いことが示されたが，注目されるのは妊娠中に抗HIV療法が施行されなかった例が50例あることである．この50例中母子感染がおこったのは1例のみであった．したがって，このことは分娩様式として帝王切開を行うことが推薦される根拠でもある．米国では血中ウイルス量が低い（1,000コピー/ml未満）妊婦では経腟分娩を推奨する意見も多いが，これは感染妊婦の数が多いことや帝切を行った場合の高い医療費とも関係があるようである．今後，わが国においても経腟分娩へのtrialも考慮されるべきであろうが，現状では現場における医療スタッフの対応の問題も含めて陣痛開始以前に予定帝切を行う選択がベターであると考える．

おわりに

先ごろHIV感染男性の精子を用いた人工授精（AIH）で児に感染が起こったことが報告され，日産婦学会では，精子からHIVを除去してのAIHを行う際には，インフォームドコンセントの確認と倫理委員会の承認を得ることを薦める通達がなされた．HIV感染症に関してはプライバシーの問題やインフォームドコンセントのあり方などいまだ医療現場のみならず社会においてもコンセンサスの得られていないことも多く，突然症例に遭遇した場合には困惑を感じるものと思われる．しかし，今後ほかの性感染症の動向とも関連して感染者の増加も予想されるため産婦人科医としてその対応についての知識の修得は是非とも必要と考える．

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SHORT COMMUNICATION

Severe perinatal hypophosphatasia due to homozygous deletion of T at nucleotide 1559 in the tissue nonspecific alkaline phosphatase gene

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Objectives Hypophosphatasia is an inherited disorder characterized by defective bone mineralization and deficiency of tissue nonspecific alkaline phosphatase (TNSALP) activity. This disorder is caused by various mutations in the *TNSALP* gene. We report here hypophosphatasia in two siblings, both of them severely affected by the perinatal (lethal) type.

Methods We diagnosed the first infant by clinical and radiologic manifestations, and laboratory findings. Laboratory findings were characterized by deficiency of serum alkaline phosphatase. Both parents and the second infant were then analyzed by molecular techniques.

Results The radiograph of the first infant showed severe hypomineralization of the skeleton. Molecular analysis of the second infant showed that this condition was caused by a homozygous single T nucleotide deletion at cDNA number 1559 (1559delT). Both parents were heterozygous carriers for this mutation, although they were not consanguineous.

Conclusion This mutation has been frequently found in Japanese hypophosphatasia patients, but this is the first observation of a homozygous deletion. This report shows that homozygosity for the 1559delT mutation of the *TNSALP* gene results in a severe lethal phenotype. Copyright © 2003 John Wiley & Sons, Ltd.

KEY WORDS: hypophosphatasia; tissue nonspecific alkaline phosphatase; gene mutation; homozygous mutation; prenatal diagnosis

INTRODUCTION

Alkaline phosphatases (ALP) are a group of nonspecific enzymes that are ubiquitous in nature and hydrolyze many types of monophosphate esters in most cells. Although the physiological function of these enzymes remains unclear, they play a role in bone matrix mineralization, perhaps by determining the steady state levels of inorganic pyrophosphate (PPi) (Hessle *et al.*, 2002). Hypophosphatasia is an inherited disorder characterized by defective bone mineralization and deficiency of tissue nonspecific alkaline phosphatase (TNSALP) activity. This disorder is highly variable in its clinical phenotype, which ranges from stillbirth without bone mineralization to premature exfoliation of deciduous teeth during adolescence and pathological fractures developing only late in adulthood (Whyte, 1994). Depending on the age at diagnosis, five clinical forms are currently recognized: perinatal (lethal), infantile, childhood, adult and odontohypophosphatasia. Typically, the earlier a patient manifests skeletal symptoms, the more severe the disorder (Whyte, 1994). While perinatal and infantile hypophosphatasia are transmitted as an autosomal recessive trait, both autosomal recessive and autosomal dominant transmission may be found in childhood, adult and odontohypophosphatasia (Whyte *et al.*, 1979; Eastman and Bixler, 1983; Eberle *et al.*, 1984; Hu *et al.*, 2000; Müller *et al.*, 2000; Lia-Baldini *et al.*, 2001). The Tissue Nonspecific Alkaline Phosphatase Gene Mutations Database (http://www.sesep.uvsq.fr/Database.html) currently includes 127 *TNSALP* gene mutations responsible for hypophosphatasia. These occur in a relatively small number of North American, Japanese and European patients (Mornet, 2000), indicating a very strong allelic heterogeneity in the disease. Most of these

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(82%) were missense mutations that account for the observed variable clinical heterogeneity. Very few mutations were recurrently found in unrelated patients and only two seem to be frequent—E174K and 1559delT in Caucasian and Japanese patients respectively (Mornet, 2000).

We report here for the first time a case of hypophosphatasia that was homozygous for deletion of T at nucleotide 1559 (1559delT). Both parents were heterozygous carriers for this mutation, although there was no evidence of consanguinity. Two siblings had the perinatal (lethal) type of hypophosphatasia. A homozygous deletion was strongly suspected for the first sibling and was conclusively shown for the second.

CASE REPORT

A 21-year-old pregnant, Japanese woman, primigravida, in a nonconsanguineous partnership was referred for fetal evaluation after a fetal anomaly was suspected by ultrasonography at 34 weeks' gestation. Ultrasound examination revealed marked hypomineralization of the skull and spine, narrowing of the chest, marked shortening of the limbs (femur length shorter than −3 SD) and polyhydramnios. At 35 weeks' gestation, a cesarean section was performed because of fetal distress. The newborn infant was female, weighing 2918 g with Apgar scores of 1 at 1 min and 3 at 5 min. The infant was resuscitated but died three hours later owing to respiratory failure. Clinical manifestations were characterized by marked shortening of the long bones and a globular boneless skull. A radiograph of the infant showed a severe hypophosphatasia phenotype (Figure 1). The autopsy indicated that the infant died as a result of respiratory failure, and no major abnormalities were reported except bone dysplasia as shown in the radiograph, pulmonary hypoplasia and chest deformity. Laboratory tests showed a serum ALP of 5 IU/L (reference range 430–1140 IU/L for infants) in the umbilical cord blood of the newborn infant, 70 IU/L (reference range 70–260 IU/L for adults) for the father and 73 for the mother. Both parents have no clinical symptoms. These findings led to the diagnosis that the newborn infant was homozygous for the hypophosphatasia-related deletion and that both parents were heterozygous carriers.

In the second pregnancy, ultrasound examinations at 14 and 17 weeks showed severe shortening and bowing of the limbs (femur length 8.7 mm and 9.9 mm respectively, shorter than −3 SD) and hypomineralization of the skull and spine. This was diagnosed as recurrence of hypophosphatasia, and the parents opted for termination of the pregnancy. To confirm the genetic condition in this family, genetic testing was offered and performed after obtaining informed consent. Fetal genomic DNA was extracted from 2 mL of umbilical cord blood. Parental and fetal DNA were then screened to detect mutations. Exon 12 of the *TNSALP* gene was screened first since, as described above, the 1559delT in this exon has been found frequently in Japanese. To amplify exon 12, PCR was performed using primers of exons 12 to 2, as originally described by Orimo *et al.* (1997). The PCR products (178 bp) were digested with the restriction enzyme *Dde*I and then electrophoresed on 4 to 20% polyacrylamide gradient gel. For the wild type sequence, *Dde*I cleaves the 178-bp fragment into 134 + 44 bp, but for the mutant allele with 1559delT, the fragment is cleaved into 120 + 44 + 14 bp (Orimo *et al.*, 2002). The results showed that both parents were heterozygous carriers and the fetus was homozygous for this deletion (Figure 2). The mutation was confirmed by sequencing both parents and the affected child (data not shown).

DISCUSSION

The variety of *TNSALP* gene mutations results in variable clinical expressivity among patients. This in turn makes it difficult to establish a correlation between genotype and phenotype because most patients are compound heterozygotes, making it difficult to determine the respective roles of missense mutations. Site-directed mutagenesis and transfection analysis of TNSALP cDNA along with computer-assisted modeling of proteins may help solve this problem by using alkaline phosphatase activity to determine the relation between the individual tested mutation and the phenotype (Fukushi *et al.*, 1998; Goseki-Sone *et al.*, 1998; Sugimoto *et al.*, 1998; Zurutuza *et al.*, 1999). To date, 10 patients with 1559delT in the *TNSALP* gene have been reported, and these only in the Japanese, never in the Caucasians (Orimo *et al.*, 1994; Orimo *et al.*, 2002). All patients were heterozygous for the deletion. Severity of the disease ranged from intrauterine death to deformity of extremities in childhood, depending on the counterpart allele accompanying the deletion (Orimo *et al.*, 2002). An expression study of the mutated *TNSALP* gene demonstrated that the protein had undetectable ALP activity (Goseki-Sone *et al.*, 1998) suggesting that 1559delT is a severe allele. Here, we report the first case of a hypophosphatasia patient homozygous for the deletion 1559delT, allowing us to directly correlate the phenotype and the genotype. The proband exhibited typical signs of the most severe form of the disease, that is, perinatal hypophosphatasia: *in utero* impaired mineralization, shortened long bones and poor mineralization of skull and spine; in addition, polyhydramnios and stillbirth by respiratory distress are frequently observed in perinatal hypophosphatasia. Thus, our report confirms that the 1559delT mutation corresponds to a severe allele responsible for perinatal lethal hypophosphatasia.

The deletion causes a frameshift downstream from leucine at codon 503, resulting in elimination of the termination codon at 508 and the addition of 80 amino acid residues to the C-terminus. This part of the molecule is involved in membrane anchoring. The absence of any residual activity observed in site-directed mutagenesis experiments (Goseki-Sone *et al.*, 1998) suggests that the mutated protein is degraded in the cytoplasm or that the residues involved in membrane attachment via phosphatidyl inositol interaction may have been hidden by the additional peptide and are therefore not accessible

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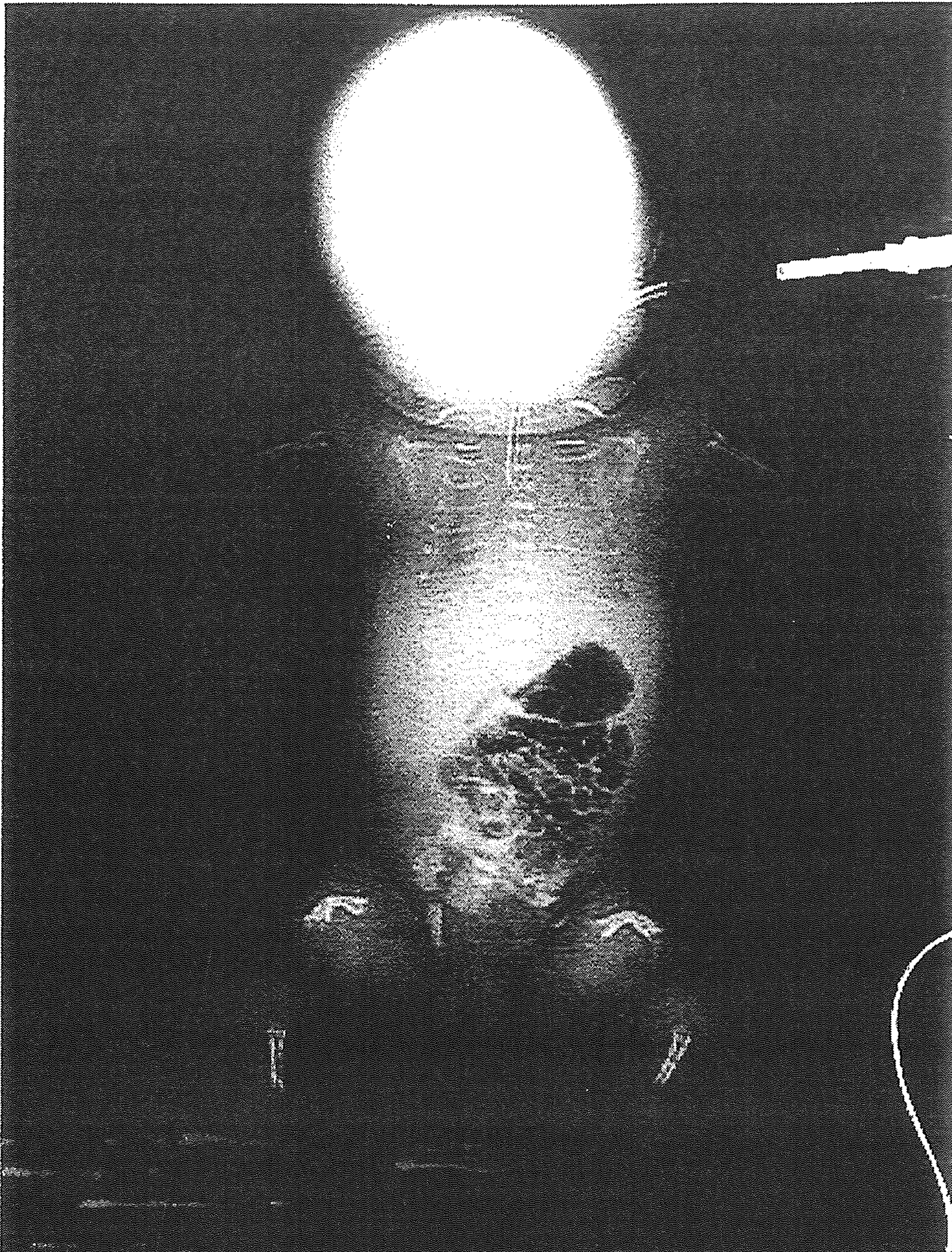

Figure 1—Radiographic findings. Marked retardation of skeletal ossification, poor mineralization of the skull, shortness and bowing of the poorly mineralized tubular bones, marked flatting of vertebral bodies, narrow chest with short hypomineralized ribs and cupping and fraying of metaphyses of long bones. These findings are consistent with a phenotype of severe form of hypophosphatasia, that is, perinatal (lethal) type

for the anchoring process. Because cells expressing the mutant protein showed immunoreactivity against TNSALP in the cytoplasm (Goseki-Sone *et al.*, 1998), the second hypothesis is more likely.

The mutation 1559delT appears to be frequent in Japanese individuals. Approximately 71% of reported Japanese hypophosphatasia patients carried this deletion corresponding to an allele frequency of approximately 36% (Orimo *et al.*, 2002). As for the Caucasian population in which the mutation E174K was previously reported to show a founder effect (Hérasse *et al.*, 2002), it would be of interest to investigate whether the deletion had a unique origin or rather multiple origins due to recurrence of *de novo* mutations. The fact that 1559delT has never been found in countries other than Japan strongly suggests a unique origin of the mutation. However, by using haplotype analysis with single-nucleotide polymorphisms of intron 8 and exon 9, Orimo *et al.* (2002) defined two distinct haplotypes associated with the deletion. The authors concluded that the deletion might have derived from more than a single founder.

Perinatal hypophosphatasia has been diagnosed *in utero* by ultrasonography performed with careful attention to the limbs as well as the skull (van Dongen *et al.*,

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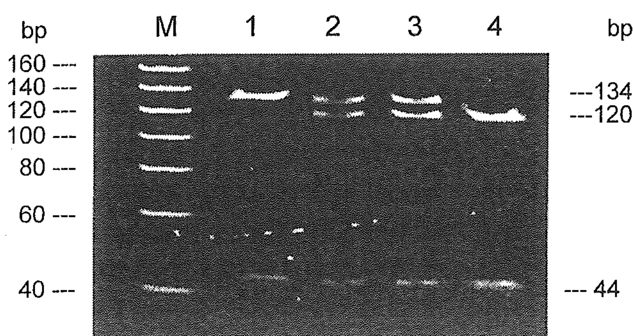

Figure 2—PCR amplification and restriction enzyme analysis of genomic DNA from a healthy control, both parents and the affected infant. PCR was performed to amplify exon 12 of the *TNSALP* gene and PCR products were digested by *Dde*I as described in the Methods. *Dde*I cleaves the 178-bp fragment into 134 + 44 bp for the wild-type sequence and 120 + 44 + 14 bp for the mutant allele with 1559delT. Lane M: 20-bp DNA ladder marker, Lane 1: healthy control. PCR fragment 178 bp was cleaved into 134 + 44 bp. Lanes 2 and 3: father and mother. PCR fragment 178 bp was cleaved into 134 + 44 bp and 120 + 44 (+14) bp. These results indicate that both parents are heterozygous carriers for 1559delT of the *TNSALP* gene. Lane 4: affected infant; PCR fragment 178 bp was cleaved into 120 + 44 (+14) bp. The parentheses indicate bands that do not appear in the figure. This result indicates that the infant is homozygous for 1559delT of the *TNSALP* gene

1990). However, occasionally hypophosphatasia fetuses *in utero* are not clearly distinguished from those affected with other skeletal dysplasias. During the first pregnancy of our patient, precise *in utero* diagnosis of hypophosphatasia was not performed because of insufficient time to confirm the diagnosis in our hospital. During the second pregnancy, diagnosis by ultrasonography was properly performed for this severe condition. When *TNSALP* gene mutations are identified in the index case, early molecular prenatal diagnosis is possible in chorionic villi, as previously reported (Henthorn and Whyte, 1995; Orimo *et al.*, 1996; Mornet *et al.*, 1999). The combination of these complementary techniques is useful for confirming the diagnosis of this disorder that presents with a wide variety of phenotypes.

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HIV 母子感染予防の臨床的研究 第4報-「HIV 母子感染予防対策マニュアル」の改訂と普及対策-

演者名

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Description about a revised edition "HIV mother-to-child transmission prevention management manual"

The purpose and a method

The "HIV mother-to-child transmission prevention management manual" unique to Japan was published in March 2000. We considered subsequent new knowledge, and the request and question which were brought near by, and we revised this manual so that it might be useful in present condition of our country. We explain per contents of this revised edition.

はじめに

HIV(Human Immunodeficiency Virus)は血液製剤を介した感染が防御され、近年は性行為感染と母子感染がその多くを占めている。幸いにもわが国の HIV 感染者数は極めて少ないが、クラミジアをはじめとする他の性行為感染症が若年者を中心に広く浸透し始めており[1]、HIV の急激な増加が危惧されている。

当初約 30%といわれていた HIV 母子感染率はこの 10 年間に講じられた様々な感染予防対策により飛躍的に改善し、わが国を始め先進諸国での母子感染率は約 2%にまで低下している[2-4]。本稿では、現在わが国で一般的に行われている HIV 母子感染予防対策につき、2002 年に改訂された**「HIV 母子感染予防の臨床研究」**班編「HIV 母子感染予防対策マニュアル」の変更点（表２）を中心に解説する。

Ⅰ. HIV 抗体スクリーニング検査

母子感染予防対策の効果はここ数年で急激に進歩し、母体の生命予後と母子感染率は飛躍的に改善されている。HIV に対する治療や母子感染予防対策を行うためには、妊娠初期からの抗 HIV 剤の投与が必要であり、従って妊娠初期の HIV 抗体検査が求められる[5]。

Ⅱ. 母子感染予防対策の実際

HIV 抗体検査をきっかけに HIV 感染が判明した無症状の妊婦に対し、わが国で一般に行われている感染予防対策の骨子は表 1 の 4 点である[7]。

（1） 妊娠中からの抗 HIV 剤の内服

1994 年、米国より **ZDV(AZT,ジドブジン)を妊娠中の母体に経口投与し、分娩中の母体に点滴静注し、更に新生児にも ZDV シロップを内服させることで母子感染率を 25.5%から 8.3%にまで低下させることが報告された[8]。** 更に 6 年後にはこのプロトコールに登録された児の就学期のデータが示され[9]、6 歳の時点でも ZDV による影響がないことが明らかとなった。ZDV は、現在のところ HIV 母子感染の防御効果を有する唯一の抗 HIV 剤と考えられている。一方近年、抗 HIV 療法は、ZDV の単剤投与から**多剤併用療法（HAART : Highly**

Active Anti-Retroviral Therapy）へと改善された。この治療は ZDV 単剤投与に比較し抗ウイルス効果が極めて高く、速やかに血中ウイルス量を感度以下にまで抑えることが可能である。ウイルス量が低いほど母子感染率は低下するといったデータも報告されており[10)]、母体の治療と母子感染防止を目的に、HAART 療法を開始することも勧められている。現在のところ HAART 療法が原因と考えられる児の異常は認められていない。しかし、この児の就学期および成人期の安全性については未だ確認されていない。妊娠中の抗 HIV 剤投与にあたっては、上記の治療法の比較を患者およびその家族に明確に提示した上で、血中のウイルス量や CD4 値を参考にどちらを選択するかを決定することが肝要である[7)]。

（2） 選択的帝王切開術[2-4)]

わが国では、児への母体体液の付着を可能な限り防御しようとの意図から、選択的帝王切開術が勧められてきた。わが国における分娩様式による母子感染率の検討では、帝王切開 1.9%、経膣分娩 29.4%と、帝王切開術による分娩は母子感染を防御することが明らかとなった[2)]。また近年欧米からも、帝王切開術＋抗 HIV 剤の妊娠中投与により母子感染率は約 2%まで抑制されると報告されている[3, 4)]。

一方、妊婦治療に HAART を行うことで血中のウイルス量を測定感度以下まで抑えることが可能となったことで、血中ウイルス量が極めて少ない例では経膣分娩でも母子感染は起こらないとの報告も散見される。多少なりとも体内にウイルスが残存する限り母児感染の可能性は否定できず、従ってひとたび児に感染をきたした場合の予後の重篤さに鑑み、現時点ではこれまでどおりの選択的帝王切開術を推奨した。

分娩時期は、わが国では子宮収縮に伴う母児間輸血により胎内感染をきたす危険を回避する目的で、妊娠 36 週までに児を娩出することが好ましいと考えられてきた。しかし、欧米では分娩時期を早めに設定してはいないにもかかわらず、同様の成績が得られていること、更に 36 週前後で出生した新生児の中には、沐浴により低体温をきたす例や、出生時の処置の間に呼吸障害を生じ気管内挿管の上、人工呼吸器管理を必要とした例も認められていることから、**分娩時期は、むしろ児の未熟性を考慮し、妊娠 36 週にこだわらずに陣痛発来前の帝王切開術がよいと考えた。**

HIV 感染妊婦の帝王切開術に際し、わが国では特別の準備・特別の術式で手術を行っている施設が多い。HBV や HCV など他の感染症と比較し手術時の対応が過剰すぎるのではないかとの意見も聞かれる。しかし、これら万全の準備を整えて行う手術も、帝王切開術にかかる保険診療の枠の中で対応可能であり、根治可能な治療法が未だ開発されていない現状では、確実に感染から身を守る手段を講じることが肝要と考えられる。

（3） 出生後の児の処置

出生時の薬剤（ 次亜塩素酸ナトリウム液（ミルトン®、ピューラックス®）、ポリビニールアルコール沃素液（消毒用イソジン®）など）を用いた沐浴や清拭、耳、鼻、口腔内の消毒、胃洗浄などは、わが国で効果的な抗 HIV 剤が何一つなかった時期に考案された感染防御策である。諸外国ではこれらの処置を行ってはいないが、わが国と同様の成績が得られている。従って**児の皮膚や粘膜を傷害する危険も併せ持つ上記薬剤の使用に関しては、言及しないこととなった。**

（4） 母乳保育の禁止[11)]

わが国は症例数が少ないため、母乳栄養による感染のリスクに関するデータはまとめられてはいないが、諸外国からの報告では母乳を介した HIV 感染が高率に認められている。乳汁分泌抑制剤のメシル酸ブロモクリプチン（パーロデル®）投与に際しては、極めて高率に出現する副作用とその対応に関しては熟知しておくべきである。

（5） 新生児への抗 **HIV** 剤予防投与

現在は ZDV シロップを生後 6 週間内服することが推奨されている。また、カリニ肺炎予防のための ST 合剤に関しても、感染児にカリニ肺炎を発症する頻度が高いこと、またその予後が極めて重篤なことから、ZDV 終了後より 1 才時まで投与することが勧められている。

終わりに

「HIV 母子感染予防対策マニュアル」（厚生労働省エイズ対策研究推進事業「妊産婦の STD 及び HIV 陽性率と妊婦の STD 及び HIV の出生児に与える影響に関する研究」班、「HIV 母子感染予防の臨床研究」班編）は、エイズ予防情報センター（http://api-net.jfap.or.jp/）ホームページの予防関連資料室に掲載されているので参照されたい。

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表 1. HIV 母子感染予防対策の骨子

(1)	妊娠中からの抗 HIV 剤の内服
(2)	選択的帝王切開術
(3)	母乳保育の禁止
(4)	新生児への抗 HIV 剤投与

表 2.「HIV 母子感染予防対策マニュアル」の主な改訂点

母体に対する予防対策
(1) 妊娠中に内服する抗 HIV 剤に関し、ZDV 単剤と多剤併用療法を比較
(2) ウイルス量が少なければ経膣分娩も可能か？⇒現状では No
(3) 帝王切開の時期：妊娠 36 週を陣痛発来前に変更
(4) 帝王切開術における 2 次感染予防処置の簡略化⇒まだ行わない

児に対する予防対策
(5) 出生時処置の簡略化（特別な胃洗浄や消毒は不要）
(6) 早産児や経口摂取困難な児に対するジドブジンの投与法
(7) カリニ肺炎予防のための ST 合剤投与の必要性

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Infection of macaques with an R5-tropic SHIV bearing a chimeric envelope carrying subtype E V3 loop among subtype B framework

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Summary. To establish simian/human immunodeficiency virus (SHIV) clones bearing a chimeric envelope carrying subtype E V3 loop among subtype B envelope, four subtype E V3 sequences were substituted into $SHIV_{MD14}$, a SHIV clone bearing an envelope derived from a CXCR4 (X4)/CCR5 (R5)-dual tropic subtype B HIV-1 strain. SHIV-TH09V3, an only V3-chimera clone capable of replicating in human and macaque peripheral blood mononuclear cells (PBMCs), was propagated in pig-tailed macaque PBMCs and in cynomolgus macaque splenic mononuclear cells. The propagated virus stocks were intravenously inoculated into respective macaque species. SHIV-TH09V3 infected both macaque species as shown by plasma RNA viremia, isolated viruses from PBMCs and plasma, and antibody production against viral proteins. To assess how the substituted V3 sequence affected coreceptor usage, SHIV-TH09V3 stocks propagated *in vitro* and after isolation from macaques were verified for their corecepor usage by GHOST cells assay. SHIV-TH09V3 maintained R5-tropic phenotype both *in vitro* and after isolation from macaques, in contrast to the X4/R5-dual tropic $SHIV_{MD14}$. This indicates the substituted V3 sequence among the backbone of $SHIV_{MD14}$ governs coreceptor usage. Future study of infecting macaques with SHIV-TH09V3 and $SHIV_{MD14}$ will focus on differences of the outcome caused by the different V3 sequences in connection with coreceptor usage.

Introduction

Multiple genetic subtypes of *Human immunodeficiency virus 1* (HIV-1) strains, now classified mainly into A through K [21], have been spreading and intermingled worldwide. Despite their distribution variety, only subtype B, originally from Europe and North America, has been accepted major focus of research.

For prophylaxis/vaccine development, making of macaque AIDS model is a prerequisite [1, 18]. Though several groups have so far established simian/human immunodeficiency viruses (SHIVs) carrying a whole envelope sequence from non-subtype B HIV-1 [4, 13, 16, 17], it was initially difficult to establish a usable SHIV because candidate SHIVs often failed to infect macaques. Assuming that a non-subtype B envelope as a whole does not easily fit the construction of an infectious SHIV clone, we selected V3 loop region out of subtype E envelopes for substitution into a SHIV bearing a subtype B envelope framework.

Selecting V3 region came from a concept that V3, at least of subtype B, is a relatively independent functional region governing cell tropism [9, 10, 25, 28, 31] and coreceptor usage [3, 5, 6, 29]. One concern was that amino acid sequences of subtype E V3 differed from those of subtype B as much as 50% [11, 15, 23, 34], possibly hindering subtype E V3 from enjoying the relative independence among a subtype B envelope framework. But this concern was considerably cleared by a study showing that subtype B HIV-1 clones chimeric with subtype E V3 maintained phenotypes of the V3 in terms of cell tropism and coreceptor usage [24]. This suggested the cross-subtype independence of V3 function, encouraging us to construct subtype E V3-chimera SHIVs.

Here, we report an establishment of a SHIV clone, designated SHIV-TH09V3, bearing a chimeric envelope with subtype E V3 among subtype B framework. SHIV-TH09V3 infected both pig-tailed and cynomolgus macaques maintaining R5-tropic phenotype dictated from the substituted subtype E V3.

Materials and methods

Construction of the recombinant DNA clones of V3-chimera SHIVs

pMD14 [26], a SHIV clone bearing a subtype B HIV-1 envelope derived from an X4/R5-dual tropic strain (HIV-1_{DH12}), was used as a backbone to generate V3-chimera SHIVs. Although there were two versions of pMD14 (pMD14YE and pMD14RQ) with minor differences, only pMD14YE was used in this study. Four subtype E V3 sequences, TH09V3, NH2V3, KH005V3 and NH1V3, were used for the chimeric substitution (Fig. 1A) as described previously [24]. Briefly, *Bgl* II-to-*Bsu*36 I DNA fragment (269 bp) encoding subtype E V3 and DH12 flanking sequences was generated by the overlap extension method [14], digested by *Bgl* II and *Bsu*36 I, and cloned back into pMD14 (Fig. 1B). The structures of the reconstituted regions were confirmed by DNA sequencing.

Western blot

HeLa cells (6×10^5 cells) were grown in 10% FBS-DMEM in a T25 flask for one day, and transfected with 3 μg of the SHIV plasmid DNA using FuGENE 6 transfection reagent (Roche Diagnostics). The cells were harvested at 48 hours after transfection, washed with

PBS, and lysed on ice for 10 min with 200 μl of RIPA buffer (50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 0.1% SDS, 1% (v/v) Nonidet P-40, 0.5% sodium deoxycholate, 0.1 mM PMSF, 1 μg/ml antipain, 1 μg/ml leupeptin and 1 mM iodoacetamide). The lysates were sonicated at 4 °C for 30 sec, followed by centrifugation at 4 °C. The supernatants were recovered and the protein concentrations were quantified by Bradford protein assay kit (BioRad). 6 μg of proteins were separated per lane by SDS-polyacrylamide gel electrophoresis and transferred to PVDF membrane (Millipore). The membrane was incubated with antibodies described below, followed by incubation with Protein A conjugated with horseradish peroxidase (Amersham Pharmacia Biotech). The antigen bands were visualized with ECL system (Amersham Pharmacia Biothch).

The following antibodies were used: plasma of a rhesus macaque that became seroconverted by infection with SIVmac239; plasma of a positive control patient from LAV BLOT 1 kit (SANOFI Diagnostics Pasteur); plasma of two patients, J28 and NH1, who became seroconverted by infection with HIV-1 subtype E [23]; RC25 monoclonal antibody (Chemo-Sero-Therapeutic Research Institute) which recognizes HIV-1 subtype B V3 loop; rabbit polyclonal antiserum raised against synthetic peptide corresponding to the consensus V3 sequence of subtype E non-syncytium-inducing (NSI) HIV-1 from Thailand [11, 15].

Preparation of the cell-free SHIV stocks

$SHIV_{MD14}$ and V3-chimera SHIVs were prepared as described previously [24]. Briefly, HeLa cells (5×10^5 cells) were grown in 10% FBS-DMEM in a T25 flask for one day, and transfected with 30 μg of the plasmid DNA using calcium phosphate coprecipitation methods. The culture supernatants were collected at 48 or 72 hours after transfection, filtered and kept at −152 °C until analysis of reverse transcriptase (RT) activity.

Replication of the SHIV stocks in human and macaque PBMCs

0.1 ml of the SHIV stocks (2×10^4 cpm of RT activity) were incubated for 16 hours at 37 °C either with Phytohaemagglutinin (PHA, 1 μg/ml)-stimulated human PBMCs (1×10^5 cells) or with Concanavalin A (ConA, 5 μg/ml)-stimulated PBMCs (1×10^5 cells) of pig-tailed macaque (*Macaca nemestrina*). The PBMCs were washed and cultivated in 0.2 ml of 10% FBS-RPMI 1640 medium with 20 Units/ml of recombinant human interleukin-2 (IL-2) in 96-well plates. The culture medium was replaced behalf with the fresh medium every two or three days, and the collected medium was stored at −80 °C until RT activity analysis.

Preparation of the in vivo *inoculation stocks of SHIV-TH09V3*

ConA-stimulated PBMCs of pig-tailed macaque and ConA-stimulated splenic mononuclear cells of cynomolgus macaque (*Macaca fascicularis*) were infected with the SHIV-TH09V3 stock and propagated in 10% FBS-RPMI 1640 medium containing IL-2. P27 Gag concentration of the culture supernatant was determined by sandwich ELISA (Coulter). 50% Tissue culture infectious dose ($TCID_{50}$) was determined by using M8166 cells [7].

Animal care

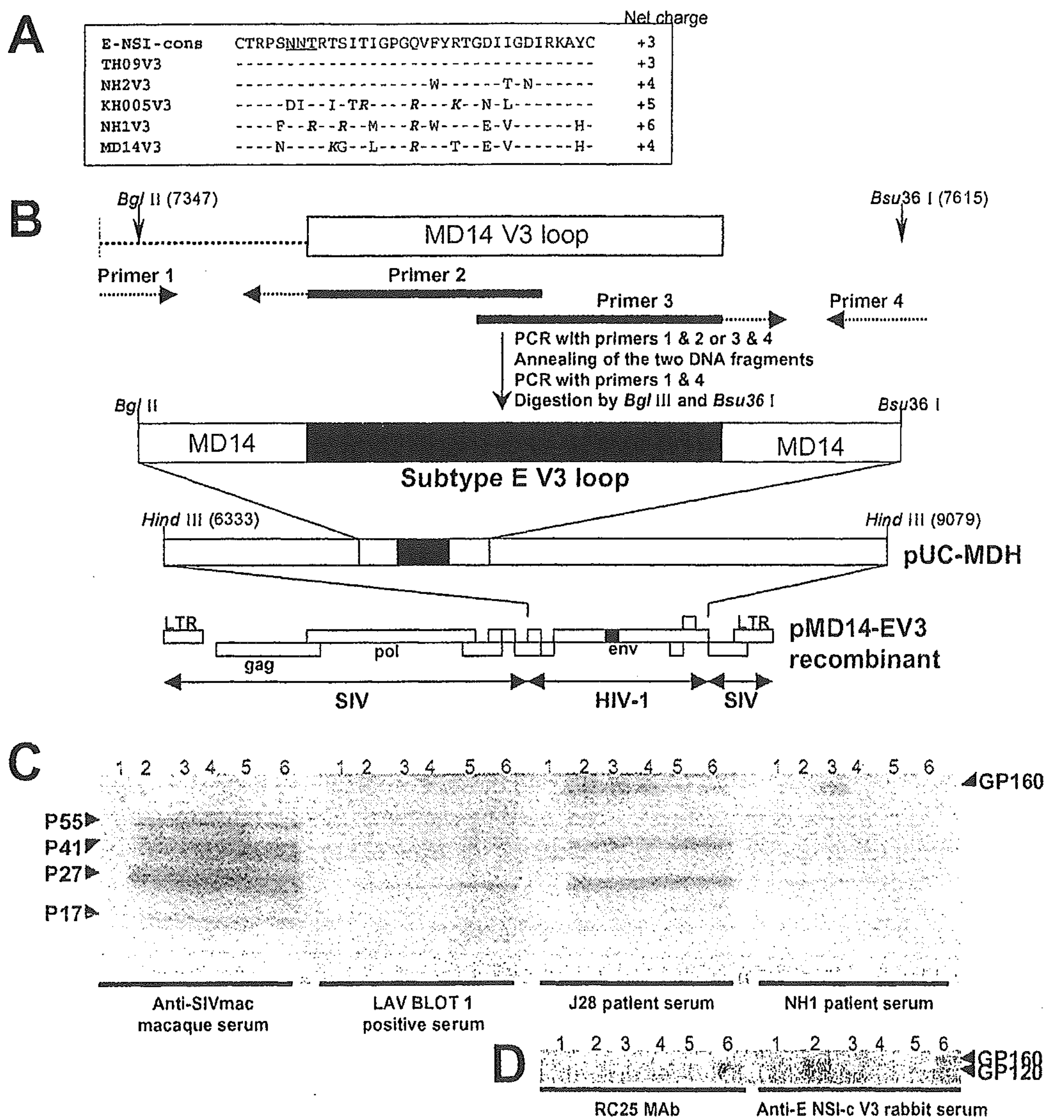
Maintenance and treatment of pig-tailed and cynomolgus macaques were strictly adhered to the guidelines of the Institutional Animal Care and Use Committee of the National institute of infectious Diseases, Japan. All the experiments were conducted in accordance with the Laboratory Biosafety Manual, World Health Organization. The macaques were anesthetized with ketamine hydrochloride for inoculation, blood sampling and autopsies.

SHIV infection of macaques

Two pig-tailed macaques (Pt-3944, Pt-3938) were intravenously inoculated with 600 $TCID_{50}$ (corresponding to 1 ng of P27 Gag) of the SHIV-TH09V3 stock propagated in pig-tailed macaque PBMCs. Two cynomolgus macaques (Cy-256, Cy-329) were intravenously inoculated with 10000 $TCID_{50}$ (corresponding to 305 ng of P27 Gag) of the SHIV-TH09V3 stock propagated in cynomolgus macaque splenic mononuclear cells.

Virus isolation from PBMCs and plasma

EDTA-treated whole blood was centrifuged to separate plasma and cellular component. The cellular component was applied for density-gradient centrifugation to purify PBMCs. 5×10^5 of PBMCs or 100 μl of plasma were cocultivated with M8166 cells in 10% FBS-RPMI medium for 4 weeks, with a weekly monitoring of P27 Gag production in the supernatant.



Quantification of plasma viral RNA

Viral RNA (vRNA) was extracted from plasma by QIAamp Viral RNA Kit (Quiagen), followed by real-time RT-PCR carried out and monitored in ABI 7700 PRISM spectrofluorometric thermal cycler (PE Biosystems) as described previously [22]. Each 25-μl reaction mixture contained the followings: 10 μl of the prepared RNA; 1 pmol/μl of each primer (5′AATGCAGAGCCCCAAGAAGAC3′ and 5′GGACCAAGGCCTAAAAAAC CC3′); 0.24 pmol/μl of probe (Fam-5′ACCATGTTATGGCCAAATGCCCAGAC3′-Tamra); 1 × TaqMan^{TM} EZ buffer; 0.3 mM of each dATP, dCTP and dGTP; 0.6 mM of dUTP; 0.25 Unit of AmpErase UNG; 2.5 Units of rTth DNA Polymerase (TaqMan^{TM} EZ RT-PCR Kit, PE Biosystems). RT-PCR condition was as follows; 50 °C for 2 min, 60 °C for 30 min, 95 °C for 5 min, followed by 50 cycles of 95 °C for 5 sec and 62 °C for 30 sec. The control RNA, whose serial dilution served standard curve, was prepared from pKS460 containing SIVmac239 gag under T7 promoter by using MEGAscript^{TM} (Ambion). RNA recovery rate from plasma was determined by doing a parallel purification of the control RNA, as a reference for calculation of plasma vRNA copy number. The limit of detection was approximately 500 RNA copies/ml.

Flow cytometry

50 μl of EDTA-treated peripheral blood was incubated with FITC-conjugated anti-CD3 monoclonal antibody (Mab) (FN-18, Biosource), PE-conjugated anti-CD4 Mab (SK-3, Becton Dickinson) and PerCP-conjugated anti-CD8 Mab (SK-1, Becton Dickinson). Erythrocytes were lysed by FACS Lysing Solution (Becton Dickinson), followed by addition of 50 μl of Flow-count beads solution (Beckman Coulter). CD4- and CD8-positive T-lymphocytes

Fig. 1. Construction of the pMD14-based V3-chimera SHIV DNAs, and protein profiles of the SHIVs. **A.** Deduced amino acid sequences of V3 loops: subtype E non-syncytium-inducing (NSI) V3 consensus sequence from Thailand [11, 15]; subtype E HIV-1 strains (TH09V3, NH2V3, KH005V3 and NH1V3) substituted for V3-chimera construction; and parental subtype B HIV-1 strain (MD14V3). Underline, N-glycosylation motif at which first N (Asparagine) is potentially glycosylated. *Italic letters*, basic amino acids exchanged with respect to the subtype E NSI V3 consensus sequence. The consensus sequence is completely the same as TH09V3. **B.** Construction scheme. Overlapping primers 2 and 3, and outer primers 1 and 4 were used to generate recombinant DNA segments carrying subtype E V3 and MD14 flanking sequences by overlap extension method [14]. The final PCR products were digested with *Bgl* II and *Bsu*36 I and cloned into the HIV-1 gp120 subclone, pUC-MDH [23]. Subsequently, the *Bgl* II-*Bsu*36 I fragment of the pUC-MDH was cloned into pMD14 to reconstitute a full-length SHIV molecular clone. **C.** Profiles of the viral proteins expressed in HeLa cells transfected with the SHIVs. Cell lysates were separated by 12% polyacrylamide gel and analyzed by Western blot with the following antibodies: anti-SIVmac macaque serum, positive control antiserum of LAV BLOT 1 kit, antiserum of J28 patient [23], and antiserum of NH1 patient [23]. Lanes: *1*, mock transfection; *2*, SHIV-TH09V3; *3*, SHIV-NH2V3; *4*, SHIV-KH005V3; *5*, SHIV-NH1V3; *6*, $SHIV_{MD14}$, respectively. Bands of Gag products (P55 precursor, P27 capside, P17 matrix) and GP160 Env are indicated. P41 band (possible Env transmembrane protein or partially-cleaved Gag product, or both) are also indicated. **D.** Differential V3 immunoreactivity between the SHIVs was further focused by Western blot analysis using two primary antibodies against V3 sequences (RC25 monoclonal antibody and a rabbit antiserum raised against the consensus V3 sequence of NSI HIV-1 E from Thailand). Bands of GP160 Env and GP120 Env are indicated

($CD3^+$) in FSC-SSC lymphocytes-gate were analyzed by FACS Caliber (Becton Dickinson). Absolute counts of the T-lymphocyte subpopulations were determined by referring the known beads count.

Seroconversion analysis

Plasma was incubated at 56 °C for 30 min, and then used as a primary antibody for LAV BLOT 1 Western blot kit.

GHOST cells assay

GHOST cells [2, 30], human osteocarcinoma cells transfected with genes for human CD4 and either human CXCR4 or CCR5, were cultivated in flasks with 10% FBS-DMEM containing 500 μg/ml of gentamycin, 50 μg/ml of hygromycin and 1 μg/ml of puromycin. Trypsin-detached GHOST cells were washed, resuspended with the medium and then seeded into 96-well plates (8×10^2 cells per a well). After an overnight cultivation, culture medium was aspirated out, followed by application of SHIV diluted serially by the medium. After an additional overnight incubation, the applied viral supernatant was aspirated out, and the wells were washed, and then 250 μl of the medium was loaded per a well. After additional 48 hours, culture supernatants were monitored for P27 Gag production.

Results

Construction and preparation of V3-chimera SHIV clones

Four V3-chimera SHIVs were constructed (Fig. 1A and 1B). TH09V3 and NH2V3 are the V3 sequences from non-syncytium-inducing (NSI) HIV-1_{TH09} [11] and HIV-1_{NH2} [23], respectively. KH005V3 and NH1V3 are those of syncytium-inducing (SI) HIV-1_{KH005} [34] and HIV-1_{NH1} [23], respectively.

SHIV stocks were prepared from supernatants of the cultures in which HeLa cells were transfected by the SHIV DNA constructs and then cultivated for 48 to 72 hours. All the viral stocks had RT activity in the range of $1.5 - 2.0 \times 10^3$ cpm/ μl. We named respective V3-chimera SHIVs as SHIV-TH09V3, SHIV-NH2V3, SHIV-KH005V3 and SHIV-NH1V3.

Protein profiles of SHIVs

Western blot analysis of the lysate of the transfected HeLa cells was performed to examine the profiles of viral proteins (Fig. 1C). Anti-SIVmac antiserum recognized SIVmac Gag products: P55 precursor, P27 capside, P17 matrix, and a possible partially-cleaved Gag P41 product. While P27 Gag was mainly detected by the positive control antiserum of LAV BLOT 1 kit, additional GP160 Env band and a 41 kDa band (possible Env transmembrane protein or a partially-cleaved Gag product, or both) were also detected by antiserum of J28 patient [23]. Although antiserum of NH1 patient [23] recognized P27 Gag evenly all through the SHIVs, its recognition of GP160 Env was extensive for SHIV-NH2V3, weak for SHIV-TH09V3, and undetectable for other SHIVs.

Differential V3 immunoreactivity between the SHIVs was further focused by two primary antibodies against V3 sequences (Fig. 1D). GP160 Env and GP120