

these AIDS-related malignancies. It is essential to explore the molecular mechanism of Vpr-induced double-strand breaks to clarify their role in HIV-1 infection and their effect on the stability of the host cell genome.

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# 【HIV-1感染に伴う染色体異常】

Chromosomal abnormalities in HIV-1 infection

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## Key words

HIV-1 vpr, NADCs; Non-AIDS defining cancers; Chromosomal abnormality

## 要約

エイズ症例では悪性腫瘍を高頻度に認める。Highly active anti-retrovirus therapy (以下HAART) の導入によりHIV-1感染症による日和見感染症のコントロールが可能になり、カポジ肉腫など癌の発症率は激減した。しかし近年、non-AIDS defining cancers (NADCs) と呼ばれる一群の悪性腫瘍が、免疫不全を示さないHIV-1感染者で報告されて以来、HIV-1感染による癌化機序が問われている。NADCsとして、皮膚がん・肛門周囲腫瘍・ホジキンリンパ腫や前立腺癌などが10万人に対して980人の頻度で発症する。なぜか? 本編ではHIV-1感染に伴って誘導される染色体異常に関する知見を紹介するとともに、私達が解析してきたVprによるゲノム不安定性についてを紹介する。

## はじめに

Highly active anti-retrovirus therapy (以下HAART) の導入により、HIV-1感染症の予後決定因子であった日和見感染症は激減した。しかし近年、HIV-1感染者に必ずしも免疫不全を伴わないnon-AIDS defining cancers (以下NADCs) が認められることが分かるとともに、NADCsがHIV-1感染者の予後を決する重要な病態として注目されている。私達は、1997年の解析当初からHIV-1を「癌ウイルス」と想定し、ウイルス感染そのものによる悪性腫瘍誘発の

可能性を検証してきた。

本編ではNADCsに関する現状を紹介し、ウイルス感染に伴うゲノム不安定性の様子とその機序について私達の知見を供覧する。

## 1. 増え続けるHIV-1感染者と ウイルス感染に伴うゲノム不安定性

厚生労働省「エイズ動向委員会」の報告によると、2004年の我が国の新規感染者数はほぼ一千人で、特に10-20代の若者を中心としながら依然増加傾向にある。複数の薬剤を組み合わせたHAARTにより、患者予後は飛躍的に改善されてはいるものの、現行HAARTでは体からウイルスを完全には駆逐することができない。即ち、HIV-1感染者のフォローは生涯必要であり、長期的な予後を考える上で、悪性腫瘍が最も重要な予後決定因子となる。

エイズ症例において高頻度に癌が発症することは以前から指摘されており、HIV-1感染者の発癌リスクは健常人よりも60倍以上高いことが提唱されてきた。しかし、このような悪性腫瘍は一般的には、エイズ病態下での免疫担当細胞の機能不全により誘発されるものと考えられ、事実、カポジ肉腫に見るように免疫不全に伴ってヘルペスウイルスが感染し、これが肉腫発症機序の一因となっている場合も

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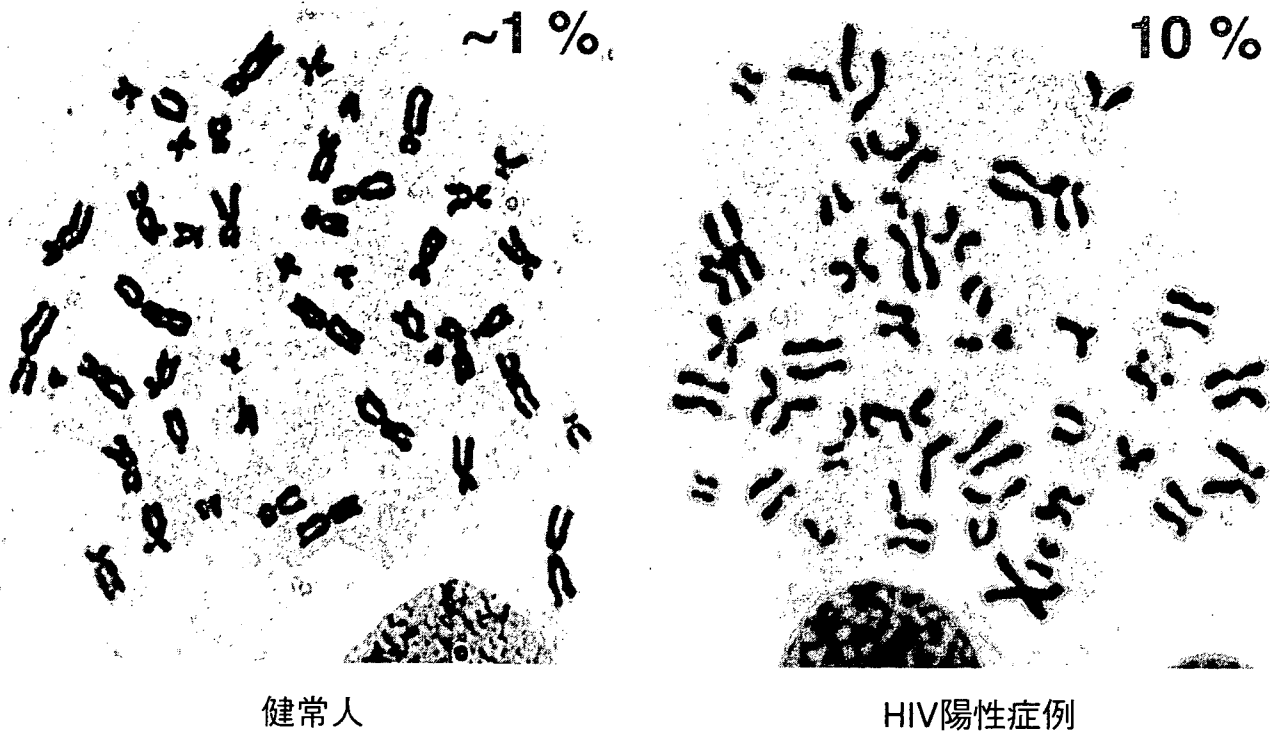


図1 HIV-1感染に伴う姉妹染色分体の早期分離。

健常人由来末梢血単核球細胞に試験管内で、ウイルスを感染させた後、分裂期染色体のギムザ染色による観察を行った。健常人由来末梢血単核球細胞の姉妹染色分体はセントロメア領域で束ねられているX字型を呈している（左図）が、感染後の細胞では、姉妹染色分体は離れて存在している（早期分離、右図）。その頻度は、健常人由来では1%未満、感染後の細胞では10%と高頻度であった。

ある。HAART導入後、著しい免疫不全病態は回避できるようになり、カポジ肉腫の頻度は激減した。しかし、近年行われたコホート研究により、免疫不全を示さないHIV-1感染者でも高頻度に癌の発症が認められることが指摘された<sup>2)</sup>。NADCsの頻度は10万人当たり約980人と試算され<sup>3)</sup>、皮膚がん、肛門周囲腫瘍、ホジキンリンパ腫や前立腺癌などが認められる。また、悪性腫瘍を初期症状として外来に訪れるHIV-1症例もあることから、今後、臨床の現場でも無視できない疾病概念となることが予測される。

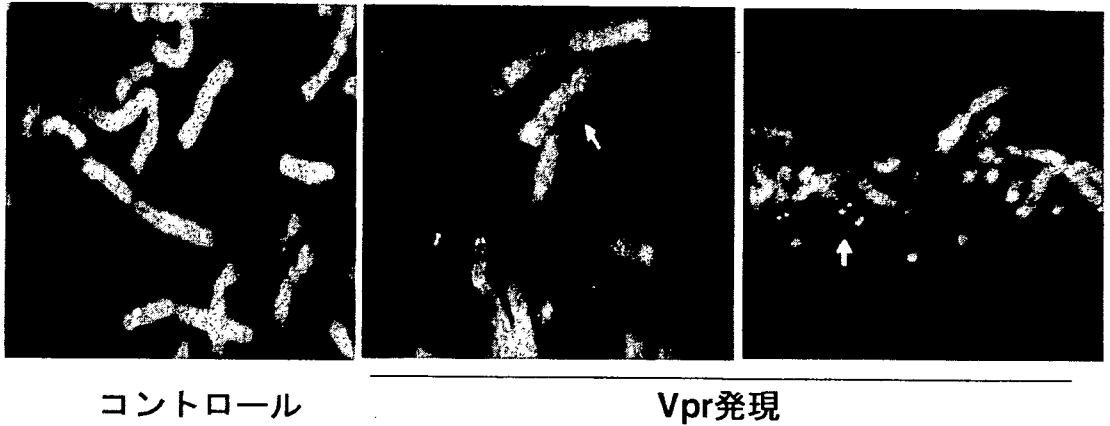
HIV-1陽性患者では、染色体転座、遺伝子再構成、p53の遺伝子変異など、様々なゲノム不安定性が検出され、悪性腫瘍発症との積極的な関連が指摘されている<sup>3),4)</sup>。また、ゲノム不安定性の要因である染色体異常がHIV-1陽性者の末梢血細胞中でも検出されている<sup>3),4)</sup>。私達はHIV-1陽性者の末梢血細胞をPHA

存在下で培養すると早期姉妹染色分体分離（PCS; premature sister chromatid separation）が誘発され（図1、右図、姉妹染色分体が解離している）、染色体の異数倍体化も生じることを見出した<sup>5)</sup>。また、健常人由来末梢血細胞に試験管内でHIV-1ウイルスを感染させた場合でも、同様にPCSが誘導されたことから、HIV-1自身に癌ウイルス様の機能があることが示唆された。この発見は、試験管内で感染させた不死化細胞がマウスに対して造腫瘍性を示した実験結果と良く合致する<sup>6)</sup>。

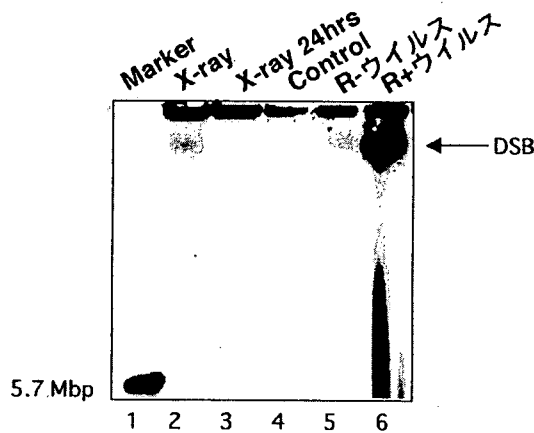
## 2. HIV-1遺伝子Vprによるゲノム不安定性

当研究部は、HIV-1遺伝子の一つであるVpr（Viral protein R）に早期から着目し、その解析を行

2a.



2b.



2c.

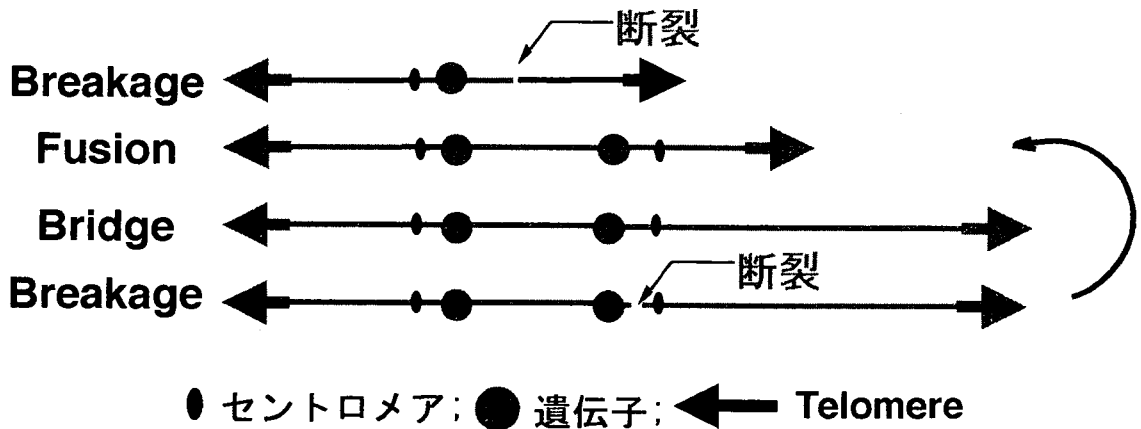


図2 VprによるDNA二重鎖切断

- 2a. Vprによる遺伝子増幅. コントロール (左), Vpr発現後 (中央及び右図) のFISH解析結果を示す。ドットが増幅した遺伝子である。単一の染色体上に幾つもの遺伝子シグナルが検出される (中央図)。また、二動原体染色体を有する細胞も検出される (右図)。
- 2b. Vprプラスウイルス (図中R+ウイルス, レーン6) を培養細胞に感染させ、パルスフィールド電気泳動法にて解析した。陽性コントロールとしてX線照射後の細胞も解析した (レーン2)。矢印で示す位置に、高分子DNAよりも早い泳動度を示すDNAの塊が検出された。
- 2c. BFBサイクルの模式図. DSBが生じると染色体の融合が起こり、二動原体染色体が形成される。分裂期に動原体が娘細胞の両極に牽引され、物理的に切断されることでDSBが生じる。この部位を起点として再度このサイクルが開始し、何回も繰り返される事で遺伝子増幅が生じる。

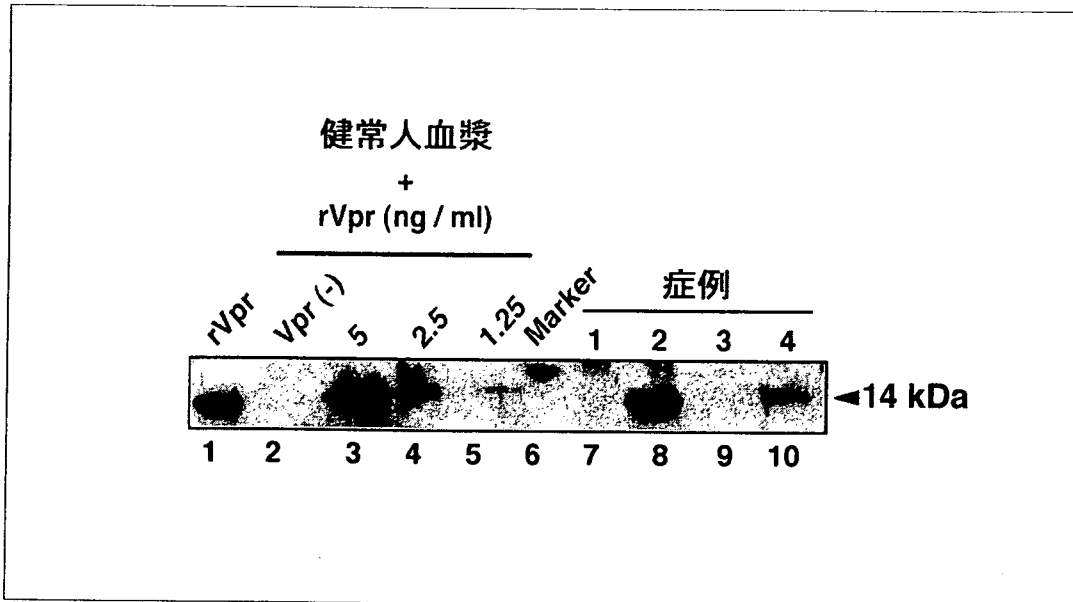


図3 HIV-1陽性者血中Vprの検出.

Vprに対する2種類の単クローン抗体を用いて免疫沈降-ウエスタン解析法を行った。52例中20例に分子量14 kDaのVprを検出した。代表的な症例を示す(レーン7-10)。その濃度は最大10 ng/ml(0.7 nM)であった。種々の量の精製rVpr標品を健常人由来血漿に添加し、同様の解析を行い、得られたシグナルの強度を比較することで患者血漿中Vpr量を半定量した(レーン2-5)。

って来た。Vprは96個のアミノ酸からなる約14 kDaの核蛋白質で、究めて多くの宿主側蛋白質と結合する。当初、Vprは細胞周期のG2期における細胞増殖抑制を示すことが知られていた。しかし、Vprの機能を詳細に解析した結果、G2期の異常に加えて、M期の異常も誘導することを見いだした。さらにVpr発現を誘導した細胞中には、染色体の数の異常や小核の形成、遺伝子増幅(図2a, 中央図)、中心体の数の異常等、様々なゲノム不安定性を認めた<sup>7)</sup>。さらにここ1-2年の解析で、VprがゲノムDNA二重鎖切断(以下DSB: double strand breaks)を誘発することを証明した<sup>8),9)</sup>。図2bは、DSBの様子を示した実験結果で、Vprプラスのウイルスを培養細胞に感染させると(レーン6)、X線照射後(レーン2)に検出されるのと同じように早い泳動度を示すDNAの塊が生成された(図2b, 矢印)。

遺伝子増幅とDSBは一見、関連性の無い現象のように見える。しかし、染色体上の広い領域にわたって生じる遺伝子増幅にはDSBが重要な役割を担っており、その機序を理解するためのモデルと

してbreakage-fusion-bridge (BFB) サイクルが提唱されている。

BFBサイクルは1942年、Barbara McClintockにより提唱されたダイナミックな染色体の動きで、トウモロコシの分裂の様子を詳細に観察することで発見された<sup>10)</sup>。図2cに簡単な模式図を示す。染色体上に損傷DNAが生じると二動原体染色体が形成される(BreakageとFusion)。そしてそれぞれの動原体は、次の細胞分裂の際に娘細胞側に牽引され、対極に移動する。その結果、娘細胞間で一旦ブリッジが形成されるが(Bridge)、分裂が進む過程でこのブリッジに断裂が生じ(Breakage)、再び二動原体染色体が形成される。

このサイクルが繰り返されることで染色体上の広い領域に亘って再構成が進む。例えば薬剤耐性遺伝子のように増殖を支持する遺伝子が染色体上に局在すれば、結果として「遺伝子増幅」となる。BFBサイクルが遺伝子増幅に関与していることを示唆する重要な所見は、二動原体染色体の存在であるが、Vpr発現により得られた遺伝子増幅陽性細胞中に二

動原体染色体を認めている(図2a, 右図)。余談だが、このようにゲノムがダイナミックに動く事を発見したMcClintockは、1983年「可動遺伝子の発見」でノーベル生理学・医学賞を受賞した。

### 3. 患者血中Vpr濃度の把握と リコンビナントVpr蛋白質(rVpr) によるDSB誘発

このような恐ろしい機能を有するVprが、HIV-1感染者の血液や脳脊髄液などの体液中に存在することが報告されてきた。しかし、その濃度については不明であった。そこで、52例の患者血漿について2種類の単クローン抗体による免疫沈降-ウエスタン解析を行った。その結果、20症例にVprを検出し、その濃度は数ng/ml (最大濃度1 nM) であった(図3)<sup>11)</sup>。さらに、大腸菌で発現・精製したrVprを数nMの濃度で健康人末梢白血球細胞の培養系に添加すると、DSBの指標であるATM (ataxia telangiectasia mutated) のリン酸化型蛋白質のフォーカス形成が誘導された<sup>9)</sup>、(星野: 未発表データ)。

HIV-1感染者で認められる悪性腫瘍の主たる病型はB細胞性リンパ腫であるが、B細胞はCD4を発現しないためウイルスは感染しない。また、EBウイルス感染を示す頻度は悪性リンパ腫症例の60%で、EBウイルス非感染者の中には免疫不全を示さない症例も見いだされている<sup>9)</sup>。即ち、B細胞性リンパ腫の発症にはEBウイルス以外の因子も関与していることが予測される。

このような背景の中で、私達はVprによる染色体異常とDSBに特に注目している。血液中に存在するVprは体中の細胞に対してDSBを誘導し得る。さらに、Vprはマクロファージからある種のサイトカインを産生させることも分かっており、このサイトカインが前癌細胞の増殖を支持する可能性も考えられる。現在、HIV感染患者における悪性腫瘍発症のモデルとして、VprによるB細胞性リンパ腫の誘発能を解析中である。HIV感染に関連するB細胞性リンパ腫発症では、c-myc遺伝子と免疫グロブリン遺伝子間の遺伝子組み替えを認める<sup>9)</sup>。このような現象はX線照射後に形成される悪性リンパ腫の発症過程でも生じる異常な遺伝子再編である。VprがX線照射のようにDSBを誘導することから、B細胞に同様な異常な染色体転座を誘発する可能性は十分に考え

られる。

### おわりに

「有効な薬剤が開発された現在ではHIV-1感染は怖くない」という誤った考え方をする若者が多いとしたら、その方々には是非、「HIV-1感染と一見関連性のないように考えられてきた悪性腫瘍の発症が、現在では重要な問題となりつつある」ということを理解して頂きたいと願う。私達が本職とするエイズ病態の研究も大切であるが、何よりも大切なことはHIV-1感染予防であることをご理解頂けたら有り難い。

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### 細胞ニュース

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# Socioeconomic and Reproductive Factors Associated with Condom Use Within and Outside of Marriage Among Urban Pregnant Women in Zambia

Chipepo Kankasa, MD, PhD<sup>1</sup>, Margaret Siwale, MD, PhD<sup>1</sup>, Francis Kasolo, MD, PhD<sup>2</sup>, Ayako Nishiyama, MD, MHS<sup>3</sup>, Hiroshi Terunuma, MD, PhD<sup>4</sup> and Naomi Wakasugi, MD, PhD<sup>3</sup>

## ABSTRACT

A cross-sectional questionnaire survey was conducted on 470 pregnant women in Lusaka, Zambia. Multivariate analysis revealed school attendance and child deaths as independently significant variables positively associated with HIV seropositivity. Among women with fidelity, HIV prevalence was not significantly lower, and condom use was much lower than among women who were having extramarital affairs. Factors significantly associated with condom use within and outside of marriage differed—age and number of live births within, and sexual transmission knowledge outside of marriage. School attendance was not effective for gaining knowledge on sexual transmission or condom use. Regular own earning was significantly effective for condom use in both groups, irrespective of school attendance. The following should be implemented intensively: effective education on HIV and sex in and out of school before early sexual debut, further implementation of family planning with emphasis on condom use, and empowering women by assisting with their economic independence. (*Afr J Reprod Health* 2005; 9[3]:128-136)

## RÉSUMÉ

**Facteurs de reproduction et socio-économiques liés à l'usage du condom dans le mariage et hors de mariage chez les femmes urbaines enceintes en Zambie.** Une enquête transversale basée sur un questionnaire a été menée auprès de 470 femmes enceintes à Lusaka en Zambie. Les analyses multivariées ont montré que la fréquentation à l'école et la mortalité infantile sont des variables importants liés à la séropositivité du VIH. Parmi les femmes fidèles, le niveau de fréquence n'était pas inférieur de manière significative et le taux de l'usage du condom était moins élevé chez elles que chez les femmes qui trompaient leurs maris. Il y avait une différence à l'égard de l'âge et nombre de naissance vivantes dans le mariage et la connaissance de la transmission sexuelle hors de mariage ou l'usage du condom. La fréquentation à l'école n'était pas effective quant à l'acquisition de la connaissance sur la transmission sexuelle ou l'usage du condom. Un salaire personnel et régulier bien efficace pour l'utilisation du condom pour les deux groupes, que l'on fréquente l'école ou non. Il faut une mise en oeuvre rigoureuse des propositions suivantes: une éducation efficace du VIH et de la sexualité dans l'école et hors de l'école avant le commencement précoce de la vie sexuelle, une réalisation davantage de la planification familiale en mettant l'accent sur l'utilisation du condom et la capabilisation de la femme en l'aidant à accéder à l'indépendance économique. (*Rev Afr Santé Reprod* 2005; 9[3]:128-136)

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KEY WORDS: Zambia, HIV, Condoms, Extramarital relationships, Income

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## Introduction

The global burden of HIV infection is increasingly shifting to women and children.<sup>1</sup> This trend is a real and serious threat, particularly in Africa where infected females outnumber infected males<sup>1,2</sup>, and the fertility rate is very high<sup>3</sup>. Zambia is no exception, since the estimated HIV prevalence rates among young people (15–24 years of age) are 16.8% – 25.2% for women and 6.5% – 9.7% for men<sup>4</sup>. Furthermore, 20% – 27% of the approximately 400,000 women who deliver every year in Zambia<sup>5</sup> are estimated to be HIV-positive. Consequently, around 30,000 to 40,000 Zambian infants are thought to contract HIV by vertical transmission every year.

Among the foremost reasons cited for the increased vulnerability of women to HIV infection is the higher efficiency of male-to-female HIV transmission than the reverse that has been shown in developed countries<sup>6,7</sup>. In addition to this universal reason, many other factors seem to play a role in African countries. The high rate of undiagnosed and untreated STI (sexually transmitted infection)<sup>8</sup> and the high frequency of transfusion of contaminated blood during high-risk deliveries under the insufficient safe blood policy<sup>9</sup> are increasing the biologic risk of HIV infection among African women.

The socioeconomic and cultural conditions faced by women, such as poverty, economic dependence, socio-sexual subordination, and power inequity, are also considered to contribute to their increased vulnerability to HIV infection<sup>10,11</sup>. Male resistance to condom use and women's inability to negotiate safer sex<sup>12</sup> are considered to be major obstacles to the reduction of non-protective sex. The promotion of condom use within marriage might be the most difficult challenge in this society, where child bearing is of great value. However, it should be more targeted, in order to reduce the advancing deadly threat of a generalized HIV epidemic in women and children.

Confronted with a very high prevalence of

HIV infection among pregnant women in Zambia, we decided to investigate the factors related to women that facilitate condom use to help women protect themselves against contracting HIV infection.

## Methods

The present study was performed at a primary-level health clinic offering antenatal services, located in an urban area of Lusaka city. The clinic covered a population of 120,000 people living in a compound named Chipata, with an estimated 6,400 annual pregnancies. The clinic is one of six antenatal clinics at which the national PMTCT (Prevention of Mother to Child Transmission) programme was conducted between 2000 and 2002, with the support of UNICEF/UNAIDS.

The pregnant women on their first attendance at antenatal care were invited to individual pre-test counselling after a brief explanation of the VCT Voluntary Counselling and Testing and PMTCT programme. Our cross-sectional questionnaire survey was given before the start of individual pre-test counselling. Zambian female staff trained for this study interviewed the women individually, using a structured questionnaire. After counselling, the consent of each woman for serologic testing of HIV was sought. Each woman was assigned a serial number in addition to the number on her clinical record sheet. HIV status was checked thereafter using the serial number, and the confidentiality of each individual was strictly respected.

The questionnaire included items aimed at defining the women's socioeconomic characteristics (age, recognition of own and husband's HIV sero-status before this VCT, years of school attendance, own earning, condom use) and reproductive profile (number of pregnancies, live births, abortions, and child deaths). The women were also asked which body fluid could transmit HIV among blood, breast milk, semen, and vaginal secretion. As for condom use, respond-



ents were asked how often their husbands and/or their partners used condoms in the past. The term 'partner' in this study was defined as sexual partner excluding husband. Each answer was classified into three categories, respectively: never use, sometimes use, and always use. Regarding sexual relationship, we classified the respondents into three groups according to their answers to two questions on condom use: 'only husband' for those who answered only the question for condom use with husband, 'husband and partner' for those who answered both questions for condom use with husband and with partner, and 'only partner' for those who answered only the question for condom use with partner. The group 'only husband' indicates, in its broad sense, women with fidelity, and the other group 'husband and partner' indicates women who were having extramarital affairs.

The sampling was conducted consecutively between November 2000 and May 2001. The HIV sero-status was determined at the laboratory attached to the clinic using an HIV-1&2 rapid test kit (ABBOTT), and confirmed using another test kit (BIONOR or GenieII). The results were announced to the women two days after, and then they received post-test counselling. Ethical approval for the study was obtained from The Zambian Government/University of Zambia Research Ethic Committee.

### Data analysis

Associations between HIV sero-status and socio-economic/reproductive characteristics were examined by the chi-square test and unpaired Student's t-test. School attendance was divided into seven categories and subjected to the following analyses. The risk factors significantly associated with HIV seropositivity were identified by univariate analyses. Multivariate logistic regression analysis adjusted for those factors was then performed to identify variables that had a significant independent association. The multivariate logistic regression analyses for con-

dom use were performed to two outcomes, one with the husband, and the other with partners. Since no subject answered that she always used condoms with her husband, we dichotomised the remaining two answers, never use and sometimes use. As for condom use with partners, the answers sometimes use and always use were combined together and used for further analysis. Statistical analyses were conducted using SPSS software, version-10.

### Results

The general characteristics of the surveyed population, together with HIV seropositivity and its risk factors, are summarized in *Table 1*. A total of 490 pregnant women agreed to be tested for HIV. The rate of HIV test acceptance was 49.1%. Twenty cases were eliminated due to lack of serologic data (i.e., missing blood sample, one; rapid test declined, two; data not recorded, 17). Consequently, 470 pregnant women aged 15 to 44 were enrolled for analyses. The overall HIV prevalence was 24.5% (95% confidence interval [CI]=20.6–28.4). Only 6.2% were aware of their own HIV status before undergoing HIV testing, including two women who knew of their HIV infection and 27 who knew that they were HIV negative. Similarly, 5.7% of women were aware of their husband's HIV status (data not shown). A feature of age-specific prevalence of HIV infection was a slightly higher rate among women aged 15–19 years than that recorded in the sentinel surveillance study conducted in the same Lusaka city in 1998 (14.8% in 1998, 16.2% in the present study)<sup>13</sup>. The age of women was associated significantly with HIV seropositivity by univariate, but not by multivariate analysis. The prevalence of HIV infection increased significantly with increase in the years of school attendance by both univariate and multivariate analyses. The rate of correct answers on the routes of HIV transmission were 97.2% for blood, 80.8% for breast milk, 65.2% for semen, and 62.6% for vaginal secretion (data not shown). Thus, a gap

**Table 1. Characteristic of pregnant women and analyses of risk factors for HIV seropositivity**

Characteristics	Univariate analysis				Multivariate analysis	
	N	%HIV +ve	OR (95%CI)	P-value	AOR (95%CI)	P-value
<b>Age (n=464)</b>						
15-19	105	16.2	1.04 (1.00-1.08)	<0.05	1.05 (1.00-1.11)	0.09
20-24	185	22.7				
25-29	108	31.5				
30-39	60	30.0				
40+	6	16.7				
<b>School attendance<sup>a</sup> (n=467)</b>						
0 yr	38	18.4	1.17 (1.01-1.36)	<0.05	1.22 (1.05-1.43)	<0.05
1-2 yr	14	14.3				
3-4 yr	67	22.4				
5-6 yr	86	23.3				
7-8 yr	163	23.3				
9-10 yr	80	31.3				
11 yr+	19	42.1				
<b>Sexual transmission knowledge<sup>b</sup> (n=468)</b>						
wrong	163	25.2	1.00			
right	305	23.9	0.94(0.60-1.46)	0.8		
<b>Own earning (n=454)</b>						
regularly	88	23.9	1.00			
irregularly	60	26.7	1.16 (0.55-2.47)	0.7		
none	306	24.2	1.02 (0.58-1.77)	1		
<b>Live births (n=468)</b>						
none	128	18.0	1.00	1.00		
1	127	29.9	1.95 (1.08-3.52)	<0.05	1.52 (0.81-2.88)	0.2
2+	213	25.4	1.55 (0.90-2.68)	0.1	0.93 (0.43-2.00)	0.8
<b>Abortions (n=470)</b>						
never	422	24.6	1.00			
experienced	48	22.9	0.91 (0.45-1.85)	0.8		
<b>Child deaths (n=470)</b>						
never	351	22.2	1.00	1.00		
experienced	119	31.1	1.58 (1.00-2.51)	0.05	1.69 (1.01-2.83)	<0.05
<b>Condom use with husband (n=449)</b>						
never	344	24.1	1.00			
sometimes	105	22.9	0.93 (0.56-1.56)	0.8		
always	0	-	-			
<b>Condom use with partner (n=167)</b>						
never	118	29.7	1.00			
sometimes	43	23.3	0.72 (0.32-1.62)	0.4		
always	6	0.0	-			

<sup>a</sup>7 categories: 0=0yr. 1=1-2yr. 2=3-4yr. 3=5-6yr. 4=7-8yr. 5=9-10yr. 6=11yr+ P<0.05: unpaired t-test

<sup>b</sup>Sexual transmission knowledge means whether women know that semen can transmit HIV.

OR: Odds ratio AOR: Adjusted odds ratio CI: confidence interval

**Table 2. Characteristics of women by sexual relationship and those associated with HIV seropositivity**

Sexual relationship	Condom use with husband				Condom use with partner			HIV seropositivity		
	N	Non use (%)	Use <sup>a</sup>	P-value	Non use (%)	Use <sup>b</sup> (%)	P-value	%HIV+ve	OR (95%CI)	P-value
Only husband	299	242 (80.9)	57 (19.1)	<0.01	-	-	0.2	22.4	1.00	
Husband and partner	150	102 (68.0)	48 (32.0)		103 (68.7)	47 (13.3)		26.7	1.26 (0.80-1.98)	0.3
Only partner	17	-	-		15 (8.2)	2 (11.8)		29.4	1.44 (0.49-4.24)	0.5

OR: Odds ratio, CI: confidence interval

<sup>a</sup> Use indicates sometimes use of condom<sup>b</sup> Use indicates sometimes or always use of condom**Table 3. Multivariate analyses for the association of women's characteristics and condom use either with husband or with partner.**

	Condom use with husband (n=423)			Condom use with partner (n=156)		
	N	OR (95%CI)	P-value	N	OR (95%CI)	P-value
Age	423	0.92 (0.86-0.98)	<0.05	156	0.98 (0.88-1.09)	0.7
School attendance <sup>a</sup>	423	1.11 (0.94-1.32)	0.2	156	1.02 (0.80-1.31)	0.9
Sexual transmission knowledge <sup>b</sup>						
wrong	143	1.00	59	1.00		
right	280	0.70 (0.43-1.14)	0.2	97	2.11 (0.97-4.61)	0.06
Own earning						
regularly	84	1.00	35	1.00		
irregularly	54	0.39 (0.16-0.96)	<0.05	15	0.11 (0.01-0.97)	<0.05
none	285	0.74 (0.41-1.32)	0.3	106	0.83 (0.34-2.04)	0.7
Live births						
none	107	1.00	53	1.00		
1	116	2.49 (1.22-5.05)	<0.05	37	0.94 (0.32-2.79)	0.9
2+	200	4.13 (1.78-9.58)	<0.01	66	1.34 (0.36-4.92)	0.7
Abortions						
never	381	1.00	140	1.00		
experienced	42	1.94 (0.96-3.94)	0.07	16	2.93 (0.91-9.49)	0.07
Child deaths						
never	313	1.00	119	1.00		
experienced	110	0.73 (0.41-1.30)	0.3	37	0.68 (0.26-1.78)	0.4
HIV serostatus						
negative	323	1.00	114	1.00		
positive	100	0.93 (0.53-1.63)	0.8	42	0.58 (0.24-1.39)	0.2

<sup>a</sup> 7 categories: 0=0yr. 1=1-2yr. 2=3-4yr. 3=5-6yr. 4=7-8yr. 5=9-10yr. 6=11yr+<sup>b</sup> Sexual transmission knowledge means whether women know that semen can transmit HIV.

OR = odds ratio adjusted for each variable listed above CI = 95% confidence interval

existed in the extent of knowledge between the non-sexual (blood or breast milk) and the sexual (semen or vaginal secretion) routes of HIV transmission ( $P<0.01$ , chi-square test). Those who

answered correctly on the non-sexual routes had a trend toward longer school attendance (blood,  $P<0.05$ ; breast milk,  $P=0.09$ ; unpaired t-test). However, no association was found regarding

the sexual routes. Furthermore, knowledge of the routes of sexual transmission did not correlate significantly with HIV seronegativity in the present study. One-third of the women had their own earnings, whether or not the earning was regular. As for the factors comprising the reproductive profile, child deaths were associated significantly with HIV seropositivity in the multivariate analysis. None of the women answered that they always use condoms, and only one-fourth reported sometimes use of condoms with their husbands. Condom use both with husband and partner tended to decrease HIV prevalence, but a significant association was not revealed.

Table 2 shows the distribution of women by sexual relationship, condom use, and HIV seropositivity in each group. Of the 466 pregnant women, 32% had extramarital sexual relationships. The HIV prevalence among women with fidelity was not significantly lower than that among women without fidelity. With respect to condom use with the husband, it was much lower among the fidelity group than among extramarital affair group ( $P < 0.01$ , chi-square test). Women involved in extramarital sexual relationships seemed to have increased condom use either with the husband or with the partner.

Table 3 shows the results of the multivariate analyses for the association of women's characteristics with condom use either with husband or with partner. Condom use with husband showed a significant association with age and number of live births (for age, odds ratio [OR]=0.92, 95% CI=0.86–0.98); for the number of live births, 1, OR=2.49, 95% CI=1.22–5.05; 2+, OR=4.13, 95% CI=1.78–9.58). On the other hand, condom use with partner was not associated with these factors. Sexual transmission knowledge tended to associate with condom use with the partner but not with the husband. Women with irregular earnings had significantly lower condom use with both husband and partner than those with regular earnings (with husband,

OR=0.39, 95% CI=0.16–0.96; with partner, OR=0.11, 95% CI=0.01–0.97).

## Discussion

The present study indicates the tendency of and the factors associated with the HIV epidemic among urban pregnant women in Zambia in 2001. The overall prevalence of HIV infection in the study population was slightly lower than that in the report of sentinel surveillance performed in 1998 (median 27.3%, min. 25.9%, max. 29.1% in antenatal care clinics-major urban areas)<sup>13,14</sup>. However, it may be noted that the prevalence in women 15–19 years of age was slightly higher than in that report.

We observed a contradictory effect of the length of education on the prevention of women contracting HIV infection. This result was not unexpected, since previous reports have found that a woman's educational attainment was linked positively with a high prevalence of HIV infection<sup>15,16</sup>. School attendance did not, in fact, increase condom use with husband or extramarital sexual partner. Furthermore, school attendance was not effective for these women to gain knowledge on sexual transmission of HIV, although it was effective for knowledge of non-sexual transmission routes, such as blood and breast milk. This suggests to us a lack of effective and consistent education regarding HIV transmission and sexual behaviour, at least within the school system. Actually, we had several cases of infected teenagers who had a correct knowledge of the sexual transmission routes. It may be possible that the knowledge was not gained before a risky event or the knowledge was the result of a risky event that led them to become infected with HIV. This again indicates insufficient or lacking education on HIV and sex both in and out of school, and that educational efforts should be implemented before the very early age of sexual debut among Zambian women<sup>17,18</sup>. Another reason for non-linkage between sexual transmission knowledge and HIV protection might be the power inequity

between men and women, which makes it exceedingly difficult for women even with sexual transmission knowledge to negotiate safer sex with their sexual partners<sup>19,20</sup>.

Concerning whether a woman's fidelity protects her from HIV infection, our result was not affirmative. Among women with fidelity (the "only husband" group), the prevalence of HIV infection was not significantly lower, and condom use was much lower than among women who were having extramarital affairs (the "husband and partner" group). This finding suggests to us that the woman's fidelity only cannot prevent HIV transmission within marriage, given together with the high rate of polygamy in Zambia<sup>21</sup>. On the other hand, significantly higher use of condoms both with the husband and with other partners among women having extramarital relations, prompts us to consider that the meaning of condom use as an HIV protector is becoming accepted and is put into practice to some extent in Zambia.

Interestingly, the factors significantly associated with condom use within and outside of marriage were very different from each other. Reproductive factors, such as the number of live births, promoted condom use with the husband, which is consistent with the previous report<sup>22</sup>. It would appear that the motivation for condom use comes after a sufficient number of childbirths. In other words, condom use within marriage can be promoted when family planning is more accepted and practiced. On the other hand, for condom use with the extramarital sexual partner, not the reproductive factors but the knowledge of the sexual transmission route of HIV was barely associated, which indicates that sexual transmission knowledge can push condom use as HIV protection in the context of which childbearing is not expected.

Besides this, it is noteworthy, in compari-

son to the inefficacy of school attendance, that regular earning by a woman was significantly associated with condom use with both husband and partner. The possibility of sex work as the source of earnings was thought to be very low, since the percentage of women with fidelity among women with regular earnings was 59% compared with 37% for women without fidelity, and this ratio was similar in both the irregular earnings and no earnings groups (data not shown). Empowering women, or correcting the power imbalance between partners, has received much attention as critical to gaining the male partners' cooperation and acceptance of condom use<sup>1,19,23</sup>. However, it has not been clearly demonstrated what constitutes a woman's empowerment and how it can be acquired. Our results suggest that even relative but stable economic independence by their own earning, irrespective of their school attendance, might be indispensable, even though education has been believed to be a prerequisite for economic empowerment. It would appear that a woman's economic independence should receive proper attention to combat the spread of HIV infection through condom use.

In conclusion, the following should be implemented intensively in the HIV/AIDS prevention programme in Zambia: effective HIV and sex education for young girls less than 15 years of age in and out of school, further implementation of family planning with emphasis on condom use, and empowering women by helping them to become economically independent.

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# Increased Risk of Intrauterine Transmission of HIV-1 Associated With Granulocyte Elastase in Endocervical Mucus

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**Background:** One of the remaining challenges in the prevention of mother-to-child transmission of HIV is to reduce the risk of the transmission during pregnancy. It remains to be investigated which factors affect intrauterine HIV transmission and how they can be identified and addressed during pregnancy.

**Methods:** Granulocyte elastase in the endocervical mucus of HIV-positive pregnant women in Zambia was measured, and its association with intrauterine transmission of HIV-1 from the mother to the fetus was investigated.

**Results:** The intrauterine transmission rate determined by polymerase chain reaction assay of DNA from neonates at birth was 15.3%. The risk for intrauterine transmission was 8.65-fold higher in women who were positive for granulocyte elastase than in those who were negative.

**Conclusion:** We suggest that the women showing positive granulocyte elastase at delivery be strongly suspected of having and if having had chorioamnionitis during pregnancy, which could affect the intrauterine transmission of HIV.

**Key Words:** HIV-1, mother-to-child transmission, chorioamnionitis, granulocyte elastase

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The baseline rate of mother-to-child transmission (MTCT) of HIV in the absence of any intervention has been shown to be higher in Africa, ranging from 25% to 42%, than in developed countries, ranging from 14% to 25%.<sup>1</sup> Transmission occurs not only during delivery but through breast-feeding<sup>2</sup>

and during pregnancy, with risks such as advanced HIV infection<sup>3,4</sup> and poor general health status<sup>5,6</sup> contributing to the higher baseline rate in Africa. Thus, the overall efficacy of an MTCT prevention regimen that focuses solely on the perinatal period<sup>7</sup> is limited. One of the remaining challenges in the prevention of MTCT is to reduce the risk of HIV transmission during pregnancy.

Several studies have shown that genital tract infections<sup>8</sup> and placental inflammation, especially chorioamnionitis (CAM),<sup>4,5,9–12</sup> facilitate MTCT, although there are reports showing no significant association<sup>6,13</sup> or an inverse result.<sup>14</sup> Most of these studies investigated the association of histologic CAM with total or intrapartum transmission rather than with intrauterine transmission. CAM may cause HIV transmission during pregnancy by transplacental and transamniotic modes of infection.<sup>10</sup> In any case, a noninvasive assay of CAM during pregnancy is required to prevent intrauterine transmission.<sup>8,10</sup>

Granulocyte elastase, a multifunctional serine protease found in endocervical mucus, has been shown to correlate well with the presence of cervicitis (first stage of CAM) and CAM and to be a sensitive marker identifying a pregnancy at risk for adverse newborn outcomes such as threatened preterm delivery, low birth weight, and chronic lung disease.<sup>15,16</sup> It has also been shown that the use of intravaginal antibiotics in pregnant women showing positive granulocyte elastase resulted in a decrease of elastase to the normal range, and no preterm delivery was observed.<sup>16</sup> This prompted us to speculate that the risk of intrauterine HIV transmission can similarly be identified if positive granulocyte elastase levels are shown to be a relevant marker for it.

## METHODS

We measured granulocyte elastase in the endocervical mucus of HIV-positive women and investigated its association with intrauterine HIV transmission. Pregnant women were consecutively recruited at delivery between June and September 2001 in the maternity ward of the University Teaching Hospital in Lusaka, Zambia, because most come to the hospital after they go into labor, deliver on the same day, and are discharged within 2 days after delivery.

A prospective longitudinal study with repeated elastase assay was difficult, because the study participants' access to antenatal care was usually absent or took place in primary health centers, where they cannot deliver. The present study

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was therefore designed as a retrospective cohort exploring past intrauterine transmission with elastase levels present at delivery.

Women in labor with intact membranes and no bleeding were enrolled after giving consent for the study content, including HIV testing for the mother and newborn. Skilled Zambian midwives recorded the data, including gestational age, labor duration, and physical measurements, and examined the placenta. Maternal HIV serostatus was determined using an HIV-1 and -2 rapid test kit (Abbott) and was later confirmed by polymerase chain reaction (PCR). To evaluate the rate of intrauterine transmission of HIV, the peripheral blood of the neonates born to HIV-positive mothers was taken within 24 hours after delivery and analyzed by PCR.<sup>17</sup> DNA was purified by the QIAamp DNA Blood Mini Kit (Qiagen). PCR analysis was performed as previously described,<sup>18</sup> using pol primers: 409 through 512, 1030 through 1050, 823 through 845, and 964 through 986. Vaginal smears were taken using a dry sterile speculum and then subjected to vaginal pH measurement using pH paper (pH-Fix 3.6–6.1; macherey.nagel) and Gram staining for microscopic diagnosis of bacterial vaginosis (BV) according to the method of Nugent. Cervical mucus was then swabbed, and granulocyte elastase activity was measured with the Teizo Elastase Test (Teikoku Hormone Manufacturing Company), which detects elastase activity greater than 1.6  $\mu\text{g/mL}$ .<sup>19</sup> The birth weights of the newborns were rounded off to every 100 g. The other numeric data were dichotomized with a median split and subjected to analyses.

Univariate and multivariate logistic regression analyses were conducted using SPSS computer software, version 10.

## RESULTS

Two hundred sixty-four women fit the criteria. Of this group, 260 agreed to HIV testing and 263 received granulocyte elastase testing. Seventy-two (27.7%) of 260 women were HIV-seropositive (95% confidence interval [CI]: 22.3 to 33.1). Eleven of 72 newborns were HIV-1-positive by PCR at birth, resulting in a 15.3% (95% CI: 7.0 to 23.6) intrauterine transmission rate. The rates of positive cervical granulocyte elastase were 25.1% (66 of all 263 women, 95% CI: 19.9 to 30.3), 29.2% (21 of 72 HIV-positive women, 95% CI: 18.7 to 39.7), and 23.5% (44 of 187 HIV-negative women, 95% CI: 17.5 to 29.6), respectively, and were not significantly different. Among HIV-positive women, the difference in basic characteristics was not significant: in elastase-positive and elastase-negative women, the women's average age was 27.3 years and 25.5 years, the average body mass index (BMI) was 26.1 and 25.9, and the newborns' average birth weight was 3010 g and 3140 g, respectively. The placentas were not examined histologically in this study, but the size of the placenta as well as its ratio to neonate body weight was significantly higher in elastase-positive women than in elastase-negative women ( $P < 0.05$  and  $P < 0.05$ ; student's  $t$  test).

Table 1 shows the rates of intrauterine HIV transmission in 72 HIV-seropositive mothers as a result of risk factors.

**TABLE 1.** Analysis of Risk Factors of Intrauterine Transmission of HIV-1 in 72 Seropositive Mothers

Factors	No. Mothers	Intrauterine Transmission Rate (per 100 births)	Univariate OR (95% CI)	<i>P</i>	No. Mothers	AOR (95% CI)*	<i>P</i>
All women†	72	15.3	—	—	—	—	—
BMI of mothers							
<25.6	34	17.6	1.0		32	1.0	
≥25.6	35	14.3	0.78 (0.21 to 2.84)	0.70	35	0.42 (0.08 to 2.26)	0.31
Duration of labor, min							
<610	34	17.6	1.0		33	1.0	
≥610	34	14.7	0.81 (0.22 to 2.94)	0.74	34	0.53 (0.11 to 2.56)	0.43
Child birth weight, g‡							
(range: 2100–4400, mean = 3100)	70	15.7	1.93 (0.48 to 7.71)	0.35	67	2.62 (0.30 to 2.32)	0.39
Size of placenta, cm							
<18.2	34	8.8	1.0		33	1.0	
≥18.2	36	22.2	3.00 (0.71 to 12.2)	0.14	34	5.00 (0.58 to 42.7)	0.14
Weight of placenta, g							
<700	34	11.8	1.0		31	1.0	
≥700	36	19.4	1.81 (0.48 to 6.85)	0.38	36	0.51 (0.07 to 3.91)	0.52
Elastase							
Negative	51	7.8	1.0		48	1.0	
Positive	21	33.3	5.88 (1.50 to 23.0)	0.01	19	8.65 (1.75 to 43.3)	<0.01
Vaginal Gram stain score							
0–3 (normal)	32	12.5	1.0		30	1.0	
4–6 (borderline BV)	24	12.5	1.00 (0.20 to 5.00)	1.00	22	1.07 (0.17 to 6.84)	0.94
7–10 (BV)	16	25.0	2.33 (0.50 to 10.9)	0.28	15	3.29 (0.54 to 20.0)	0.20

\* $n = 67$ , adjusted for each variable listed.

†All HIV-positive women for whom PCR testing of HIV-1 in the newborn was available.

‡Newborn birth weights rounded to every 100 g.

According to previous reports in the literature, we chose the following risk factors to be evaluated: BMI, duration of labor as maternal side factors, and neonate birth weight, size and weight of the placenta as neonate side factors. We also added the BV score because BV is known to be a pathologic condition leading to CAM.

The intrauterine transmission rate was 33.3% (7 of 21 women, 95% CI: 0.13 to 0.53) among mothers with positive cervical elastase, which was significantly higher than the rate of 7.8% (4 of 51 women, 95% CI: 0.005 to 0.15) among those with negative cervical elastase (odds ratio [OR] = 5.88, 95% CI: 1.50 to 23.0). This trend remained in the multivariate analysis (adjusted odds ratio [AOR] = 8.65, 95% CI: 1.73 to 43.3).

### DISCUSSION

The present study was not prospective but retrospective, and the power of the study was fair but not sufficient. The power was anticipated before the study as more than 0.8 ( $1-\beta = 0.839$ ,  $\alpha = 0.05$ ) and calculated as 0.718 after the study. A prospective study that includes an intervention with antibiotics for elastase-positive and HIV-positive women and determination of its effect on intrauterine HIV transmission is still necessary.

The results of our study suggest that women with positive elastase at delivery are strongly suspected of having and of having had CAM during pregnancy, which could affect the intrauterine transmission of HIV. Regardless of the presence of antiretroviral therapy or of the viral load level in pregnant woman, the placental barrier, weakened by its inflammation, could increase the chance of HIV entering the fetal blood. As shown previously in the case of threatened preterm delivery, the elastase assay could be a predictive and noninvasive test of placental inflammation that could be applied to identify women at risk for HIV transmission during pregnancy. It should be further investigated in which situations and when elastase screening should be implemented and whether, together with an inexpensive intervention with antibiotics, it could help to reduce MTCT when antiretroviral therapy for pregnant women is absent or limited.

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## 海外における医療・検査事情

## ザンビア共和国のエイズと結核の状況—ラボラトリーが担う役割

HIV / AIDS and tuberculosis in Zambia —The role of laboratory systems.

わかすぎ  
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## I. エイズをめぐる世界の動向

抗生物質とワクチンという予防と治療の2つの武器の発見によって、感染症克服の明るい地平が開かれた現代においてなお、多くの開発途上国ではいまだに感染症ペシミズムの時代が続いている。たとえば先進国では抗結核薬によって結核が激減した一方で、多くの患者が集中する開発途上国では、結核は死の宣告に近い。1991年WHOはこのような結核状況の南北差を明らかにし、費用が10ドルで済む直接監視短期治療法(DOTS: Directly Observed Treatment, Short-course)を提案し世界の結核対策として推進してきたが、なお開発途上国を中心に結核の死亡者は毎年150万人にのぼる。

そしてHIV/AIDSが今同様な南北格差状況を示し、結核と同じく圧倒的に多くの感染者が開発途上国、貧困国に集中している。そのためウイルスの発見後20年を超えて、開発途上国、特にサブサハラアフリカではエイズ発症者・死亡者が蓄積増加し、数千万人の生命がすでに奪われてしまった。予防に力点が置かれてきた開発途上国のエイズ対策も、予防のみに頼るのではなく感染者へのケア・治療を行うことが客観的必要性となってきている。1996年以降行われたHAART (Highly Active AntiRetroviral Therapy) が先進国の感染者に生存の延長、QOLの回復という目に見える効果を示してからさらには、その必要性が叫ばれ、2000年の日本主導の沖縄感染症対策イニシャチブ、2002年世界エイズ・結核・マラリア対策基金(グローバルファンド)発足、

2003年WHOによる3 by 5イニシャチブ(2005年までに世界で300万人のHIV感染者に治療を)へとつながってきた。

例えばブラジル政府は、自国でARVジェネリック薬を製造・供給するとともに、全エイズ患者のARV治療を保証する対策を取っている。これに伴い若者を中心とする新感染者が大幅な減少をみたといわれている。エイズ患者の治療をすることは生存を延長するので新感染者を増やすかもしれないという思惑に反して、予防行動の促進がみられたのである。このようなBalanced Prevention & Careの方向は、今後、「12の予防と9の治療・ケア対策を行うことによって、2010年までに2,900万人の新感染の発生を防げる。」(Lancet 360: 73, July 2002)とした論文などに基づいている。そのために必要な資金は年間70~100億ドル必要と見積もられ、グローバルファンドもまだその目標額に到達してはいないが、2004年6月時点で、世界88カ国、150プロジェクトに対し13億ドル約束され、これまで4.2億ドルが拠出された。このような国際社会の努力を背景として、開発途上国のHIV感染者のARV治療は次第に現実のものになってきている。

## II. ザンビアのエイズと結核の現状(図1)

南部アフリカの中央に位置し、周辺を8つの国に囲まれた人口1,020万人のザンビア共和国は、1964年イギリスより独立を果たしたが、現在、国民1人当たりのGNPは320USドルで、1日1ドル以下で生活する人が国民の85%を占める、重債務貧困国

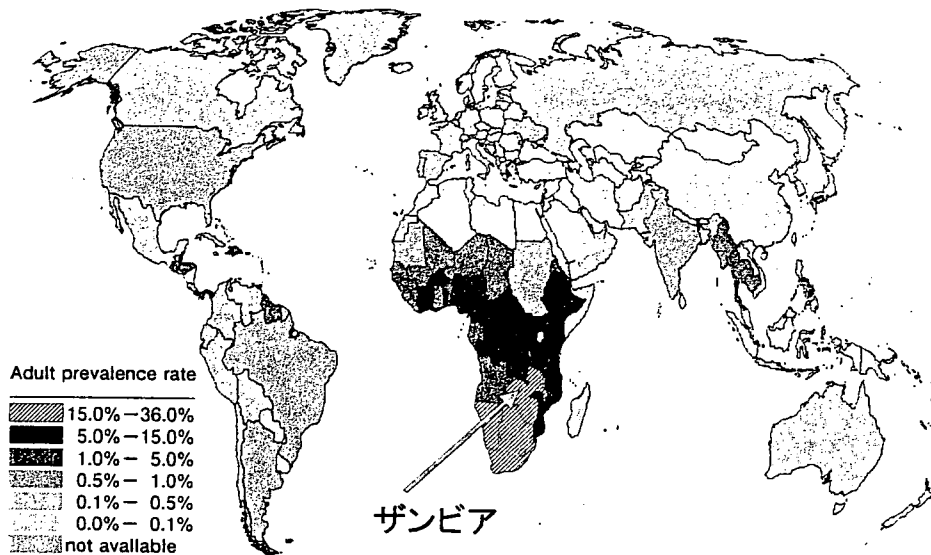


図1 A global view of HIV infection  
39.4 million people living with HIV/AIDS as of end 2004

である。

ザンビアの乳児死亡率は108（出生1,000対）、5歳未満児死亡率192（出生1,000対）、妊産婦死亡率750（出生10万対）と母子保健指標の悪さが目立つが、疾病構造の特徴は、感染症の比重が大変大きい、典型的な途上国型で、中でもエイズ、結核、マラリアの罹患率・死亡率の高さは特筆に値する。UNAIDSによれば、ザンビアでは毎年10万人以上のエイズ死亡があり、2000年末では成人（15～49歳）の21.5%、約100万人のHIV感染者（うち女性59万人）がいると推定された（世界5位）が、2003年末は16%（都市部25%、農村部13%）とされている。この減少は予防対策の効果を示すというよりは、エイズ死亡の増加による、実質生存感染者率の低下と考えたほうがよい。実際、社会生産基盤を担う年齢の男女、妊婦、母親、政府官僚、教師、看護師などのエイズ発症、死亡の話は頻繁に聞くようになっている。また若い女性・妊婦の高い感染率と、出生の多さの結果、毎年40万の出産のうち10万がHIV陽性妊婦の出産、これに対し抗レトロウイルス薬（ARV）によって予防介入できているのは1万人に満たないので、毎年3～4万人のHIV陽性児が出生していると推定される。母親ないし両親をエイズで失ったエイズ孤児も年々増加し、70万人に達するであろうといわれている。

一方、HIV感染の猛威に伴い、結核が急速な再興感染症となっている。ザンビアの結核罹患率は

1985年に105（人口10万対）であったものが、2000年には512（人口10万対）と5倍の急増をしている。年間患者数は2002年には5万人であり、結核発症者の7割がHIV陽性である（すなわちHIV感染を原因として結核の感染・発症につながったと考えられる）という。これもまた世界トップクラスの二重感染率の多さである。また肺結核患者は1988～1992年の4年間で倍増しており、そのうちスメア陽性は42%である。多剤耐性結核は1.4%とまだあまり高値は示していない。DOTS対策は2001年より開始され国内数十ヶ所で展開されているが、いまだ大変弱体で、WHO目標の85%治癒率に及ばず、55%の治癒率にとどまっている。最近、JICAによるラボラトリープロジェクトがコミュニティボランティアとともにHIV/TB二重感染者の治療（Community-based DOTS & HAART）を開始したが、このような介入対策以前のこの地域では、DOTS実施中の死亡が24%にのぼり、治癒率も64%である。

### III. JICA・UTHプロジェクトからのエイズ・結核対策への貢献-全国ラボラトリー機能の強化

JICAはザンビア政府の要請に基づき1989年4月～1995年3月まで、続いて1995年4月～2000年3月までの二期にわたってプロジェクト型技術協力