Relevance of Early Detection of HIV Type 1 SI/CXCR4-Using Viruses in Vertically Infected Children

Cintia M. Crudeli,¹ Paula C. Aulicino,¹ Carlos A. Rocco,¹ Rosa Bologna,² Andrea Mangano,¹ and Luisa Sen¹

Abstract

The aim of the study was to investigate the prevalence and persistence of syncytium-inducing (SI) strains in HIV-1-infected children along time of infection and to evaluate the influence of antiretroviral therapy and host factors on viral tropism. This is a retrospective analysis carried out in 267 HIV-1 vertically infected children from an Argentinean cohort. The viral phenotype was screened in MT-2 cells and coreceptor usage confirmed by the GHOST cell assay. Also, CD4⁺ T cell count, viral load, antiretroviral therapy, and human CCR5-Δ32 and CCR2-64I genotypes were analyzed. A high frequency of HIV-1 SI/CXCR4-using variants (22%) was found among children within the first trimester of life, reaching 46% after 10 years of infection. At acute infection, zidovudine prophylaxis did not significantly affect the proportions of SI HIV-1 strains, while their presence was favored by the CCR5⁺/Δ32 genotype. Interestingly, the majority of the early SI strains did not persist over time, probably due to a higher susceptibility to antiretroviral (ARV) treatment or immunologic pressure. At the chronic stage, SI variants emerged even in the presence of HAART reaching 36% at 120 months of infection. Also the HIV-1 SI phenotype was associated with lower CD4⁺ T cell counts all along the course of infection. These findings highlight the need to evaluate the presence of SI/CXCR4 variants early at primary infection. This will make it possible to optimize the use of CCR5 inhibitors in children who are apparently carriers of the R5 virus preventing early therapeutic failure due to the reemergence of SI strains from reservoirs.

Introduction

VIRAL AND HOST FACTORS CAN modify the course of HIV-1 infection. Among viral determinants HIV-1 phenotypic variants have been associated with different degrees of virulence *in vivo*. According to cell tropism, they are classified as T cell tropic (T-tropic) or macrophage-tropic (M-tropic). T-tropic variants are syncytium-inducing (SI) and highly cytopathic, while M-tropic viruses are non-syncytium-inducing (NSI) and less virulent.^{1,2} Based on coreceptor usage, HIV-1 variants are classified as CCR5-using (R5), CXCR4-using (X4), or dual-tropic viruses (R5X4), with a straightforward correlation between T cell tropism, SI capability, and CXCR4 usage.^{3,4} M-tropic NSI strains are R5 and predominant at viral transmission. In contrast, T-tropic strains are SI/CXCR4-using variants and tend to emerge in approximately 50% of infected adult and pediatric patients with advanced disease.^{5,6}

HIV-1 natural infection in children differs from that of adults. In infants, time to AIDS has a bimodal distribution. Around one-fourth to one-third of infected children show a

more aggressive infection, which progresses to AIDS within the first 2 years of life, probably due to an immature immune system at the time of infection.^{7,8} The other three-fourths of infected children have a disease progression similar to that of adults. Shortly after transmission, most infants are infected with NSI/R5 variants.^{9–12}

We have previously observed that the presence of HIV-1 SI variants at acute infection, with rapid replication capability, is indicative of a poor prognosis favoring a rapid onset of AIDS in vertically infected children.¹³ The presence of SI/CXCR4-using viruses at an early stage of vertical transmission may be particularly relevant in pediatric immunopathogenesis, specially at birth due to an extremely active thymus with an unusual high number of potential target cells.^{14–16}

Other factors such as antiretroviral (ARV) therapy and host genotype related to coreceptor usage may affect viral tropism. To date, their influence on the viral phenotype in infected children and adults remains controversial and information is scarce.^{17–21}

The aims of our study were to investigate the prevalence and persistence of SI strains in HIV-1-infected children along

¹Laboratorio de Biología Celular y Retrovirus-CONICET and ²Servicio de Infectología, Hospital Nacional de Pediatría "Prof. Dr. J. P. Garrahan," Buenos Aires, Argentina.

the time of infection, and to evaluate the effect of ARV therapy and host genotype on HIV-1 variants with different tropism.

Materials and Methods

Study population

A retrospective evaluation of HIV-1 tropism was performed in 267 HIV-1 vertically infected children, born between 1986 and 2002, admitted at the "Garrahan Pediatric Hospital," Buenos Aires, Argentina. A total of 107 infants were studied at primary infection (≤ 12 months of age) and 160 at the chronic stage (> 12 months old). No information was available to distinguish between intrauterine, during delivery, and/or breast-feeding transmission.

At acute infection, information on zidovudine (ZDV) prophylaxis according to the AIDS Clinical Trials Group (ACTG) 076 protocol²² was available in 88 of the 107 children. Forty-three infants received ZDV prophylaxis without breast-feeding as follows: (1) in 16 mother-infant pairs, the mothers received ZDV at pregnancy (last trimester) and at delivery, and the baby for the first 6 weeks of life; (2) in 10 pairs, the mother received ZDV at delivery and the infant during the first 6 weeks of life; and (3) in 17 cases, only the baby received ZDV for 6 weeks after birth. Forty-five infants did not receive prophylactic ARV therapy, and of them, 18 were breast-fed. The first HIV-1 phenotype determination was performed before starting HAART in 88 infants. At the chronic stage (> 12 months old), 160 HIV-1-infected children were studied, and at the time of HIV-1 phenotype determination, 35 were naïve and 125 were receiving HAART [median time of 31 months, inter quartile range (IQR) = 13–56 months].

For phenotypic switch studies, 10 patients with SI variants at acute infection were prospectively evaluated, with a median follow up time of 25 months (IQR:13.5–37 months). Informed consent from the parents or legal guardians was obtained. The study was reviewed and approved by the Ethics Committee of the Hospital.

Isolation of HIV-1 from peripheral blood mononuclear cell cocultures

HIV-1 was isolated by cocultivation of cells as previously described by the AIDS Clinical Trials Group.²³ Briefly, peripheral blood mononuclear cells (PBMCs) from both the patient and HIV-1-seronegative blood donors prestimulated for 24–72 hours with 5 μ g/ml of phytohemagglutinin (PHA) (Difco Laboratories) were cocultured at a final concentration of 2×10^6 cells/ml. Cocultures were maintained for 28 days in RPMI 1640 medium (Gibco BRL, Invitrogen) supplemented with 20% heat inactivated fetal bovine serum (FBS), 5 U/ml interleukin (IL)-2 (Sigma Aldrich), and 10 μ g/ml gentamicin (Gibco BRL Invitrogen). Measurement of HIV-1 p24 Ag of coculture supernatants was performed with a commercial assay kit (Vironostika HIV-1 Antigen, BioMérieux).

Determination of syncytium-inducing viral isolates using MT-2 cells

The SI assay was performed as described by Japour *et al.*²⁴ In 96-well plates, supernatants from HIV-1 positive cocultures were added to four wells containing 5×10^4 MT-2 cells/well in RPMI 1640 supplemented with 10% FBS and 10 μ g/ml gentamicin. SI primary viral isolates and a CXCR4-tropic

HIV-1_{LAI}/PBMC laboratory adapted strain (from NIH AIDS Reference and Reagent Program) were used as positive controls and wells without virus and with NSI isolates were considered as negative controls. Syncytium formation of MT-2 cells was examined under a microscope every 3 days for 28 days. To confirm the infection of MT-2 cells, at the end of the culture, HIV-1 proviral DNA of MT-2 cell lysates was amplified directly, as described by Albert and Fenyo.²⁵ To ensure infectivity of the viral inoculum, a parallel control assay was performed adding the viral inoculum in wells containing 2×10^5 PHA-stimulated PBMCs from HIV-1-seronegative donors. Supernatants from all the wells were measured for HIV-1 p24 Ag. When the viral inoculum infected PBMCs but was unable to induce syncytium in MT-2 cells, the viral isolate was scored as NSI.

Determination of coreceptor usage

Viral infection was performed as described by Vodrós and Fenyo.²⁶ GHOST cell lines transfected with CD4⁺ and viral coreceptors (kindly provided by Dr. H. Salomon, Centro Nacional de Referencia de SIDA) were maintained in DMEM medium (Hyclone, Biodynamics) containing 10% FBS and antibiotics. One day before infection, 24-well plates were prepared with 1.5×10^5 cells/well, followed by infection with virus standardized for TCID₅₀. Cultures were observed under a fluorescent microscope and p24 Ag was determined. In all cases, CCR5-tropic (HIV-1_{BaL}) and CXCR4-tropic (HIV-1_{LAI}/PBMC) laboratory adapted strains were used as control of coreceptor usage. CXCR4 using was confirmed by the inhibition of viral infection with AMD3100 (Sigma Aldrich), 10 μ g/ml, a CXCR4 inhibitor.

CCR5 and CCR2 genotyping

Of the 267 patients, 259 were genotyped for CCR5- $\Delta 32$ and 246 for CCR2-64I by RFLP-PCR, as previously described.²⁷

Clinical parameters

In 240 children, CD4⁺ T cell counts were measured using flow cytometry (FACSort; Becton Dickinson, CA). The data were collected close (< 6 months) to the time of the phenotypic study. Clinical and immunological stages were determined according to the 1994 criteria of the U.S. Centers for Disease Control and Prevention (CDC) classification for children.²⁸

Viral load measurements

Plasma viral load was determined by the HIV-1 RNA QT Nuclisens (Organon Teknika, Boxtel, Netherlands), AmpliCor HIV-1 Monitor test, or COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (Roche Diagnostic Systems, Branchburg, USA), depending on the kit available at the time of measurement.

Statistical analysis

The effects of age, ZDV prophylaxis, HAART, CCR5- $\Delta 32$, and CCR2-64I on the HIV-1 phenotype were tested with logistic regression analysis. Group contrasts for CD4⁺ T cells percentage/absolute counts and viral load were evaluated with the Mann-Whitney test. In all cases, two-tailed tests were performed with a significance level of 0.05.

FIG. 1. Distribution of HIV-1 phenotype from vertically infected children at different ages or times of infection. Filled columns are syncytium-inducing (SI) variants and n =the number of patients SI/non-syncytium-inducing (NSI). A patient with SI viral isolates was plotted as SI at the age observed; when a patient had more phenotypic characterizations as NSI during follow-up, we considered the first one.

Results

Proportion of SI and NSI HIV-1 variants in children at different times of infection

HIV-1 isolates from 267 vertically infected patients, ranging from 1 to 220 months of age, were evaluated for SI capability in MT-2 cells. The viral phenotype was determined once in 170 children, and more than once (2–9 times) in the other 97 infants. Children were stratified according to age (time of infection). The frequency of SI HIV-1 isolates varied with time of infection, being 22% within the first 3 months after birth, with a sustained drop until 12 months of age (Fig. 1). Afterward, it was followed by an increase, reaching 46% of SI variants after 10 years of infection.

To confirm CXCR4 coreceptor usage, 35 SI isolates derived from MT-2 cultures were tested in GHOST transfected cell lines. Of them, 33 were R5X4, and the remaining 2 SI MT-2 isolates were able to infect the GHOST cells only through the CCR5 coreceptor. The absence of infection in CXCR4 GHOST cells of the two latter SI isolates could be due to a higher sensitivity of the MT-2 assay (which expresses only the

CXCR4 coreceptor) for detection of low levels of SI strains,²⁹ based on different levels of expression of coreceptors between MT-2 and GHOST transfected cell lines.³⁰

In addition, viral isolates from 10 children of our cohort were simultaneously tested by Trofile and MT-2 assays with a full concordance of both methods (all 10 SI strains were X4R5 dual/mix by Trofile).

Effect of ARV drugs on viral tropism

We next analyzed whether ARV prophylaxis/treatment might have influenced the selection of SI variants at different stages of infection. Thus, we divided the patients into acute and chronic stages. Viral isolates from a total of 88 infected infants under 12 months old at acute infection were studied comparing those who had received ZDV as preventive treatment ($n=43$) and those who had not ($n=45$). During the first 6 months of life, the percentage of infants with SI variants did not differ regardless of having received preventive therapy or not, indicating that, at early stages of infection, preventive ZDV did not affect the viral phenotype (Table 1).

TABLE 1. HIV-1 PHENOTYPIC CHARACTERISTICS ACCORDING TO WHETHER THE CHILDREN HAD RECEIVED OR NOT RECEIVED ANTIRETROVIRAL THERAPY AT DIFFERENT STAGES OF INFECTION

Stage of infection ^a	Age (months old)	ARV	Viral phenotype		Total n	p ^b
			NSI n (%)	SI n (%)		
Acute infection	≤6	No	27 (84)	5 (16)	32	0.906
		Yes	24 (77)	7 (23)	31	
	>6 to 12	No	10 (77)	3 (23)	13	
		Yes	12 (100)	0 (0)	12	
Total of patients (n=88)	≤12	No	37 (82)	8 (18)	45	0.015
		Yes	36 (84)	7 (16)	43	
Chronic infection	>12 to 60	No	21 (88)	3 (12)	24	
		Yes	34 (69)	15 (31)	49	
Total of patients (n=160)	>60	No	10 (91)	1 (9)	11	
		Yes	46 (60)	30 (40)	76	
	>12	No	31 (88)	4 (12)	35	
		Yes	80 (64)	45 (36)	125	

^aPatients ≤12 months old include infants who had received zidovudine exclusively for 6 weeks, and in patients older than 12 months, at a chronic stage of infection, children receiving HAART.

^bAge-stratified logistic regression.

ARV, antiretroviral; NSI, non-syncytium-inducing; SI, syncytium-inducing.

Between 6 and 12 months of age, SI variants were absent in infants who had previously received ZDV prophylaxis, although the association was not statistically significant ($p=0.9$).

We further evaluated the impact of HAART on the viral phenotype in a total of 160 children older than 1 year. At the moment of study, 125 children were receiving HAART and 35 were naïve of ARV (due to a delay in HIV diagnosis). Because age can be a confounding factor the children were subdivided into two groups: ≤ 60 and >60 months old, following PAHO guidelines,³¹ which consider children older than 60 months immunologically similar to adults. The proportion of SI variants was significantly higher in patients under therapy than in those who did not receive any ARV ($p=0.015$), independently of the age stratum (Table 1). The marked reduction of SI variants in naïve children older than 12 months old may be due to a shorter asymptomatic period than those carrying NSI variants.

Likewise, the association between HAART and SI phenotype in children at the chronic stage was significant after correcting for a monotone effect of age, immunological stage, and viral load ($p=0.019$, data not shown).

SI to NSI phenotype switch at early stages of infection

We further investigated the effect of ARV therapy on the HIV-1 SI to NSI phenotype switch in the acute stage. Consecutive viral tropism was evaluated in 10 patients before and close to initiation of ARV treatment (Table 2). Two viral tropism determinations were performed in infants #354 and #579 prior to the start of therapy. In both cases, the viral phenotype spontaneously switched from SI to NSI at 3 and 29 months, respectively. Moreover, infant #579 preserved the same NSI tropism after 34 months of ARV therapy with one log₁₀ reduction of viral load. In the other six infants, the initial SI variants switched to NSI as early as 3 months after initiation of HAART, with a mean of 12.3 months under ARV. In the remaining two infants (#75 and #545), SI variants persisted despite the treatment. Infant #75 remained SI and died at 18 months of age, with a severe immunodeficiency. Paradoxically, in patient #545, the SI variants detected at 5 months of age did not switch after initiation of HAART, with persistent high viral loads, normal CD4⁺ T cells, and free of AIDS up to 121 months. These observations indicate that the majority of the SI variants are susceptible to phenotype switch

TABLE 2. SEQUENTIAL HIV-1 ISOLATES FROM PATIENTS WHO INITIALLY HARBORED SYNCYTIIUM-INDUCING VARIANTS

Patient ID	Age (months)	ZDV (with=1; without=0)	Viral phenotype (NSI/SI)	Treatment (time of ARV in months)	Viral load (log ₁₀ RNA copies/ml)	CD4 ⁺ T cell (%/μl)
#354	3	0	SI	None	6.36	3
	29		NSI	None	5.18	ND
#579	1	1	SI	None	>5.9	ND
	3		NSI	None	5.9	14
	38		NSI	ZDV-ddI-NFV (34)	4.69	ND
#495	3	1	SI	None	6	34
	8		NSI	ZDV-3TC-NFV (4)	4.2	48
#507	4	0	SI	None	5.34	8
	8		NSI	ZDV-3TC-NFV (3)	3.08	ND
#371	1	0	SI	None	4.8	17
	15		NSI	ZDV-ddI (13)	5.26	19
	34		NSI	3TC-d4T-NFV (32)	4.11	25
#526	8	0	SI	None	5.72	18
	17		NSI	ZDV-ddI-NFV (7)	4.41	40
	21		NSI	ZDV-ddI-NFV (11)	4.99	ND
#462	5	0	SI	None	5.67	19
	11		NSI	ZDV-ddI-NFV (4)	4.86	19
	15		NSI	ZDV-ddI-NFV (8)	4.81	18
	20		NSI	ZDV-ddI-NFV (13)	4.91	29
#109	7	0	SI	None	>5.8	32
	15		NSI	ZDV-ddI (8)	4.86	18
	32		NSI	ZDV-ddI-NFV (25)	4.58	26
	57		NSI	d4T-3TC-EFV (50)	4.73	ND
#75 ^a	2	1	SI	None	>5.8	ND
	4		SI	None	ND	5
	11		SI	ZDV-ddI-RTV (4)	>5.8	6
#545	5	1	SI	None	5.44	30
	10		SI	ZDV-3TC-NFV (3)	5.65	30
	37		SI	ZDV-3TC-NFV (30)	3.84	42
	108		SI	ABC-ddI-KLT (101)	5.61	17

^aPatient 75 died at 18 months of age.

ZDV, zidovudine; ARV, antiretroviral; NSI, non-syncytium-inducing; SI, syncytium-inducing; ND, not determined; ddI, didanosine; NFV, nelfinavir; 3TC, lamivudine; d4T, stavudine; EFV, efavirenz; RTV, ritonavir; ABC, abacavir; KLT, lopinavir-ritonavir.

TABLE 3. RELATIONSHIP BETWEEN VIRAL PHENOTYPE, CLINICAL PARAMETERS, AND CCR5/CCR2 GENOTYPES AT DIFFERENT STAGES OF INFECTION

	Acute infection			Chronic infection		
	NSI	SI	p	NSI	SI	p
HIV viral load (log ₁₀ RNA copies/ml)						
Median	5.64	5.55	0.181	4.25	4.70	0.026
Interquartile range	5.14–5.9	4.53–5.82		3.45–4.84	3.93–5.38	
CD4 ⁺ T cell percentage						
Median	26	18	0.027	23	13	0.010
Interquartile range	19–34	6–31		17–28	5–26	
CD4 ⁺ T cell count (cells/ μ l)						
Median	1631	738	0.069	816	493	0.035
Interquartile range	865–2278	227–1992		960–1282	63–913	
CCR5 genotype, n (%)						
CCR5 ⁺ /+	85 (88)	12 (12)	0.028	93 (70)	40 (30)	0.625
CCR5 ⁺ /D32	3 (50)	3 (50)		15 (65)	8 (35)	
CCR5 D32/D32	—	—		—	—	
CCR2 genotype, n (%)						
CCR2 ⁺ /+	60 (87)	9 (13)	0.408	71 (68)	33 (32)	0.617
CCR2 ⁺ /64I	23 (82)	5 (18)		25 (74)	9 (26)	
CCR2 64I/64I	3 (75)	1 (25)		5 (71)	2 (29)	

either spontaneously or by HAART during acute pediatric infection.

Association of HIV-1 tropism and clinical parameters

We next explored whether the HIV-1 phenotype is associated with clinical parameters, and whether it may differ between the acute and chronic stages of infection. In infants younger than 12 months of age, viral loads were extremely high regardless of the viral phenotype. The median for patients with SI viruses was 5.55 log₁₀ copies/ml and for NSI variants was 5.64 log₁₀ copies/ml. However, at chronic infection, the viral loads were higher in children with SI variants than in those with NSI isolates ($p=0.026$) (Table 3). Evaluation of the viral phenotype in association with immunological parameters showed that children under 12 months harboring NSI strains had higher CD4⁺ T cells in percentage and absolute counts than those carrying the SI variants.

Therefore, at early and chronic stages of infection, the SI phenotype was significantly associated with lower CD4⁺ T cell counts ($p=0.010$). Furthermore, different stages of immunodeficiency were evaluated by a logistic regression analysis confirming that the SI phenotype is strongly correlated with severe immunodeficiency ($p\leq 0.001$). These findings indicate that along the whole course of infection the viral tropism is associated with the immunodeficiency of the patients.

Association between CCR5-Δ32, CCR2-V64I polymorphisms, and HIV-1 tropism

Since CCR5-Δ32 heterozygosity may contribute to a selective pressure for NSI/R5 viruses to alternatively use CXCR4 as a coreceptor, we evaluated its influence on the frequency of HIV-1 SI isolates at different stages of infection (Table 3). At primary infection, we observed that SI isolates were significantly more frequent in CCR5⁺/Δ32 heterozygous children than in CCR5⁺/+ homozygotes (60% vs. 14%, respectively, $p=0.028$). However, this association was not observed in children at chronic infection.

The evaluation of CCR2-V64I polymorphism revealed no association with viral tropism at either stage of infection (Table 3). These results indicate that at early stages of HIV-1 infection acquired by perinatal transmission, children heterozygous for the 32-bp deletion in the CCR5 allele are more prone to harbor SI variants.

Discussion

In the present study, we observed a high proportion of SI/CXCR4-using variants at primary infection in HIV-1 vertically infected children, apparently not affected by ZDV prophylaxis. In acute infection, SI variants can switch to NSI spontaneously or after initiation of HAART. However, HAART was not able to prevent the late reemergence of SI strains. In addition, the CCR5⁺/Δ32 genotype favored the early presence of SI variants, but had no influence on the appearance of SI strains at the late stage of infection.

Several studies have indicated that most infants at the acute stage are infected with NSI/R5 viruses.^{9–12} Notably, in our study, a high proportion of vertically infected children carried SI variants within the first months of life. The presence of SI variants, at as early as 1 month of age, suggests a rapid switch from NSI to SI viruses after transmission,³² or a direct perinatal transmission of SI/CXCR4-using variants. Huang *et al.*³³ and Salvatori *et al.*¹⁰ have documented that viruses with different phenotypes, including CXCR4-using viruses, are potentially transmissible from the mother to the infant. Unfortunately, in our study, samples from the mothers were not available.

Therefore, we could not determine whether SI variants were transmitted or suffered a premature phenotypic switch. However, these two possible explanations are not mutually exclusive. The infection with SI variants can also be a common event for other routes of transmission. In recent studies in adult cohorts, including HIV-1 seroconverters and naïve patients, CXCR4-using variants were found in proportions as high as 20%.^{34–36} The high proportion of SI viruses at the early

stage of infection might be due to a preferential selection of CXCR4-using variants by trophoblast cells, favoring their passage *in utero*.^{10,37} Likewise, in the newborn, other conditions, such as the predominant expression of the CXCR4 coreceptor in thymocytes, and detectable CXCR4 in neonatal PBMCs and monocytes, with very low levels of CCR5, may promote the selection of SI variants.^{38–40} Furthermore, CCR5 expression in immune cells varies with age, being undetectable at birth,^{41,42} creating a potential beneficial scenario for the replication of SI variants.

Another relevant finding was that the SI viral phenotype at the early stage of infection was more common among CCR5⁺/Δ32 heterozygotes than among CCR5⁺/+ homozygotes. However, this association was not found at the chronic stage. These observations suggest that CCR5⁺/Δ32 heterozygosity could act as a selective pressure at primary infection, favoring transmission of CXCR4-using variants.⁴³

In contrast to our results, different investigators did not find SI variants during the first year of infection, even in symptomatic children.^{44,45} These discrepancies can be attributable to regional HIV-1 subtype bias and/or to the sensitivity of the methods used. It has been suggested that different HIV-1 subtypes can vary in their ability to use the CCR5 or CXCR4 coreceptors. Among HIV-1 group M viruses, subtype D and CRF01_AE have shown a higher prevalence of CXCR4-using variants when compared to other subtypes,^{46,47} although these differences are controversial.^{48,49} In our population, BF recombinants represent almost 85% of the circulating strains,⁵⁰ but we found no evidence of a preferential coreceptor usage between BF and B subtypes (unpublished data). Therefore, the higher frequency of SI variants at acute stages of infection seems to be independent of HIV-1 subtype in our group of vertically infected children.

On the other hand, different methods for phenotype determinations show broad sensitivity ranges. Current assays of viral tropism include two versions of the MT-2 assay (PBMC and MT-2 coculture or MT-2 infected with viral stocks), transfected cell lines, and the algorithm from the V3 loop of the *env* gene.^{51–53} It has been reported that the MT-2 assay used (with viral stocks) has a 98% concordance with the Trofile assay,²⁹ which is the only tropism test approved for screening the phenotypic variants prior to administration of CCR5 antagonists. In agreement, a complete concordance between the Trofile and MT-2 assays was observed in our group of patients tested.

Furthermore, we found that ZDV as preventive therapy did not affect the presence of SI variants after birth. Nevertheless, the absence of SI variants after 6 months of age in children with ARV prophylaxis was noteworthy. Although we found no statistical differences as compared with children without ZDV prophylaxis, the small number of patients of this age stratum did not make it possible to discard a difference with statistical power above 0.8 (the desired sample should have $n > 79$ for the observed side effect). The fact that early circulating SI/CXCR4-using variants can spontaneously disappear suggests that they have a higher susceptibility than NSI/R5 strains to immune control, allowing NSI/R5 variants to emerge and predominate in the infection.^{54,55}

It should be noted that at chronic stages of infection, only 35 of 160 children persisted without treatment because of a delayed diagnosis. The majority of these asymptomatic naïve patients carried NSI strains. Probably, children who are car-

riers of SI variants soon after birth are more frequently symptomatic and thus diagnosed and treated at an earlier stage of infection. A different scenario was observed in children under HAART with a higher prevalence of SI variants consistent with data on adult cohorts.^{17,56} indicating that HAART did not prevent the appearance of SI strains at late stages of infection. The SI emergence may be related to a reduced expression of CCR5 in patients under treatment,^{57,58} and/or to an increase in naïve CD4⁺ T cell subsets, with high levels of CXCR4.⁵⁹

As previously described,^{1,60} we observed a strong association between SI tropism and immunodeficiency regardless of the time of infection. However, according to different authors, the immune damage by SI/CXCR4-using viruses may differ with the time of infection. At primary infection, SI strains can directly affect thymic function¹⁶ with a decrease in the number of CD4⁺ T cells, while at late stages the impairment can be mainly at the level of secondary lymphoid tissue. Further studies are needed to elucidate this issue.

These findings highlight the need to evaluate the presence of SI/CXCR4 variants at primary infection. These observations may have important clinical and therapeutic implications, especially during early HIV-1 infection, since the phenotypic determination at the acute stage is crucial to provide a more effective and accurate treatment. This will make it possible to optimize the use of CCR5 inhibitors and prevent early therapeutic failure in children who are apparently carriers of the R5 virus due to the reemergence of SI strains from reservoirs.

Acknowledgments

We thank Ms. Natalia Beltramone, Mrs. Carmen Gálvez, and Mrs. Silvia Marino for their technical assistance. Cintia Crudeli executed the laboratory work; Paula Aulicino, Carlos Rocco, and Andrea Mangano actively participated in discussions and critical reading; Carlos Rocco performed the statistical analyses; Rosa Bologna is the infectious disease specialist and performed the follow-up of the pediatric cohort; and Luisa Sen directed the investigations. All authors contributed to the writing and revision of the manuscript. We acknowledge the NIH AIDS Research and Reference Reagent Program for providing the HIV-1_{LAI}/PBMC strain used in this work. This study was partially supported by the Argentine Awards from Agencia Nacional de Promoción Científica y Tecnológica (PICT 2004-25830) and from Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 6057).

Author Disclosure Statement

No competing financial interests exist.

References

1. Koot M, Keet IPM, Vos AHV, *et al.*: Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4⁺ cell depletion and progression to AIDS. *Ann Intern Med* 1993; 118:681–688.
2. Richman D and Bozzette SA: The impact of the syncytium-inducing phenotype of human immunodeficiency virus on disease progression. *J Infect Dis* 1994;169:968–974.
3. Bjordal A, Deng H, Jansson H, *et al.*: Coreceptor usage of primary human immunodeficiency virus type 1 isolates

- varies according to biological phenotype. *J Virol* 1997;71:7478–7487.
4. Dragic T, Litwin V, Allaway GP, *et al.*: HIV-1 entry into CD4⁺ cells is mediated by the chemokine receptor CC-CKR5. *Nature* 1996;381:6667–6673.
 5. Tersmette M, de Goede REY, Al BJM, *et al.*: Differential syncytium-inducing capacity of human immunodeficiency virus isolates: Frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *J Virol* 1988;62:2026–2032.
 6. Poveda E, Briz V, Quiñones-Mateu M, and Soriano V: HIV tropism: Diagnostic tools and implications for disease progression and treatment with entry inhibitors. *AIDS* 2006;20:1359–1367.
 7. Duliege AM, Messiah A, Blanche S, *et al.*: Natural history of human immunodeficiency virus type 1 infection in children: Prognostic value of laboratory tests on the bimodal progression of disease. *Pediatr Infect Dis J* 1992;11:630–635.
 8. European Collaborative Study 1991: Children born to women with HIV-1 infection: Natural history and risk of transmission. *Lancet* 1991;337:253–260.
 9. van't Wout AB, Kootstra NA, Mulder-Kampinga GA, *et al.*: Macrophage-tropic variants initiate human immunodeficiency virus type 1 infection after sexual, parenteral, and vertical transmission. *J Clin Invest* 1994;94:2060–2067.
 10. Salvatori F and Scarlatti G: HIV type 1 chemokine receptor usage in mother-to-child transmission. *AIDS Res Hum Retroviruses* 2001;17:925–935.
 11. Scarlatti G, Hodara V, Rossi P, *et al.*: Transmission of human immunodeficiency virus type 1 (HIV-1) from mother to child correlates with viral phenotype. *Virology* 1993;197:624–629.
 12. Church JD, Huang W, Mwatha A, *et al.*: HIV-1 tropism and survival in vertically infected Ugandan infants. *J Infect Dis* 2008;197:1382–1388.
 13. Kopka J, Batalla M, Mangano A, *et al.*: Relevance of viral phenotype in the early AIDS outcome of pediatric HIV-1 primary infection. *Pediatr Res* 2002;52:475–480.
 14. Douek DC, McFarland RD, Keiser PH, *et al.*: Changes in thymic function with age and during the treatment of HIV infection. *Nature* 1998;396:690–695.
 15. Gaulton G, Scobie J, and Rosenzweig M: HIV-1 and the thymus. *AIDS* 1997;11:403–414.
 16. Correa R and Munoz-Fernandez MA: Viral phenotype affects the thymic production of new T cells in HIV-1-infected children. *AIDS* 2001;15:1959–1963.
 17. Johnston E, Zijenah L, Mutetwa S, *et al.*: High frequency of syncytium-inducing and CXCR4-tropic viruses among human immunodeficiency virus type 1 subtype C-infected patients receiving antiretroviral treatment. *J Virol* 2003;77:7682–7688.
 18. Galán I, Jiménez JL, González-Rivera M, *et al.*: Virological phenotype switches under salvage therapy with lopinavir-ritonavir in heavily pretreated HIV-1 vertically infected children. *AIDS* 2004;18:247–255.
 19. D'Aquila RT, Sutton L, Savara A, Hughes MD, and Johnson VA: CCR5/Dccr5 heterozygosity: A selective pressure for the syncytium-inducing human immunodeficiency virus type 1 phenotype. *J Infect Dis* 1998;177:1549–1553.
 20. Ometto L, Bertorelle R, Mainardi M, *et al.*: Polymorphisms in the CCR5 promoter region influence disease progression in perinatally human immunodeficiency virus type 1-infected children. *J Infect Dis* 2001;183:814–818.
 21. Mosier DE, Picchio GR, Gulizia RJ, *et al.*: Highly potent RANTES analogues either prevent CCR5-using human immunodeficiency virus type 1 infection in vivo or rapidly select for CXCR4-using variants. *J Virol* 1999;73:3544–3550.
 22. Connor EM, Sperling RS, Gelber R, *et al.*: Pediatric AIDS Clinical Trials Group Protocol 076 Study Group 1994: Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. *N Engl J Med* 1994;331:1173–1180.
 23. Hollinger FB, Bremer JW, Myers LE, Gold JW, and McQuay L: Standardization of sensitive human immunodeficiency virus coculture procedures and establishment of a multicenter quality assurance program for the AIDS Clinical Trials Group. *J Clin Microbiol* 1992;30:1787–1794.
 24. Japour AJ, Fiscus SA, Arduino JM, *et al.*: Standardized microtiter assay for determination of syncytium-inducing phenotypes of clinical human immunodeficiency virus type 1 isolates. *J Clin Microbiol* 1994;32:2291–2294.
 25. Albert J and Fenyo EM: Simple, sensitive, and specific detection of human immunodeficiency virus type 1 in clinical specimens by polymerase chain reaction with nested primers. *J Clin Microbiol* 1990;28:1560–1564.
 26. Vödrös D and Fenyo EM: Quantitative evaluation of HIV and SIV co-receptor use with GHOST(3) cell assay. *Methods Mol Biol* 2001;304:333–342.
 27. Mangano A, Theiler G, Sala L, *et al.*: Distribution of CCR5-Delta 32 and CCR2-64I alleles in an Argentine Amerindian population. *Tissue Antigens* 2001;58:99–102.
 28. U.S. Centers for Disease Control and Prevention 1994: Classification system for human immunodeficiency virus (HIV) infection in children under 13 years of age. *Morb Mortal Wkly Rep* 1994;43:1–10.
 29. Coakley E, Reeves JD, Huang W, *et al.*: Comparison of human immunodeficiency virus type 1 tropism profiles in clinical samples by the Trofile and MT-2 assays. *Antimicrob Agents Chemother* 2009;53:4686–4693.
 30. Lee B, Sharron M, Montaner L, *et al.*: Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages. *Proc Natl Sci Acad USA* 1999;96:5215–5220.
 31. Antiretroviral therapy for children in Latin America and the Caribbean. PAHO 2008, Sec. 1:87.
 32. Resino S, Gurbindo D, Bellón Cano JM, *et al.*: Predictive markers of clinical outcome in vertically HIV-1-infected infants. A prospective longitudinal study. *Pediatr Res* 2000;47:509–515.
 33. Huang W, Eshleman SH, Toma J, *et al.*: Vertical transmission of X4-tropic and dual-tropic HIV-1 in five Ugandan mother-infant pairs. *AIDS* 2009;23:1903–1908.
 34. Brumme ZL, Goodrich J, Mayer HB, *et al.*: Molecular and clinical epidemiology of CXCR4-using HIV-1 in a large population of antiretroviral-naïve individuals. *J Infect Dis* 2005;192:466–474.
 35. de Mendoza C, Rodriguez C, García F, *et al.*: Prevalence of X4 tropic viruses in patients recently infected with HIV-1 and lack of association with transmission of drug resistance. *J Antimicrob Chemother* 2007;59:698–704.
 36. Frange P, Galimand J, Goujard C, *et al.*: High frequency of X4/DM-tropic viruses in PBMC samples from patients with primary HIV-1 subtype-B infection in 1996–2007: The French ANRS CO06 PRIMO Cohort Study. *J Antimicrob Chemother* 2009;64:135–141.

37. Lagaye S, Derrien M, Menu E, *et al.*: Cell-to-cell contact results in a selective translocation of maternal human immunodeficiency virus type 1 quasispecies across a trophoblastic barrier by both transcytosis and infection. *J Virol* 2001;75:4780–4791.
38. Naif HM, Li S, Alali M, *et al.*: CCR5 expression correlates with susceptibility of maturing monocytes to human immunodeficiency virus type 1 infection. *J Virol* 1998;72:830–836.
39. Fear WR, Kesson AM, Naif H, Lynch GW, and Cunningham AL: Differential tropism and chemokine receptor expression of human immunodeficiency virus type 1 in neonatal monocytes, monocyte-derived macrophages, and placental macrophages. *J Virol* 1998;72:1334–1344.
40. Kitchen SG and Zack JA: CXCR4 expression during lymphopoiesis: Implications for human immunodeficiency virus type 1 infection of the thymus. *J Virol* 1997;71:6928–6934.
41. Tuttle DL, Coberley CR, Xie X, *et al.*: Effects of human immunodeficiency virus type 1 infection on CCR5 and CXCR4 coreceptor expression on CD4 T lymphocyte subsets in infants and adolescents. *AIDS Res Hum Retroviruses* 2004;20:305–313.
42. Shalekoff S, Gray GE, and Tiemessen CT: Age-related changes in expression of CXCR4 and CCR5 on peripheral blood leukocytes from uninfected infants born to human immunodeficiency virus type 1-infected mothers. *Clin Diagn Lab Immunol* 2004;11:229–234.
43. Shalekoff S and Tiemessen CT: CCR5 delta32 heterozygosity is associated with an increase in CXCR4 cell surface expression. *AIDS Res Hum Retroviruses* 2003;19:531–533.
44. Casper C, Naver L, Clevestig P, *et al.*: Coreceptor change appears after immune deficiency is established in children infected with different HIV-1 subtypes. *AIDS Res Hum Retroviruses* 2002;18:343–352.
45. Spencer LT, Ogino MT, Dankner WM, and Spector SA: Clinical significance of human immunodeficiency virus type 1 phenotypes in infected children. *J Infect Dis* 1994;169:491–495.
46. Huang W, Eshleman SH, Toma J, *et al.*: Coreceptor tropism in human immunodeficiency virus type 1 subtype D: High prevalence of CXCR4 tropism and heterogeneous composition of viral populations. *J Virol* 2007;81:7885–7893.
47. Frange P, Chaix ML, Raymond S, *et al.*: Low frequency of CXCR4-using viruses in patients at the time of primary non-subtype-B HIV-1 infection. *J Clin Microbiol* 2010;48:3487–3491.
48. Cilliers T, Nhlapo J, Coetzer M, *et al.*: The CCR5 and CXCR4 coreceptors are both used by human immunodeficiency virus type 1 primary isolates from subtype C. *J Virol* 2003;77:4449–4456.
49. Zhang L, Carruthers CD, He T, *et al.*: HIV type 1 subtypes, coreceptor usage, and CCR5 polymorphism. *AIDS Res Hum Retroviruses* 1997;13:1357–1366.
50. Aulicino PC, Bello G, Guimaraes ML, *et al.*: Longitudinal analysis of HIV-1 BF1 recombinant strains in vertically infected children from Argentina reveals a decrease in CRF12_BF pol gene mosaic patterns and high diversity of BF unique recombinant forms. *Infect Genet Evol* 2011;11:349–357.
51. Low AJ, Dong W, Chan D, *et al.*: Current V3 genotyping algorithms are inadequate for predicting X4 co-receptor usage in clinical isolates. *AIDS* 2007;21:17–24.
52. Liesnard C, Delforge ML, Tchetcheff M, *et al.*: Importance of method in the determination of syncytium-inducing phenotype of human immunodeficiency virus type 1 clinical isolates. *J Virol Methods* 1997;64:137–145.
53. Whitcomb JM, Huang W, Fransen S, *et al.*: Development and characterization of a novel single-cycle recombinant-virus assay to determine human immunodeficiency virus type 1 coreceptor tropism. *Antimicrob Agents Chemother* 2007;51:566–575.
54. Baur A, Schwarz N, Ellinger S, *et al.*: Continuous clearance of HIV in a vertically infected child. *Lancet* 1989;2:1045.
55. Rübsamen-Waigmann H, Williams WR, Bretram U, and Von Briessen H: Reversal of HIV-phenotype to fulminant replication on macrophages in perinatal transmission. *Lancet* 1989;2:1155–1156.
56. Holtkamp N, Otteken A, Findhammer S, *et al.*: Unexpected coreceptor usage of primary human immunodeficiency virus type 1 isolates from viremic patients under highly active antiretroviral therapy. *J Infect Dis* 2000;181:513–521.
57. Gervaix A, Nicolas J, Portales P, *et al.*: Response to treatment and disease progression linked to CD4⁺ T cell surface CC chemokine receptor 5 density in human immunodeficiency virus type 1 vertical infection. *J Infect Dis* 2002;185:1055–1061.
58. Nicholson JK, Browning SW, Hengel RL, *et al.*: CCR5 and CXCR4 expression on memory and naive T cells in HIV-1 infection and response to highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2001;27:105–115.
59. Resino S, Resino R, Maria Bellón J, *et al.*: Clinical outcomes improve with highly active antiretroviral therapy in vertically HIV type-1-infected children. *Clin Infect Dis* 2006;43:243–252.
60. Muñoz-Fernández MA, Obregón E, Navarro J, *et al.*: Relationship of virologic, immunologic, and clinical parameters in infants with vertically acquired human immunodeficiency virus type 1 infection. *Pediatr Res* 1996;40:597–602.

Address correspondence to:

Luisa Sen
Hospital de Pediatría J. P. Garrahan
Laboratorio de Biología Celular y Retrovirus
Combate de los Pozos 1881
(1245), Ciudad Autónoma de Buenos Aires
Argentina

E-mail: lsen@garrahan.gov.ar; senluisa@gmail.com