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Biological Phenotype of Human Immunodeficiency Virus Type 1 Clones at Different Stages of Infection: Progression of Disease Is Associated with a Shift from Monocytotropic to T-Cell-Tropic Virus Populations

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The composition of human immunodeficiency virus type 1 (HIV-1) clonal populations at different stages of infection and in different compartments was analyzed. Biological HIV-1 clones were obtained by primary isolation from patient peripheral blood mononuclear cells under limiting dilution conditions, with either blood donor peripheral blood lymphocytes or monocyte-derived macrophages (MDM) as target cells, and the biological phenotype of the clones was analyzed. In asymptomatic individuals, low frequencies of HIV-1 clones were observed. These clones were non-syncytium inducing and preferentially monocytotropic. In individuals progressing to disease, a 100-fold increase in frequencies of productively HIV-1-infected cells was observed as a result of a selective expansion of nonmonocytotropic clones. In a person progressing to AIDS within 19 months after infection, only syncytium-inducing clones were detected, shifting from MDM-tropic to non-MDM-tropic over time. From his virus donor, a patient with wasting syndrome, only syncytium-inducing clones, mostly non-MDM-tropic, were recovered. Parallel clonal analysis of HIV-1 populations in cells present in bronchoalveolar lavage fluid and peripheral blood from an AIDS patient revealed a qualitatively and quantitatively more monocytotropic virus population in the lung compartment than in peripheral blood at the same time point. These findings indicate that monocytotropic HIV-1 clones, probably generated in the tissues, are responsible for the persistence of HIV-1 infection and that progression of HIV-1 infection is associated with a selective increase of T-cell-tropic, nonmonocytotropic HIV-1 variants in peripheral blood.

Human immunodeficiency virus type 1 (HIV-1) isolates differ with respect to syncytium-inducing (SI) capacity, replication rate, and cell tropism (1, 3, 8, 33). In stable asymptomatic individuals, only low-replicating isolates which do not induce syncytia in primary T cells and can not be transmitted to the H9 T-cell line are detected. H9 cell-line-tropic SI isolates emerge in the course of infection, presumably as a consequence of a progressively failing anti-HIV-1 immune response (23, 24, 38), and their appearance is strongly associated with subsequent rapid $CD4^+$ cell decline and clinical progression (34, 35).

Next to $CD4^+$ T cells, monocytes and macrophages are major targets for HIV-1 infection (4, 10, 11, 18, 26). Non-SI (NSI) isolates appear to be much more monocytotropic than SI isolates and can be detected at all stages of HIV-1 infection (22, 30). Since in most individuals early after infection only NSI variants are observed (34), we postulated that SI clones which may have been present in the inoculum and which lack monocytotropism can be suppressed by the competent anti-HIV-1 response mounted by the host early after infection, and that monocytotropic/NSI clones are essential for the persistence of HIV-1 infection in the early asymptomatic phase (30, 33, 34). Indeed, in tissue, macrophages are the predominant infected cells (10), and the isolation of monocytotropic HIV-1 variants from peripheral blood in which $CD4^+$ T cells are the major infected cell population (21, 28) suggests that peripheral blood T cells are infected by progeny from HIV-1-infected tissue macrophages.

To study whether monocytotropic HIV-1 variants are indeed most predominant in the asymptomatic phase of infection, dynamics of virus populations were studied at the clonal level in two asymptomatic and two symptomatic individuals. Furthermore, to analyze whether upon infection of a new individual T-cell tropic variants are eliminated and monocytotropic variants persist, an accidental donor-recipient pair was studied. Finally, the hypothesis that monocytotropic variants persist in tissue during all stages of infection was analyzed by parallel virus isolation from cells present in bronchoalveolar lavage fluid and peripheral blood from a patient with AIDS. The results of these studies confirm the importance of monocytotropic clones for the persistence of HIV-1 infection especially during the early asymptomatic phase and moreover demonstrate a qualitative shift to more T-cell-tropic clones along with a selective expansion of nonmonocytotropic clones with progression of HIV-1 infection.

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MATERIALS AND METHODS

Subjects. Primary virus isolation studies were performed on blood samples obtained from four men. Two subjects were asymptomatic (CDC II) and two were symptomatic (CDC IV C2) at the moment blood samples were taken. In addition, we studied (i) an individual accidentally infected with a minute amount of blood and (ii) his donor, a patient with wasting syndrome (CDC IV A) (19, 27). For comparison of HIV-1 clones from different tissue compartments, bronchoalveolar cells and peripheral blood were obtained from an AIDS patient with Kaposi sarcoma who was suspected to have pulmonary disease.

Preparation of patient cells. Heparinized blood (30 ml) was taken from each patient, and peripheral blood mononuclear cells (PBMC) were isolated by Percoll density gradient centrifugation. Bronchoalveolar lavage was performed with a total volume of 100 ml of physiologic salt solution. Cells were isolated by Percoll density gradient centrifugation and subsequently depleted for $CD8^+$ cells by use of magnetic beads (Dynal). Cells were stored until use in liquid nitrogen.

Monocyte isolation and culture. Monocytes, >95% pure, were prepared from PBMC of HIV-1-seronegative plasmapherese donors as described previously (9) and cultured in vitro to obtain monocyte-derived macrophages (MDM). In brief, PBMC were obtained from heparinized venous blood by isolation on a Percoll density gradient and then further enriched for monocytes by centrifugal elutriation. Monocytes were cultured for 5 days at a cell concentration of 10^6 monocytes per ml in endotoxin-free (17) Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% pooled human serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). Cells were plated in 24-well plastic tissue culture plates (Nunc) at 1 ml per well and were maintained at 37°C in a humidified atmosphere supplemented with 5% CO_2. Cultures were kept for 4 to 5 weeks, and medium was changed every week.

PBL. PBMC were suspended at 5×10^6 cells per ml in IMDM supplemented with 10% fetal calf serum (Hyclone), penicillin (100 U/ml), streptomycin (100 μg/ml), and phytohemagglutinin (PHA; 1 μg/ml). After 2 days, only the nonadherent cells were harvested and the PBL thus obtained, in which CD4 primary T cells are the major if not the only target cell type, were infected and resuspended at a concentration of 10^6 cells per ml in 8 ml of medium (without PHA) supplemented with partially purified interleukin-2. Medium was changed every 4 days, and fresh 2-day PHA-stimulated PBL from a seronegative blood donor were added to the cultures once a week.

H9, MT2, and Sup-T1 cell lines. H9, MT2, and Sup-T1, all $CD4^+$ T-lymphocyte cell lines were maintained at 5×10^5 cells per ml in IMDM with 10% newborn calf serum (GIBCO), penicillin (100 U/ml), and streptomycin (100 μg/ml).

Clonal isolation of HIV-1 on PBL or MDM. Virus was isolated from selected HIV-1-seropositive individuals by direct limiting dilution of patient cells (36). Patient cells were cocultivated with healthy donor PBL (10^4 patient cells with 10^5 PBL per well) in 96-well microtiter plates or with healthy donor MDM (4×10^4 patient cells per 10^6 MDM per well) in 24-well plates. Culture conditions were similar to those described above. Virus replication in the culture supernatant was detected by a p24 capture enzyme-linked immunosorbent assay (ELISA) (37). Positive cultures were expanded for biological phenotyping and stock preparation. The number of HIV-1-producing patient cells per well was estimated by the formula $u = -\ln F_o$ (Poisson distribution), in which F_o is the fraction of negative cultures per total number of cultures (20). Virus was assumed to be derived from a single infected cell if the fraction of positive cultures did not exceed 37% of the total number of cultures. Since the mean proviral copy number per infected cell in blood of HIV-1-infected individuals was estimated to be about 1 (28, 32), such cultures were considered to contain clonal HIV-1 isolates.

Virus detection. Virus production was assessed in a p24 antigen capture ELISA (37). Triton X-100-treated culture supernatant samples were added to microtiter plates (Nunc) coated with an anti-p24 antibody which was previously demonstrated to recognize all HIV-1 isolates (37). Bound p24 was detected with horseradish peroxidase-labeled rabbit anti-p24 immunoglobulin (7). A culture was considered positive when in at least two successive supernatants p24 levels were higher than two times the negative control. Three times a week, the PBL cultures were checked for syncytium formation as described previously (33). Each time, at least 10 microscope fields (magnification, ×100) were observed.

Preparation of virus stocks. Positive PBL cultures were expanded by coculture of the virus-producing cells with 8×10^6 PHA-stimulated PBL in 8 ml of medium. For the expansion of MDM cultures and for the determination of SI capacity, PHA-stimulated PBL were added to the cultures; after overnight cocultivation, PBL were harvested and further cultured with fresh PHA-stimulated PBL. PBL cultures were maintained as described above. Virus-containing supernatant was harvested after 10 days, stored at −80°C, and used for determination of cell line tropism. The 50% tissue culture infective dose ($TCID_{50}$) of clones isolated on MDM was determined in the original supernatant without intervention of a single PBL passage.

RESULTS

Shift from predominant monocytotropic to predominant T-cell-tropic HIV-1 variants during a progressive clinical course. To investigate the prevalence of HIV-1 clones and their biological properties at different stages of HIV-1 infection, we studied four seropositive individuals, two asymptomatic (CDC II) and two with clinical progression of HIV-1 infection (CDC IV C2). HIV-1 biological clones were obtained from patient PBMC by virus isolation under limiting dilution conditions with either PBL or MDM from the same blood donor as target cells. From the two asymptomatic individuals (patient 96 [P96] and P119) in two experiments, only low frequencies of HIV-1 clones (4 to $7/10^6$ $CD4^+$ T cells) were observed when PBL were used as target cells. A high proportion (50 to 53%) of the clones thus obtained could be cell free transmitted to MDM (Table 1). On the other hand, when clonal isolation was performed with MDM as target cells, much higher frequencies of clones were obtained (20 to $76/10^6$ $CD4^+$ T cells; Table 1). Thus, only a minority of the clones in these asymptomatic individuals were nonmonocytotropic, and the relative efficiency of recovery on PBL and MDM suggested a preference for monocytes over T cells for the majority of the clones.

A reverse phenomenon was observed in the two symptomatic individuals, P168 and P169. Compared with the asymptomatic individuals, an almost 200-fold-increased recovery rate of HIV clones on PBL was observed (900 to $1,300/10^6$ PBMC; Table 1). However, only few of these clones (1 to 5%) could be transmitted to MDM (Table 1). The yield of HIV-1 clones by primary isolation on MDM was comparable to the yield in asymptomatic subjects (16 to

TABLE 1. Analysis of shift in frequencies of monocytotropic and T-cell-tropic HIV-1 clones along with progression of disease

Patient	CDC stage	Frequency of infected cells/10^6 $CD4^+$ cells estimated by clonal isolation on:		Proportion (%) of MDM-tropic clones in PBL-tropic population[a]	Maximal proportion (%) of MDM-tropic clones[b]
		MDM	PBL		
P96	II	20	7	53	87
P119	II	76	4	50	98
P168	IV CII	40	890	3.4	5
P169	IV CII	16	1,316	1.3	1

[a] Estimated by cell-free transmission of MDM-tropic clones to MDM of four different donors.
[b] Calculated with the assumption that MDM-tropic clones in a PBL-tropic pool do not overlap with the clones primarily isolated on MDM.

$40/10^6$ $CD4^+$ T cells), but the contribution of these clones to the total pool was low, since in these individuals at least 95% of the clones were nonmonocytotropic. These results thus point to an increase in frequencies of HIV-1-infected cells as a result of a selective expansion of T-cell-tropic clones.

A proportion of the clones that were derived from both the asymptomatic and the symptomatic individuals and were primarily isolated on PBL demonstrated the capacity to replicate in MDM (Table 1). Since it is unclear to what extent this monocytotropic population overlaps with the population of clones primarily isolated on MDM, we calculated the population of clones with a preference for monocytes over T cells, assuming that the overlap was either complete or, alternatively, nonexistent. With the assumption of nonexistent overlap, the frequencies of preferential MDM-tropic clones ranged from 16.5 to $73.5/10^6$ $CD4^+$ cells in the asymptomatic individuals, compared with 0 to $10/10^6$ $CD4^+$ cells for the symptomatic individuals. In the case of complete overlap, the frequencies were within the same range for both the asymptomatic and the symptomatic subjects (16 to $76/10^6$ $CD4^+$ cells). Irrespective of the assumption of existent or nonexistent overlap, the maximal proportion of preferential MDM-tropic clones to the total population of clones was much higher in the asymptomatic individuals (87 to 97%) than in the symptomatic individuals (1 to 5%).

HIV-1 clones in asymptomatic individuals are predominantly monocytotropic and lack SI capacity and T-cell-line tropism. Monocytotropism of a majority of biological HIV-1 clones isolated from the four seropositive individuals on primary MDM could be confirmed by successful cell-free transmission to MDM (26 of 30 clones tested; Table 2). Subsequent passage to PHA-stimulated PBMC revealed that except for the clone derived from symptomatic individual P169, none of the monocytotropic biological HIV-1 clones induced syncytia or any other cytopathic effect. Furthermore, all of these MDM-tropic NSI HIV-1 clones lacked the capacity to replicate in permanent T-cell lines Sup-T1, H9, and MT-2 (Table 2).

Biological phenotyping of the HIV-1 clones isolated on PHA-stimulated PBL revealed that in individuals P96, P119, and P168, only NSI clones were present, whereas from individual P169, both NSI and SI clones were recovered. The biological HIV-1 clones were then compared for relative infectivity for MDM and primary T cells. Parallel determination of $TCID_{50}$ values on PBL and MDM revealed that most clones, irrespective of the target cell used for primary isolation, replicated equally well or better in PBL than in MDM (Table 3). However, the difference in $TCID_{50}$ observed by titration of the monocytotropic HIV-1 clones on MDM or PBL was significantly lower for clones recovered on MDM (1 to 2 logs) than for clones recovered on PBL (2 to 6 logs). Absolute $TCID_{50}$s on MDM were higher for MDM-derived clones, whereas $TCID_{50}$s on PBL were higher for the clones initially recovered on PBL.

Shift from predominantly T-cell-tropic to MDM-tropic HIV-1 populations upon transmission to a new individual. The accidental exposure of a seronegative individual to a minute amount of blood from a patient with end-stage HIV-1-related disease has been described (19, 27). To test whether upon transmission to a new individual SI T-cell-tropic variants are eliminated and monocytotropic variants persist, the presence of HIV-1 clones in both donor and recipient was

TABLE 2. Sequential passage of HIV-1 clones initially isolated on MDM or PBL

Patient	Cell type	No. clones tested[a]	Sequential cell-free passage to:			SI capacity in PBL	Sup-T1, H9, or MT2 tropism
			MDM	PBL	MDM		
P96	MDM	5	+	+	+	−	−
		2	−	−	−	−	−
	PBL	9	+	+	+	−	−
		21	−	NT[b]		−	
P119	MDM	10	+	+	+	−	−
		6	+	−	−	−	−
		1	+	−	+	−	−
		2	−	−	−	−	−
	PBL	5	+	+	+		−
		1	−	NT	−	−	
P168	MDM	3	+	+	+	−	−
	PBL	1	+	+	+	−	−
		13	−	NT		−	−
P169	MDM	1	+	+	+	+	+
	PBL	1	+	+	+	−	−
		12	−	NT		−	−
		1	+	+	+	+	+
		4	−	NT		+	+

[a] Clones are grouped according to the pattern of transmission observed.
[b] NT, not tested.

TABLE 3. Comparison of $TCID_{50}$ on MDM and PBL of primary HIV-1 clones isolated on PBL or MDM

Primary HIV-1 clones isolated on:	Patient	Clone	$TCID_{50}$/ml on:	
			MDM	PBL
MDM	P96	M.A5	$10^{3.9}$	$10^{3.4}$
		M.B3	$10^{4.1}$	$10^{4.6}$
		M.C4	$10^{4.1}$	$10^{3.9}$
	P119	M.A2	$10^{4.1}$	$10^{4.6}$
		M.A5	$10^{3.7}$	$10^{4.4}$
		M.A6	$10^{4.1}$	$10^{4.4}$
		M.C2	$10^{4.2}$	$10^{4.7}$
		M.C3	$10^{3.0}$	$10^{4.4}$
		M.D3	$10^{3.7}$	$10^{4.6}$
	P168	M.A2	$10^{4.1}$	$10^{4.7}$
	P169	M.A5	$10^{4.1}$	$10^{4.6}$
PBL	P96	P.A3	$10^{2.4}$	$10^{5.6}$
		P.B11	$10^{2.6}$	$10^{5.4}$
		P.E12	$<10^{1.9}$	$10^{4.8}$
	P119	P.A5	$10^{3.1}$	$10^{4.4}$
		P.B11	$10^{2.8}$	$10^{4.8}$
		P.D12	$10^{2.9}$	$10^{4.6}$
	P168	P.B9	$10^{2.1}$	$10^{5.8}$
		P.C10	$<10^{1.9}$	$10^{5.6}$
	P169	P.B8	$<10^{1.9}$	$10^{5.8}$
		P.D2	$<10^{1.9}$	$10^{5.7}$

analyzed. Clonal isolates were recovered from a PBMC sample obtained from the virus donor 9 months before the date of transmission. The properties of the HIV-1 clones obtained at that time point resembled those of the clones of the two symptomatic individuals described above (Table 4). No HIV-1 clones could be obtained by coculture with MDM. All clones obtained on PBL were SI, and only 3 of 12 clones recovered on PBL could be cell free transmitted to MDM of at least one of four donors tested (Table 4).

Zidovudine treatment of the recipient within 1 h after infection did not result in prevention of infection (19), and HIV-1 became detectable by virus isolation 30 days after exposure. The frequency of infected cells as determined by clonal isolation on PBL decreased following seroconversion (5, 6) and then increased again from day 105 of infection onward. At day 105, zidovudine was withdrawn because of side effects. A progressive decline of $CD4^+$ T-cell numbers was observed, and extrapulmonary tuberculosis developed at day 623 of infection.

Like the clones of the virus donor, all HIV-1 clones in the recipient were SI. However, in contrast to the virus donor, at day 41 of infection, HIV-1 clones could be rescued from PBMC of the recipient by coculture with MDM, and 75% of the clones recovered on PBL were tropic for MDM. In this rapidly progressing patient, already at day 105 a shift toward more T-cell-tropic clones had occurred, and most of the clones detected at that time were nonmonocytotropic (Table 4).

HIV-1 clones derived from bronchoalveolar lavage are highly monocytotropic. Direct detection of proviral DNA in PBMC subsets of infected individuals has demonstrated that monocytes in the peripheral blood seldom harbor the virus and that $CD4^+$ T cells are the major virus reservoir (28). In agreement with this finding, we observed that monocytes become susceptible to HIV-1 infection only during their differentiation into macrophages, probably only after their migration into the tissues (31). Also, the finding that all monocytotropic clones obtained in this study could replicate at least equally well in primary T cells (Table 3) is compatible with a T-cell origin of these clones. On the basis of these findings, we postulated that T cells in the peripheral blood harboring monocytotropic HIV-1 variants had been infected in the tissues by progeny of infected macrophages (31). To test this hypothesis, we compared the biological properties of HIV-1 clones derived from cells in bronchoalveolar lavage fluid and peripheral blood sampled simultaneously from an AIDS patient (Table 5).

The clones detected in the patient PBMC again were mostly nonmonocytotropic, as observed for the symptomatic individuals described above (Tables 1 and 4). In contrast, recovery of HIV-1 clones from cells in bronchoalveolar fluid was equally efficient with MDM and PBL, and 75% of the clones isolated on PBL could be transmitted to MDM. Comparison of infectivity for MDM and PBL showed that bronchoalveolar lavage-derived clones, in contrast to

TABLE 4. Comparison of HIV-1 clones in a donor-recipient pair

Donor or recipient	Time scale of blood sampling[a]	CD4 counts (10^9/l)	No. of clones isolated on:		Frequency of infected $CD4^+$ cells/10^6 estimated by clonal isolation on:		Minimal proportion (%) MDM-tropic clones of total[b]	SI capacity in PBL
			MDM	PBL	MDM	PBL		
Donor 704	−248	0.21	0	12	<1	100	25	+
Recipient P127	0[c]	ND[d]	ND	ND	ND	ND	ND	
	+13	0.9	ND	0	ND	<0.5	ND	+
	+30[e]	0.4	ND	48	ND	161	ND	+
	+41	0.65	9	41	30	119	80	+
	+58	0.72	ND	13	ND	86	ND	+
	+105[f]	0.29	ND	3	ND	10	ND	+
	+168	0.31	4	11	9	270	3	+
	+232	0.23	ND	9	ND	166	ND	+
	+301	0.13	ND	46	ND	687	ND	+

[a] Days after accidental transmission.
[b] Total frequency of MDM-tropic clones estimated by cell-free passage of primary HIV-1 clones isolated on PBL to MDM of four different blood donors together with clones primarily isolated on MDM.
[c] Start of zidovudine treatment within 45 min after virus exposure.
[d] ND, not determined.
[e] Seroconversion.
[f] End of zidovudine treatment.

TABLE 5. Comparison of frequency of monocytotropic HIV-1 clones in peripheral blood bronchoalveolar lavage

Origin of patient cells	Frequency of infected cells/10^6 patient cells by clonal isolation on[a]:		Proportion (%) MDM tropic clones of total	$TCID_{50}$/ml for MDM-tropic clones in[b]	
	MDM	PBL		MDM	PBL
Peripheral blood	8	37	34	$10^{3.1-3.3}$	$10^{4.8-5.2}$
Bronchoalveolar lavage	9	12	85	$10^{4.4-4.6}$	$10^{4.5-4.8}$

[a] After depletion for CD8 cells, the bronchoalveolar cell population consisted of 53% $CD14^+$ cells (macrophages) and 47% $CD3^+$ cells (T cells).
[b] Values represent ranges of five different clones.

MDM-tropic PBMC clones, preferentially infected monocytes. Indeed, the $TCID_{50}$ (MDM)/$TCID_{50}$ (PBL) ratio of the bronchoalveolar lavage-derived clones was higher than the ratio of any of the PBMC-derived clones examined in this study.

DISCUSSION

Previously we demonstrated the presence of monocytotropic NSI HIV-1 variants during all stages of infection (30). Here we analyzed the presence of monocytotropic HIV-1 variants in more detail and performed primary virus isolation after limiting dilution of patient PBMC. An inverse correlation between a high proportion of cells infected with monocytotropic HIV-1 variants and progression of disease was demonstrated. In two asymptomatic individuals (CDC II), monocytotropic HIV-1 clones accounted for >85% of the total pool of HIV-1 clones, whereas in two individuals classified as CDC IV C2, only <5% of the clones demonstrated the capacity to replicate in MDM. Although a more than 100-fold increase in the total frequency of HIV-1-infected cells was observed in symptomatic individuals compared with asymptomatic individuals, the frequency of cells infected with monocytotropic HIV-1 clones remained relatively constant. Apparently the increase in frequencies of HIV-1-infected cells is due to a selective expansion of non-MDM-tropic HIV-1 clones. It is unclear to what extent the population of clones primarily isolated on MDM overlaps with the MDM-tropic clones primarily isolated on PBL. Within the total population of monocytotropic clones, we calculated the population of clones with a preference for monocytes over T cells, assuming that the overlap was either complete or nonexistent. In both cases, the percentage of preferential monocytotropic clones was lower in the symptomatic individuals (0 to 1% and 4 to 5%) than compared in the asymptomatic individuals (85 to 87% and 96 to 98%), demonstrating a quantitative shift toward preferential T-cell-tropic clones also within the pool of monocytotropic clones. Thus, within the pool of MDM-tropic clones, a shift from preferential MDM tropism toward preferential T-cell tropism can be observed parallel to an increase in the frequency of infected cells with predominantly T-cell-tropic variants.

Increasing virus load with progression of disease has been described (14). Furthermore, in about half of the individuals progressing to AIDS, a conversion from the NSI to the SI phenotype can be observed (34). This finding together with the monocytotropism regained by some late-stage SI clones

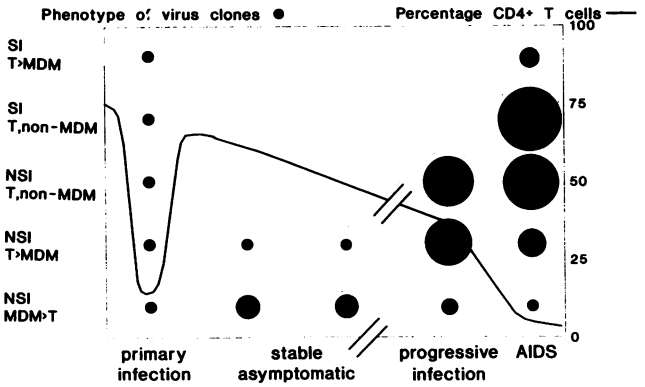

FIG. 1. Schematic representation of the prevalence of HIV-1 variants with different tropism at distinct stages of HIV-1 infection. The model is inferred from data presented in this report and elsewhere (5, 6, 12, 27, 36). Increases in virulence of the phenotype are given along the *y* axis. T>MDM, preferential T-cell tropic; T, non-MDM, T-cell tropic, nonmonocytotropic; MDM>T, preferential monocytotropic. Circle sizes approximately represent the relative frequency of a given virus phenotype.

(704, Table 4; P169, Tables 1 and 3) further supports the notion of increased virulence during the course of HIV-1 infection (3, 30).

Although SI isolates are detected in half of the asymptomatic individuals progressing to AIDS, in the early asymptomatic phase, only NSI variants are observed in over 95% of HIV-1-infected individuals (16, 27, 34). SI and NSI variants are both transmissible (27). In one case, we detected only NSI isolates in a recipient infected by a person with SI variants (27). Other observations have confirmed that SI variants may disappear early after seroconversion (2, 12). This finding suggests that upon transmission to a new individual, the SI variants, which may have been present in the inoculum, are eliminated in the recipient by the then relatively uncompromised immune system (15). The fact that low-expressing NSI variants generally appeared to be much more monocytotropic favors the hypothesis that by their capacity to replicate in monocytes, these variants may be adapted for survival in early HIV-1 infection, when the anti-HIV immune response is thought to be most effective (5, 6, 13, 38). The capacity of host immune surveillance to suppress highly virulent HIV-1 variants may be a crucial determinant for the length of the clinical latency period between moment of seroconversion and development of AIDS (27). Figure 1 schematically summarizes the prevalence of HIV-1 clones with distinct phenotypes in the course of infection, based on data from this and previous studies. The pattern probably is representative for persons in whom SI variants emerge during progression to AIDS, although obviously variation among individuals may exist.

The significance of monocytotropic HIV-1 variants during early infection was further demonstrated by primary isolation studies on a donor-recipient pair in which virus was accidentally transmitted (19). In the donor (P704), a terminal HIV-1-infected patient, a low frequency of monocytotropic HIV-1 variants was present, whereas in the recipient (P127), a high frequency of monocytotropic HIV-1 clones could be demonstrated. In contrast to what would be expected on the basis of our previous observations (30), all clones of this individual exhibited SI capacity. The apparent inability to eliminate SI variants in the new recipient might, in part, be explained from their monocytotropism. The immediate fast

decline of CD4 cell number (27) and the subsequent rapid progression toward AIDS of P127 (27) and the previously described P320, in whom monocytotropic SI variants were also present (12, 29), support this hypothesis. Furthermore, the observation that some end-stage patients with AIDS, such as P704, harbor monocytotropic SI HIV-1 variants might explain why individuals who become infected by patients with AIDS have a higher risk for rapid disease progression (25, 39). All peripheral blood-derived HIV-1 clones were more infectious in PBL, irrespective of the target cell used during primary isolation. Furthermore, none of the peripheral blood-derived monocytotropic clones demonstrated monocytotropism comparable to that of HIV-1 clone Ba-L, which was originally isolated from a lung biopsy (10). Here, comparable frequencies of cells infected with monocytotropic HIV-1 variants in either bronchoalveolar cells or peripheral blood were demonstrated. In bronchoalveolar cells, however, the monocytotropic HIV-1 variants were a major portion of the total pool of HIV-1 clones (75%). Furthermore, bronchoalveolar cell-derived HIV-1 clones were most infectious on MDM, whereas PBMC-derived clones replicated better in PBL.

These results demonstrate the importance of monocytotropic HIV-1 isolates for persistence during early infection and for dissemination of HIV-1 to compartments outside the peripheral blood. Macrophages in tissue compartments probably serve as a reservoir from which new virus variants are generated that cause slow decrease of immune surveillance, in time allowing the emergence of more virulent SI variants that are associated with disease progression. The high proportion of monocytotropic variants in early infection indicates that these and their host cells should be the target for new therapeutic strategies.

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