Table 1. HIV-1 RNA in plasma and cerebrospinal fluid (CSF), CD4+ cell count, neurological findings and treatment.

Date	October 13, 1995	March 2, 1996	August 21, 1996	December 9, 1996	January 8, 1997
CSF HIV-1 RNA (*)	ND	ND	1252653 (**)	ND	2895
Plasma HIV-1 RNA (*)	151000	74654	< 200	< 200	< 350
CD4 T cells	19 (2%)	3 (1%)	105 (15%)	177 (11%)	137 (11%)
CD4/CD8	0.02	0.01	0.26	0.27	0.28
Anti HIV therapy:	None	ZDV+3TC + INDINAVIR (Jun. 9, 1996)	D4T+3TC +SAQUINAVIR +RITONAVIR (Oct. 9, 1996)		
ZDV (1989–1993)					
ZDV+ddI (1994–1995)					
ZDV+3TC (1995)					
Neurologial findings:	HIV Polyneuropathy				
	HIV myelopathy				

(*), Copies/ml (Monitor HIV Roche). (**), Checked by 2 methods. ND, Not done; ZDV, zidovudine; D4T, stavudine; 3TC, lamivudine.

On 9 October 1996, quadruple therapy with stavudine (80 mg/daily) plus 3TC (300 mg/daily plus saquinavir (1200 mg/daily) plus ritonavir (1200 mg/daily) was started. After 2 months of this drug regimen we observed a decrease in painful paresthesias, reapparance of the patellar reflexes and a marked improvement in the clinical status and gait ataxia. HIV-1 RNA in the CSF decreased by about 2.65 $\log_{10}$ (Table 1). We assessed whether genotypic resistance to indinavir had occurred. None of the protease sequence differences between CSF virus from this patient and the HIV-1_{LAI} isolate corresponded to recognized indinavir resistance mutations.

To our knowledge this is the first observation of polyneuropathy and myelopathy related to HIV-1 infection worsening in a patient treated using ZDV – 3TC – indinavir with plasma HIV-1 RNA load decreasing below the cut-off value (<200 copies/ml). This observation suggests that the CNS can be a sanctuary for HIV. In the phase II trials with a triple combination, such as ZDV – 3TC – indinavir, approximately 90% of patients had plasma HIV-1 RNA concentrations sustained below the level of detection for more than 48 weeks. However, markers of HIV-1 replication in the peripheral blood may not adequately reflect the degree of CNS or peripheral nerve involvement [2,3].

This observation demonstrates that HIV-related neuroglogical complications can occur despite using the optimal combination of nucleosides and protease inhibitors to produce a major reduction of HIV-1 RNA in the plasma and without emergence of drug-resistant mutant in CSF and plasma. Therapeutic strategies against HIV may fail because of pharmocokinetic factors such as poor drug diffusion across the blood-brain barrier. A combination of two protease inhibitors (ritonavir and saquinavir) may be an appropriate strategy for HIV-related neurological disorders. The therapeutic goal of suppressing viral replication for years, if not decades, needs the same level of virus suppression in lymphoreticular tissues and CSF. CSF levels of antiretroviral drugs and CSF viral load should be included in the management of such patients.

G. Pialoux, S. Fournier, A. Moulignier, J.-D. Poveda*, F. Clavel* and B. Dupont, *Hôpital de l'Institut Pasteur, 209 rue de Vaugirard, and *Institut Pasteur, Paris, France.*

Date of receipt: 4 April 1997; accepted: 15 April 1997.

References

1. Janssen RS, Nwonyanwu OC, Selik RM, Stehr-Green JK: **Epidemiology of HIV encephalopathy in the United States.** *Neurology* 1992, **42**:1472–1476.
2. Douglas Pratt R, Nichols S, McKinney N, Kwok S, Dankner WM, Spector SA: **Viological markers of HIV-1 in cerebrospinal fluid in infected children.** *J Infect Dis* 1996, **174**:288–293.
3. Sei S, Stewart SK, Farley M, *et al.*: **Evaluation of HIV-1 RNA levels in cerebrospinal fluid and viral resistance to zidovudine in children with HIV encephalopthy.** *J Infect Dis* 1996, **174**:1200–1206.

Macrophage-tropism of HIV-1 isolates of different genetic subtypes

Monocytes play an important role in the pathogenesis of HIV-1 infection [1-5]. However, there is controversy as to whether all or only some HIV-1 isolates of particular biological phenotype have the ability to replicate in monocytes [4,5]. Part of the inconsistency could be explained by the presence of a large number of quasi-species in each isolate; moreover, poor purification of monocytes by plastic adherence only and the different concentrations of viral stocks used in various studies could also play a role. In addition, most studies so far have been performed on subtype B strains, and thus little is known on the capacity of HIV-1 strains belonging to other genetic subtypes to replicate in monocytes. We therefore investigated the *in vitro* susceptibility of highly purified monocytes, precultured under standardized conditions to productive infection with various HIV-1 strains, and derived clones representing different genetic subtypes with different phenotypic properties.

A panel of 20 primary bulk HIV-1 isolates and 63 biological clones [43 non-syncytia-inducing (NSI) and 20 syncytia-inducing (SI)] belonging to different genetic subtypes: three subtype A, two subtype B, one subtype C, five subtype D, one subtype E, three subtype F, two subtype H, and three group O viruses was selected and analysed. Monocytes were purified from peripheral blood mononucler cells (PBMC) of HIV-seronegative donors by E-rosetting followed by plastic adherence, thus eliminating all other contaminating cells. This procedure resulted in more than 99% monocytes, as assessed by flow cytometry analysis after staining with anti-CD3 and anti-CD4. Cells were maintained in culture for 7 days and were then infected with 1000 median tissue culture infective doses ($TCID_{50}$) of virus. Infection was monitored by weekly examining supernatant for production of p24 antigen using an in-house enzyme-linked immunosorbent.

The 20 primary HIV-1 isolates had several replicative patterns: six replicated to high titres; three produced moderate levels of p24 antigen; four replicated to low titres; five resulted in silent infection with detected virus replication only demonstrable following cocultivation with uninfected phytohaemagglutinin-stimulated PBMC; and for two isolates virus production could not be demonstrated at any time over a 6-week culture period and no proviral DNA could be detected by polymerase chain reaction. No consistent replication pattern was observed across the different genetic subtypes, and several patterns were observed with the same genetic subtype (data not shown). When the input inoculum of virus was increased to 10 000 $TCID_{50}$, all the 20 primary isolates replicated in cultured monocytes, a finding that suggests that susceptibility of monocytes of HIV-1 infection is inoculum dose-dependent.

We also examined the capacity of HIV-1 biological clones to replicate in blood monocytes. The number of biological clones that were able to replicate in monocytes at the multiplicity of infection of 1000 $TCID_{50}$ was higher among those with NSI phenotype (38 out of 43; 88.4%) than those with SI phenotype (13 out of 20; 65%). In addition, the majority of NSI clones yielded a faster and higher virus production, in contrast to SI clones, which replicated to low titres. These observations are in agreement with those of Schuitemaker *et al.* [4], but are not consistent with those of Valentin *et al.* [5], and may reflect the differences in methods used to isolate and maintaining monocytes in culture.

The V3 loop of these isolates was further analysed, a histidine residue at position 13 and the motif Tyr-Xaa-Thr-Xaa-Xaa-Xaa-Ile-Gly-Asp from positions 21 to 29 on the carboxy-terminal side of the V3 crown of primary HIV-1 isolates were correlated to monocyte tropism. A positively charged amino acid (arginine or lysine) was also found at positions 11 and/or 25 in all the SI clones analysed, but in none of the NSI clones, regardless of subtype. The non-charged amino-acid residues (serine, glycine) and negatively charged amino acids (aspartic acid or glutamic acid) were found at positions 25 in all NSI clones. Furthermore, the overall net charge of the V3-loop sequence in SI clones was higher than that in NSI clones (mean net charge, 6.25 and 3.7 for SI and NSI clones, respectively).

In conclusion, this study shows that HIV-1 strains belonging to different genetic subtypes exhibit similar capacity to replicate within cells of the monocyte-macrophage lineage. We also confirm and extend previous reports, showing that NSI biological clones replicate more efficiently in monocytes than SI clones and that V3 loop displays a lower net charge, due to the absence of positively charge amino acids in crucial positions. The question whether these charateristics relate to the recently observed preferential usage of co-receptors by various HIV-1 isolates and clones remains to be investigated.

Acknowledgment

The authors are indebted to Professor L. Muylle and his staff on the Red Cross Blood Transfusion Centre, Antwerp, Belgium for the regular supply of blood of healthy donors.

E. Karita, J. N. Nkengasong*, B. Willems, G. Vanham, K. Fransen, L. Heyndrickx, W. Janssens, P. Piot and G. van der Groen,
*Department of Microbiology, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium and *Virology Laboratory, Projet RETRO-CI, Abidjan, Côte d'Ivoire.*

Sponsorship: Supported in part by grant 3-301.96 from the National Fonds voor Wetenschappelijk Onderzoek, and grant 30226696 and GO134-97 from the Fonds Geneeskundig Wetenschappelijk Onderzoek, Brussels.

Date of receipt: 3 August 1996; revised: 11 April 1997; accepted: 18 April 1997.

References

1. Scuitemaker H: **Macrophage-tropic HIV-1 variants: initiators of infection and AIDS pathogenesis?** *J Leuk Biol* 1994, **56**:218–224.
2. Collman R, Hassan NF, Walker R, *et al.*: **Infection of monocyte-derived macrophages with human immunodeficiency virus type 1 (HIV-1). Monocyte-tropic and lymphocyte-tropic strains of HIV-1 show distinctive patterns of replication in a panel of cell types.** *J Exp Med* 1989, **170**:1149–1163.
3. von Briesen H, Andreesen R, Rübsamen-Waigmann H: **Systematic classification of HIV biological subtypes on lymphocytes and monocytes/macrophages.** *Virology* 1990, **178**:597–602.
4. Schuitemaker H, Koostra NA: DeGoede REY, De Wolf F, Miedema F, Tersmette M: **Monocytotropic human immunodeficiency virus 1 (HIV-1) variant detectable in all stages of HIV infection lack T-cell line tropism and syncytium-inducing ability in primary T-cell culture.** *J Virol* 1991, **65**:850–360.
5. Venentin A, Albert J, Fenyö EM, Asjö B: **Dual tropism for macrophages and lymphocytes is a common feature of primary human immunodeficiency virus type 1 and 2 isolates.** *J Virol* 1994, **68**:6684–6689.