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# Virologic, Immunologic, and Clinical Follow-Up of a Couple Infected by the Human Immunodeficiency Virus Type One, Group O

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The pathogenic course (virologic, immunologic, and clinical changes) of infection due to human immunodeficiency virus type one (HIV-1) group O viruses is unknown at present. To address this issue, serial HIV-1 isolates from a married couple (patients A and B) infected with a group O virus were analyzed to determine the temporal association between disease status and alterations in several parameters including plasma viral burden as measured by semiquantitative polymerase chain reaction, changes in CD4+ T cells, presence of neutralizing antibodies, and the ability to induce syncytia on the MT2 cells. For patient A who has been asymptomatic for at least 8 years, both the absence of syncytium-inducing (SI) variants and the presence of autologous and heterologous neutralizing antibodies correlated with a clinically healthier status. In contrast, a switch from NSI to SI variants was observed in patient B in 1990, followed by an expanded in vitro host range, increased viral burden, and a sharp decrease in CD4+ T cells 4 years later. Moreover, plasma obtained from this patient uniformly failed to neutralize both autologous and heterologous viruses. These observations in patient B correlated with a slightly unfavorable clinical status. Based on our preliminary results, it appears that the pathogenic course of infections due to group O viruses is similar to that reported previously for infections due to group M viruses. ***J. Med. Virol. 51:202–209, 1997.***



**KEY WORDS:** pathogenic course; HIV-1; viral burden

## INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) is caused by two types of viruses; the human immunodeficiency virus type one (HIV-1) and type two (HIV-2) [Barré-Sinoussi et al., 1993; Gallo et al., 1984; Clavel et al., 1986], HIV-1 being the more pathogenic type [Looney et al., 1990; Shpaer and Mullins, 1993]. These viruses are characterized by extensive alteration in their nucleotide sequence [Wong-Staal et al., 1995]. As a consequence, HIV-1 isolates have been subdivided into group M with at least eight genetic subtypes (A through H)[Myers et al., 1993] and group O [De Leys et al., 1990; Gurtler et al., 1994; Vanden Haesevelde et al., 1994]. HIV-1 group O isolates are highly divergent from group M isolates, differing by about 50–60% in the *env* gene [Gurtler et al., 1994; Vanden Haesevelde et al., 1994]. Hallmarks in a typical pathogenic course (virologic and immunologic changes) of individuals infected by HIV-1 group M viruses, particularly subtype B viruses, include development of neutralization escape variants and a change of biologic properties of the infecting virus from non-syncytium inducing (NSI) to syncytium-inducing (SI) phenotypes [Tersmette et al., 1989; Connor et al., 1993]. These changes correlate usually with CD4+ T-lymphocyte depletion, increased viral burden, and poor disease prognosis [Tersmette et al., 1989; Connor et al., 1993]. However, the pathogenic course of HIV-1 group O-infected individuals is unknown at present. Cross-sectional studies have, nevertheless, demonstrated some unique biologic and antigenic properties of HIV-1 group O viruses. Some variants fail to induce antibodies detectable by certain classical enzyme-linked immunosorbent assay [ELISAs; Loussert-Ajaka et al., 1994; Schable et al., 1994], and these viruses are resistant to TIBO nonnucleoside products[Descamps et al., 1995]. Moreover, sera from individuals infected by HIV-1 group O viruses lack consistently neutralizing antibodies to HIV-1 group M viruses [Nyambi et al., 1995], whereas 34% of individuals infected by group M viruses neutralize some group O viruses[Nkengasong et al., 1995].

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Insights into the pathogenic course of HIV-1 group O viruses may be gained by examining longitudinally the relationship between changes in viral burden, dynamics in viral phenotype, CD4+ T-cell numbers, and the clinical status of individuals infected by HIV-1 group O viruses. The outcome of such studies might be beneficial to the clinical management of individuals infected by these viruses. We report on virologic, immunologic, and clinical observations of sequential blood samples obtained from a couple infected by a group O variant for at least 8 years.

## CASE REPORTS

The couple infected by the HIV-1 group O virus described in this study was first identified in 1987 [De Leys et al., 1990]. The nucleotide sequence of the virus (a prototype of HIV-1 group O) has previously been fully analyzed [De Leys et al., 1990; Vanden Haesevelde et al., 1994], and represents the prototype of HIV-1 group O viruses.

### Patient A

HIV-1 ANT70 is a virus isolate obtained from one of the pair, hereinafter referred to as patient A, a 25-year-old female. She seroconverted in March 1987, was pregnant in 1988, but the child was HIV negative. At diagnosis she was classified as group A1 according to the Centers for Diseases Control (CDC) staging system [Centers for Disease Control, 1992]. She is still clinically asymptomatic.

### Patient B

The sexual partner of patient A, hereinafter referred to as patient B, is a 37-year-old male. The exact time when he became seropositive is unknown, however. His first serologic positive test for anti-HIV-1 antibody was in 1987. At diagnosis he presented with lymphadenopathy and was classified as group A1 of the CDC staging system [Centers for Disease Control, 1992]. Up till April 1995 both patients refused antiretroviral therapy, but since May 1995 patient A has been enrolled in a clinical trial with combination therapy (Zidovudine, Saquinavir, and Zalcitabine).

## MATERIALS AND METHODS

### Viral Phenotypic Analysis

Virus was isolated from both patients' peripheral blood mononuclear cells (PBMCs) by cocultivation with phytohemagglutinin (PHA)-stimulated PBMC (stimulated for 2 days) from seronegative blood donors as previously described [Castro et al., 1988]. HIV-1 strains obtained from initial culture were propagated by short-term (7–10 days) passage and then $10^4$ $TCID_{50}$ of each of the sequential viruses from the couple were used in infecting $2 \times 10^6$ cells of selected continuous cell lines (human T-cell lines: Molt-4 clone 8 provided by M. Hayami, CEMSS provided by P. Nara, and MT-2 cells provided by M. Koot). Syncytium formation of the HIV-1 isolates was determined using the MT-2 assay, essentially as described by Koot et al. [1992]. HIV-1 cultures were considered to exhibit syncytia if one multinucleated giant cell was observed per field for four examined fields of the light microscope (magnification of 100).

Monocytes and enriched CD4+ T cells were also used. Monocytes were first separated from PBMC by E-rosetting, followed by plastic adherence for 2 hr at 37°C. The adherent cells were then cultured in the absence of IL-2 for 7 days before inoculation. This procedure resulted in more than a 99% pure monocyte population, with less than 1% being contaminating T cells, as determined by FACSCAN analysis.

Cultures with virus supernatant positive for more than 3 weeks were considered to be positive. Cultures were scored as negative if they remained negative in the p24 antigen test after 28 days.

### Replicative Kinetics in Enriched CD4+ T Lymphocytes

Sequential isolates from the couple were evaluated for growth kinetics in enriched CD4+ T lymphocytes. Normal donor PBMCs were enriched for CD4+ T lymphocytes by first depleting monocytes by plastic adherence for 2 hr at 37°C and then removing CD8+ cells by immunomagnetic separation according to the manufacturer's instructions (Dynal, Inc., Oslo, Norway). The cells were subsequently stimulated for 48 hr with PHA and transferred to growth medium containing IL-2 before inoculation with $10^4$ $TCID_{50}$ of each virus. After 24 hr, the cells were washed three times and resuspended in a final volume of 2 ml of growth medium. Culture supernatant was analyzed for HIV-1 p24 antigen every 2 days for a period of 2 weeks.

### CD4+ and CD8+ T Cell Count

$CD4^+$ cells were enumerated by standard flow cytometry using a FACSCAN flow cytometer (Beckinson San Jose, CA) and commercially available monoclonal antibodies (Beckinson, San Jose).

### Semiquantitative RNA Polymerase Chain Reaction (PCR)

Semiquantitative RNA PCR was carried out as described previously [Van Kerckhoven et al., 1994]. In brief, RNA was extracted from 100 μl of plasma by the method of Boom et al [1990]. The extracted RNA was suspended in 50 μl of Tris-EDTA buffer to which 0.1 μl of RNA Guard (32 U/μl, Pharmacia) and 0.5 μl of dithiothreitol (0.1 M) had been added. Positive and negative control plasma samples were extracted simultaneously as controls.

Reverse transcription was performed by adding 9 μl of the 50 μl of RNA-buffer solution to 11 μl of reverse transcriptase reaction mixture (Moloney murine leukemia virus reverse transcriptase; Life Technologies) at 37°C for 1 hr with random primers, as described by De Beenhouwer et al. [1992]. Five of the 20 μl of cDNA product was amplified by nested PCR using primer pairs in the *pol* region as described previously [Fransen et al., 1994]. The amplified product was subjected to electrophoresis on agarose gel and was stained with

ethidium bromide, after which the DNA was visualized by transillumination at 254 nm. Negative and positive controls were included in each run.

Three 10-fold dilutions were analyzed for each of the cDNA products with an initial positive result in the nested PCR system. The number of cDNA copies was estimated from the last dilution in the series that was reactive by PCR, assuming that at least one copy of RNA generated one cDNA sequence. Data are expressed as HIV-1 RNA copies per 1 ml of plasma.

### Neutralization Assay

Virus stocks, titration, and the neutralization assay were all done on PHA-stimulated primary human PBMCs essentially as described previously [Nyambi et al., 1995; Nkengasong et al., 1994]. Tissue culture supernatants of PBMC infected with virus (50 $TCID_{50}$) and twofold serial dilutions of heat-inactivated (56°C for 30 min) serum were incubated for 1 hr at 37°C in a 5% $CO_2$ atmosphere before addition of $5 \times 10^4$, 3-day PHA-stimulated PBMCs for 2 hr. The cells were washed and incubated in RPMI 1640 IL-2 medium (GIBCO, Paisley, Scotland, UK) supplemented with 10% fetal calf serum (FCS), 0.03% L-glutamine (Janssen Chimica, Geel, Belgium), and antibiotics. Virus replication was assessed after 7 days by an in-house p24 antigen-capturing ELISA for determining the endpoint. Neutralizing titers were defined as the reciprocal of the highest serum dilution giving a complete reduction in absorbance value in the HIV-antigen assay compared with the negative controls. HIV-1 serum-neutralizing titers of 1:10 or greater were considered positive.

### V3-Loop Peptide ELISA

The sequential sera obtained from both patients were diluted in carbonate buffer (pH 9.6)(eight steps of twofold dilutions starting with a 1:200 dilution). The V3-loop peptide ELISA was carried out subsequently as described previously [Nkengasong et al., 1994], using a 26 amino acid long V3-loop peptide (CNDIQEMRIGPMAWYSMGIGGTAGNS) of the ANT70 virus.

## RESULTS

### Clinical Evolution of Patients A and B

In November 1995, patient A was still asymptomatic. The sexual partner (patient B) presented three episodes of furunculosis; the first in September 1991, two others in 1992, and an episode of intertrigo in January 1995. However, he never experienced prolonged fever or diarrhea. In November 1995, he was still in relatively good health and without weight loss.

### Relationship Between Changes in Viral RNA Burden and CD4+ T Cells

Changes in CD4+ T-cell counts and viral burden for sequential PBMC and plasma samples for the couple infected with HIV-1 group O virus are summarized in Figure 1. Patient A seroconverted in 1987. Analysis of viral burden by semiquantitative plasma PCR methods

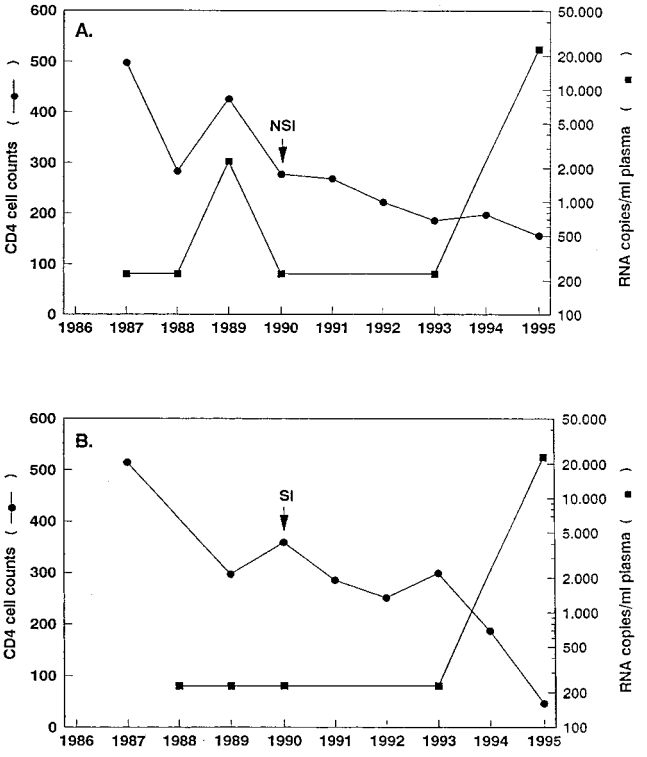


Fig. 1. Changes in CD4+ T-lymphocyte count (dark round symbols), PCR plasma viral load (dark square symbols) in patients **A** and **B** infected with group O virus. Viral load is expressed as number of RNA copies per milliliter. The arrow labeled SI in patient **B** indicates the year (1990) that there was a NSI-to-SI switch in phenotype.

demonstrated an initial low and constant numbers of viral copies (230 copies of RNA per milliliter of plasma), consistent with what has been reported for group M-infected persons [Van Kerckhoven et al., 1994; Burns and Desrosiers, 1992]. However, the level of viral burden increased by 10-fold in 1989 (2,300 copies per milliliter; Figure 1A). Interestingly, this patient appeared to have controlled her virus, as the level of viral burden fell shortly afterwards to the initial quantity of 230 copies of RNA per milliliter. Five years later, the viral burden rebounds 100-fold (23,000 copies of RNA per milliliter; fig. 1A). This sharp increase in viral burden was not followed by any change in the biologic phenotype, since the virus remained NSI. The abilities to suppress SI did not, however, prevent progressive decline in CD4+ in this patient (Fig. 1A). Her CD4+ counts decreased steadily from 500/$mm^3$ per year. It is noteworthy that CD8+ T-cell counts in this individual were remarkably high during the entire study period (average CD8+ T-cell counts of 1,406/$mm^3$).

In patient B, the level of viral burden appeared to vary inversely with CD4+ T-cell counts and changes in biologic phenotype (Fig. 1B). From 1988, the viral burden in this patient was very stable and also low as in the partner (230 copies per milliliter of plasma). However, by 1995 the number of HIV-1 RNA copies had

TABLE I. Phenotypic Analysis of Sequential HIV-1 Isolates From a Couple Infected With Group O Viruses

| | | Replicative capacity[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | T-cell lines | | | Primary cells | | | |
| | | CEMSS | MOLT-4 | MT2 | PBMC | Monocytes | | |
| Virus isolate | Date[b] | | | | | p24 antigen | PCR | Syncytium formation inMT-2 cells |
| Patient A | | | | | | | | |
| A1 | 3/87 | + | — | — | + | — | — | — |
| A2 | 3/88 | + | + | — | + | + | + | — |
| A3 | 9/90 | + | — | — | + | + | + | — |
| A4 | 1/93 | + | — | — | + | + | + | — |
| A5 | 1/95 | — | — | — | + | + | + | — |
| Patient B | | | | | | | | |
| B1 | 3/88 | — | — | — | + | + | + | — |
| B2 | 6/89 | + | — | — | + | + | + | — |
| B3 | 9/90 | + | + | + | + | + | + | + |
| B4 | 1/93 | + | + | + | + | + | + | + |
| B5 | 1/95 | + | + | + | + | + | + | + |

[a]Replication of the sequential HIV-1 group O isolates was determined by measuring HIV-1 p24 antigen in culture supernatants as described in Materials and Methods. The sensitivity of our in-house p24 antigen test for group O viruses is comparable to group M viruses.
[b]Date of sampling of the virus isolates.

increased enormously to 100-fold higher levels (23,000 copies of RNA per milliliter of plasma; Fig. 1B). This increased level of viral burden preceded a marked decline in CD4+ T cells from 200/mm$^3$ to 48/mm$^3$ (Fig. 1B). It is noteworthy that SI variants appeared 4 years prior to a sharp decrease in CD4+ T cells in 1993. The average CD8+ T-cell count was 522/mm$^3$, threefold lower than in the partner.

Recently, two studies have implicated the rapid turnover of HIV-1 in CD4+ T-cell depletion [Ho et al., 1995; Wei et al., 1995]. The rate of CD4+ T-cell depletion could thus provide an indication of the virulence of a given HIV variant. The average rate of decline of the patients' CD4+ T-cell counts was 64 and 74 counts per year for patients A and B, respectively, an observation consistent with published findings on group M viruses [Phillips et al., 1991].

### Biologic Phenotype on T and Primary Cells

To determine whether changes in viral burden and CD4+ T-cell counts stated above correlated with the appearance of distinct HIV-1 phenotypes as observed with HIV-1 group M viruses, successive isolates from the two patients were assessed for their biologic properties in vitro. Normal donor PBMCs, monocytes, and some T-cell lines were infected with $10^4$ $TCID_{50}$ of each viral isolate. All the isolates obtained from patient A were NSI and had a restricted host range, replicating only in the CEMSS cell line (Table I).

In contrast, in patient B, distinct differences were seen in the ability of sequential isolates to infect the T-cell lines. HIV-1 isolates (B1 and B2) obtained earlier (1988 and 1989) from patient B were uniformly NSI and incapable of establishing productive infection in MT-2 cells. Conversely, isolates (B3, B4, and B5) obtained subsequently (1990 to 1995) readily infected this cell line, and were all strongly syncytium including (Table I). Thus, a switch from NSI to SI occurred in 1990 in patient B (Fig. 1B), concurrent with a broadening in the cell tropism of the ensuing isolates, which infected other T cells and induced strong syncytia in both the CEMSS and MOLT cell lines (data not shown).

Some studies have reported infection of MT-2 cells without syncytium formation [Forte et al., 1994]. None of the isolates in this study infected MT-2 cells in the absence of syncytium formation, as PCR performed using pol primers [Fransen et al., 1994] on all the MT-2 cultures was only positive in those cultures that were p24 antigen positive and SI.

### Replicative Kinetics on CD4+ T Lymphocytes

In patient A whose viruses did not exhibit an NSI-to-SI switch in biologic phenotype, replicative kinetics of the initial isolate derived in 1987 showed remarkably high levels of HIV-1 replication compared with the most recently obtained isolates (Fig. 2A), suggesting that NSI isolates are not necessarily slow/low variants. Moreover, it replicated to levels well higher than CA13, a SI variant belonging to *env* subtype H (group M) [Nkengasong et al., 1994]. The replicative kinetics on enriched CD4+ T cells for isolates obtained from patient B, before and after the NSI-to-SI phenotypic switch observed in MT-2 cells were variable. Some SI isolates (B3) grew to relatively high titers whereas others (B4 and B5) did not (Fig. 2B).

### Neutralization of Sequential Autologous and Heterologous HIV-1 Group O Isolates

In patient A, a striking pattern of autologous neutralization of sequential sera and isolates was observed. Sera (a1, a2, and a3) obtained initially could not neutralize the patients' corresponding autologous isolates (Table II). However, a few years later, antibodies that neutralized the escaped viruses were observed in sera a4 and a5. Interestingly, the most recently collected serum (a5) neutralized its autologous isolate and all the other isolates with relatively high titers (Table

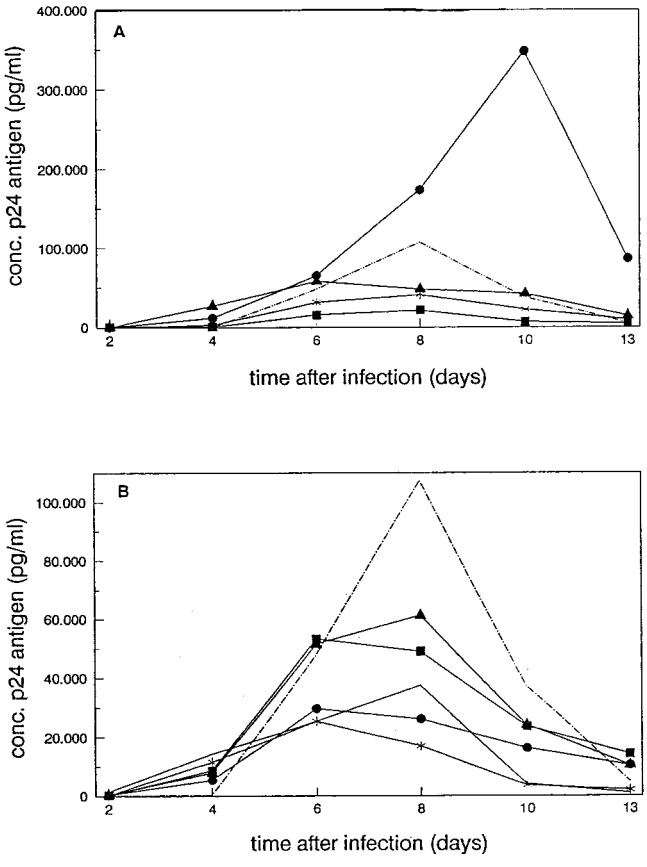


Fig. 2. Growth kinetics in CD4+ cultures of sequential isolates obtained from both patients **A** and **B** were evaluated as described in Materials and Methods. p24 antigen (expressed as picograms per milliliter) were monitored every 2 days for 13 days. A1 ●, A3 ■, A4 ▲, A5 *, and B1 ●, B2 ■, B3 ▲, B4 *, B5 (straight line without any symbols) represent sequential viral isolates obtained from patients **A** and **B,** respectively. CA 13 (–·–·) conc., concentration.

II). All the sequential viruses of this patient were of the NSI phenotype.

Interestingly, in patient B the sequential sera rarely neutralized their consecutive isolates (Table II) which was unusual, and thus, required further investigation. Experiments were therefore conducted to address two likely reasons: that this patient had a somewhat diminished humoral response, and that patient B's sequential isolates constituted a set of intrinsic neutralization-resistant viruses. Our findings were mixed; comparable anti-V3 antibody titers were measured in sera of both patients, with titers ranging from 1:2,500 to 1:6,000. Sera obtained from patient B were next used to neutralize sequential isolates derived from patient A and other HIV-1 group O viruses (CA9 and VI686). The sequential sera failed consistently to neutralize patient A's viruses and the other group O viruses (heterologous viruses) (Table II). There was no evidence of gross intrinsic resistance of patient B's viruses. Sequential isolates (B2, B3, B4, and B5) obtained from this patient were neutralized by sera (a3, a4, and a5) collected later from patient A, with titers ranging from 1:20 to 1:160 (Table II).

## DISCUSSION

That HIV-1 group O viruses differ significantly in their nucleotide sequence composition from HIV-1 group M viruses raises important questions regarding the possible existence of well-defined group-specific differences, such as pathogenesis. This contention is supported by several previous observations. Indeed, Shpaer and Mullins [1993] have shown that the pathogenesis of primate retroviruses correlates highly with the ratio of nonsynonymous (pn) to synonymous (ps) nucleotide changes, especially in the envelope gene, which determines most of the biologic and antigenic properties. This ratio (pn/ps) is significantly greater in group O than in group M viruses [Loussert-Ajaka et al., 1995]. The Pn/ps ratio is also significantly greater in the HIV-1 group M than in the HIV-2 group, and has been shown to correlate well with pathogenesis. In particular, contrary to HIV-1, HIV-2 isolates have a lower pathogenicity and transmission, CD4+ cell killing [Looney et al., 1990], lower viral burdens [Simon et al., 1993], and a lesser rate of progression to advanced HIV-related disease [Marlink et al., 1994]. Furthermore, individuals infected by HIV-2, unlike HIV-1, develop autologous neutralizing antibodies suggested to be markers for less virulence [Björling et al., 1993; Tamalet et al., 1995]. We therefore reasoned that individuals infected with group O viruses may have a more aggressive pathogenic course than those infected with group M viruses.

However, in many ways, the pathogenic course observed in this study is similar to that of group M viruses, albeit its limitation to two individuals. Patient A is still asymptomatic, despite being seropositive for at least 8 years. This is consistent with the 8–10 year median clinical latency seen in individuals with group M infections. This favorable clinical picture appears to correlate well with several viral and host parameters in our study. First, this individual developed "catch up" high-titered autologous neutralizing antibodies later on in the infection. This is, however, not a unique finding for group O infections. Autologous virus-neutralizing antibodies prevail in HIV-2-infected individuals [Björling et al., 1993; Tamalet et al., 1995], and have been suggested to be markers for less virulence. Though rare, autologous-neutralizing antibodies have been reported in individuals infected with HIV-1 group M viruses, appearing at different rates and to a very different extent [Arendrup et al., 1992; Wrin et al., 1994].

Second, it is noteworthy that no NSI-to-SI switch was observed in this patient, even though the rate of CD4+ T-cell decrease was remarkably similar (except in 1994–1995) to patient B whose virus switched from the NSI to the SI phenotype. These findings, together with the presence of autologous-neutralizing antibodies and remarkable high levels of CD8+ T cells, correlated with a favorable course of the disease in this patient. Studies correlating emergence of SI variants, neutralization escapes viruses, and disease stage are

TABLE II. Neutralizing Antibodies in Sequential Serum Samples of Patient A (ANT 70) and B (Partner) Against Sequential Autologous and Heterologous Isolates

| Virus isolates | Date | Reciprocal of neutralizing antibody titer in patient A | | | | |
|---|---|---|---|---|---|---|
| | | a1 | a2 | a3 | a4 | a5 |
| A1 | 3/87[a] | <10 | 40 | 320 | 640 | 160 |
| A2 | 3/88 | <10 | <10 | 40 | 40 | 40 |
| A3 | 9/90 | <10 | <10 | <10 | 160 | 80 |
| A4 | 1/93 | <10 | <10 | 80 | 160 | 160 |
| A5 | 1/95 | <10 | 320 | 320 | 160 | 1280 |
| B2 | 6/89 | <10 | <10 | 80 | 160 | 10 |
| B3 | 9/90 | <10 | <10 | 40 | 160 | 40 |
| B4 | 9/93 | <10 | <10 | 20 | 320 | 20 |
| B5 | 1/95 | <10 | <10 | 40 | 160 | 20 |
| | | Reciprocal of neutralizing antibody titer in patient B | | | | |
| | | b1 | b2 | b3 | b4 | b5 |
| B1 | 3/88 | 20 | <10 | <10 | 20 | <10 |
| B2 | 6/89 | <10 | <10 | 10 | <10 | <10 |
| B3 | 9/90 | <10 | <10 | <10 | <10 | <10 |
| B4 | 1/93 | <10 | <10 | <10 | <10 | <10 |
| B5 | 1/95 | <10 | <10 | <10 | <10 | <10 |
| A3 | 9/90 | ND[b] | <10 | <10 | <10 | <10 |
| A4 | 1/93 | ND | <10 | <10 | <10 | <10 |
| A5 | 1/95 | ND | <10 | <10 | <10 | <10 |
| VI686[c] | | ND | <10 | <10 | <10 | <10 |
| CA9[c] | | ND | <10 | <10 | <10 | <10 |

[a]Date of sampling of virus and plasma.
[b] ND, Not done.
[c] Heterologous HIV-1 group O viruses.

thus far rare. Our observations are very consistent with those of Tsang et al. [1994], who first reported the correlation between the presence of autologous-neutralizing antibodies, lack of emerging variants with SI phenotype, and a healthier clinical state.

The virologic, immunologic, and clinical picture of patient B had several parallel aspects to individuals infected with subtype B variants within group M viruses. For instance, in HIV-1 group M infection, isolates from patients with late-stage disease are mostly of rapid/high phenotype, and frequently acquire an expanded host cell range in vitro when compared with slow/low isolates from asymptomatic individuals [Tersmette et al., 1989; Connor et al., 1993]. In sequential determination of host cell range in vitro, we found a clear difference for isolates obtained early and those obtained later from patient B, who had a poorer prognosis, to establish productive infection in cell lines. Only sequential HIV-1 group O isolates obtained later in this patient infected the MT-2 cell line and were SI. In addition, the appearance of the SI phenotype preceded by a few years the rapid onset of CD4+ T-cell decline concurrent with a significant increase in the levels of HIV-1 RNA viral burden.

It was noteworthy that in patient B a consistent lack of neutralizing antibodies was observed to both autologous and heterologous isolates throughout the 8-year study period. Clearly, this lack of neutralizing antibodies was not the result of a diminished humoral immune response; rather, it is more likely to reflect the inability of a specific subset of humoral immune cells to produce neutralizing antibodies. This assessment is supported by the observation that sequential sera from patient B had anti-V3-loop antibody titers comparable to those of the sexual partner. Moreover, the viruses from patient B did not represent a set of resistant isolates to neutralization, as they were neutralizable by sera from patient A.

Because of the possible role played by monocytes in HIV-1 pathogenesis, infectivity of group M viruses to these cells has been extensively studied with conflicting observations [Schuitemaker et al., 1992; Foire et al., 1994; Valentin et al., 1994]. With rare exceptions, all the viruses obtained from the two patients infected monocytes as demonstrated by p24 antigen and PCR positivity. Our findings, though preliminary, suggest that nucleotide sequence diversity may not affect monocyte infectivity. The study of in vitro and in vivo features of distinct viruses is important because subtype-specific biologic properties might exist, with relevance to pathogenesis. However, we [Nkegasong et al., 1995] and others [Leitner et al., 1995] have not demonstrated any in vitro biologic differences in comparing some HIV-1 subtypes within group M. A recent report in Thailand has, however, suggested that subtype E variants are preferentially transmitted compared with subtype B viruses [Kunanusont et al., 1995].

In summary, this is the first demonstration of a direct association between viral burden and CD4+ T-cell numbers, changes in viral biologic phenotype, presence of neutralizing antibodies, and clinical status in a detailed longitudinal study of individuals infected by group O variants. Our preliminary findings suggest

that the pathogenic course of individuals infected with HIV-1 group O and M may be similar. However, larger prospective studies are needed to provide more insights into the natural course of group O infections. This will, however, be difficult due to the infrequency of occurrence of these variants; only about 3–5% of seropositive individuals from the epicenter of these viruses are attributed to group O viruses [Nkengasong et al., 1993].

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