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The Relationship between HIV Type 1 Disease Progression and V3 Serotype in a Rural Ugandan Cohort

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ABSTRACT

Antigenic properties of the V3 region are reflected by HIV-1 serotypes. These may represent biological properties of the virus. We serotyped HIV-1 in 142 serum samples from participants in a rural Uganda cohort who seroconverted between August 1991 and December 2001. Clinical progression was assessed using Cox proportional hazards and Kaplan–Meier methods. Of 112 (79%) samples successfully serotyped, 36% were serotype A, 17% serotype B, 18% serotype C, and 29% serotype D. Median follow-up time, age at enrollment, and first CD4 count were similar in each serotype group. Clinical progression was faster for serotype D than other serotypes to AIDS or death, death, and CD4 count <200 cells/mm^3 (all $p < 0.05$). HIV-1 V3 serotypes are associated with variations in the pathogenicity of HIV-1 and should be taken into account when studying the biological relevance of HIV-1 diversity.

INTRODUCTION

One characteristic of HIV-1 disease is the variation in the progression rates among different patients. These variations have been mostly attributed to either viral or host factors.[1] Most studies reported, however, are from patients in the West infected with HIV-1 subtype B, with sparse information from Africa where there are diverse HIV-1 subtypes and highest HIV-1 prevalence.

So far nine different HIV-1 group M genetic subtypes have been identified (A–D, F–H, J, and K).[2] These genetic subtypes have been identified by a comparison of nucleotide sequences in different parts of the HIV-1 genome, especially the *env* and *gag* regions. In addition, there are recombinant viruses that belong to two or more distinct genetic subtypes in different regions of the genome.[2]

The role of genetic subtypes in disease progression, vaccine development, and transmission has been of great research interest.[3–10] Though currently the broad view is that genetic subtypes may not necessarily have major biological significance, in our two previous studies[5,6] we have reported that genetic subtype D might be associated with faster disease progression compared with A. Kanki *et al.*[11] have also shown that genetic subtype A women were at eight times less risk of progression to AIDS than women with other subtypes.

One other way of characterizing HIV-1 is the use of V3 serotyping for epidemiological studies and identifying new genetic subtypes.[12–17] This is a system of grouping HIV-1 on the basis of similarities of antibody binding patterns to V3 synthetic peptides. One advantage of this technique is that it is simpler and less expensive than genetic subtyping, but more importantly serotypes reflect antigenic properties of the V3 region of HIV-1, which in turn may represent biological properties of the virus. The V3 region is highly antigenic and important in the pathogenesis of HIV-1 and host immunity.[1] Serological assays measuring functional binding antibody to whole viruses or epitopes have been used widely in epidemiological studies of numerous viral infections.[18] Though in some infected individuals the serotypes derived match the genetic subtype of the infecting virus within the V3 envelope, this is not always the case. For example, while in Thailand and Brazil, V3 serotyping correlates very well with the circulating *env* genetic subtypes,[12–14,19] in East Africa cross-reactivity is very common[12,14,20] and V3 peptides have to be complimented with gp41 peptides to better discriminate the serotypes[17] (C.-P. Pau, personal communication). We set out to investigate the relationship between the rate

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of HIV-1 disease progression and V3 serotypes in a population of 142 individuals in a rural Ugandan cohort.

MATERIALS AND METHODS

Subjects and sample collection

The Medical Research Council (MRC) Programme on AIDS in Uganda established a Natural History Cohort (NHC) of HIV-1-infected individuals and HIV-1-uninfected controls living in rural southwestern Uganda in 1990.[21] Participants are recruited from a large population study where the dynamics of HIV-1 infection are examined by annual census and questionnaire and serological surveys.[22] The NHC includes seroincident cases with a known date of seroconversion, estimated as the midpoint between the dates of the last seronegative and first seropostive test results, and seroprevalent cases, identified as already HIV-1 positive at the start of the cohort in 1990.[23] All participants in this cohort are invited to attend the study clinic every 3 months to provide a detailed medical history and undergo a physical examination and laboratory investigation. The participants also attend the clinic for investigation and treatment at other times when ill. All participants were encouraged to visit HIV voluntary counselling and testing services that are provided in the study village.

Clinical follow-up

A WHO clinical stage[24] was ascribed to each routine clinic visit by a computer program using clinical and laboratory data from that visit, and relevant previous visits. WHO stage 4 is equivalent to AIDS. CD4 lymphocyte counts are measured every 6 months using FACSCOUNT (Becton Dickinson International, Belgium). Compliance at routine visits has been good with >90% of the patients seen at least once a year. Deaths of cohort participants are reported by local home visitors, and even if the person has moved and not been seen for several years, information about death comes from family members residing in the study area. For this study, the period of observation was from August 1991 to December 2001. During this period, 142 participants with known dates of seroconversion were followed.

Blood samples were drawn at each visit (every 3 months) and serum was stored at −20°C. In addition, most of these individuals had their HIV-1 genetic subtypes determined using DNA sequencing or heteroduplex mobility assay.[5,25]

V3 peptide serology

V3 peptides (MRC AIDS Reagent Project, appendix 1) derived from subtype A, B, C, and D were coated on ELISA plates at different concentrations (1, 0.1, and 0.01 μg/ml). The coating buffer was 20 mM sodium carbonate (BDH), pH 9.6. The plates were washed after 48 hr incubation at room temperature. The plates were then blocked using phosphate-buffered saline (PBS) (Sigma Chemicals Company, Germany), pH 7.4, containing 5% dry skimmed milk (Marvel, Premier Beverages, Stafford, U.K) and 10% heat-inactivated newborn calf serum (Sigma). Serum samples were incubated at a dilution of 1:100 in blocking buffer with 0.1% Tween 20. After incubation at 37°C for 1.5 hr the plates were washed, then antibody bound to the peptide was detected by antihuman IgG peroxidase conjugate (Sigma) and *ortho*-phenylenediamine dihydrochloride (OPD) substrate (Sigma) and the optical density (OD) read at 492 nm.

Twenty HIV-1-uninfected control samples were included on each plate to calculate a cutoff (CO) value. The CO value was determined as the mean of the negative controls plus 3 standard deviations. The OD/CO value was determined. The serotype of a sample was the peptide giving the highest OD/CO ratio at the lowest peptide concentration[12,17] approximating to antibody avidity.

Statistical methods

Characteristics of patients were compared between persons infected with V3 serotypes A, B, C, and D. Age was categorized as <40 and ≥40 years. This was done to create meaningful categories describing the study population as "young" and "old." CD4 counts were categorized into <200, 200–499, and ≥500 cell/mm^3. These characteristics were compared between V3 serotypes A, B, C, and D using χ^2 or Fisher's exact test, while continuous characteristics were compared using the Kruskal–Wallis test of association.

Three different indicators of clinical progression were estimated from date of seroconversion to date of death, date when entering stage 4 or date of death, and date of the first CD4 lymphocyte count <200 cells/mm^3. Survival times between individual serotypes were compared using Kaplan–Meier survival techniques. Hazard ratios (HR) and the 95% confidence intervals were estimated using Cox regression, adjusting for age category and sex. Statistical significance of HR was assessed using the likelihood ratio test.

All data analysis was performed using STATA Version 6 (STATA Corporation, College Station, TX).

The study is based on the NHC, which received ethical approval from the Uganda Virus Research Institute and the National Council for Science and Technology. All subjects gave informed written consent.

RESULTS

Serotyping

During the study period, 160 HIV-1 seroincident participants were recruited into the NHC. Only 142 samples from these cases were available for testing, 15 participants were not bled, and another 3 samples were not available. One hundred and twelve (79%) of these were successfully serotyped as A, B, C, or D using a panel of HIV-1 V3 region peptides. The distribution of serotypes was 40 (36%) serotype A, 19 (17%) serotype B, 20 (18%) serotype C, and 33 (29%) serotype D. Repeatability was assessed by retesting 23 samples blindly by the same person. Twenty (87%) gave the same serotype results. There were 30 samples (21%) that were untypable, 5 of them had cross-reactivity while 25 had very weak reactivity to the panel of peptides used. In this study, 43 serotyped and 15 untypable samples had genetic subtype data based on the V3 *env* DNA sequence available as shown in Table 1. For the typable samples, the concordance between serotype and genetic subtype was 70%, taking A/C and B/D as the same serotypes.

Table 1. Correlation between DNA Genetic Subtypes and V3 Serotypes

	DNA sequence genetic subtype				
	A	*B*	*C*	*D*	*Total*
V3 serotype					
A	10	1	1	4	16
B	3	0	0	1	4
C	2	0	2	5	9
D	0	1	0	13	14
Untypable	8			7	15
Total	23	2	3	30	58

Population characteristics

The distribution of age at seroconversion ($p = 0.99$), sex ($p = 0.79$), and first CD4 count after seroconversion ($p = 0.28$) did not differ significantly between those successfully serotyped and those who were untypable (data not shown). The median time from last HIV-1 negative test to first HIV-1 positive test for those serotyped was 1.02 years [interquartile range (IQR), 0.91–2.03]. The distribution of age at estimated date of seroconversion, sex, and first CD4 count is shown by serotype in Table 2. There were no significant differences between serotypes. Comparison of age at enrollment and follow-up time by HIV-1 V3 serotype did not differ significantly between serotypes, $p = 0.49$ and $p = 0.76$, respectively (data not shown).

Median time from seroconversion to first CD4 lymphocyte count overall was 1.5 years (IQR, 0.8–2.4 years), and from seroconversion to last CD4 lymphocyte count was 5.0 years (IQR, 2.5–5.5 years). There were no significant differences by serotype.

Clinical progression of incident cases infected with HIV-1 V3 serotypes A, B, C, and D

The Kaplan–Meier survival curves from estimated date of seroconversion to the end points AIDS or death, death, and CD4 count <200 cells/mm^3 are shown by serotype in Figure 1. The estimated median survival times for each end point in months from seroconversion are presented by serotype in Table 3. There were significant differences in median survival time for individual serotypes for all three end points. In a subsidiary analysis, using Cox's proportional hazards model, serotype D had a faster median progression to all end points than individual non-D serotypes (Table 4). A further analysis comparing serotype D with non-D serotypes combined showed serotype D had a significantly faster median progression to all end points [hazard ratios (95% CI) 2.3 (1.3–4.0) for AIDS or death, 2.4 (1.3–4.4) for death, and 2.1 (1.2–3.7) for CD4 count <200 cells/mm^3.

DISCUSSION

Our study has demonstrated that HIV-1 V3 serotypes might have biological significance. Clinical progression in our participants with serotype D was significantly faster than for other serotypes for all end points. Our study was based on participants of a clinical cohort with well-documented and short seroconversion intervals and regular clinical and laboratory follow-up. There is one other study that has reported that progression to AIDS was significantly faster in patients infected with serotype B than serotype B-Br.[26]

We do not think this observation in our study is solely a reflection of genetic subtype differences since we have reported

Table 2. Description and Comparison of Characteristics of Subjects by HIV-1 V3 Serotype

	HIV-1 V3 serotype				
	A (n = *40*)	*B* (n = *19*)	*C* (n = *20*)	*D* (n = *33*)	p *value*
Estimated age at seroconversion (years)					
<40	30 (75%)	17 (89%)	15 (75%)	27 (82%)	0.57
≥40	10 (25%)	2 (11%)	5 (25%)	6 (18%)	
Median (IQR)	28 (22–39)	24 (19–29)	27 (23–38)	31 (22–37)	0.44
Sex					
Male	22 (55%)	9 (47%)	10 (50%)	20 (61%)	0.79
First CD4 count (cells/mm^3)					
<200	1 (3%)	0 (0%)	0 (0%)	4 (12%)	0.12
200–499	12 (30%)	3 (16%)	5 (25%)	4 (12%)	
≥500	27 (68%)	16 (84%)	15 (75%)	25 (76%)	
Median (IQR)	693 (468–964)	785 (581–938)	66 (510–873) 7	692 (500–1035)	0.75
Median time (in years) between date of seroconversion and first CD4 count (IQR)	1.9 (1.1–2.8)	1.4 (0.9–2.2)	1.4 (0.7–2.3)	1.1 (0.6–2.2)	0.31
Median years of follow-up time (IQR)	5.2 (3.3–8.2)	5.2 (4.5–8.5)	5.7 (4.2–8.2)	6.4 (3.8–8.8)	0.76

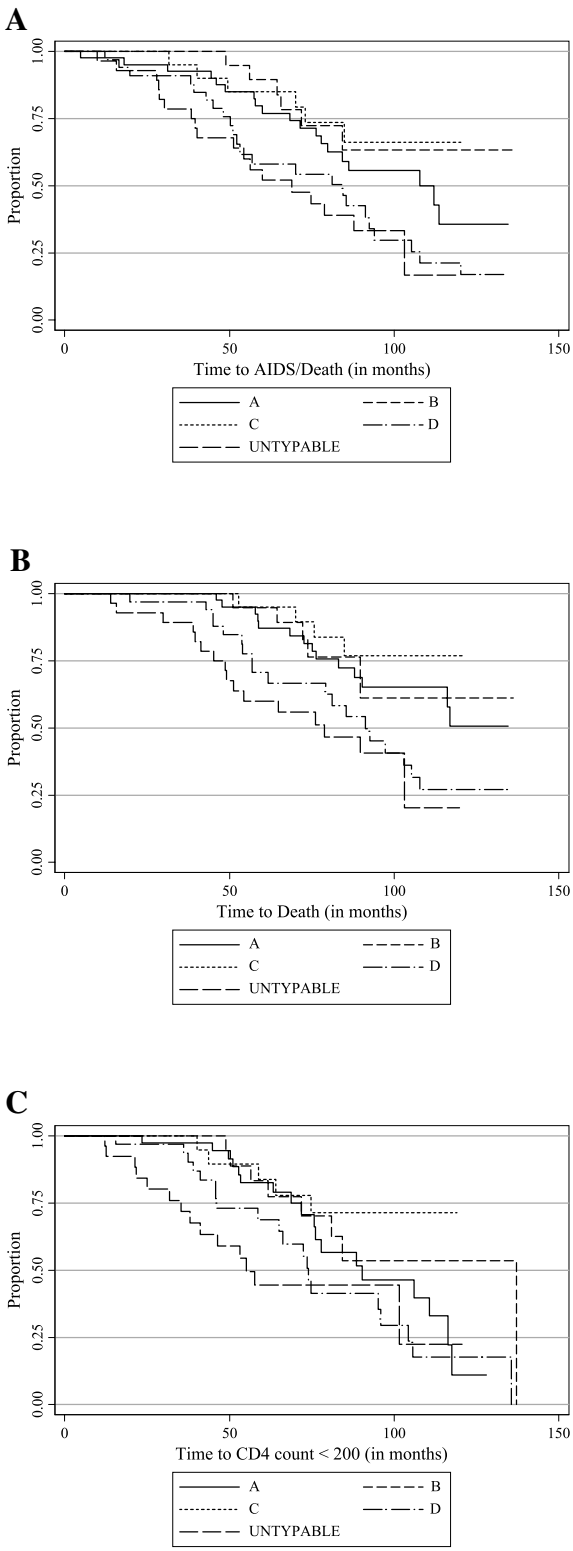

FIG. 1. Survival curves by serotype for the different end points (analysis time in months). Clinical progression was significantly faster for untypable individuals than for individuals successfully serotyped: (**A**) median survival to death or AIDS (69 vs. 105 months; $p = 0.014$, (**B**) to death (79 vs. 117 months; $p = 0.003$), and (**C**) CD4 count <200 (55 vs. 95 months; $p = 0.031$).

before in another cohort only a 76% correlation between V3 serotypes and genetic subtypes[17] considering A/C and B/D genetic subtypes as the same serotypes. In this cohort, we also had 70% correlation between serotypes and envelope genetic subtypes, with cross-reactivity of A/C and to a lesser extent B/D peptides as reported before,[12,17] however, there were many genetic subtype D samples (39%) that cross-reacted with A/C peptides as well. The study group in this paper extends the follow-up and expands the study group in one of our previous reports on subtype and disease progression.[5] The more significant results in the present study, therefore, may be a reflection of a better correlation between biological properties and serotypes than genetic subtypes.

Interestingly, the 30 untypable samples showed faster disease progression compared to the typable samples. The reason for the weak reactivity to the peptides was not advanced disease stage since the median time since seroconversion and the median first CD4 counts at enrollment for these and the typable ones were similar (data not shown). This faster progression in those with weak reactivity could imply that failure to mount an immune response to the V3 region might have a detrimental clinical outcome or these viruses possess other unique antigenic properties. This observation might also be a weakness of the study, in that apart from serotypes there are other properties such as antibody levels that may influence our observations. The other possible reason for the nonreactivity of some samples might be the choice of the pepides used. We used the single consensus peptides that we have previously evaluated as useful serotyping reagents and recognized by most samples from Uganda.[14] We might have had reactivity for these samples if other peptides were used and this would be worth exploring further. There are also other reports where other peptides including homologous peptides have failed to serotype.[27,28] That less than 80% of samples could be serotyped potentially introduces some bias into this study. However, available information on individuals suggests that there was no particular association between successful serotyping and any individual genetic subtype.

Genetic subtypes have failed to consistently show biological differences;[4,29] this observation in our study indicates that serotypes might represent antigenic properties and serotypes should be considered when looking for biological properties of virus. However, since V3 peptides are linear and most antigenic properties of gp120 arise from the three-dimensional structure, it is difficult to explain our observations. One study has reported that only a small proportion of cross-reactive anti-gp120 antibodies in HIV^+ sera is capable of reacting with linear epitopes,[30] but another study using beta-turn analysis indicated that divergent V3 loop apical residues showed a good correlation of probable beta-turn occurrence with strong peptide seroreactivity,[31] implying that serotypes may indeed represent antigenic properties of the V3 loop.

Although V3 serotyping does not correlate very well with genetic subtype among certain populations, such as Africa, this relatively simple method may still be useful in describing the diversity of strains in a given area. If serotypes indeed represent the antigenic properties of the V3 loop, then this may have implications for understanding the biological relevance of HIV-1 diversity, not only with disease progression but also transmission and vaccine development and HIV-1 molecular epidemiology therefore should be complemented with

Table 3. Survival Analysis of Subjects by HIV-1 V3 Serotype from Estimated Date of Seroconversion

End point	*HIV-1 V3 serotype*	*Number of events*	*Number of samples*	*Hazard rate (/1000 pyo)*	*Median survival time (IQR) (in months)*	p *value*
AIDS or death	Untypable	18	29	10.3	69 (40–103)	0.005
	A	19	40	5.8	112 (68–[a])	
	B	6	19	3.8	>136 (72–[a])	
	C	6	20	3.6	>121 (73–[a])	
	D	23	33	9.9	84 (50–108)	
Death	Untypable	16	29	8.6	79 (49–103)	0.002
	A	14	40	3.9	>135 (83–[a])	
	B	5	19	3.1	>136 (90–[a])	
	C	4	20	2.2	>121 ([a]–[a])	
	D	19	33	7.4	91 (57–[a])	
CD4 count <200 cells/mm^3	Untypable	14	27	9.9	55 (35–102)	0.0143
	A	18	40	6.5	90 (72–116)	
	B	8	19	5.3	137 (72–137)	
	C	5	20	3.1	>119 (75–[a])	
	D	20	33	9.3	74 (46–104)	

[a]Insufficient events to estimate median survival time and/or interquartile range.

serotypes. Finally in our cohort, serotypes might have to be considered when evaluating factors affecting HIV-1 disease progression. Our observations, however, need further confirmation in other cohorts with more than one serotype.

APPENDIX

Peptides provided by the MRC AIDS Reagent project, made by peptide and protein research. Washington Singer Labs, University of Exeter, Exeter, U.K. Peptide A = KSVHIGPGQAFYAT; peptide B = RQRTHIGPGQALYTT; peptide C = KSIRIGPGQTRYAT; and peptide D = KSIHIGPGRAFYTT.

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Table 4. Cox Regression Survival Analysis Comparing Serotype D with A, B, and C Unadjusted and Adjusted for Age at Seroconversion and Sex

End point	*Serotype*	*Unadjusted hazard ratio*	p *value*	*95% confidence interval*	*Adjusted hazard ratio*	p *value*	*95% confidence interval*
AIDS or death	Untypable	1.14	0.681	0.6–2.1	1.16	0.637	0.6–2.2
	A	0.55	0.055	0.3–1.0	0.51	0.034	0.3–0.9
	B	0.37	0.029	0.1–0.9	0.43	0.067	0.2–1.1
	C	0.33	0.017	0.1–0.8	0.29	0.008	0.1–0.7
	D	1					
Death	Untypable	1.32	0.42	0.7–2.6	1.59	0.182	0.8–3.1
	A	0.48	0.036	0.2–1.0	0.48	0.038	0.2–1.0
	B	0.42	0.082	0.2–1.1	0.53	0.216	0.2–1.4
	C	0.27	0.016	0.1–0.8	0.24	0.010	0.1–0.7
	D	1					
CD4 count <200 cells/mm^3	Untypable	1.19	0.622	0.6–2.4	1.26	0.514	0.6–2.5
	A	0.66	0.212	0.3–1.3	0.62	0.143	0.3–1.2
	B	0.44	0.059	0.2–1.0	0.49	0.107	0.2–1.2
	C	0.29	0.013	0.1–0.8	0.30	0.016	0.1–0.8
	D	1					

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