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Low-cytopathic infectious clone of human immunodeficiency virus type I (HIV-I)

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Single genotypic variants of HIV-I, contained in a parental cytopathic HIV-I isolate, were isolated by molecular cloning and propagated in susceptible cells. Two such HIV-I clones, designated N1T-E and N1T-A, exhibited similar restriction endonuclease maps but strikingly different biological activities. Infection of T lymphocytes or monocytes by clone N1T-E was characterized by slow kinetics and lack of significant cytopathic effects, but high reverse transcriptase activity levels in culture supernatants of chronically-infected cells. Clone N1T-A, like the parental HIV-I isolate, exhibited fast kinetics of infection in T cells and monocytes and strong cytopathicity in these cells. Full characterization of the low-cytopathic virus in comparison to the structurally similar cytopathic clone may facilitate the elucidation of the molecular basis of HIV cytopathogenicity.

HIV-I variant; Cytopathogenicity; Restriction map; Biological activity

1. INTRODUCTION

Human immunodeficiency virus type-I, the primary etiologic agent of the acquired immunodeficiency syndrome [1], is believed to cause immunodeficiency in part by virtue of its cytopathic interaction with CD4-positive human T lymphocytes [2]. Recent reports have suggested that HIV-I isolates from individuals with mild or no symptoms of disease may be less cytopathogenic than viruses from AIDS patients [3–5]. Attenuated cytotoxicity has been demonstrated for two different isolates of HIV-II [6,7], an HIV-I-related but different retrovirus associated with a form of immunodeficiency predominantly found in West Africa [8]. Here we describe an infectious molecular clone of HIV-I with low cytopathogenicity in human T cells and monocytes. The clone was isolated from a HIV-I-producer cell line CEM/HIV/N1T [9], which has previously been shown to contain multiple HIV-I genotypes [10] and to produce a typical cytopathic virus [9,10]. Our results suggest that low-cytopathic and highly-cytopathic HIV-I clones can coexist in a single virus producing culture and, presumably, in one individual.

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Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; IF, indirect immunofluorescence staining; RT, reverse transcriptase

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2. MATERIALS AND METHODS

2.1. *Cells and viruses*

CD4-positive T cell lines used in these studies were obtained as follows: CEM cells [11] were the gift from L. Montagnier; the HIV-lysis resistant subclone of CEM, CR10, was established in this laboratory [9]; HUT-102 B2 cells [12] were received from W. Green, and the human monocytoid cell line, U-937 [13], was obtained from the American Type Culture Collection (ATCC, Rockville, MD). Suspension cell lines were maintained in RPMI-1640 medium (Gibco Laboratories, Grand Island,

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NY) supplemented with 10% fetal bovine serum (FBS) and antibiotics. The N1T isolate of HIV [9,10] was propagated in CEM or CR10 cells; the single genotypic N1T virus clones (see below) were maintained in CEM cells. For infection studies, virus isolates were concentrated 100-fold, tested for RT activity and infectivity, and stored at −80°C until use.

2.2. *Molecular cloning of HIV proviruses*

Standard techniques described by Maniatis et al. [14] were employed. Briefly, genomic DNA from CEM/HIV/N1T cells [9,10] was prepared by a cesium chloride/guanidinium isothiocyanate method. Cellular DNA was digested to completion with the restriction enzyme *Xba*I, size fractionated on 10–40% sucrose density gradient, and cloned into the λJ1 phage vector (gift of J.I. Mullins). Recombinant clones were identified using ^{32}P-labeled HIV-I DNA probe N1G-G [10], purified by plaque-hybridization. Proviral sequences were then subcloned in pUC18, propagated and analyzed for biological activity by transfection and rescue of infectious progeny virus.

2.3. *Infectivity studies*

For suspension cell cultures, 1×10^6 cells were adsorbed with the appropriate HIV clone (400000 cpm RT activity/1×10^6 cells/1 ml) for 1 h at 37°C. Cells were then washed once with phosphate-buffered saline (pH 7.4), resuspended in culture medium and cultured under standard conditions. At the designated time intervals, 1 ml aliquots were removed to determine total cell count, viability, RT activity in culture supernatant and HIV antigen expression in cells by the IF method.

2.4. *Analytical assays, chemicals, and radiochemicals*

HIV infection was monitored as described previously [9] by detecting the presence of HIV antigens in infected cells using the IF method and/or by measuring the level of RT in culture supernatants. Enzymes for recombinant DNA experiments were purchased from New England Biolabs, Inc. (Beverly, MA) and Bethesda Research Laboratories (Gaithersburg, MD) and FITC-conjugated anti-human IgG from Tago Immunochemicals (Burlingame, CA). All radiochemicals were obtained from New England Nuclear Corp. (Boston, MA), and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

3. RESULTS AND DISCUSSION

3.1. *Isolation of genetically polymorphic infectious molecular clones of HIV-I present in a single virus isolate*

HIV-I/N1T was isolated from peripheral blood lymphocytes of a patient with lymphadenopathy as described [15]. Virus was propagated in CEM cells or in chronically-infected CR-10 cells [9] and characterized as a T cell-tropic, highly-cytopathic retrovirus closely resembling prototypic HIV-I strains [9,10,15]. As shown for several other HIV-I and HIV-II isolates [6,16,17], HIV-I/N1T virus was found to contain multiple virus genotypes [10]. A recombinant DNA phage library prepared from HIV-I/N1T-producer cells yielded 21 HIT-I positive clones, 6 of which were analyzed and five of them were found to contain a full-length proviral DNA. Five of the 6 clones could be distinguished by restriction endonuclease mapping, and all but one were biologically active. Thus, like the previously described HIV-I/IIIB and HIV-2$_{ST}$ isolates [6,17], HIV-I/N1T virus contains multiple different genotypes that are capable of producing infectious progeny virus. Fig.1 shows restriction endonuclease maps of two such N1T virus clones, N1T-A and N1T-E, described in this communication. Similarity in restriction patterns, in particular in the 5′-half of N1T-A and N1T-E genomes, suggests close genetic relationship between these variants, as previously reported for other multiple proviral DNA clones from individual patients [16].

3.2. *Presence of low-cytopathic HIV-I/N1T clone among highly cytopathic variants in the same producer cell line*

Comparative functional analysis of the different N1T provirus DNA clones revealed that 4 of 5 clones, termed N1T-A, -B, -C and -D, produced progeny viruses whose biological properties were similar to the parental isolate; namely, they exhibited rapid kinetics of infection and induced massive cell fusion and cytolysis. An example of such a study using N1T-A clone and CEM cells is shown in fig.2A. In contrast, an equivalent dose of the 5th clone, termed N1T-E, did not induce cell aggregation or any substantial cell lysis and fusion (fig.2B). An infection kinetics study, using T lymphoid cell lines CEM and HUT-102, and a monocytic cell line U-937, confirmed that N1T-E exhibited significantly attenuated cytopathicity as compared to N1T-A (table 1). The limited cytopathicity of N1T-E was not due to low infectivity of this variant, because all 3 cell lines were comparably susceptible to infection with both variant viruses, as shown by the virus-specific IF assay and detection of RT in culture supernatants. However, only E virus-infected cells survived primary infection and remained chronically infected (table 1). Noncytopathic infection of HUT-102 cells with N1T-E virus is particularly striking, because HTLV-I-carrying T cell lines are known to be exquisitely susceptible to HIV-induced lysis and fusion [18].

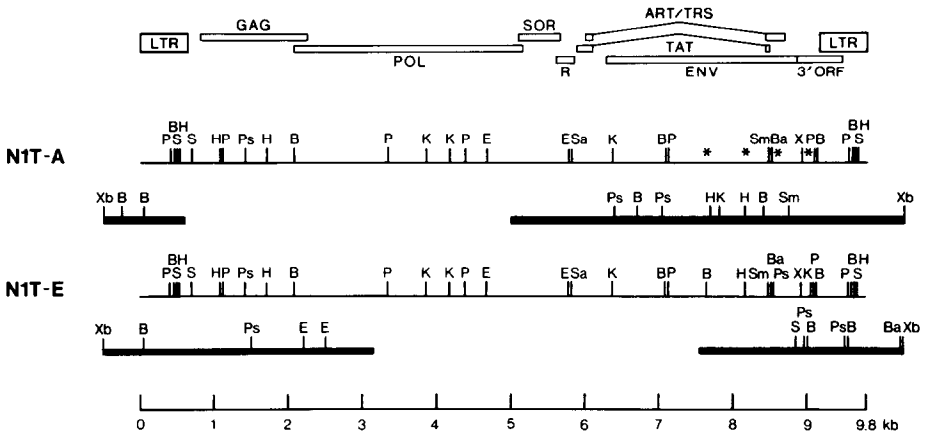

Fig.1. Restriction endonuclease map of cytopathic and low-cytopathic molecular clones of HIV-I. All clones contain a full-length HIV-I provirus (upper thin line) flanked by cellular sequences (lower bold line). Asterisks denote sites absent in N1T-A virus. Restriction enzyme cleavage sites: Ba, *Bam*HI; B, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; Ps, *Pst*I; P, *Pvu*II; S, *Sac*I; Sa, *Sal*I; Sm, *Sma*I; Xb, *Xba*I; X, *Xho*I.

One of the advantageous consequences of the attenuated cytopathic property of the N1T-E virus is that cells that normally succumb to the cytopathic effects of HIV, such as the 3 cell targets listed in table 1, can become chronic carriers and producers of the virus (table 1, fig.3). Hence, potential effects of chronic HIV infection on cellular gene expression [19] and cell function [20] can be evaluated without the background of cytolysis. It is also of interest that CEM cells chronically infected with N1T-E replicated virus to much higher levels (as judged by the supernatant RT levels) than the respective cultures of N1T-A or parental N1T virus (fig.3). This result suggests that massive virus replication is not, by itself, sufficient for cell killing by HIV.

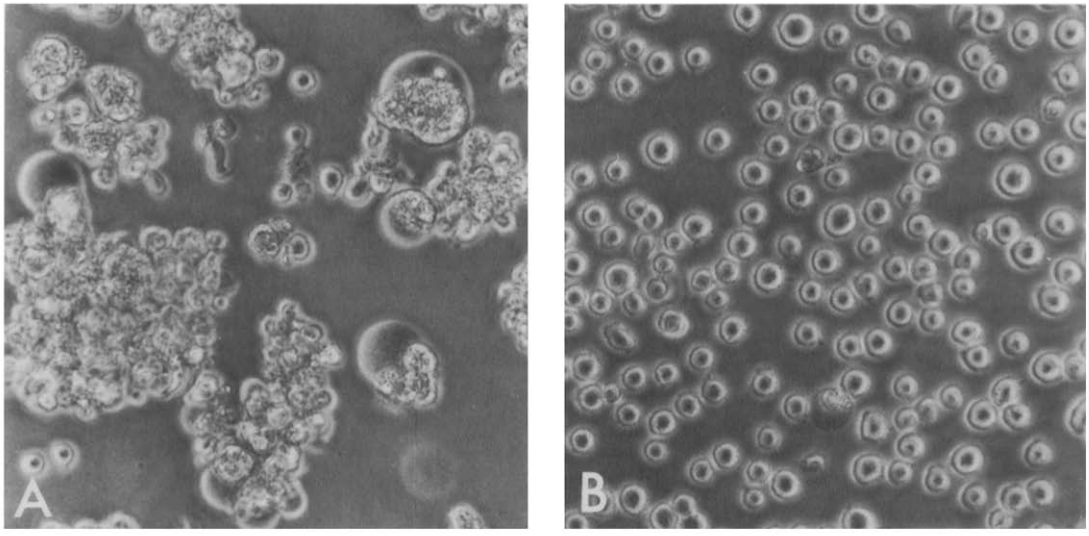

Fig.2. Effect of a cytopathic and low-cytopathic HIV-I clone on morphology of infected cells. CEM cells were exposed to (A) N1T-A or (B) N1T-E variant of HIV-I/N1T (400000 cpm of viral RT activity/1 $\times$ 10^6 cells per ml) and observed for cell aggregation, formation of multinucleated giant cells, and cell degeneration 3 days after infection.

Table 1

Infectivity and cytopathogenicity of N1T-E and N1T-A clones tested in human T-lymphoid and monocytoid cell lines

Cell line	Virus clone	Time after primary infection								
		1 week			3 weeks			7 weeks		
		V[a]	HIV-Ag	RT	V	HIV-Ag	RT	V	HIV-Ag	RT
HUT-102	N1T-E	81.2	<1	5.9	97.5	90.2	4.7	N.D.	N.D.	N.D.
HUT-102	N1T-A	38	91.7	8.0	0	N.D.	N.D.			
U-937	N1T-E	75	<1	3.4	96.1	<1	1.7	95	84.9	21.4
U-937	N1T-A	22.2	63.3	3.2	22.2	76.4	2.1	0	N.D.	N.D.
CEM[b]	N1T-E	99	<1	3.3	84.3	81	214.8	90.8	93	702.3
CEM[b]	N1T-A	36	66.3	7.8	12.5	71.8	32.1	0	N.D.	N.D.

[a] V, viability

[b] Sample times for CEM cells were 1, 2 and 6 weeks

Cells were exposed to N1T-A or N1T-E virus and tested at the designated times after infection as described in section 2. Cell viability, determined by trypan blue exclusion method, is expressed here as % living cells; HIV-Ag represents % cells staining positive for HIV-I antigens by an IF assay; RT represents viral reverse transcriptase activity in culture supernatant, expressed as cpm $\times 10^{-3}$. Note that cells which are not viable by trypan blue exclusion criterion can still stain positive for HIV antigens. N.D., not done

The basis for the observed low cytopathicity of N1T-E virus is unclear. One possibility is a defect at the level of virus entry, as suggested for a non-cytopathic HIV-II_{ST} [6]. Such a defect would imply that only a small proportion of virus preparation (i.e., nondefective particles) would be able to enter cells at any given time. Higher multiplicity of infection of an input virus should then correlate with increased cytopathicity, but that was not the case with HIV-II_{ST} [6] or N1T-E described here (not shown). Alternatively, the defect could be at the level of virus expression during some stage after virion penetration. This view is supported by the fact that only a small proportion of cells expressed viral antigens until 1–2 weeks after infection (table 1), whereas viral transcripts could be detected 4 days postinfection (not shown). Delayed expression of viral functions or viral latency have been proposed as one of the mechanisms responsible for HIV-I persistence in resting T lymphocytes [21,22]. Antigenic stimulation of such cells results in accelerated expression or reactivation of the latent viral genome [22]. Analysis of N1T-E viral gene expression during early stages of infection could allow us to distinguish between these two possibilities.

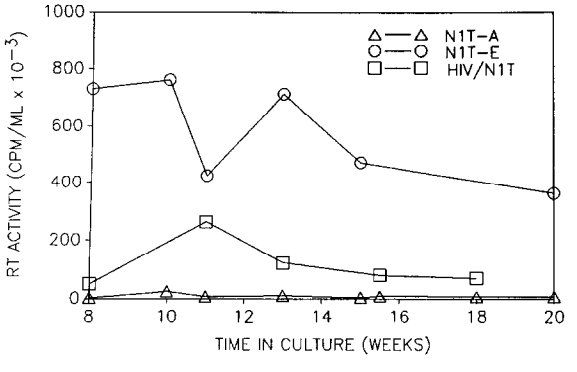

Fig.3. Replication of HIV/N1T variants in chronically infected cells. N1T-E virus was propagated in CEM cells; the highly cytopathic N1T-A and parental N1T viruses were propagated in lysis-resistant CR-10 cells [9].

4. CONCLUSIONS AND IMPLICATIONS

The present work describes, for the first time, a molecular clone of HIV-I with attenuated cytopathicity. Recent reports on noncytopathic HIV-II isolates [6,7] implied a correlation with the generally mild course of immunodeficiency in West African patients infected with this virus [8]. Our low-cytopathic HIV-I/N1T-E clone originated from a patient with lymphadenopathy [15], who remains relatively healthy. At the same time, this individual also carries highly cytopathic variants of HIV-I which are closely related to the N1T-E virus. It is impossible to conclude from the present data how those diverse variants may have evolved. However, comparative analysis of the cytopathic

and noncytopathic N1T viral clones may help to reveal the molecular basis for HIV-I cytopathicity.

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REFERENCES

[1] Piot, P., Plummer, F.A., Mhalu, F.S., Lamboray, J.-L., Chin, J. and Mann, J.M. (1988) Science 239, 573–579.
[2] Fauci, A.S. (1988) Science 239, 617–622.
[3] Von Briesen, H., Becker, W.B., Henco, K., Helm, E.B., Gelderblom, H.R., Brede, H.D. and Rubsamen-Waigmann, H. (1987) J. Med. Virol. 23, 51–66.
[4] Asjo, B., Morfeldt-Manson, L., Albert, J., Biberfeld, G., Karlsson, A., Lidman, K. and Fenyo, E.M. (1986) Lancet ii, 660–662.
[5] Cheng-Mayer, C., Seto, D., Tateno, M. and Levy, J.A. (1988) Science 240, 80–82.
[6] Kong, L.I., Lee, S.-W., Kappes, J.C., Parkin, J.S., Decker, D., Hoxie, J.A., Hahn, B.H. and Shaw, G.M. (1988) Science 240, 1525–1529.
[7] Evans, L.A., Moreau, J., Odehouri, K., Legg, H., Barboza, A., Cheng-Mayer, C. and Levy, J.A. (1988) Science 240, 1522–1525.
[8] Kanki, P.J., M'Boup, S., Ricard, D., Barin, F., Denis, F., Boye, C., Sangare, L., Travers, K., Albaum, M., Marlink, R., Romet-Lemonne, J.-L. and Essex, M. (1988) Science 236, 827–831.
[9] Casareale, D., Stevenson, M., Sakai, K. and Volsky, D.J. (1987) Virology 156, 40–49.
[10] Sakai, K., Casareale, D., Sonnabend, J. and Volsky, D.J. (1986) Ann. Inst. Pasteur/Virol. 137E, 311–315.
[11] Foley, G.E., Lazarus, H., Dredd, J., Faber, S., Uzman, B.G., Boone, B.A. and McCarthy, R.E. (1965) Cancer 18, 522–529.
[12] Dukovich, M., Wano, Y., Thi Vich Thuy, L., Katz, P., Cullen, B.R., Kehri, J.H. and Greene, W.C. (1987) Nature 327, 518–522.
[13] Sundstrom, C. and Nilsson, K. (1976) Int. J. Cancer 17, 565–577.
[14] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1986) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[15] Casareale, D., Dewhurst, S., Sonnabend, J., Sinangil, F., Purtilo, D.T. and Volsky, D.J. (1985) AIDS Res. vol.I, 407–421.
[16] Hahn, B.H., Shaw, G.M., Taylor, M.E., Redfield, R.R., Markham, P.D., Salahuddin, S.Z., Wong-Staal, F., Gallo, R.C., Parks, E.S. and Parks, W.P. (1986) Science 232, 1548–1553.
[17] Looney, D.J., Fisher, A.G., Putney, S.D., Rusche, J.R., Redfield, R.R., Burke, D.S., Gallo, R.C. and Wong-Staal, F. (1988) Science 241, 357–359.
[18] Harada, S., Koyanagi, Y. and Yamamoto, N. (1986) Science 229, 563–566.
[19] Stevenson, M., Zhang, X. and Volsky, D.J. (1987) J. Virol. 61, 3741–3748.
[20] Gupta, S. and Vayuvegula, B. (1987) J. Clin. Immunol. 7, 486–489.
[21] Folks, T., Powell, D.M., Lightfoote, M.M., Benn, S., Martin, M.A. and Fauci, A.S. (1986) Science 231, 600–602.
[22] Zagury, D., Bernard, J., Leonard, R., Cheynier, R., Feldman, M., Sarin, P.S. and Gallo, R.C. (1988) Science 231, 850–852.