

Cryptic Nature of Envelope V3 Region Epitopes Protects Primary Monocytotropic Human Immunodeficiency Virus Type 1 from Antibody Neutralization

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Characterization of biological and immunological properties of human immunodeficiency virus type 1 (HIV-1) is critical to developing effective therapies and vaccines for AIDS. With the use of a novel CD4⁺ T-cell line (PM-1) permissive to infection by both monocytotropic (MT) and T-cell-tropic virus types, we present a comparative analysis of the immunological properties of a prototypic primary MT isolate of HIV-1 strain JR-CSF (MT-CSF) with those of a T-cell-tropic variant (T-CSF) of the same virus, which emerged spontaneously in vitro. The parental MT-CSF infected only PM-1 cells and was markedly resistant to neutralization by sera from HIV-1-infected individuals, rabbit antiserum to recombinant MT-CSF gp120, and anti-V3 monoclonal antibodies. The T-CSF variant infected a variety of CD4⁺ T-cell lines, contained positively charged amino acid substitutions in the gp120 V3 region, and was highly sensitive to antibody neutralization. Neutralization and antibody staining of T-CSF-expressing cells were significantly inhibited by HIV-1 V3 peptides; in contrast, the MT strain showed only weak V3-specific binding of polyclonal and monoclonal antibodies. Exposure of PM-1 cells to a mixture of both viruses in the presence of human anti-HIV-1 neutralizing antiserum resulted in infection with only MT-CSF. These results demonstrate that although the V3 region of MT viruses is immunogenic, the target epitopes in the V3 principal neutralizing domain on the membrane form of the MT envelope appear to be cryptic or hidden from blocking antibodies.

Human immunodeficiency virus type 1 (HIV-1), the etiologic agent of AIDS (3, 15), has been cultivated in primary CD4⁺ T cells, mononuclear phagocytes, and tumor cell lines. Various HIV-1 phenotypes can be distinguished on the basis of properties of the virus in culture. In general, HIV-1 isolates that preferentially infect CD4⁺ T-cell lines are able to induce formation of syncytia and have been referred to as syncytium-inducing (SI) viruses (13, 22, 39). T-cell-tropic isolates grow with high replication rates (38) and exhibit high sensitivity to inactivation by soluble CD4 (sCD4) (10, 26), and their detection in clinical samples is correlated with the decline in CD4⁺ T-cell number and progressive loss of T-cell immune function (17, 33–35, 39). In contrast, monocytotropic (MT) HIV-1 isolates usually do not infect CD4⁺ T-cell lines or induce formation of multinucleated giant cells in vitro; they therefore have been referred to as non-SI viruses (13, 22, 39). Relative to T-cell-tropic isolates of HIV-1, MT viruses grow with low replication rates (39), are resistant to inactivation by sCD4 (10, 26), and are the virus types more frequently associated with in vivo transmission (45) and with the asymptomatic clinical status of HIV-1-infected individuals (17, 33–35, 39). Recently, several investigators have presented preliminary evidence that primary/MT isolates (field isolates) are remarkably resistant to antibody neutralization using hyperimmune sera from vaccinees or HIV-1-infected individuals (9).

A close correlation exists between HIV-1 phenotype and the

molecular structure of the third variable domain (V3 loop) of the virus envelope protein, gp120 (13, 20, 30). Viral genetic constructs in which the V3 structure was altered showed changes in cell tropism and replication rate (6, 8, 11, 12). The V3 loop also represents the dominant antibody neutralization site of gp120 (for a review, see reference 44), and its sequence variability is important in determining virus susceptibility to neutralizing antibodies (21). Thus, the V3 loop has been termed the HIV-1 principal neutralizing domain.

In this report, we present biological, immunological, and partial genetic data directly comparing a prototype MT isolate of HIV-1, JR-CSF (7), with a T-cell-tropic variant selected in tissue culture. Antibody neutralization assays revealed a marked shift, from an antibody-resistant phenotype characteristic of the MT viruses to an antibody-sensitive T-cell-tropic phenotype. Antibody resistance of the primary isolate was related to reduced exposure of immunodominant V3 domain epitopes.

MATERIALS AND METHODS

Reagents, viruses, and antisera. Mouse monoclonal antibodies (MAbs) 50.1 and 59.1 (43), directed to the V3 loop of the MN strain of HIV-1 (epitopes RIHIG and GPGRAF, respectively), were generously donated by A. Profy (Repligen, Cambridge, Mass.). The mouse V3 domain-specific MAbs 1026 and 1034 were raised against gp120 of HIV-1 MN and cross-react with recombinant JR-CSF gp120 as well as with the consensus V3 peptide (reference 29 and unpublished results). Peptide mapping and the sensitivity of antibody binding to protease-mediated cleavage between arginine and alanine in the GPGRAF sequence indicated that both MAb 1026 and

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MAB 1034 recognize the tip of the V3 domain (data not shown). Mouse MABs SIM-2 and SIM-3, directed to CD4 (31), and the cell-free supernatants containing HIV-1 JR-CSF (7) or the T-cell-tropic HIV-1 MN strain (37) were obtained from the AIDS Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases (contributions of James Hildreth, Irvin Chen, and Robert Gallo, respectively). The rabbit antiserum PB69 was generated against HIV-1 JR-CSF recombinant gp120 (28), which is identical in sequence to MT-CSF used in this study. Serum samples were obtained from HIV-1-infected individuals and from healthy donors, inactivated at 56°C for 30 min, and stored at -70°C until use.

Cells. The CD4⁺ T-cell lines PM-1, H9 (32), MT-2 (18), and SUP-T1 (38) were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml), streptomycin (100 µg/ml), 2 mM glutamine, and 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES).

Virus infections. The primary JR-CSF isolate (MT-CSF) and the T-cell-tropic variant (T-CSF) infect and persistently replicate in PM-1 cells, which are permissive to infection by and replication of primary isolates and laboratory-adapted strains of HIV-1 (23). PM-1 and H9 cells were infected by mixing cells with virus suspensions as previously described (7). Newly infected cells usually formed multinucleated syncytia after 3 to 4 days in culture, developing into chronically infected cell lines by 2 weeks.

Indirect immunofluorescence. Cells chronically infected with HIV-1 (2×10^5) were incubated with antiserum from HIV-1-infected individuals (1:100 dilution) or mouse MABs (5 µg/ml) for 30 min at room temperature in 100 µl of culture medium. Cells were washed twice and then incubated with fluorescein isothiocyanate (FITC)-conjugated goat F(Ab')₂ fragments to human immunoglobulins A, G, and M (Cappel) or FITC-conjugated goat F(Ab')₂ to mouse immunoglobulin G (Cappel) for 30 min at room temperature. Cells were washed, fixed with 1% paraformaldehyde, and analyzed with a FAC-Scan fluorescence-activated flow cytometer (Becton-Dickinson). The effect of V3 loop peptides on human antibody binding was investigated by incubating antiserum with synthetic peptides (50 µg/ml) for 1 h before cell staining. Peptide sequences were as follows: consensus peptide, CTRPNNTRKSIHIGPGRAFYTTEIIGDIRQAHG; HIV-1 MN peptide, CNKRKRIHIGPGRAFYTTEIIGDIRQAHG; and HIV-1 strain RF peptide, CNTRKSITKGPGRVIYATGQ (American Bio-Technologies). A peptide from the third domain of the CD4 molecule (CD4B; donated by F. Robey, National Institute of Dental Research), with the sequence TFDLKNKEVSVKRVTDQPKL, was used as a control.

Syncytium formation and neutralization assays. Infected PM-1 cells (10^4 per well) were incubated for 1 h at 37°C under 5% CO₂ in a flat-bottom microtiter plate with 100 µl of culture medium (without phenol red) in the absence or presence of human antiserum to HIV-1, rabbit PB69 antiserum, or mouse MAB 1026 or 1034 at the indicated concentrations. Target cells (5×10^4 in 100 µl) were then added, and 24 h later, the wells were evaluated for the presence of multinucleated giant cells. Cytopathic effect (CPE) was determined colorimetrically after 72 h, by the addition of 50 µl of 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT; Sigma) and measurement of optical density (OD) at 450 nm as previously described (36). The CPE was calculated on the basis of the OD measured in wells in which the anti-CD4 MABs SIM-2 and SIM-4 were added at saturating concentrations to block syncytium formation and preserve 100% cell viability.

The percentages of CPE and of inhibition of CPE were calculated by using the following equations:

$$\% \text{ CPE} = 1 - \left(\frac{\text{CPE}}{\text{total}} \right) \times 100$$

$$\% \text{ inhibition CPE} = \frac{\text{Ab} - \text{CPE}}{\text{total} - \text{CPE}} \times 100$$

where OD values were from wells containing HIV-1-infected cells and uninfected T-cell lines (CPE), HIV-1-infected cells and T-cell lines in the presence of MABs SIM-2 and SIM-4 (total), and HIV-1-infected cells and T-cell lines in the presence of anti-HIV-1 polyclonal sera or MABs (Ab). Values for CPE and the percentage of syncytium-forming cells correlated in each experiment. For peptide blocking experiments, V3 loop peptides (10 µg/ml) were incubated for 1 h at 37°C with 100 µl of diluted neutralizing antiserum before the addition of infected cells. For neutralization assays with infectious cell-free supernatants, 200 tissue culture infectious doses (defined here as the endpoint in a twofold serial dilution of an infectious supernatant which is positive for syncytium formation in PM-1 cells after 6 days) of virus were incubated for 1 h at 37°C with antiserum. Uninfected cells (2×10^4 per well) were added, and the plates were incubated for 6 to 7 days. CPE was measured as described above. Viral p24 antigen in culture supernatants was quantitated with an immune complex dissociation kit (ICD-Prep; Coulter) followed by an HIV-1 p24 antigen capture assay (Coulter).

DNA sequencing of the HIV-1 JR-CSF *env* gene. Lysates of the cell lines stably infected with HIV-1 JR-CSF were subjected to PCR amplification with the primers 5'-CCA ACC CAC AAG TAG TAT TGG-3' and 5'-ACC ATC TCT TGT TAA TAG CAG CCC-3', which generate a product from nucleotides 6471 to 7615 of the *env* gene (4). DNA sequence was determined with a set of sequencing primers derived from the PCR primers and from conserved regions of the envelope (27). Sequencing was performed on an Applied Biosystems 370A DNA sequencer. The sequences of the additional primers were 5'-GGG ATC AAA GCC TAA AGC CAT G-3', 5'-TAC AAT GTA CAC ATG GAA TT-3', 5'-TGG CAG TCT AGC AGA AGA A-3', 5'-GAA TTT TTC TAC TGT AAT TC-3', 5'-GAA TTA CAG TAG AAA AAT TCC CCT CC-3', 5'-TTC TTC TGC TAG ACT GCC A-3', and 5'-AAT TCC ATG TGT ACA TTG TA-3'.

RESULTS

MT strains of HIV-1 characteristically do not replicate in CD4⁺ T-cell lines. Propagation of these isolates in vitro is usually accomplished through viral passage in normal monocytes or peripheral blood lymphocytes. In this study, in order to directly compare virus isolates, we used the CD4⁺ T-cell line PM-1 for continuous replication of the molecularly cloned MT isolate JR-CSF. This cell line is permissive to infection with both MT (for example, JR-FL and Ba-L) and T-cell-tropic (for example, IIIB and MN) HIV-1 phenotypes (23). After chronic infection with the primary JR-CSF isolate (MT-CSF), this cell line expresses large amounts of gp120 and is able to fuse with uninfected PM-1 cells, forming multinucleated cells.

After 5 months of continuous culture in PM-1 cells, HIV-1 JR-CSF broadened in cell tropism, from a PM-1 cell-restricted virus to one capable of infecting a variety of CD4⁺ tumor cell lines, a biological property characteristic of T-cell-tropic isolates. To establish a cell line chronically infected only with

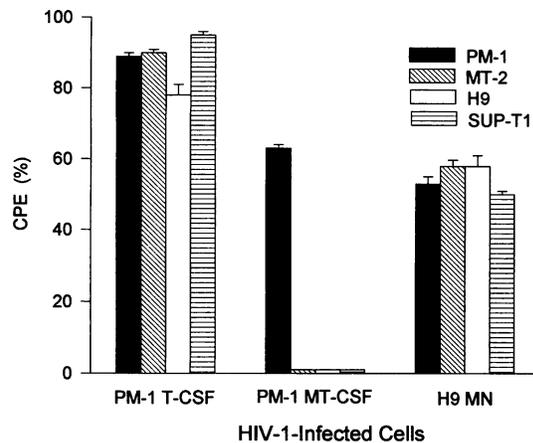


FIG. 1. Tropism of MT-CSF and T-CSF HIV-1 isolates. PM-1 cells infected with MT-CSF or T-CSF (10^4 per well) were incubated with PM-1, MT-2, H9, or SUP-T1 cells (5×10^4 per well), and CPE was evaluated colorimetrically after 72 h as described in Materials and Methods. Data are means \pm standard errors for a representative experiment. The tropism of HIV-1 MN (MN-infected H9 cells) was also determined for comparison.

T-cell-tropic virus, we infected H9 cells with culture supernatants containing the T-cell-tropic variant (T-CSF) and then transferred the resulting virus back to PM-1 cells. The resultant T-CSF-infected PM-1 cells were then assayed with several T-cell lines for syncytium formation and CPE to determine the cell tropism of the virus. T-CSF induced syncytia in and was cytopathic to PM-1, MT-2, H9, and SUP-T1 cells, whereas the MT-CSF parental strain formed syncytia and was cytopathic only in PM-1 cells (Fig. 1). The two virus isolates showed similar replication rates in PM-1 cells (data not shown).

T-cell-tropic viruses contain characteristic positively charged amino acids in the V3 region (6, 8, 11–13). We therefore determined the DNA sequence that spans the gp120 V3 region from both variant and parental viruses. Several differences were apparent in the V3 region between the MT-CSF and T-CSF isolates (Table 1): Ser-298, Thr-301, Ser-304, and Thr-315 in MT-CSF were replaced by Asn, Lys, Arg, and Lys, respectively, in T-CSF. The Thr-301-to-Lys substitution disrupts the glycosylation recognition sequence (NXT) and should eliminate N-linked carbohydrate at this site. T-CSF also contained an Ile insertion between positions 304 and 305 of MT-CSF. Thus, the V3 domain of T-CSF contained three more positive residues (Lys, Arg, and Lys) than the parental MT V3 domain, and these changes occurred at positions that have been shown to be critical for development of the T-cell-tropic or SI phenotype (6, 8, 11–13).

Amino acid substitutions in the V3 region or in the other envelope sites may alter the conformation of the envelope, possibly affecting its exposure and antigenicity. To examine the relative exposure of the V3 loops of MT-CSF and T-CSF, we measured the binding of MAbs directed to the gp120 V3 loop

TABLE 1. Amino acid sequences of the gp120 V3 domains of MT-CSF, T-CSF, and MN^a

Strain	Sequence ^a
MT-CSFS N N T R K S . I H I G P G R A F Y T T G E
T-CSFN N N K R K R I I H I G P G R A F Y K T G E
MNN Y N K R K R . I H I G P G R A F Y T T K N

^a Sequences were aligned according to Myers et al. (27) from amino acid position 298 of MT-CSF. The MN sequence is shown for comparison. T-CSF contains an insertion (I), and the dots were placed for correct alignment.

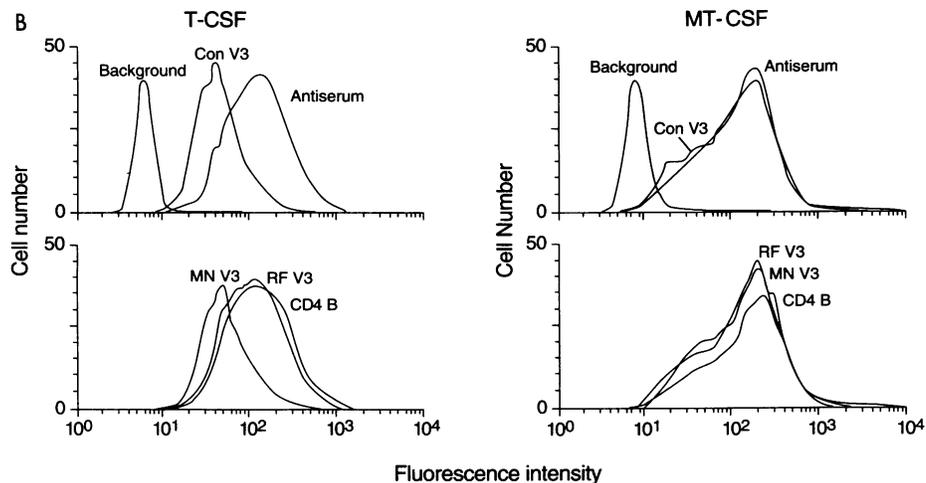
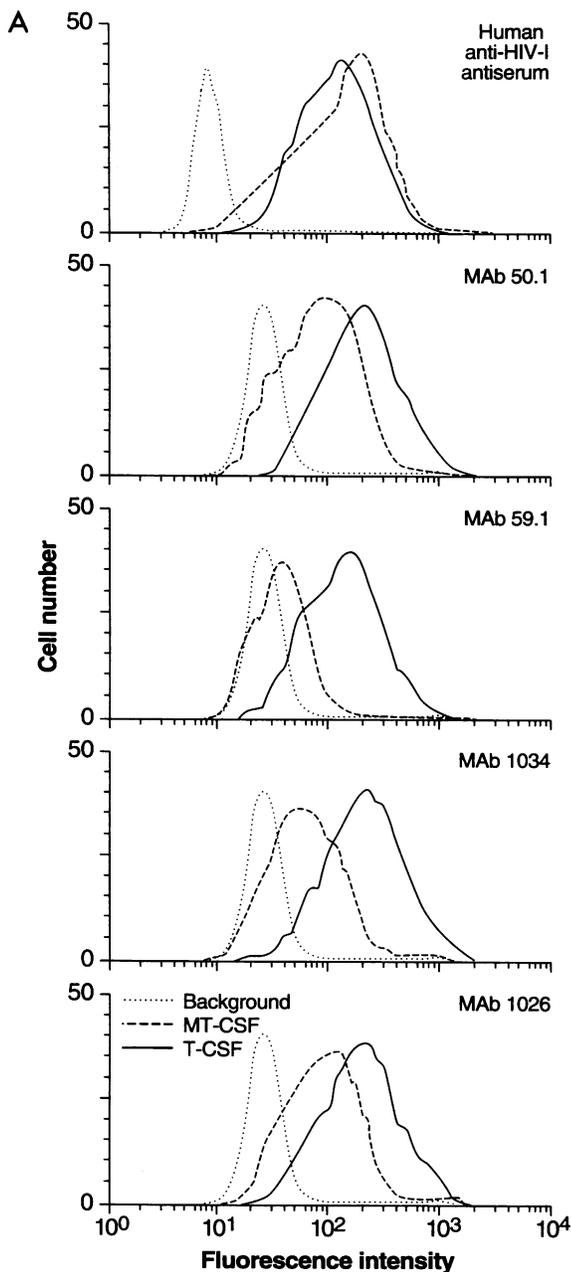
of HIV-1 MN, as well as of human antiserum to HIV-1, to infected cells. Mouse MAbs 50.1, 59.1, 1026, and 1034 bound with 3- to 15-fold-higher intensity to PM-1 cells infected with T-CSF than to MT-CSF-infected cells, whereas the human antiserum exhibited similar fluorescence intensities with both infected cell lines (Fig. 2A).

We next evaluated human antibody recognition of the V3 region by testing the effect of V3 peptide competition on human antibody binding to virus envelope expressed at the cell surface. Staining of the surface of T-CSF-expressing cells was markedly reduced by either the consensus V3 (identical to MT-CSF V3 except for a Ser-to-Asn substitution at position 298) or MN V3 peptide (Fig. 2B). In contrast, antibody binding to MT-CSF-infected cells was inhibited only partially by the consensus V3 peptide and was not inhibited by the MN V3 peptide. HIV-1 RF and a control peptide showed minimal effects on antibody binding to both isolates.

The low relative binding of monoclonal and polyclonal antibodies to the V3 domain on the primary virus isolate suggested that the MT and T-cell-tropic viruses may also differ in sensitivity to antibody neutralization. The effects of antisera from asymptomatic HIV-1-infected individuals were examined in neutralization assays with infected cells or infectious cell-free supernatants. Six of seven human antisera at a 1:50 dilution completely inhibited the CPE of the fusion event between T-CSF-infected and uninfected PM-1 cells (Fig. 3A). In contrast, the same antisera inhibited the CPE of MT-CSF-infected cells by no more than 20 to 40%. Two of the antisera were tested in twofold serial dilutions with cell-free virus (Fig. 3B). Both antisera significantly blocked the CPE of T-CSF even at a 1:1600 dilution, whereas inhibition of the CPE of the MT-CSF was apparent only at a 1:100 dilution. We also tested MAbs 1026, 1034, and 50.1 in neutralization assays. All MAbs inhibited the CPE of PM-1 cells infected with T-CSF as well as of MN-infected H9 cells; however, none of the MAbs inhibited syncytium formation and the subsequent CPE initiated by PM-1 cells chronically infected with MT-CSF (Fig. 3C).

We also evaluated the susceptibility of T-CSF and MT-CSF to neutralization by a polyclonal rabbit antiserum (PB69) to recombinant gp120 of the primary JR-CSF isolate, which is identical in sequence to MT-CSF used in this study. The CPE of T-CSF was almost completely inhibited by PB69 (1:50 dilution), whereas that of HIV-1 MN was inhibited by 70% (Fig. 4B). In contrast, PB69 inhibited the CPE of MT-CSF by only 10%.

FIG. 2. (A) Polyclonal human antibody and monoclonal anti-V3 antibody binding to PM-1 cells chronically infected with MT-CSF or T-CSF. Infected cell lines were incubated with human antiserum to HIV-1 or a mouse MAb (50.1, 59.1, 1034, or 1026) to V3, washed, and then incubated with appropriate FITC-conjugated secondary antibodies. After fixation, the cells were analyzed with a FACScan flow cytometer. (B) Effect of prior treatment of human anti-HIV-1 antiserum with V3 peptides on antibody binding to PM-1 cells infected with MT-CSF or T-CSF. Human antiserum to HIV-1 was incubated with a peptide (50 μ g/ml) corresponding to the consensus (Con), MN, or RF V3 sequence or with a control peptide corresponding to the third domain of CD4 (CD4B) before exposure to infected cells and indirect immunofluorescence assay.



To determine the contribution of V3-specific antibodies to the neutralizing activity of human and rabbit antisera, we incubated antisera with V3 loop peptides before the neutralization assay. Neutralization of T-CSF by human (Fig. 4A) or rabbit (Fig. 4B) antisera was significantly inhibited in the presence of either the consensus (MT) or the MN V3 peptide. The quantitation of p24 antigen in culture supernatants from syncytium formation assays showed comparable results (data not shown).

To examine whether neutralizing antibodies would affect virus selection when both phenotypes were present in the same inoculum, we incubated both cell-free MT-CSF and T-CSF supernatants (200 tissue culture infectious doses) with serum from healthy control or HIV-1-infected individuals before exposure to uninfected PM-1 cells. After 1 month in culture, the V3 genotype and the phenotype of the resultant virus were determined (Table 2). For virus supernatants incubated with control antiserum, only T-CSF was subsequently detected in the infected cells. For virus supernatants incubated with anti-HIV-1 antiserum, only MT-CSF was detected in the chronically infected cultures.

DISCUSSION

In this report, we present a comparative analysis of an MT virus and its T-cell-tropic variant. We have shown that the transition from an MT type to a T-cell-tropic HIV-1 markedly affects the immunological properties of the resultant variant virus. The emergence of a T-cell-tropic variant correlated with an increase in susceptibility to antibody neutralization relative to the original isolate, a primary MT virus. Associated with this phenotype transition, an increase in the exposure of immunodominant V3 sites occurred, with a resulting shift in the antibody-resistant MT phenotype to an antibody-sensitive T-cell-tropic phenotype. On the basis of our results, we suggest that MT isolates of HIV-1 are resistant to neutralizing antibodies as a consequence of low exposure of neutralizing epitopes in the V3 domain.

Emergence of the T-cell-tropic JR-CSF variant was first associated with an ability to continuously replicate in H9 cells and to infect cell lines that are susceptible only to T-cell-tropic strains of HIV-1. We detected four amino acid substitutions (positions 298, 301, 304, and 315) and one insertion (between positions 304 and 305) in the gp120 V3 loop of T-CSF, resulting in the addition of three positively charged residues.

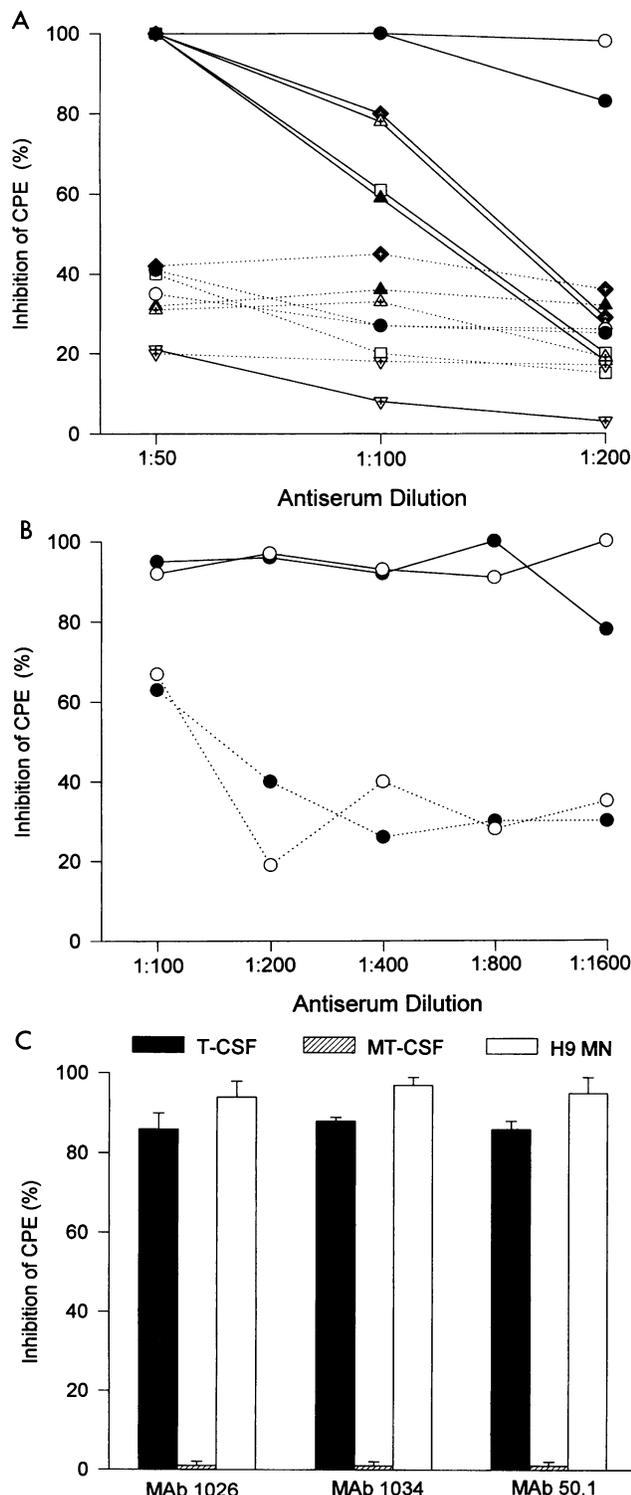


FIG. 3. Abilities of antisera from HIV-1-infected individuals and mouse anti-V3 MAbs to neutralize MT-CSF and T-CSF HIV-1 isolates. (A) PM-1 cells infected with MT-CSF or T-CSF (10^4 per well) were incubated with human antiserum to HIV-1 at various dilutions before the addition of uninfected PM-1 cells (5×10^4 per well). The CPE was evaluated after 72 h, and percent inhibition of CPE was calculated. Data are from a representative experiment. Each symbol represents one of seven antisera tested. Solid lines, T-CSF; dotted lines, MT-CSF. Control normal human serum had no significant

Both tropism and replication rate are related to the structure of the V3 domain (6, 8, 11, 12). Given that replacement of negatively charged by positively charged amino acids at only two positions in this domain is sufficient to produce T-cell-tropic (or SI) variants (6, 8, 11–13), we suggest that the mutations in the V3 sequence of T-CSF played a role in the observed phenotype switch. Future studies with recombinant viruses, substituting the V3 mutations back in the MT virus, are required to verify this hypothesis.

Associated with the transition of the MT to the T-cell-tropic phenotype, we detected changes in the exposure of V3 region epitopes. Thus, the extent of binding of anti-V3 loop MAbs was greater to PM-1 cells infected with T-CSF than to MT-CSF-infected cells. Because MAb 59.1 recognizes a linear epitope (GPGRAF) (43) common to both T-CSF and MT-CSF, the difference in binding to cell surface envelope for this MAb cannot be attributed to changes in the linear epitope sequence. Similarly, the fine specificities of MAbs 1026 and 1034 indicate that both bind to the tip of the V3 loop; in addition, they bind to recombinant (MT type) JR-CSF gp120 as well as to the consensus V3 peptide (reference 29 and unpublished results). Overall, these data support the hypothesis that the V3 region of the MT isolate either differs in conformation from that of T-CSF or is cryptic (sterically hidden), possibly because of loop interactions with other regions of the envelope or with other cell surface molecules. The binding of MAb 50.1, which recognizes the HIV-1 MN epitope RIHIG (43) and, probably, the corresponding sequence in the T-CSF V3 domain (RIIHIG), may depend on both linear and conformational properties not present in the MT V3 loop. The high binding intensity of the MAbs to T-CSF indicates that the conformations of the V3 loop are similar in both T-CSF and MN, probably as a result of similar contents or positions of positively charged residues. Indeed, both viruses have positively charged residues at positions 301 (lysine) and 304 (arginine), and in addition, lysine is present at position 316 in T-CSF and position 317 in MN.

The ability of V3 peptides to block the binding of human polyclonal anti-HIV-1 antibodies to cells infected with T-CSF, but not to MT-CSF-infected cells, is consistent with the MAb data and also indicates that the V3 domain of T-CSF is exposed and is the major target for polyclonal antibodies. The MT V3 domain is less accessible to polyclonal antibodies and makes a minor contribution to total antibody binding. The weak but detectable binding of anti-V3 antibodies to MT-CSF indicates that a minor fraction of the V3 sites is accessible to antibodies, thus suggesting that the MT-V3 site may be conformationally flexible but skewed toward an inaccessible conformation.

inhibitory effect (data not shown). (B) Infectious cell-free supernatants were incubated with human antiserum to HIV-1 at various dilutions before the addition of uninfected PM-1 cells (2×10^4 per well). CPE was measured after 6 to 7 days of incubation. Data are from a representative experiment, with each symbol representing one of two antisera tested. Solid lines, T-CSF; dotted lines, MT-CSF. Control normal human serum did not produce significant inhibition (data not shown). (C) PM-1 cells infected with MT-CSF or T-CSF were incubated with MAb 1026 (5 μ g/ml), 1034 (10 μ g/ml), or 50.1 (10 μ g/ml) before addition of uninfected PM-1 cells and determination of CPE as described for panel A. Data are means \pm standard errors from a representative experiment. The effects of the MAbs on the CPE of MN-infected H9 cells are shown for comparison. A control MAb to the C4 region of HIV-1 strain IIIB had no significant inhibitory effects at 10 μ g/ml (data not shown).

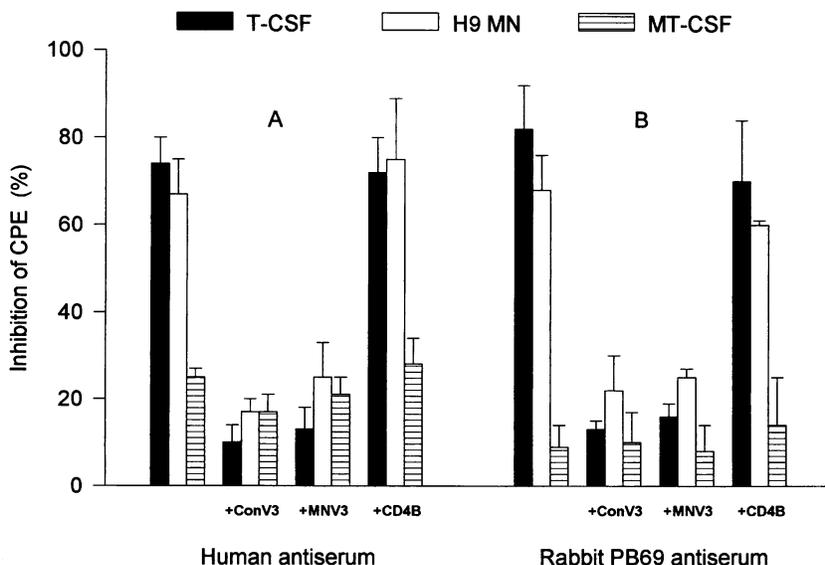


FIG. 4. Effect of prior incubation of antiserum with V3 loop peptides on neutralization of MT-CSF and T-CSF isolates. Human antiserum to HIV-1 (1:200 dilution) or rabbit antiserum PB69 (1:50 dilution) was incubated with the HIV-1 consensus (Con) or MN V3 loop peptide or the control CD4B peptide at a concentration of 10 μ g/ml. Peptide-treated antisera were then incubated with PM-1 cells infected with MT-CSF or T-CSF or H9 cells infected with MN. After subsequent addition of uninfected PM-1 cells, the CPE was evaluated. Values are means \pm standard errors of four (A) or three (B) experiments. Control, normal human, or rabbit sera did not produce significant inhibition of CPE (data not shown).

Neutralization assays with polyclonal human and rabbit antisera and anti-V3 MAbs revealed that T-CSF was sensitive and MT-CSF was resistant to blocking antibodies. The target for neutralizing activity of the polyclonal antisera toward T-CSF was shown to be the V3 domain, as demonstrated by inhibition of neutralization after prior incubation of antisera with HIV-1 consensus or MN V3 peptides. One explanation for MT-CSF resistance is that a major fraction of envelope on the surface of the MT-CSF-infected cells is not bound by anti-V3 antibodies and thus is free to mediate cell-to-cell fusion. It has been reported that a human MAb (447-52-D) was able to neutralize the MT isolate SF-162, although less efficiently than eight other lymphotropic HIV-1 isolates (16). Because of the use of different methods, it is difficult to directly compare that finding with those presented in this report, but it is possible that the SF-162 V3 loop is more exposed to antibodies than is the V3 domain of the JR-CSF strain. Other envelope epitopes, outside the V3 domain, may contribute to neutralizing activity (5, 14, 25, 42), as shown by the low level of

neutralization to both MT and T-cell-tropic viruses after treatment of antisera with V3 peptides.

The neutralization results with the rabbit antiserum prepared against recombinant gp120 of the primary JR-CSF virus indicate that the MT V3 loop, despite its poor exposure on the membrane form of the envelope proteins, is able, either in the native form of gp120 or after processing, to elicit neutralizing antibodies. These antibodies recognize and block the T-cell-tropic virus, but they neutralize only weakly the MT isolate, against which they were raised. The potent V3-specific neutralizing activity of human antisera against T-CSF suggests either that the V3 epitopes on MT viruses are immunogenic or that infected individuals harbor T-cell-tropic variants that induce anti-V3 reactivity. The data obtained with the rabbit antiserum to MT-CSF gp120 are consistent with the view that MT viruses may serve as an early driving force in the course of HIV infection in stimulating neutralizing antibodies to the MT V3 domain, which cross-react with the T-cell-tropic V3 site. Resistance of the MT virus to antibodies generated against the MT envelope suggests that vaccines based on primary or MT envelopes as the antigen may not provide any more activity than do vaccines based on T-cell-tropic envelopes.

Antibodies to the V3 loop are prevalent in HIV-1-infected individuals (19), and it is reasonable to assume that they can offer protection against T-cell-tropic variants. On the basis of our observation that a human neutralizing antiserum completely blocked the infectivity of T-CSF, but not that of MT-CSF, when both phenotypes were present in the same inoculum, we predict that anti-V3 antibodies may not prevent HIV-1 infection and dissemination if the harbored virus is present in the primary/MT form. MT virus phenotypes are most prevalent in asymptomatic infected individuals (17, 33-35, 39) and are the most frequently transmitted form of HIV-1 in vivo (45). It is possible that sensitive T-cell-tropic variants are blocked by antibodies during the asymptomatic period of the disease, which could result in low transmission of this

TABLE 2. V3 genotype and phenotype of HIV-1 JR-CSF recovered after infection of PM-1 cells with MT-CSF and T-CSF isolates that had been incubated in the presence of serum from control or HIV-1-infected individuals^a

Inoculum	Genotype/phenotype	
	Control serum	Anti-HIV-1 antiserum
MT-CSF	MT/MT	MT/MT
T-CSF	T/T	-/-
MT + T-CSF	T/T	MT/MT

^a MT-CSF, T-CSF, or a mixture of both viruses was incubated for 1 h with serum from control or HIV-1-infected individuals (1:50 dilution) and then used to infect PM-1 cells. After 1 month in culture, the V3 genotype was determined by PCR amplification and direct sequencing of the V3 region, and phenotypes of harbored viruses were analyzed. T, T-cell tropic.

phenotype and reduced immunopathogenesis mediated by T-cell-tropic strains. However, the persistent replication of MT viruses, together with continuous development of variants resistant to autologous antibodies (1, 2, 24, 40, 41), would result in progressive immune damage, further depletion of neutralizing antibodies and cell-mediated responses, and eventually the emergence of more aggressive viruses that initiate progression to AIDS.

Our results help to explain the marked resistance of primary/MT virus isolates to antibody neutralization mediated by hyperimmune sera from HIV-1-infected individuals and vaccinees (9). They further illustrate that a major obstacle to vaccine effectiveness may be the cryptic nature of neutralization epitopes in the virus phenotype predominantly transmitted in vivo.

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