Thrombotic microangiopathy associated with unusual viral sequences in HIV-1-positive patients

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Abstract

Background. Thrombotic microangiopathy (TMA) is a rare disorder caused by endothelial cell damage. TMA has been associated with the human immunodeficiency virus 1 (HIV-1) infection, yet only a minority of all HIV-1 patients develops TMA. Since HIV-1 has been shown to interact with endothelial cells, we investigated whether certain mutations in the HIV-1 envelope protein are associated with the development of TMA in HIV-1-infected patients.

Methods. Plasma was obtained from nine HIV-1-positive patients with TMA. Viral loads were determined from the samples and compared with the clinical data. Viral envelope protein sequences from the regions known to be responsible for viral tropism were isolated, sequenced and compared with known HIV-1 isolates. The isolates were expressed as synthetic fusion proteins; binding of these fusion proteins to CD4+ cells as well as to endothelial cell lines was investigated.

Results. The viral loads in patients with HIV/TMA were highly variable with no correlation to the clinical status. Most patients carried macrophage-tropic viral envelope protein sequences and an unusual insertion was found in the V2 variable region. The isolates showed increased CD4 binding, but a direct binding to endothelial cells was not observed.

Conclusions. Although TMA is generally diagnosed in patients with advanced HIV-1 infection, viral loads per se were not predictive of TMA in this study. While a direct interaction with endothelial cells was not detectable, specific viral envelope mutations were found in a region known to influence viral tropism. Hence, viral-specific factors might contribute to the pathogenesis of HIV-associated TMA.

Keywords: human immunodeficiency virus; thrombotic microangiopathy

Introduction

Thrombotic microangiopathy (TMA) occurs as a complication of infection with the human immunodeficiency virus 1 (HIV-1), occasionally as the first manifestation of HIV-1 infection [1]. TMA is caused by microvascular endothelial cell damage, which leads to intraluminal platelet thrombus formation. The resulting occlusion of small vessels causes ischaemic lesions in the capillary beds involved. The clinical consequences of the endothelial injury are a microangiopathic haemolytic anaemia, thrombocytopenia, neurological symptoms and renal failure. In the diarrhoea-associated form of TMA, the aetiological agent has been identified as verotoxin-producing enterohaemorrhagic Escherichia coli and organ involvement is clearly related to the distribution of endothelial receptors, which bind and internalize these toxins.

For HIV-associated TMA, such a detailed molecular pathogenesis is still lacking, but direct interactions of the virus with the endothelium could play a role in the pathogenesis of TMA [2]. Although endothelial cells do not express the CD4 antigen, which constitutes the primary HIV-1 receptor, HIV-1 infection of endothelial cells has been reported [3]. CD4 binding is normally a prerequisite for the conformational changes of the HIV-1 envelope protein gp120, which create a binding site for the HIV-1 co-receptor and mediate cell attachment and fusion with the host cell [4]. The chemokine receptors CCR5 and CXCR4 have been identified as...
the major HIV-1 co-receptors. Macrophage tropic viruses, frequently isolated from asymptomatic HIV-1 carriers, use CCR5, whereas T-cell tropic viruses, emerging late in the course of the disease, enter CD4+ cells through CXCR4. In vitro studies have demonstrated that the infection of CD4− cells can take place through the co-receptor in concert with other cell-surface molecules [5]. The HIV-1 co-receptors are expressed on a wide range of cell types and have been found on different endothelial cell lines, including microvascular endothelial cells [6]. It has also been shown that the gp120-mediated apoptosis in cultured endothelial cells requires the co-receptor CXCR4 [7]. Yet, these observations do not explain why only a small percentage of all HIV-1 patients develops TMA. We hypothesized that changes in the gp120 HIV-1 envelope protein might facilitate the generally inefficient interaction with CD4− cells [5] and, thus, induce endothelial cell damage leading to TMA.

We, therefore, isolated the viral envelope protein sequence domains involved in co-receptor binding from nine HIV-1 patients with TMA and report now that these sequences differ from the majority of HIV-1 envelope protein isolates.

Subjects and methods

Patients

Plasma samples obtained during the initial plasmapheresis therapy for HIV-associated TMA were available from all patients treated either at the Beth Israel Hospital, Boston (seven cases) or at the Brigham & Women’s Hospital, Boston (one case) between 1990 and 1996. In one additional patient who died before plasmapheresis was initiated, a serum sample was obtained. All samples were frozen and stored at −20°C at the time of collection. In all cases, the TMA diagnosis was based on the clinical picture and the typical laboratory findings of a microangiopathic haemolytic anaemia with the peripheral blood smear showing erythrocyte fragmentation (Table 1). Biopsies were not obtained because of thrombocytopenia.

Viral load

Viral loads were determined with the Amplicor™ HIV Monitor test kit (Roche Diagnostic Systems) following the instructions of the manufacturer. The results were corrected for potential dilution by simultaneous measurement of blood urea nitrogen in serum and the pheresis samples.

RNA isolation

Extraction of viral RNA from the samples was performed using silica beads. Briefly, 200 µl of each plasma sample were incubated with 900 µl guanidinium thiocyanate containing lysis buffer and 40 µl of acid-treated size-fractioned silica particle (Sigma) solution for 10 min at room temperature. The silica beads were pelleted by centrifugation and washed twice with wash buffer containing guanidinium thiocyanate, followed by two washes with 70% ethanol and one wash with acetone. The pellets were dried for 15 min at 37°C and the RNA was eluted by resuspending the silica beads in 60 µl TE buffer containing 0.5 U/µl RNase inhibitor (Promega) and incubating for 10 min at 56°C followed by centrifugation to pellet the silica.

RT–PCR, cloning and sequencing

The V2 and V3 regions of the HIV envelope gp120 gene, which are involved in HIV co-receptor binding, were amplified from the extracted RNA in a nested polymerase-chain reaction (PCR). For the first PCR, the ES8 and ED5 primers of the Heteroduplex Mobility Analysis HIV-1 env Subtyping Kit (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, Bethesda, USA) were utilized amplifying the V1 through V5 regions of the envelope gene. Reverse transcription (RT) was performed for 30 min at 42°C in a 50 µl reaction consisting of the 25 µl RNA sample, RT buffer (Gibco), 40 U M-MLV reverse transcriptase (Gibco), 0.2 mM dithiothreitol, 0.68% Nonidet P-40, 500 µM deoxynucleoside triphosphates and 250 ng of the antisense ES8 oligonucleotide (5'-CAC TTC TCC AAT TGT CCC TCA-3'). The reaction was stopped by heating the mixture to 99°C for 5 min. A fragment encoding the regions V1 through V5 of the envelope gene. Reverse transcription (RT) was performed for 30 min at 42°C in a 50 µl reaction consisting of the 25 µl RNA sample, RT buffer (Gibco), 40 U M-MLV reverse transcriptase (Gibco), 0.2 mM dithiothreitol, 0.68% Nonidet P-40, 500 µM deoxynucleoside triphosphates and 250 ng of the antisense ES8 oligonucleotide (5'-CAC TTC TCC AAT TGT CCC TCA-3'). The reaction was stopped by heating the mixture to 99°C for 5 min. A fragment encoding the regions V1 through V5 of the envelope gene.

Table 1. Clinical and laboratory data of the HIV-1-infected TMA patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Platelets (µl)</th>
<th>Haemoglobin (mg/dl)</th>
<th>Erythrocyte fragmentationa</th>
<th>LDH (U/l)</th>
<th>Creatinine (mg/dl)</th>
<th>Neurological abnormalities</th>
<th>Clinical status</th>
<th>Viral load (copies/ml)</th>
<th>Outcome from TMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39000</td>
<td>7.9</td>
<td>Yes</td>
<td>694</td>
<td>1.2</td>
<td>Seizure</td>
<td>AIDS</td>
<td>5078</td>
<td>Recovery</td>
</tr>
<tr>
<td>2</td>
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<td>7.2</td>
<td>Yes</td>
<td>409</td>
<td>2.5</td>
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<td>AIDS</td>
<td>5040</td>
<td>Death</td>
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<tr>
<td>3</td>
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<td>8.3</td>
<td>Yes</td>
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<td>1.7</td>
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<td>AIDS</td>
<td>517939</td>
<td>Recovery</td>
</tr>
<tr>
<td>4</td>
<td>24000</td>
<td>8.3</td>
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<td>2385</td>
<td>2.2</td>
<td>Altered mental status</td>
<td>AIDS</td>
<td>261622</td>
<td>Relapses</td>
</tr>
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<td>8.1</td>
<td>Yes</td>
<td>1436</td>
<td>1.3</td>
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<td>Asymptomatic</td>
<td>59788</td>
<td>Recovery</td>
</tr>
<tr>
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<td>1.8</td>
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<tr>
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<td>3505</td>
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<tr>
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<td>9000</td>
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<td>1536</td>
<td>2.3</td>
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<td>Asymptomatic</td>
<td>133257</td>
<td>Recovery</td>
</tr>
<tr>
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<td>377</td>
<td>1.1</td>
<td>None</td>
<td>AIDS</td>
<td>219845</td>
<td>No response</td>
</tr>
</tbody>
</table>

aOn the peripheral blood smear. bHaematocrit (%) was substituted for haemoglobin.
50 µl RT reaction mix, 250 ng ED5, PCR buffer (Boehringer), 1 mM MgCl₂ and 2 U Taq polymerase (Taq 2000; Stratagene). Forty cycles were performed with an annealing temperature of 55°C and an extension time of 2 min. Five microlitres of this PCR product served as a template for the amplification of the V2 and V3 regions (25 cycles, 55°C annealing temperature, 1 min 15 s extension time) using 500 ng of each of the internal primers FP2 (5'-CGC CGG ACG CTG AAA AAA CTG CAG TTT CAA TAT-3') and RP1 (5'-CGC GGG GCC GGC GCC AGT AGA AGA ATT CCC TTC AAC AA-3') in a 50 µl reaction (PCR buffer, 200 µM deoxynucleoside triphosphates, 1.5 mM MgCl₂, 1 U Taq polymerase). The presence of an ~660 bp PCR product was confirmed with gel electrophoresis. The PCR product was subcloned into the pcDNA3 vector (Invitrogen) using standard techniques. Double-stranded sequencing was performed on all clones using the dideoxy method. The viral genotype with regard to tropism for macrophages vs transformed T-cell lines was determined from the predicted amino acid sequences of the clones, according to proposed genotype determination criteria [8]. The criteria involve amino acid substitutions in the V2 and V3 regions influencing the overall charge of the domains and the glycosylation pattern of the V2 region.

**gp120–IgG fusion protein**

The PCR fragments were cloned into a vector expressing a synthetic gp120 envelope protein fused to the CH₂–CH₃ domains of human IgG [9]. This vector contained the gp120 sequence of the MN isolate optimized for expression in eukaryotic cells and modified to include several unique restriction sites. A PstI and an EcoRI site were used for the subcloning of the patient-derived V2–V3 fragments. The chimeric proteins were expressed in HEK 293T cells after transient transfection with 20 µg of each plasmid DNA per 10 cm dish, using the calcium phosphate method. The fusion protein-containing supernatants were harvested 72 h after transfection.

An α1-acid glycoprotein–IgG₁ fusion protein, generated in the same way, was used as a negative control.

**Immunoprecipitation and western blotting**

For immunoprecipitation, 500 µl of supernatant was incubated with 30 µl of protein G (GammaBind Sepharose; Pharmacia) at 4°C overnight. The immune complexes were washed twice in 20 mM Tris pH 7.5, 1 mM EDTA, 0.2 M NaCl, resuspended in 30 µl SDS-sample buffer (100 mM Tris pH 6.8, 4% SDS, 0.2% bromophenol blue, 20% glycerol, 10% mercaptoethanol) and separated on a 10% SDS–PAGE gel. After transfer to a PVDF membrane (Poly Screen; NEN), the gp120–IgG fusion proteins were detected utilizing a horseradish peroxidase-conjugated antibody against human IgG 1:10 000 (Amersham) in combination with chemiluminescence (Pierce).

**Cell culture**

All cell cultures were grown in a humidified chamber at 37°C in 5% CO₂. HEK 293T cells and HeLa CD4+ cells (clone 1022, AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) were cultured in Dulbecco’s modified Eagle’s medium (Bio Whittaker) supplemented with 10% iron-supplemented calf serum (Sigma). Immortalized human microvascular endothelial cells (HMEC-1, originally obtained from Dr Thomas J. Lawley) were grown in EBM (Clonetics) supplemented with 10% fetal bovine serum (HyClone), 10 ng/ml hEGF (Collaborative Biomedical Products) and 1 μg/ml hydrocortisone (Sigma). A spontaneously immortalized HUVEC cell line (ECV304, ATCC) was cultured in Medium 199 (Bio Whittaker) supplemented with 10% fetal bovine serum.

For adhesion assays, HEK 293T cells, transiently transfected with either CD4 or a control plasmid (green fluorescent protein), were detached from the culture dishes with 0.5 mM EDTA in phosphate-buffered saline (PBS), centrifuged at 1000 r.p.m. for 5 min and resuspended in PBS at a concentration of 3 000 000 cells/ml.

**Adhesion assay**

Bacterial culture plates (Easy Grip Petri Dish; Falcon) were coated with goat anti-human IgG Fc antibody (Cappel) at 20 µg/ml for 2 h at room temperature, blocked with 2 mg/ml bovine serum albumin (Sigma) in PBS for 3 h and then incubated with 1 ml of supernatants, containing ~100 ng/ml fusion protein for 1 h. After brief washing with PBS, the coated dishes were incubated with 3 000 000 cells in 1 ml for 30 min. The plates were washed twice and three fields were counted under the microscope using an ocular micrometer (Unitron). To some assays, recombinant soluble CD4 (sCD4; R. Sweet, SmithKline Beecham; AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) was added at a concentration of 20 µg/ml. sCD4 was either mixed with the fusion protein containing supernatants for 30 min at room temperature before immobilizing them on the plates or added to the plates separately for 30 min before incubating them with the cells.

**Results**

**Patient characteristics and viral loads**

Viral RNA was isolated from plasma or serum samples, respectively, of nine consecutive patients with HIV-associated TMA. In all patients, the reason for hospital admission was a microangiopathic haemolytic anaemia with varying degrees of thrombocytopenia and lactate dehydrogenase (LDH) elevations (Table 1) along with clinical symptoms suggestive of TMA. Six patients had focal neurological abnormalities or mental status changes and six patients had impaired renal function with elevated serum creatinine levels. Only one patient had no detectable renal or central nervous system abnormalities suggestive of TMA end organ manifestations. Three patients were taking antiretroviral medication (AZT, 4DT, 3TC) at the time and the majority of patients was being treated with antiviral or antibacterial agents for opportunistic infections, but none had received substances typically associated with drug-induced TMA [2] when they were admitted with HIV-associated TMA.

Viral load measurements revealed a wide range of viral particle numbers and no correlation with the
Figure 1. HIV-1 viral loads at start and end of plasmapheresis. Comparison of viral load measurements at the beginning and the end of the first plasmapheresis in six patients. Viral loads are displayed on a logarithmic scale.

clinical status (Table 1). Three patients were asymptomatic or undiagnosed regarding their HIV-1 infection until they presented with TMA. Viral loads in these patients were high, ranging from 59,788 to 208,872 copies/ml. In contrast, several patients with manifest acquired immunodeficiency syndrome (AIDS) before or at the onset of TMA had low viral loads (minimum: 5078 copies/ml). The highest viral load (51,939 copies/ml) was observed in a patient with advanced AIDS. This patient required salvage splenectomy in the course of his TMA treatment. Five other patients responded to plasmapheresis and medical therapy (steroids and antiplatelet agents) alone, one of which continued to have low-grade thrombocytopenia, despite treatment. Two patients died, one of them before the initiation of plasmapheresis therapy. Again, no direct correlation was observed between viral load and response to treatment or outcome.

In six patients, viral loads were not only measured at the beginning, but also at the end of the first plasmapheresis. Figure 1 shows that the number of viral particles increased during treatment in half the cases, remained in the same range in two patients and viral particles increased during treatment in half the plasmapheresis. Figure 1 shows that the number of copies/ml was observed in a patient with advanced AIDS. This patient required salvage splenectomy in the course of his TMA treatment. Five other patients responded to plasmapheresis and medical therapy (steroids and antiplatelet agents) alone, one of which had three TMA relapses thereafter. One patient continued to have low-grade thrombocytopenia, despite treatment. Two patients died, one of them before the initiation of plasmapheresis therapy. Again, no direct correlation was observed between viral load and response to treatment or outcome.

V2 and V3 gp120 envelope sequences

The second and third variable region (V2 and V3) and the adjacent constant domains (C2 and C3) of the HIV-1 envelope gp120 gene were isolated by RT–PCR and sequenced for each of the nine patients. These regions interact with the co-receptors and determine viral tropism [4]. The co-receptor binding site involves the conserved bridging sheet that lies between the protruding V1/V2 and V3 loops as well as some residues in V3 itself; the V2 loop also influences co-receptor use [10]. Sequence analysis revealed that the conserved cysteines essential for the formation of disulphide bridges were present in all isolates. All but one isolate (sample 2) appeared to encode for a functional envelope protein. Figure 2 shows the peptide sequences for V2 and V3, respectively, in comparison to a consensus sequence derived from four representative HIV-1 isolates (HXB2, BAL1, JR-CSF and JR-FL). All patient isolates (except sample 2) had an insertion of variable length (1–23 amino acids) in the carboxy terminal hypervariable region of V2. The majority of the samples contained two or more potential N-linked glycosylation sites within this region, predicting a macrophage tropic genotype [8].

The V3 region is generally more conserved in length and amino acid sequence than V2. In our study, only one patient isolate (sample 7) differed from the others in length, consisting of 34 amino acids instead of 35. The conserved GPG motif at the tip of the V3 loop was present in seven samples; in samples 2 and 8, this motif was changed to dqG and eqG, respectively. Using the genotype determination criteria [8], all clones except sample 8 were classified as macrophage tropic.

Expression of the gp120-fusion proteins

The cloned gp120 sequences were expressed as chimeric proteins fused to the CH2–CH3 domains of human IgG1 in HEK 293T cells. Expression was verified by western blot analysis (Figure 3); similar levels of expression were obtained for all samples except patient 2, probably as the result of the frameshift mutation, and patient 1, where the fusion protein was poorly expressed.

Adhesion of CD4+ cells and endothelial cells to the gp120-fusion proteins

To test whether elongation of the V2 region of the gp120 envelope protein affects binding to the principal HIV receptor CD4 [11], we analysed the adhesion of two different cell lines expressing CD4 to the individual gp120-fusion proteins immobilized on a bacterial Petri dish. Figure 3 shows that binding was detectable in six out of eight fusion proteins. The number of adherent cells was higher with all these patient-derived proteins than those observed using the parental syn gp120mn. Cellular adhesion depended upon the expression of CD4; mock-transfected 293T cells did not bind to the gp120 fusion protein (data not shown). Conversely, CD4-expressing cells did not bind to a control fusion protein containing the α1-acid glycoprotein instead of gp120.

Two endothelial cell lines, the microvascular human endothelial cell line HMEC-1 and the macrovascular human endothelial cell line ECV304 were tested for their ability to adhere to gp120 protein derived from patients with TMA. None of the isolated gp120 proteins were able to bind these two cell types. To investigate whether binding of gp120 to co-receptor can be triggered in the presence of soluble CD4 [12], all...
adhesion assays were also performed in the presence of sCD4 added to the gp120-fusion protein-containing supernatants before coating the plates or to the immobilized fusion proteins before incubation with the cells. No changes in adhesion were observed in the presence of sCD4.

**Discussion**

TMA seems to manifest at a more advanced stage in the HIV-1 infection and a sharp decline in the incidence of TMA has been observed since the introduction of highly active antiretroviral therapy [13]. Viral loads, which are used to predict the clinical course and to evaluate the response to therapy, would, therefore, be expected to be high in HIV patients with TMA. To date, no data had been published measuring viral loads or CD4 cell counts in patients with HIV-associated TMA.

Our viral load measurements revealed that the HIV-induced microangiopathy is associated with highly variable amounts of circulating viral particles and viral loads did not decrease following plasmapheresis. In one patient, whose plasma was available from his last plasmapheresis treatment for TMA, even a sharp increase of viral titres was observed, further supporting the notion that viral loads per se do not constitute a risk factor for HIV-associated TMA.

Since TMA appears to develop only in a small subgroup of HIV patients, specific host or viral factors have to be postulated to explain this rare complication of HIV-1 infection. To investigate whether certain HIV-1 envelope protein gp120 mutations mediate an increased viral endothelial tropism in HIV-associated TMA, selected regions of the gp120 envelope protein were subjected to sequence analysis. Since both the V2 and V3 region of the envelope gene contain co-receptor binding residues and the V2 loop is involved in viral tropism [8], a region of the envelope protein containing...
these two domains was isolated and sequenced for each patient.

Application of established genotype determination criteria revealed macrophage-tropic isolates for the majority of the patients [8]. Even though macrophage-tropic viruses are usually observed in asymptomatic HIV-1 carriers or in the earlier phases of HIV disease, six of our patients had manifest AIDS at the time of TMA diagnosis and only three were asymptomatic or undiagnosed.

The most striking feature of the isolated viral sequences was an insertion of 1–23 amino acids in the V2 carboxy terminal hypervariable region when comparing the sequences to a consensus sequence derived from four known HIV-1 isolates. To avoid a selection bias due to the high variability of the HIV genome, reference sequences were used for comparison rather than an individual control group from HIV-1 patients without TMA. Despite the known HIV sequence variability, V2 insertions have only been described in a minority of HIV-1 envelope protein sequences [10,14–16]. They have been linked to the switch of macrophage-tropic viruses to the T-cell line tropic isolates that appear as the disease progresses [14], but their appearance seems to be transient [16]. In two studies [10,15], the appearance of V2 extensions correlated with the maintenance of macrophage tropism and slow disease progression. Since there is evidence that V2 is involved in the determination of co-receptor use and viral tropism, the observed V2 insertions could affect endothelial cell tropism.

The V3 region is generally much more conserved. Nevertheless, isolates from two patients revealed a mutation of two residues at the conserved GPG motif at the tip of the V3 loop. Substitutions of these residues have been found only in a small number of patients [17]. Because the tip of the V3 region constitutes the primary neutralizing determinant of the viral envelope, substitutions in this region are likely to affect the host immune response to the virus.

To examine whether the isolated V2 and V3 sequences modulate functional interactions with the putative host cells, these regions were inserted into a synthetic gp120 envelope protein and expressed as gp120–IgG fusion proteins. Expression of full-length gp120–IgG protein was achieved in eight out of nine patients. The individual fusion proteins were subsequently tested for their ability to bind various cell lines in an adhesion assay.

Adhesion of two different CD4+ cell lines was observed for six out of eight gp120–IgG fusion proteins. The CD4 binding site, composed of several discontinuous epitopes involving the V4 variable region, remained unchanged in all gp120 fusion proteins. However, the affinity for CD4 varies in different isolates and has been found to be significantly lower in primary macrophage-tropic viruses than in laboratory strains [18]. It has also been shown that length polymorphism in V2 affects the accessibility of the CD4 receptor binding site [11] and that V2 can modify the sensitivity of the virus to CD4 neutralization [19]. In accordance with the findings of Fox et al. [11], who observed increased binding to CD4 in gp120 mutations containing V2 insertions, six of our gp120 fusion proteins displayed higher CD4 affinities than the parental syndp120mn fusion protein. On the other hand, the fusion proteins with the two longest V2 domains of all, failed to bind CD4.

We could not demonstrate direct binding of a microvascular endothelial cell line or immortalized human umbilical vein cells to the gp120 fusion proteins. It is conceivable that the two tested cell lines did not display the appropriate receptor. Recently, the presence of the C-type lectin DC-SIGN, which is able to bind HIV independently of CD4 [20], has been shown on microvascular endothelial cells [6]. In addition, binding of gp120 might also require prior activation of endothelial cells [21].

Taken together, our data show that TMA develops in a subgroup of HIV-1 patients without correlation to their viral titres. The HIV-1 envelope protein sequences isolated from nine TMA patients display unusual...
characteristics, which have been associated with macrophage tropism, slow disease progression and strong selection pressure. These mutations are typically found in asymptomatic HIV carriers at the time of conversion from macrophage to T-cell tropism and display an increased affinity for CD4 in vitro. Although the patient group was clinically heterogeneous, it is interesting to speculate that this conversion might be associated with increased binding and perhaps activation of T cells. Future work will have to examine whether gp120 proteins with these V2 mutations facilitate interaction with endothelial cells and endothelial tropism or damage endothelial cells through indirect mechanisms, for example, an abnormal T-cell response.

Conflict of interest statement. None declared.

References

18. Dhar ES, Li XL, Moudgil T, Ho DD. High concentrations of recombinant soluble CD4 are required to neutralize primary human immunodeficiency virus type 1 isolates. Proc Natl Acad Sci USA 1990; 87: 6574–6578

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