JOURNAL OF VIROLOGY, Mar. 2001, p. 2706–2709 Vol. 75, No. 6
0022-538X/01/$04.00+0 DOI: 10.1128/JVI.75.6.2706–2709.2001

Macrophage Tropism of Human Immunodeficiency Virus Type 1 Facilitates In Vivo Escape from Cytotoxic T-Lymphocyte Pressure

M. SCHUTTEN,[1] C. A. VAN BAALEN,[1] C. GUILLON,[1] R. C. HUISMAN,[1] P. H. M. BOERS,[1] K. SINTNICOLAAS,[2] R. A. GRUTERS,[1,3] AND A. D. M. E. OSTERHAUS[1]*

Institute of Virology, University Hospital Rotterdam, 3015 GE Rotterdam,[1] and Laboratory for Histocompatibility and Immunogenetics, Bloodbank Rotterdam, 3015 CN Rotterdam,[2] The Netherlands, and UMR103, CNRS bioMerieux, ENS de Lyon, Lyon, France[3]

Received 10 October 2000/Accepted 8 December 2000

Early after seroconversion, macrophage-tropic human immunodeficiency virus type 1 (HIV-1) variants are predominantly found, even when a mixture of macrophage-tropic and non-macrophage-tropic variants was transmitted. For virus contracted by sexual transmission, this is presently explained by selection at the port of entry, where macrophages are infected and T cells are relatively rare. Here we explore an additional mechanism to explain the selection of macrophage-tropic variants in cases where the mucosa is bypassed during transmission, such as blood transfusion, needle-stick accidents, or intravenous drug abuse. With molecularly cloned primary isolates of HIV-1 in irradiated mice that had been reconstituted with a high dose of human peripheral blood mononuclear cells, we found that a macrophage-tropic HIV-1 clone escaped more efficiently from specific cytotoxic T-lymphocyte (CTL) pressure than its non-macrophage-tropic counterpart. We propose that CTLs favor the selective outgrowth of macrophage-tropic HIV-1 variants because infected macrophages are less susceptible to CTL activity than infected T cells.

The predominant biological phenotype of human immunodeficiency virus type 1 (HIV-1) isolates changes during the course of infection. Early after seroconversion, usually only macrophage-tropic, non-syncytium-inducing (NSI) variants are found. With progression to AIDS, HIV-1 isolates tend to lose their capacity to infect macrophages and may gain the ability to induce syncytia (SI) (10, 14). It has been well documented that only macrophage-tropic viruses persist directly after seroconversion (21), even when a mixture of variants was transmitted (2, 8). Data obtained in the simian immunodeficiency virus macaque model have suggested that Langerhans cells or macrophages are the primary target cell after sexual transmission (12). It has been proposed that these primary target cells act as a selective barrier against variants that are not capable of infecting them, i.e., SI variants (12, 20). This physical barrier is not effective if HIV enters the body via other routes, e.g., through blood transfusion, needle-stick accidents, or intravenous drug abuse. Also, in those cases in which macrophages are less likely to be the sole primary target cell type, the selective outgrowth of macrophage-tropic/NSI viruses is observed (2, 12).

Therefore, it must be assumed that additional mechanisms select against non-macrophage-tropic variants after the virus has entered the body. HIV-1-specific cytotoxic T lymphocytes (CTLs) have been shown to exert strong selective pressure on HIV-1 quasispecies during seroconversion (1, 7) and are thus a likely candidate. CTL pressure on replication of non-macrophage-tropic and macrophage-tropic variants was analyzed in a previously described xenograft versus host disease (GvHD) mouse model, because it supports high-level replication of both virus types in their characteristic target cells (6).

* Corresponding author. Mailing address: Institute of Virology, Erasmus Medical Centre Rotterdam, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands. Phone: (31)-10-4088066. Fax: (31)-10-4089485. E-mail: osterhaus@viro.fgg.eur.nl.

MATERIALS AND METHODS

Animals. XID mice (CBA/HNOlaHsd; Harlan Nederland BV, Zeist, The Netherlands) received total body irradiation with syngeneic bone marrow support. Human peripheral blood mononuclear cells (PBMC) were isolated from the whole blood of HLA B14-matched seronegative individuals by Ficoll gradient. After one wash step, cells were administered intraperitoneally (i.p.) at 3×10^6 to 5×10^6 cells per gram of mouse body weight, which results in an acute GvHD situation within 6 to 14 days (6). After PBMC were administered, CTLs (10^7 per mouse) were injected i.p. together with 10^4 IU of recombinant human interleukin-2. This was repeated every other day in accordance with the optimal dose determined in previous studies of passively transferred CTLs in the HuPBL-SCID mouse model (19).

One hour after reconstitution, mice were challenged with 30 50% mouse infectious doses of the respective HIV-1 or HIV-2 isolates i.p. Six days after grafting and infection of the human PBMC were done, the first signs of the acute GvHD reaction were observed, after which the mice were sacrificed. Cells from the peritoneal lavages were analyzed for viral load using an infectious center test. To this end, the cells were titrated in duplicate starting at 2×10^6 cells per well using fivefold dilution steps and cultured in the presence of HIV-permissive feeder cells. The lowest number of cells required to detect virus by reverse transcriptase assay after 7 days of culture was taken as a measure of the viral load.

Viruses. The HIV strains selected for the present studies were HIV-1 ACH 320.2A.1.2 (molecularly cloned, primary, SI, non-macrophage tropic; in short, HIV-1 #1.2) and HIV-1 ACH 320.2A.2.1 (molecularly cloned, primary, NSI, macrophage tropic; in short, HIV-1 #2.1). These closely related viruses were isolated from participant ACH320 from the Amsterdam cohort studies (ACH) of HIV infection and AIDS in homosexual men, as previously described (3). As a control, HIV-2 RH2 to 5 A10 (biologically cloned, primary, NSI, macrophage-tropic; in short, HIV-2 #RH2–5) from the Rotterdam cohort of HIV-2-infected persons (5) was used. Replication of the viruses in $CD4^+$ T cells has been described previously (17). Primary sequences of the second exon of Rev, which includes the TCC108 epitope, were determined as previously described (16).

CTL clones. Two CTL clones, TCC108 and TCC112, obtained via limiting dilution from participant ACH709 from the ACH, have been described in detail

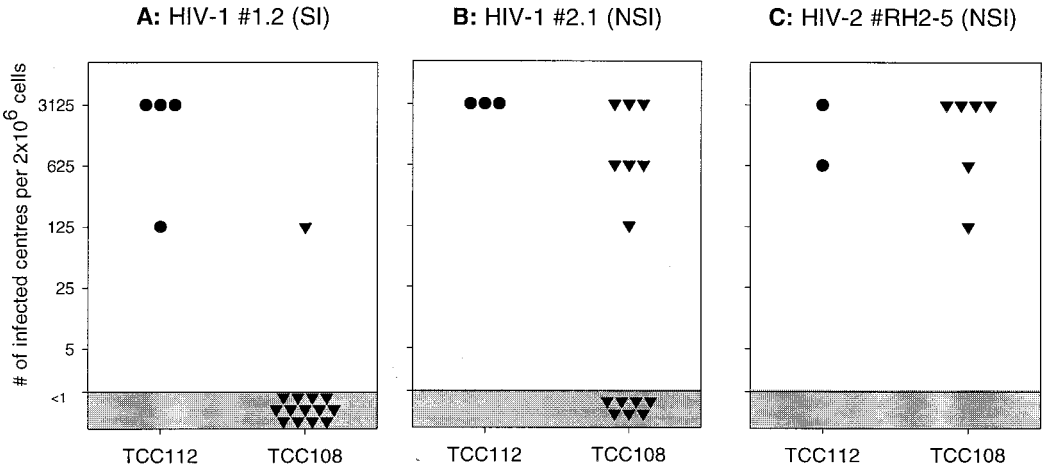

FIG. 1. Viral load determined by an infectious center test in HIV-1-infected (#1.2 [SI]; #2.1 [NSI]) and HIV-2-infected (#RH2–5 [NSI]) GvHD mice that had received either the non-HIV-specific (TCC112) or the HIV-1 Rev-specific (TCC108) $CD8^+$ CTL clone. The lowest amount of cells required to detect virus was taken as a measure of viral load. Each symbol represents the viral load of an individual mouse.

(16, 17). Both clones are $CD4^-$ $CD8^+$ as determined by flow cytometry. TCC112 did not lyse autologous $CD4^+$ T cells (TCL2H7) infected with HIV-1. TCC108 was shown to recognize HIV-1 amino acids 67 to 75 of the Rev protein (SAEP VPLQL) in the context of HLA B14. CTL clones were administered to the mice 7 to 10 days after in vitro stimulation. The presence of TCC108 cells in the lavages and their functionality were determined by flow cytometry and a chromium release assay (15, 17). In vitro CTL assays on autologous B and T cells have been described previously (17). In vitro CTL assays were performed for 4 h at an effector-to-target ratio of 10 to 1 and a peptide concentration of 10 μM.

RESULTS

HIV variants replicate readily in human PBMC in GvHD mice, without suppression by a non-HIV-specific $CD8^+$ clone and with the same tropism as those used in vitro. All viruses in this study established infection in GvHD mice in the presence of non-HIV-specific TCC112 cells: high numbers of HIV-1- or HIV-2-infected cells were reisolated despite the presence of TCC112 cells (Fig. 1), similar to the results from previous studies where no $CD8^+$ cells were added (11). No differences in the viral load were observed for HIV-1 variants #1.2 and #2.1 in this respect (Fig. 1A and B). Combined CD68 immunohistochemistry and HIV RNA in situ hybridization on tissues from GvHD mice showed that $CD68^+$ cells did not contain RNA from SI variant #1.2. By contrast, RNAs from NSI variants #2.1 and #RH2–5 were easily detected in $CD68^+$ cells (18), indicating that these viruses did replicate in macrophages in vivo in accordance with their in vitro tropism (5, 9).

Macrophage-tropic HIV-1 escapes more easily from a Rev-specific CTL clone in vivo. In the presence of HIV-1 Rev-specific CTL (TCC108), clear differences in the numbers of infected cells were observed, depending on the virus used. Replication of HIV-2 #RH2–5, which does not contain the CTL epitope, was not suppressed by the HIV-1 Rev-specific CTLs (Fig. 1C). The non-macrophage-tropic primary isolate HIV-1 #1.2 (SI) was efficiently suppressed in 13 of 14 animals (Fig. 1A). By contrast, high numbers of infected cells were found in 7 of 14 animals infected with the macrophage-tropic primary virus HIV-1 #2.1 (NSI) (Fig. 1B). Regardless of whether virus could be detected in these mice, the numbers of functional TCC108 cells in the peritoneal lavages were comparable in all animals, as determined by flow cytometry and chromium release assays (data not shown).

HIV variants escape from specific CTL pressure by mutations in the minimal epitope. The HIV-1 strains that had been passaged through these GvHD mice (Table 1) were subsequently screened for mutations in Rev. For this purpose, the second exon of Rev, including the minimal epitope for TCC108, was amplified by PCR and sequenced (16). No mutations were observed in viruses passaged through mice which had received the non-HIV-specific TCC112 cells (Table 1). By contrast, viruses that could be recovered from mice despite the

TABLE 1. In vitro characterization of HIV-1 variants after passage in GvHD mice

HIV-1 strain[a]	Origin[b]	Minimal epitope[c]	% Lysis in chromium release assay[d]	% $p55^+$ cells[e]
#1.2nm1	#1.2 + TCC112	SAEPVPLQL	44 ± 2	8
#2.1nm1	#2.1 + TCC112	SAEPVPLQL	44 ± 2	40
#1.2rm1	#1.2 + TCC108	S<u>E</u><u>E</u>PVPLQL	1 ± 4	136
#2.1rm1	#2.1 + TCC108	SAE<u>H</u>VPLQL	4 ± 3	113
#2.1rm2	#2.1 + TCC108	SAE<u>S</u>VPLQL	70 ± 0	28
#2.1rm3	#2.1 + TCC108	S<u>V</u>EPVPLQL	74 ± 4	2
#2.1rm4	#2.1 + TCC108	SAEPVP<u>F</u>QL	NT[f]	
#2.1rm5	#2.1 + TCC108	S<u>L</u>EPVPLQL	NT	
#2.1rm6	#2.1 + TCC108	SAEPVP<u>F</u>QL	NT	
#2.1rm7	#2.1 + TCC108	SAEPVP<u>F</u>QL	NT	

[a] nm, not mutated; rm, Rev mutated.

[b] The virus strains were obtained by short-term coculture of mitogen-stimulated PBMC depleted of $CD8^+$ cells with cells isolated from the peritoneal cavity of GvHD mice.

[c] Predicted amino acid sequence of the minimal epitope is given, and mutations relative to the sequence of the parental strains are underlined. Nine-amino-acid-long peptides were generated on the basis of the predicted amino acid sequences of the minimal epitope of their respective viruses.

[d] Lysis of a B14-matched Epstein-Barr virus-transformed B-cell line pulsed with 10 μM indicated peptide was determined in a chromium release assay as previously described (16).

[e] TCL 2H7 cells were infected with the HIV-1 reisolates and subsequently cultured in the absence (no clone) or presence of TCC108. After 11 days, the percentage of HIV-1 p55-expressing $CD4^+$ cells was determined by flow cytometry. The number of $p55^+$ cells is expressed as a percentage of positive cells compared with $p55^+$ cells in the absence of TCC108 cells (100%).

[f] NT, not tested.

presence of Rev-specific TCC108 cells, all proved to have a mutation in the minimal epitope SAEPVPLQL (Table 1), but not outside the epitope region (not shown). These data indicate that TCC108 cells exerted selective pressure on HIV-1 replication in an antigen-specific and MHC class I-restricted manner.

Some of the escape mutants are no longer recognized in in vitro assays. Nine-mer peptides mimicking the various wild-type or mutant Rev epitopes were tested for in vitro recognition by TCC108, when presented on an autologous B-cell line. Peptides corresponding to the index epitope, as found in HIV-1 #1.2nm1 and HIV-1 #2.1nm1 (wild type for the Rev epitope), were efficiently recognized. Accordingly, virus replication was suppressed when autologous T cells were infected with HIV-1 #1.2nm1 or HIV-1 #2.1nm1 and cultured in the presence of TCC108 (Table 1). HIV-1 #1.2rm1 and HIV-1 #2.1rm1, which could be isolated despite the presence of TCC108 in vivo, were no longer suppressed in these in vitro cultures. As expected, the two peptides corresponding to the TCC108 epitope from these viruses were not recognized in vitro.

Other escape mutants are still recognized in vitro, but not in GvHD mice. Unexpectedly, two other viruses that had escaped in vivo CTL pressure (HIV-1 #2.1rm2 and HIV-1 #2.1rm3) were still suppressed by TCC108 in in vitro cultures (Table 1), despite a mutation in their Rev epitope. Accordingly, the synthetic peptides representing these mutant epitopes were still recognized when presented on autologous B cells (Table 1). To confirm that HIV-1 #2.1rm2 and HIV-1 #2.1rm3 were indeed CTL escape variants in vivo, GvHD mice grafted with HLA B14-matched human PBMC were challenged with HIV-1 #2.1rm2 and HIV-1 #2.1rm3 in the presence of TCC108. Virus could be isolated from all the mice, and no additional mutations were observed, indicating that these viruses had indeed escaped from CTL pressure in vivo (data not shown).

DISCUSSION

Here we have used the GvHD mouse model to study interactions between CTLs and different HIV variants. In contrast to data obtained in the HuPBL-SCID model (19), we found no evidence for non-HLA-restricted suppression of HIV replication (11). As anticipated, replication of viruses containing the wild-type Rev epitope was suppressed by specific CTLs in an HLA-restricted manner and virus could escape from this pressure by mutation of the minimal epitope.

Thus, we defined a model system to study the interactions between CTL and HIV-1 variants, mimicking interactions in early HIV infection. Macrophage-tropic HIV-1 #2.1 was more efficient in escaping CTL pressure than its closely related non-macrophage-tropic counterpart HIV-1 #1.2. Which factors, other than tropism, could have contributed to this more successful escape from CTL pressure? Differences in the fidelity of the reverse transcriptase enzymes of these clones are not a likely explanation, given their overall close relatedness and similarity (4). Furthermore, the primary sequences of the CTL epitope itself and of the flanking regions are identical for these clones (4). This excludes differences in processing and presentation of the epitope for these HIV-1 variants.

How may the macrophage tropism of transmitted viruses contribute to escape from the immune pressure exerted by CTLs? HIV-1 #2.1 could have escaped from CTL pressure more easily if it had more replication cycles to acquire mutations than HIV-1 #1.2, i.e., if infected macrophages were less susceptible to CTL activity than infected T cells. Macrophages migrate easily into peripheral tissues, which may protect them from CTL activity, since CTLs can only affect target cells in their immediate proximity. In addition, T cells and macrophages differ in the expression levels of adhesion molecules, which may also influence the CTL-target cell interaction. Finally, the processing of antigens may differ among cells of different lineages or depend on the activation state of cells (13). This may also help explain the somewhat enigmatic observation that some variants were recognized in T cells in vitro but not in PBMC in vivo.

Irrespective of the mechanism involved, CTLs appear to control wild-type macrophage-tropic virus replication less efficiently than non-macrophage-tropic virus replication in vivo. Reduced pressure on macrophage-tropic variants allows for extra replication cycles, enabling the virus to acquire mutations that help it escape from CTL recognition (1) and establish chronic infection. We therefore propose that macrophages, in addition to acting as a barrier for non-macrophage-tropic HIV-1 variants at the port of entry (12), serve as a sanctuary from CTL activity for macrophage-tropic variants.

ACKNOWLEDGMENTS

We acknowledge the participants of the Amsterdam Cohort Studies on AIDS for their continuous cooperation and the donors of the Rotterdam Blood Bank for providing HLA-typed PBMC. We thank H. Blaak, K. Wolthers, K Stittelaar, and D. J. Serdijn for critical comments and advice during the writing of this paper.

This work was supported by grant 1314 from the Dutch AIDS Foundation. C.G. has a Marie Curie fellowship, grant ERB BMH4-CT-98-5079.

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