Identification of a Novel Human Immunodeficiency Virus Strain Cytopathic to Megakaryocytic Cells

By Myriam S. Kunzi and Jerome E. Groopman

Impaired megakaryocytopoiesis may be a contributing factor to thrombocytopenia associated with human immunodeficiency virus (HIV) infection. Because HIV isolates differ in their host range and pathogenicity, we investigated whether HIV strains with demonstrable cell tropism and increased cytopathicity for megakaryocytes could be derived from the blood of thrombocytopenic HIV-infected individuals. We derived a strain, HIV-WW, from the peripheral blood of an individual with severe thrombocytopenia and found the virus to be highly and specifically cytotoxic to CMK and DAMI megakaryocytic cells. CMK and DAMI cells were not permissive for the virus and HIV-WW induced cytopathicity for these megakaryocytic cells did not depend on viral replication. The CD4 N-terminus-binding domain of the HIV gp120 envelope protein did not appear to be involved in determining the cytopathic phenomenon. HIV may impair megakaryocytopoiesis through interactions at the cell surface in some cases rather than through viral entry and intracellular replication.

MATERIALS AND METHODS

Cells and virus. The DAMI cell line, derived from the peripheral blood of a patient with megakaryoblastic leukemia, was a gift from S. Greenberg (Brigham and Women’s Hospital, Boston, MA). These cells were maintained in Iscove’s modified Dulbecco’s medium (IMDM; GIBCO Laboratories, Grand Island, NY) with 10% heat-inactivated horse serum (HS; GIBCO), penicillin (P; 25 U/ml), streptomycin (S; 250 μg/mL; GIBCO), and L-glutamine (G; 2 mM/L; Mediatech, Washington, DC). The CMK cell line, derived from a child with megakaryoblastic leukemia, was a gift from T. Sato (Chiba University, Japan). H9 (HUT78) cells were obtained from the American Type Culture Collection (Rockville, MD; catalog no. CRL1593). CMK, H9, and U937 cells were maintained in RPMI 1640 (GIBCO), 10% heat-inactivated fetal bovine serum (FBS; GIBCO), and PGM.

HIV-WW virus was obtained from an asymptomatic patient with severe thrombocytopenia. We found that HIV-WW was specifically cytotoxic to megakaryocytic CMK and DAMI cells but not to T-lymphocytic or monocytic cells. The cytotoxic effect on both CMK and DAMI cells did not require HIV-WW replication, suggesting a cytopathic effect at the megakaryocyte cell surface.

From The Mapplethorpe Laboratory for AIDS Research, Division of Hematology/Oncology, Department of Medicine, New England Deaconess Hospital, Harvard Medical School, Boston, MA. Submitted July 2, 1992; accepted February 4, 1993.

Supported by Public Health Service Grants No. HL42112 and HL46668 from the National Institutes of Health.

Address reprint requests to Jerome E. Groopman, MD, Division of Hematology/Oncology, New England Deaconess Hospital, 110 Francis St., 4A, Boston, MA 02215.

© 1993 by The American Society of Hematology.

From www.bloodjournal.org by guest on January 10, 2018. For personal use only.
HIV STRAIN CYTOPATHIC FOR MEGAKARYOCYTES

A 4,000
I100
3,000
1,000
Days post-inoculation

B 4,000
3,000
2,000
1,000
Days post-inoculation

Fig 1. HIV-WW cytopathicity to CMK and DAM1 cells. Cells (2 X 10⁵) were challenged with HIV-WW (100 ng p24 antigen inoculum). Supernatant samples were harvested at the indicated times and assayed for viral p24 antigen. Viral p24 antigen levels are expressed in picograms per milliliter. Supernatant samples from mock-infected cultures were used as negative control. Cell viability was assessed by trypan blue exclusion. Each value represents the average of two independent measurements. This is one representative experiment of five independent experiments. (A) CMK cells. (B) DAM1 cells. (C) Viability: (e) p24.

Fig 2. HIV-WW is not cytopathic to H9 (HUT78) or U937 cells. Cells were challenged with HIV-WW as described in Fig 1. Cell viability was assessed by trypan blue exclusion. Each value represents the average of two independent measurements. (A) H9 cell cultures. (B) U937 cell cultures. (C) Viability: (e) p24.

Polymerase chain reaction (PCR) analysis. Total cellular DNA was extracted as previously described.11,12 HIV sequences were amplified by the PCR twice for 25 cycles, as previously described,11 using nested HIV gag-specific primer pairs. The first primer pair, SK100/SK104,3 amplifies a 280-bp region of gag that is conserved among the HIV-1 isolates and HIV-2 ROD. The second primer pair, SK38/SK39,34 amplifies an 115-bp region of gag conserved among the HIV-1 isolates. DNA isolated from HIV-1 111B-infected cells was used as a positive control and DNA extracted from mock-infected cell was used as a negative control. The specificity of the PCR products was verified by liquid hybridization to the 5'-end radiolabeled HIV-specific probe SK19.31,32 Amplification of β-globin genomic sequences31,32 was carried independently with the primer pair PC03/PC0435 to assess DNA integrity and content. The sensitivity of the PCR assay was determined by dilution of chronically HIV-1-infected THP-1 monocytic cells with uninfected THP-1 cells. The assay is sensitive to 10⁻⁴ dilution of infected cells, corresponding to 100 HIV-1 infected cells in a sample of 10⁶ cells per milliliter.31,32

RESULTS

HIV-WW was recovered from an asymptomatic patient with severe thrombocytopenia and amplified by short-term coculture with PHA-stimulated PBMCs. To assess the tropism of this primary HIV strain, CMK, DAMI, H9, and
U937 cells were challenged at $2 \times 10^5$ cells/well with 300 $\mu$L of virus stock containing 100 ng of HIV p24 antigen. HIV-WW was found to be cytotoxic to both the megakaryocytic Dami and CMK cells, but not to the T-lymphocytic H9 cells or the monocytic U937 cells. The viability of Dami and CMK cells, as assayed by trypan blue exclusion, decreased sharply from above 90% to below 50% within 10 days after exposure to HIV-WW. Total cell death in these cultures was observed within 17 days (Fig 1A and B). Cell viability remained above 90% in control CMK and Dami cultures exposed to an equivalent inoculum of PHA-stimulated, uninfected PBMC supernatant. In contrast, the viability of H9 and U937 cells remained constant and above 90% during the same course of exposure to HIV-WW (Fig 2A and B).

To determine whether the observed cytotoxicity was due to HIV-WW specifically or more generally to exposure to supernatant from HIV-infected PBMCs, we removed virus by filtration or heat-inactivated virus before inoculation. Dami cells were challenged with an equivalent HIV inoculum depleted of virions by filtration twice through a 0.2-$\mu$m cellulose acetate membrane. In another experiment, CMK or Dami cells were challenged with HIV-WW after heat-inactivation of virus at 56°C for 30 minutes. Removal by filtration or inactivation by heating of HIV-WW each restored cell viability in the megakaryocytic cell cultures (Fig 3A and B).

HIV infection in CMK or Dami cells using the laboratory strains HIV-1 IIIB or HIV-2 ROD15,36 has not been...
HIV STRAIN CYTOPATHIC FOR MEGAKARYOCYTES

3339

Fig 5. HIV-WW cytopathicity is blocked by neutralizing serum. CMK or DAMI cells were challenged with HIV-WW, as described in Fig 1, in the absence (●) or presence (□) of neutralizing serum (200 μL). Control CMK and DAMI cell cultures were mock-infected (□□) in the presence of neutralizing serum. Cell viability was assessed by trypan blue exclusion. Each value represents the average of two independent measurements. (A) CMK cell cultures. (B) DAMI cell cultures.

shown to result in any loss of cell viability. Therefore, we further investigated whether the cytopathic effect of HIV-WW on megakaryocytic cells was specific to that isolate or a general property of primary HIV-1 isolates. We challenged CMK and DAMI cells with two primary isolates obtained from nonthrombocytopenic patients. HIV-1 JR-CSF, a PBMC tropic isolate, was isolated from the cerebrospinal fluid of a patient with AIDS dementia27,28 whereas HIV-RJ9435 was recovered from an asymptomatic HIV-seropositive individual with neutropenia. Like HIV-WW, these two viruses were amplified by short-term culture in PHA-stimulated PBMCs. CMK, DAMI, H9, and U937 cells were exposed to a 300-μL inoculum of HIV-1 JR-CSF or HIV-RJ9435 under the same conditions used with HIV-WW. Neither HIV-1 JR-CSF nor HIV-RJ9435 induced cytotoxicity in CMK or DAMI cultures (Fig 4A and B). Cell viability in H9 and U937 cultures remained above 90% (data not shown). Thus, the cytotoxicity to megakaryocytic cells of inocula derived from HIV-WW-infected PBMC cultures was specific to that virus and not seen with two other primary isolates from nonthrombocytopenic patients. In addition, we found that cytotoxicity was abrogated (Fig 5A and B) when CMK and DAMI cells were challenged with HIV-WW in the presence of a broadly neutralizing serum from an HIV-seropositive donor. This serum was capable of neutralizing under the same conditions both HIV-1 JR-CSF and HIV-RJ9435 infections in PBMCs to 82% and 91%, respectively.

We then investigated whether viral replication played a role in the observed cytotoxicity. CMK or DAMI cells were exposed to HIV-WW in the presence of 250 nmol/L, 500 nmol/L, and 1 μmol/L 3'-azido-3' deoxythymidine (AZT) to inhibit viral replication. Treatment with AZT did not protect CMK or DAMI cells from the cytopathic effect of HIV-WW. AZT was not toxic to the megakaryocytic cells, as less than 10% cell death was observed in the absence of virus in control cultures treated with AZT at the same concentrations.

Fig 6. HIV-WW cytopathicity is not blocked by anti-Leu3A. CMK and DAMI cells were challenged with HIV-WW, as described in Fig 1, in the absence (●) or presence (□□) of the CD4-specific monoclonal antibody anti-Leu3A at 5 μg/mL. Control CMK or DAMI cell cultures were mock-infected (□□) in the presence of anti-Leu3A at the same concentration. (A) CMK cell cultures. (B) DAMI cell cultures.
Early events of viral integration and replication were studied by PCR analysis of total cellular DNA isolated at 2, 24, 48, and 72 hours postinoculation. To ensure detection of HIV-WW, despite possible heterogeneity at the molecular level, we selected a very highly conserved region of the HIV genome for amplification, using two gag-specific nested pairs of primers. Although the β-globin gene, used as an internal standard, was detected in equal amount in each of the amplification reactions, we found no HIV-specific sequences in the DNA isolated from CMK, DAMI, H9, or U937 cells. Thus, the megakaryocytic CMK and DAMI cells were not permissive for HIV-WW and the megakaryocyte-specific cytopathicity of the virus was not the result of viral replication. The sensitivity of the PCR assay was such that 1 infected cell in 10⁶ cells could be detected. Viral infection, therefore, likely did not occur, or at least occurred at a level below this level of detection and would not explain the observed cytopathic effects. The small amounts of HIV p24 antigen detected early in culture thus represented residual inoculum.

The binding of the viral envelope glycoprotein to the cellular CD4 receptor is a first and critical step in HIV infection. We therefore investigated whether anti-Leu3A, a CD4-specific monoclonal antibody, could inhibit HIV-WW cytotoxicity by blocking the binding of gp120 to the N-terminus of CD4. Cells were preincubated with antibody (5 μg/mL) before inoculation with virus. Anti-Leu3A monoclonal antibody did not abrogate HIV-WW-induced cytopathicity in CMK or DAMI cultures (Fig 6A and B). In contrast, 90% of the cells were viable in control mock-infected CMK cultures with or without anti-Leu3A antibody. These findings indicate that the gp120 epitope that binds to the CD4 N-terminus was not a determinant in HIV-WW cytopathicity to megakaryocytic cells. Moreover, binding to the CD4 N-terminus was not sufficient in itself to trigger megakaryocytic cell death, because binding with anti-Leu3A antibody to CD4 did not result in cytopathicity.

DISCUSSION

Impaired megakaryocytopoiesis may be a contributing factor to the thrombocytopenia associated with HIV infection. The presence of the HIV genome in bone marrow megakaryocytes of some seropositive patients has been reported. Primary marrow megakaryocytes have a life span of a few days in culture, thus limiting the feasibility of studies of HIV infection in primary cells. Therefore, experiments have been performed in cell lines of megakaryocytic lineage to establish a model of retrovirus-megakaryocyte interactions. However, such studies have been performed to date using laboratory strains of HIV, such as HIV-1 HXB and HIV-2 ROD. The properties of laboratory strains of HIV may differ significantly from primary isolates, as was recently shown by the failure of soluble CD4 to neutralize primary isolates in vitro at concentrations that effectively blocked infection with laboratory strains of HIV-1.

We derived an HIV strain from a patient with severe thrombocytopenia and found the virus, HIV-WW, to be highly and specifically cytotoxic to megakaryocytic cells. In contrast, two other primary HIV-1 isolates not associated with clinical thrombocytopenia were not cytotoxic to the megakaryocytic CMK or DAMI cells. Most strains derived directly from HIV-infected patients by short-term culture in PBMCs are not capable of infecting productively permanent cell lines, whereas laboratory isolates do replicate in such cell lines. Consistent with this finding, the megakaryocytic CMK and DAMI cell lines were incapable of sustaining HIV-WW replication. PCR amplification detected no HIV viral sequences in these cells after challenge with HIV-WW, with the sensitivity of the PCR assay at 1 infected cell in 10⁶ cells. Similarly, treatment with AZT to block replication did not protect the cells from the cytopathic effect of HIV-WW. Despite the absence of replication, the cytopathic properties of HIV-WW were dramatic and specific for cells of megakaryocytic origin and not seen in cells of T-lymphocytic or monocytic origin.

There is considerable heterogeneity in cellular tropism, cytopathicity, and kinetics of replication among the various strains of HIV. Although the viral gene products nef, tat, vif, vpr, vpu, and vpx may play an important role in controlling infectivity and viral replication, mounting evidence points to the envelope glycoprotein as a key determinant of cellular tropism and cytopathicity. Arguably, a low level of HIV envelope components may have been retained in the filtrated viral inoculum and, indeed, we observed that, although cell viability was restored to a great extent, cytopathic effects were not entirely abrogated after filtration. We found, as well, that HIV-WW-induced cytopathicity in megakaryocytic cell lines was not abrogated by anti-Leu3A monoclonal antibody, thereby indicating that the domain of the HIV gp120 envelope protein that binds to the N-terminus of CD4 was not involved in this phenomenon. Recently, regions of gp120 distinct from the CD4-binding domain have been shown to be critical in determining cell tropism, thus suggesting that interactions between several distinct envelope domains may be necessary for viral entry. Identification of the molecular determinants of viral phenotype is essential to an understanding of the pathogenesis of HIV. To this end, we have constructed a genomic library from HIV-WW infected PBMCs to isolate a clone of this novel HIV strain. Studies of its genotype and, in particular, a detailed analysis of the sequence and structure of its envelope glycoprotein should provide insights into the mechanism of its cytopathicity for megakaryocytic cells. Moreover, these studies may show megakaryocyte surface structures distinct from CD4 that specifically interact with components of the viral envelope.

ACKNOWLEDGMENT

We thank Dr S.M. Greenberg for providing us with DAMI cells and Dr T. Sato for CMK cells. We are grateful to H. Chhay and Dr R.A. Byrn for their assistance.

REFERENCES

HIV STRAIN CYTOPATHIC FOR MEGAKARYOCYTES


19. Feno EM, Albert J, Asjo B: Replicative capacity, cytopathic effect and cell tropism of HIV. AIDS 3:55, 1989 (suppl 1)


megakaryocytic cells is enhanced by tumor necrosis factor-α. Blood 79:2670, 1992

37. Daar 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 87:6574, 1990


Identification of a novel human immunodeficiency virus strain cytopathic to megakaryocytic cells

MS Kunzi and JE Groopman