CD4+ T-Lymphocyte Depletion in Human Lymphoid Tissue Ex Vivo Is Not Induced by Noninfectious Human Immunodeficiency Virus Type 1 Virions

A. W. Sylwester, J.-C. Grivel, W. Fitzgerald, J. L. Rossio, J. D. Lifson and L. B. Margolis

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A. W. SYLWESTER, J.-C. GRIVEL, W. FITZGERALD, J. L. ROSSIO, J. D. LIFSON, AND L. B. MARGOLIS

Laboratory of Cellular and Molecular Biophysics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892-1855, and Laboratory of Retroviral Pathogenesis, AIDS Vaccine Program, SAIC Frederick, Frederick, Maryland 21702-1201

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We tested infectious human immunodeficiency virus type 1 (HIV-1), noninfectious but conformationally authentic inactivated whole HIV-1 virions, and purified gp120 for the ability to induce depletion of CD4⁺ T cells in human lymphoid tissues ex vivo. Infectious CXCR4-tropic HIV-1, but not matched inactivated virions or gp120, mediated CD4⁺ T-cell depletion, consistent with mechanisms requiring productive infection.

The critical events in human immunodeficiency virus (HIV) disease occur in human lymphoid tissue, where HIV infection is associated with a cumulatively massive depletion of CD4⁺ T lymphocytes. Since HIV was first cultured, it has been known that lytic infection can directly kill infected cells in vitro. Other killing mechanisms, both direct and indirect, have also been proposed (1, 4, 5, 7, 11-14, 16, 18-23, 26-30). It remains controversial, however, whether and to what extent uninfected cells are killed. It has been proposed that some indirect cell killing may be mediated by interactions between bystander cells and HIV surface molecules (2, 6, 15, 17, 24, 26, 31). We combined a unique ex vivo culture system and a novel means of inactivating HIV type 1 (HIV-1) to test whether depletion of CD4⁺ T cells in human lymphoid tissue depends on viral replication or can be triggered by virions or viral proteins, even in the absence of productive infection.

We have shown previously that blocks of human lymphoid tissue cultured ex vivo support productive infection with HIV-1 without exogenous stimulation (8-10). In this system, infection with T-cell/CXCR4-tropic (X4) (3) but not with macrophage/CCCR5-tropic (R5) (3) HIV-1 isolates results in CD4⁺ T-lymphocyte depletion (9). We recently described inactivation of the infectivity of HIV-1 by a novel method which preserves the conformational and functional integrity of virion surface proteins (25). Treatment of virions with 2,2'-dithiodipyridine (aldrithiol-2 [AT-2]; Sigma Chemical Co., St. Louis, Mo.) (100 mM for 1 h; 37°C) inactivates virions by covalent disruption of zinc fingers in the viral nucleocapsid protein (25), required in multiple steps of the viral life cycle. Afterwards, AT-2 is diluted 27,000-fold from treated virions with a centrifugal concentrator with a 500-kDa-cutoff membrane (Amicon, Beverly, Mass.). Experiments with virus-free culture medium spiked with AT-2 showed that the residual concentration of AT-2 did not mediate detectable effects on histoculture cell viability, HIV replication, or B-cell function (as assayed by trypan blue exclusion, HIV p24 enzyme-linked immunosorbent assay [ELISA], or total immunoglobulin G ELISA, respectively.)

We compared X4-tropic infectious virus, AT-2 inactivated virions, and purified gp120 for the ability to deplete CD4⁺ T cells from ex vivo human tonsil histocultures. HIV-1 strain LAV.04 and purified HIV-1 gp120 (glycosylated or nonglycosylated, from X4 strains LAV.04 and SF2) were obtained through the AIDS Research and Reference Reagent Program, and HIV-1 strain LAI (HIV-1) was obtained from the AIDS Vaccine Program (National Cancer Institute, Frederick, Md.). AT-2 treatment eliminated detectable infectivity from virus.

FIG. 1. Inactivated and infectious HIV in cultures of human lymphoid tissue ex vivo. The solid line shows accumulation of virus in culture medium of tissue productively infected with HIV-1 LAV.04. Data are the average of 18 experiments. Infection with HIV-1 IIB in four experiments gave a similar average curve and resulted in similar CD4⁺ T-cell depletions. The stepped dashed line shows the addition of inactivated virus to culture medium every third day to approximate the productive infection curve. The straight dashed line shows the addition of a constant amount of inactivated virus to culture medium every third day to approximate the peak of productive infection.
demonstrated both by titrations with peripheral blood mononuclear cells, confirming an earlier report (25), and by the finding that tonsil tissues inoculated with inactivated virus did not secrete p24 into culture supernatants (measured by ELISA [AIDS Vaccine Program]).

Tonsil cultures were inoculated with either live or inactivated LAV.04 or LAI (IIIB). Infectious virus was applied to 2-mm blocks of human tonsil tissue as a single dose on day 1 of culture, with approximately 400 50% tissue culture infectious doses per block, as described previously (8). The kinetics of HIV replication in this culture system are shown in Fig. 1. Inactivated virus was applied to cultures by either of two protocols. In one, inactivated virus was applied on day 1 and every third day in amounts chosen to approximate the typical growth of viral infection with the R5 virus isolate SF162, which itself does not deplete CD4<sup>+</sup> T cells (9).

Activation of HIV-1 by AT-2 results in noninfectious virus that is capable of authentic binding to and fusion with target cell membranes but incapable of initiating reverse transcription (25). Such virions should be capable of mediating many of the interactions with cell membranes presumed to be responsible for indirect mechanisms of CD4<sup>+</sup> T-cell loss (2, 6, 7, 12, 27). It is conceivable that indirect mechanisms of cell killing require concentrations of virus higher than those examined here and that such higher concentrations might occur in microenvironments directly adjacent to productively infected cells. However, no depletion of CD4<sup>+</sup> T cells was observed, even with exposure to a 100-fold-increased concentration of inactivated virus, which simulated retention of the equivalent of 3 days of peak virus production entirely within the tissue. The absence of CD4<sup>+</sup> T-cell depletion under even these extreme conditions strongly implies that CD4<sup>+</sup> T-cell killing requires productive HIV infection in ex vivo tonsil histocultures and, by inference, in human lymphoid tissue in vivo.

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