CONCISE COMMUNICATION

In Vivo Cytolysis and Fusion of Human Immunodeficiency Virus Type 1–Infected Lymphocytes in Lymphoid Tissue

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Lymphoid tissue was examined to see whether in vivo cytopathic effects of human immunodeficiency virus (HIV) infection on lymphocytes could be detected. Transmission electron microscopy of mechanical suspensions prepared from lymph nodes showed both replication and phagocytosis of HIV particles by macrophages. Phagosomes contained cellular debris and virions, some of which were undergoing digestion. Neutrophils also contained HIV particles intermixed with cellular debris in phagosomes. Immunohistochemistry revealed whole Gag p24-positive lymphocytes and p24-positive cellular debris within the cytoplasm of paraacortical macrophages. Lysing p24-positive lymphocytes were also seen. In the paracortex, p24-positive multinucleated lymphocytes with up to 5 nuclei were seen. In situ hybridization for HIV RNA in combination with immunohistochemistry for HAM56, a macrophage-specific marker, revealed colabeled cells. Thus, HIV-positive lymphocytes undergo lysis in lymphoid tissue. The cellular debris is phagocytized by macrophages, which themselves can replicate HIV. HIV-positive lymphocytes fuse in lymph nodes to form multinucleated cells.

Materials and Methods

Immunohistochemistry. Immunohistochemistry was carried out for HIV Gag p24 protein on 5 lymph nodes and 1 tonsil as follows.

Formalin-fixed paraffin sections were cut at 3 μm, mounted on silanated slides, dried at 58°C for 1 h, and deparaffinized through xylene and graded alcohol to water. Sections were pretreated with microwave antigen retrieval in 10 mM citrate buffer for 3 cycles of 4 min each at 70°C. After cool-down and a water rinse, the slides were treated with 3% hydrogen peroxide in methanol for 15 min, followed by a water rinse. Protein blocking was done for 30 min, followed by incubation with mouse anti-HIV p24, clone Kal-1 (Dako, Carpinteria, CA) at a dilution of 1:20 for 30 min at room temperature. Slides were then rinsed in PBS containing 0.05% Tween 20 for a total of 15 min. Horseradish peroxidase–conjugated goat anti-mouse secondary antibody (Dako) was applied at 1:100 for 30 min at room temperature. Slides were then rinsed in PBS-Tween for at least 15 min, visualized with 3,3′-diaminobenzidine chromogen (Dako), and counterstained with hematoxylin. Appropriate HIV-positive and -negative controls were included with each staining assay.

In situ hybridization. In situ hybridization for HIV-specific RNA was done as previously described, alone and in combination with immunohistochemistry for the macrophage-specific marker HAM56, on 4 lymph nodes [10, 11]. Transmission electron microscopy. Crude mechanically disrupted suspensions of 5 lymph nodes were fixed in glutaraldehyde, pelleted, gelled in agar, postfixed in OsO4, dehydrated in graded ethanol and propylene oxide, and embedded in Spurr’s epoxy. One-micron semithin plastic sections were cut and stained with the combined methylene blue, azure II, basic fuchsin stain for light microscopic selection of blocks to be thinned for transmission electron microscopy of mechanical suspensions prepared from lymph nodes showed both replication and phagocytosis of HIV particles by macrophages. Phagosomes contained cellular debris and virions, some of which were undergoing digestion. Neutrophils also contained HIV particles intermixed with cellular debris in phagosomes. Immunohistochemistry revealed whole Gag p24-positive lymphocytes and p24-positive cellular debris within the cytoplasm of paraacortical macrophages. Lysing p24-positive lymphocytes were also seen. In the paracortex, p24-positive multinucleated lymphocytes with up to 5 nuclei were seen. In situ hybridization for HIV RNA in combination with immunohistochemistry for HAM56, a macrophage-specific marker, revealed colabeled cells. Thus, HIV-positive lymphocytes undergo lysis in lymphoid tissue. The cellular debris is phagocytized by macrophages, which themselves can replicate HIV. HIV-positive lymphocytes fuse in lymph nodes to form multinucleated cells.

An unrelenting decline in CD4 lymphocytes is the essence of human immunodeficiency virus (HIV) disease [1, 2]. One of the means by which HIV is thought to kill CD4 cells in the host is by viral cytolysis, a phenomenon that is readily documented in vitro. Surprisingly, there is no published visual proof that HIV virions actually occurs in vivo. There is also no direct in vivo evidence that HIV-positive cells in the host are targeted and killed by cytotoxic T cells, another mechanism readily demonstrated to occur in vitro. Furthermore, despite ample evidence for the formation of HIV-positive multinucleated macrophages in vivo, in the central nervous system, tonsils, and adenoids, there is no evidence that CD4 lymphocytes fuse in vivo, as they do in vitro, to form syncytial giant cells that go on to lyse [3–5]. Considering the amount of virus production and the number of HIV-infected CD4 lymphocytes that are estimated to turn over on a daily basis, it is surprising that histologic evidence for these phenomena are lacking in the host [6–9]. Thus, lymphoid tissue was examined to see whether cytopathic effects of HIV infection on lymphocytes can be detected in vivo.
microscopy. Thin sections were prepared, stained with uranyl acetate and lead citrate, and viewed on an LEO EM10 transmission electron microscope (LEO Electronmicroscopy, Thornwood, NJ) at 60 kV [10].

Results

Cells expressing Gag p24 protein. Lymphoid tissue from HIV-positive patients varied widely in the number of mononuclear cells expressing HIV Gag p24 protein and HIV RNA (figure 1A). Gag p24-positive lymphocytes were present within both germinal centers, where they cytologically resembled other activated follicular cells, and within the paracortex, where they varied from small to large. Gag p24-positive cells were rarely seen in subcapsular or medullary sinuses. The smaller p24-positive lymphocytes had a high nuclear-to-cytoplasmic ratio, centrally located round nuclei, and considerable heterochromatin. The large p24-positive lymphocytes had a lower nuclear-to-cytoplasmic ratio, irregular nuclei with relatively little heterochromatin, and conspicuous nucleoli. p24 staining of lymphocytes varied from a light brown blush to a dense cytoplasmic ring of positivity to complete obscuring of the entire cell cytoplasm and nucleus (figure 1B). Some p24-positive lymphocytes appeared to be undergoing cytolysis, as indicated by their fragmentation into multiple small p24-positive pieces of cytoplasm (figures 1B–1D). No p24-positive apoptotic cells were identified.

Large p24-positive cells were seen with eccentric irregular nuclei characteristic of macrophages (figures 1E, 1F). p24-positive macrophages were seen in close proximity to and occasionally within the lumen of paracortical blood vessels. Multinucleated p24-positive lymphocytes with up to 5 nuclei were identified in the paracortex (figures 1G, 1H).

Macrophages containing phagocytized p24-positive material. Phagocytic paracortical macrophages with abundant cytoplasm and large open pleomorphic nuclei contained p24-positive material in their cytoplasm (figure 2A). In addition to the debris, there were whole internalized p24-positive lymphocytes within macrophages (figures 2B, 2C). The phagocytized particulate p24-positive material was scattered throughout the cell cytoplasm. Occasional macrophages containing p24-positive debris were also seen within vessels.

Although tingible body macrophages in the germinal centers were regularly seen containing apoptotic cells, the latter were never p24-positive. The tingible body macrophages did contain granular p24-positive material in their cytoplasm, but less than was seen in paracortical macrophages (figure 2D).

Cells colabeled for HIV RNA and HAM56. Paracortical cells colabeled for HIV RNA expression and HAM56 antigen, an immunohistochemical macrophage marker (figures 2E, 2F).

Transmission electron microscopy. Macrophages were relatively abundant in some lymph node cell suspension specimens. They had irregular ruffled surfaces, eccentric kidney-shaped nuclei, prominent Golgi zones, subplasmalemmal linear densities, and phagosomes containing cellular debris. Mature HIV virions were found intermixed with cellular debris in macrophage phagosomes. There was occasionally more than 1 virion in a single vacuole, as well as more than one vacuole containing virions in the cytoplasm of the same cell. Mature virions with indistinct morphology were encountered in phagosomes and likely represented particles undergoing lysosomal digestion. Mature and rare budding virions were also seen in Golgi vacuoles that were free of cellular debris, consistent with viral replication by macrophages.

Neutrophils were identified that contained phagosomes with mature virus particles intermixed with cellular debris. A single lysing neutrophil contained several phagosomes containing debris and virions. Occasional lymphocytes had budding and free mature particles associated with their plasma membranes. No virus was seen associated with apoptotic lymphocytes, either free or located within tingible body macrophages. Many interdigitating dendritic cells with Birbeck granules were present in the suspension preparations; however, no HIV particles were seen associated with their surface or within their cytoplasmic vacuoles. A few bi- and trinuclear lymphocytes were identified in the suspension preparations, but they lacked HIV particles.

Discussion

There are 2 possible, but not mutually exclusive, sources for the p24-positive debris identified in the paracortical macrophages: phagocytized material from lysing HIV-infected lymphocytes or complement- and antibody-bound virus complexes to germinal center follicular dendritic cell processes. The most direct evidence that HIV-infected lymphocytes are a likely source of phagocytized material comes from the identification of both whole p24-positive lymphocytes and p24-positive cellular debris within macrophages. Additional support comes from the identification of p24-positive lymphocytes actually undergoing fragmentation, infected lymphocytes in close proximity to the positive phagocytic cells, and the identical immunohistochemical staining quality of the p24-positive lymphocytes, macrophages, and phagocytized debris.

Further support for the hypothesis that HIV-positive cells are a likely source of p24-positive material comes from the transmission electron microscopic finding of phagocytized cellular debris intermixed with HIV particles within macrophage phagosomes. This finding correlates with the punctate nature of the immunohistochemical staining of the p24-positive debris in the cytoplasm of macrophages. The HIV-expressing macrophages that were seen here, and previously in the tonsil and adenoid studies, are p24-stained throughout their cytoplasm. This is in contrast with the phagocytized material in macrophages, which has a particular appearance and is often concentrated in the Golgi region [3].

The turnover of p24 antigen on processes of follicular den-
Figure 1.  
A, In situ hybridization for human immunodeficiency virus (HIV) RNA of lymph node rich in both HIV-positive mononuclear cells (arrows) and germinal centers (arrowheads). Magnification, ×25.  
B–D, Lymphocytes in the act of lysing and shedding pieces of their p24-positive cytoplasm (arrows); 2 lymphocytes in B are almost entirely obscured by p24 staining (arrowheads). E, F, 2 large p24-positive cells with complicated nuclei (arrows), consistent with macrophages; lymphocyte in panel E is totally obscured by p24 stain (arrowhead). G, H, p24-positive multinucleated lymphocytes containing 3 and 5 nuclei, respectively. Magnifications in panels B–H, ×800.
Figure 2. A. Particulate p24-positive debris in cytoplasm of 2 macrophages. Note large irregular macrophage nuclei (arrows). B, C. At least 1 (B) and clearly 2 (C) p24-positive lymphocytes phagocytized by macrophages (arrows). Macrophage nuclei are irregular and eccentrically located (arrowheads). Vessel (B, lower right) is in close proximity to macrophage. D. Particulate (arrow) and diffuse (arrowheads) p24-positive material within cytoplasm of tingible body macrophage. E, F. Macrophages colabeled for HAM56 by immunohistochemistry (brown) and human immunodeficiency virus RNA by in situ hybridization (black). All magnifications, ×800.

Dendritic cells is a relatively slow process, measured in months or even years [11, 12]. The fate of this material is unclear. The relatively little p24-positive material seen within tingible body macrophages could account for at least some of it, considering its long half-life. It is also possible that some of the antigen finds its way both into paracortical macrophages and into the circulation. The fate of the HIV-positive germinal center mononuclear cells is also unclear, although some could end up in paracortical macrophages; others could be phagocytized by tingible body macrophages and slowly digested.

Multinucleated lymphocytes are the defining in vitro feature of syncytium-inducing HIV virotypes. It has been speculated that the Warthin-Finkeldey-type giant cell common to hyperplastic lymph nodes from HIV-infected patients might be the in vivo counterpart of the in vitro phenomenon [13]. However, it was recently demonstrated not only that these syncytial giant
cells are not expressing HIV but that they are most likely of follicular dendritic cell rather than of lymphocyte origin. They likely result from a focal contraction or collapse of the follicular dendritic cell network. Without the p24 immunohistochemistry used in this study, the multinucleated cells in these lymph node preparations would not have been appreciated. The preservation of the cytologic detail in these stains allowed us to delineate multinucleated cells with up to 5 nuclei. The relevant questions should perhaps be why multinucleated cells are not seen more often and why they are relatively small compared with what is seen in vitro. Perhaps such cells are very unstable and lyse before being able to reach the larger sizes seen in vitro.

This is the first direct visual proof that cytolysis of HIV-infected lymphocytes occurs in vivo in lymphoid tissue and that the debris can be phagocytized by macrophages. It is also the first direct evidence that HIV-positive lymphocytes fuse in vivo to form small multinucleated cells.

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References


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