

THE IMPACT OF TREATMENT ON THE PRODUCTION AND STORAGE OF HIV IN LYMPHOID TISSUE

Clinicians who manage the care of HIV-infected individuals rely primarily on the peripheral CD4+ lymphocyte count and the level of plasma HIV RNA to monitor the effects of antiretroviral treatment. However, virus and CD4+ cells in the peripheral circulation represent only the tip of the iceberg. Most HIV viral replication and the resulting changes in the immune cell populations occur in the lymphoid tissues. At the San Francisco course Ashley T. Haase, MD, reviewed the research conducted in his laboratory of the kinetics of HIV and immune cell populations in the tissues of HIV-infected individuals before and after antiretroviral treatment.

Interactions Between HIV and Host Cells

Characteristics of Retrovirus Infection

Retroviruses are so named because during viral infection genetic information flows from RNA to DNA. The HIV genome is composed of RNA, and the viral-encoded enzyme reverse transcriptase catalyzes the production of DNA provirus from the viral RNA template. From the provirus stage there are two possible outcomes, integrated nonproductive infection and productive infection. In latent infection there are nearly undetectable amounts of RNA within the cell, but viral genetic information persists as proviral DNA integrates into the host genome. There is little if any viral gene expression. In productive infection there is extensive viral gene expression. The cell produces thousands of copies of viral RNA, millions of copies of viral capsid protein, and hundreds of HIV virions. Productive retrovirus infection is associated with disease as infected cells die by a number of mechanisms; latent infection is sub-clinical.

In the HIV-infected individual, many cells harbor provirus in a silent state.

These latently-infected cells can become productively-infected and produce large amounts of virus, or with limited gene expression can express sufficient viral protein that the cell becomes a target for cytotoxic lymphocytes. The cells that remain latently infected will eventually die, but this may take years. Latent infection may involve lymphocytes, macrophages, and perhaps other cell types.

The Storage Pool for HIV in Lymphoid Tissue

Another mechanism of persistence has been described based on in situ studies of lymphoid tissue. In the lymph node germinal centers the follicular dendritic cells (FDCs) continue to act as antigen repositories that activate B lymphocytes to develop and secrete antibodies. In the HIV-infected individual the FDCs contain large numbers of HIV virions coated with antibodies, including neutralizing antibodies, that essentially form immune

complexes. These virion-antibody complexes are attached in large numbers extracellularly to the FDCs processes by complement and complement receptors.

The FDCs with the attached HIV particle immune complexes constitute a large storage pool of virus. In addition, FDCs are themselves infected and have the capacity to reactivate a productive infection. Thus the germinal centers of lymph node is a large storage reservoir of HIV, as well as a source of productive HIV infection.

Kinetics of HIV Infection

Within the lymphoid tissue of the HIV-infected individual there is a virtual HIV factory (Figure 1). The main production of HIV occurs in productively-infected T-cells and macrophages. The source of these cells is the bone marrow/thymus progenitor pool and the peripheral precursor pool undergoing self-renewal. Some virus is derived from the latently-infected

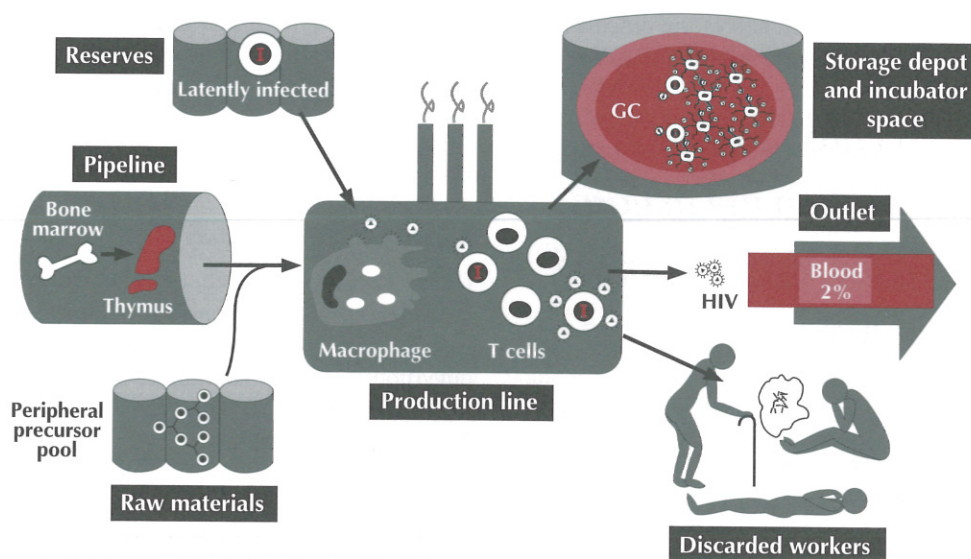


Figure 1. A schematic depicting the source of HIV and infected cell, and the eventual fate of virus and cells during HIV infection.

cell pool as these cells are activated and productively become infected. Mature HIV particles are stored in the FDCs and some move into the blood. A large number of infected cells die either directly as a consequence of HIV infection, or secondary to apoptosis.

In Situ Techniques

Considerable progress has been made in understanding the kinetics of HIV by using in situ hybridization. This technique involves a radioisotope-labeled probe, which has a sequence that is specific for the target RNA. Tissue sections are made using standard histologic techniques of thin slicing of tissue, and the tissue sections are reacted with the probe. A photographic emulsion is laid over the section and where probes have reacted with target RNA, the radioisotope which causes silver grains to form in the emulsion. When developed, the exposed grains overlying the target sequences are visible.

Immunohistochemistry involves a similar process wherein the probe consists of an antibody specific for an antigen such as the CD4 molecule on the cell membrane. The antibody is tagged with an enzyme or a fluorescent marker, and upon reacting the marker with substrates or light, those cells that have the target antigen are stained.

Distribution of HIV in Lymphoid Tissue

HIV particles attached to the processes of the FDC are quite spread out along the

In chronic HIV infection the majority of the virus is located in lymphoid germinal centers, associated with follicular dendritic cells.

surface of these large cells and so in autoradiographs show a diffuse pattern of hybridization signal. Productively-

infected cells produce large numbers of virions within the small area of one cell, resulting in a focal hybridization pattern. In the HIV-infected individual, lymphoid tissue stained with probe specific for HIV will show both of these patterns (Figure 2).

The number of copies of viral RNA can be quantified by counting the silver grains in an autoradiograph. A camera and a computer are used to count the grains and calculate the copies of RNA in a given area of tissue. By further calculation involving the volume of specific tissues, the number of HIV virions within a single lymph node or in the entire lymph system can be estimated.

Quantity of HIV in Infected Individuals

The lymphoid tissue carries approximately 98% of the viral burden. There are about 10^8 viral RNA copies per gram of lymph tissue or about 10^{11} copies in the entire body, most of which is associated with the FDCs. Only approximately 2×10^9 copies are found in the productively-infected mononuclear cell pool of macrophages and T-lymphocytes. Thus the vast majority of tissue-associated virus is HIV that is already produced and associated with FDCs in the germinal centers.

Effects of Antiretroviral Treatment on HIV Kinetics

Results of a Clinical Study

Dr Haase next described a clinical study involving antiretroviral naive HIV-infected individuals who started zidovudine/lamivudine/ritonavir. Tonsillar samples for biopsy were taken at baseline (pretreatment), day 2, week 3, and week 24.

Triple-therapy resulted in a rapid decline in plasma HIV RNA levels by more than 2 logs, and in many patients the plasma RNA levels fell below the detection limit of the assay. Figure 3 shows the changes seen in autoradiographs of tonsillar tissue sections taken at

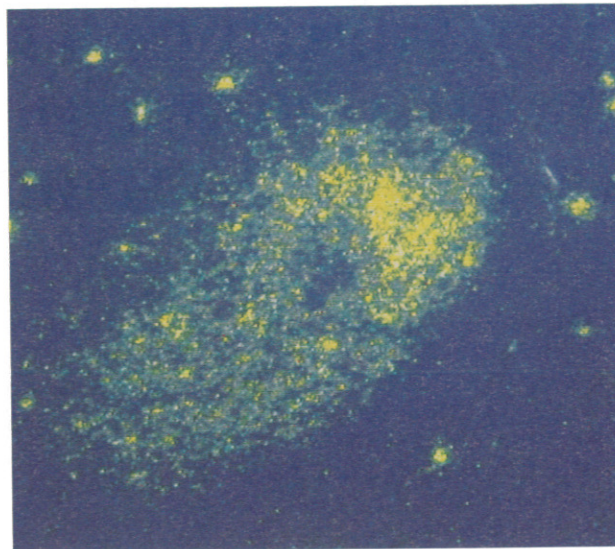


Figure 2. Autoradiograph of a section of lymph node from an individual chronically infected with HIV. The diffuse bright pattern on the right represents HIV virions stored in the follicular dendritic cells in the germinal center. The discrete bright patches on the left represent individual foci of productively infected cells.

baseline and at week 3 of treatment. Before treatment (Figure 3a) there is a large, diffuse pattern of grains representing the FDC-associated virus in the follicles, as well as areas of discrete, focal

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signal in the interfollicular area representing productively-infected mononuclear cells. After 3 weeks of therapy there was a marked reduction in viral burden in the tonsil (Figure 3b). There was a reduction in the productively-infected mononuclear

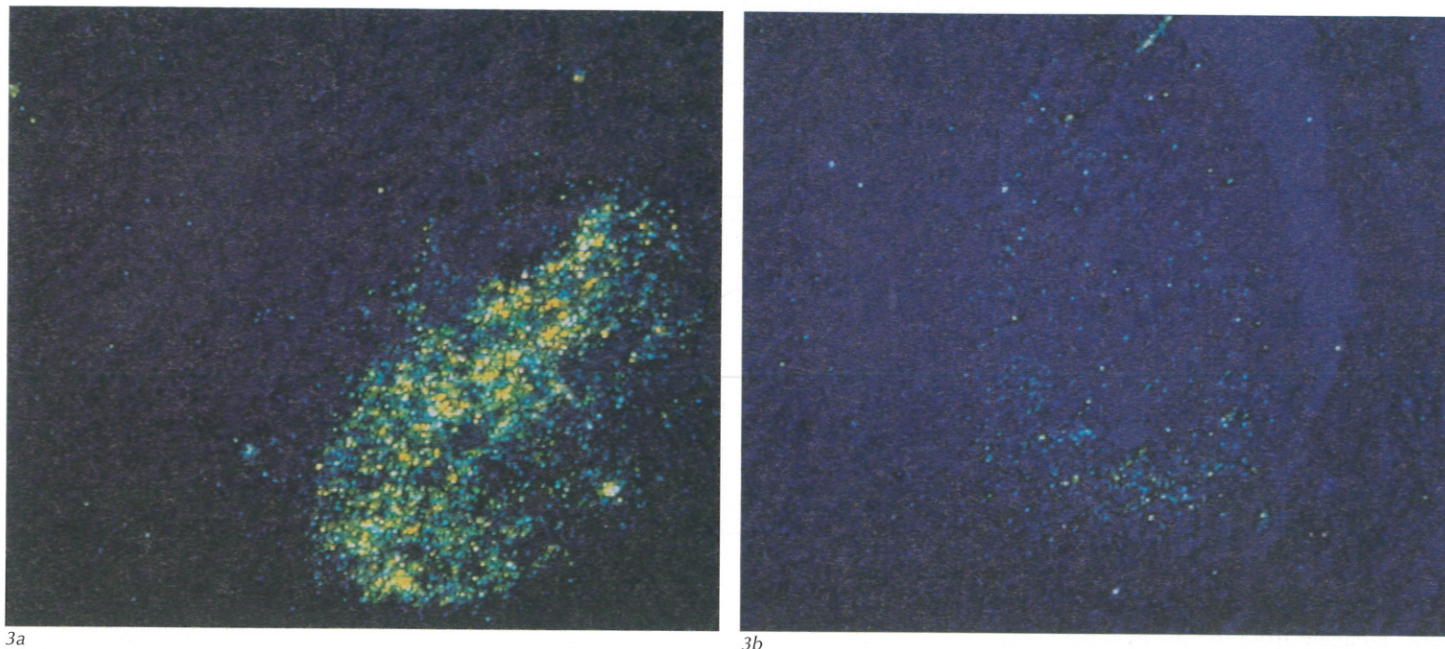


Figure 3. Autoradiograph of tonsillar tissue. 3a; HIV RNA stored in the germinal center of the tonsil prior to treatment. 3b; A germinal center of tonsil after 3 weeks of triple antiretroviral therapy. Reproduced with permission from Cavert W et al. Science. 1997. Copyright 1997 American Association for the Advancement of Science.

cell pool and a reduction in the virus associated with the FDCs.

Computer-aided photographic quantification of viral burden in the tonsillar sections revealed bi-phasic kinetics, (Figure 4). Between the baseline sample and day 2 there was a rapid decrease in HIV RNA load, with a half-life of about 1 day. A second phase with a half-life of about 14 to 15 days was seen between day 2 and week 3. The productively-infected mononuclear cell population lost about 1.0 log of virus by day 2, and another 0.5 log between day 2 and week 3. What was surprising, according to Dr Haase, was that the viral load in the FDC pool paralleled that seen in the productively-infected cell pool (Figure 4). The viral RNA levels associated with the FDC also showed a bi-phasic curve, with half-lives similar to those described for the productively-infected mononuclear cell pool.

After 6 months of treatment, the viral load in FDC was reduced from about 10^8 copies/gram of tissue to less than 10^4 copies/gram, the limit of detection of the assay, in 8 of the 10 patients. By increasing the sensitivity of the autoradiographic technique, it was shown that even after 6 months of treatment there was still HIV RNA associated with the FDCs of each germinal center in the lymph node. The

highly productive mononuclear cells producing 175 virions per cell were now gone, but there were mononuclear cells producing on average 2 to 5 virions per cell. These data show that after 6 months of continuous treatment with triple

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antiretroviral therapy, between 10^7 and 10^8 virions still persist in the entire body. HIV DNA was also still detectable, suggesting that latently-infected cells persist after treatment.

Interpretation of the Post-Treatment Kinetic Data

These data suggest that there are two pools of cells producing virus. One pool

produces the largest number of virions with a rapid turnover and a half-life of about 1 day. The other pool has a longer half-life of about 14 days and produces considerably less virus. It is believed that this second pool consists of CD3+ cells, but the exact nature of these cells has not yet been elucidated. Calculations based on the above kinetic data collected from treated patients shows that the average productively-infected mononuclear cell produces 175 virions. The turnover of these mononuclear cells is about 8×10^7 cells each day.

Dr Haase summarized changes that occurred in the CD4+ cellular compartments after triple therapy. In the uninfected individual about 2% of CD4+ cells (or about 5×10^9 cells) are in the peripheral circulation and about 98% of CD4+ cells 2×10^{11} are in the lymphoid tissue. About 45% of the CD4+ cells are naive, and a small percentage undergo apoptosis or enter a self-renewal pool. During HIV infection, prior to the initiation of treatment, about 1% of CD4+ cells (10^9 cells) are in the blood and 99% (10^{11} cells) are in lymphoid tissue. The naive cell component decreases to about 24% of the CD4+ population and the rate of apoptosis and self-renewal increases 2- to 3-fold. In the infected patient there is an

additional loss of CD4+ cells due to HIV infection, estimated to be 8×10^7 cells per day.

After 6 months of treatment the peripheral CD4+ cell count increases as expected, doubling from 10^9 to 2×10^9 total cells. This yields an apparent rate of increase of about 10^9 cells per day, but in fact the actual measured replacement rate in lymphoid tissue is calculated to be 10^8 cells per day. This suggests that the increase in the CD4+ cell count in the blood is due primarily to redistribution of lymphocytes rather than new production. The proportion of naive CD4+ cells increases, and the apoptosis rate and the re-population rate decrease. These data suggest that most of the new cells produced after antiretroviral drug treatment emanate from the renewal pathway, that is the bone marrow and the thymus, and consist of naive CD4+ lymphocytes.

Even during the later stages of HIV infection the immune system may have the capacity to recover.

Thus during the chronic stage of HIV infection this source of new lymphocytes does not appear to be irreversibly damaged and there may be the capacity for the immune reactive cell populations

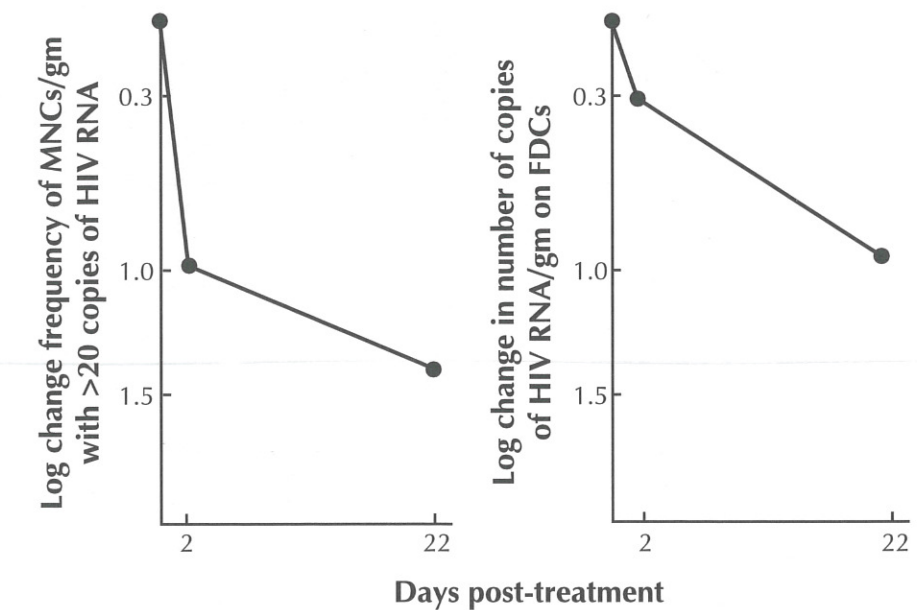


Figure 4. Changes in HIV-infected productive mononuclear cells (MNCs)/gram of tonsil tissue, and HIV RNA viral load/gram of tonsil tissue at 2 days and 3 weeks after the start of triple antiretroviral therapy. Adapted with permission from Cavert W et al. *Science*. 1997. Copyright 1997 American Association for the Advancement of Science.

to recover. This provides hope that significant immune reconstitution is possible even in the later stages of infection.

If the rate of production of naive CD4+ cells by the thymus and bone marrow is about 8×10^7 to 8×10^8 cells per day, and the rate of turnover of productively HIV-infected cells is the same, then during HIV infection the rate of cell death due directly to viral infection must equal the rate at which the cells can be replaced. Since there are other causes of CD4+ cell death in addition to that resulting directly from viral infection, the

total CD4+ cell population decreases, and this accounts for the immune depletion in HIV infection. At steady state HIV infection the cell renewal processes can just barely keep up with the rate of destruction of cells by HIV. The immune system is essentially running in place.

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Suggested Readings

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