Lymph nodes reveal HIV’s hiding place—scientists call for new drugs

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Potent combination HIV therapy (commonly called ART or HAART) can significantly prolong life. In high-income countries such as Canada, Australia and the U.S. and regions such as Western Europe, doctors increasingly estimate that young adults diagnosed today and who begin ART shortly thereafter and who take their medicines every day exactly as directed and who have minimal co-existing health conditions have a good chance of living into their 70s.

Scientists in the U.S. have collaborated on a project to study how well ART penetrates the body. They have found that while ART is good at getting into cells in the blood and greatly reduces production of HIV in the blood, HIV-infected cells continue to make viruses deep inside the body. This particularly happens in parts of the immune system called lymph nodes and lymphatic tissues. These findings have many potential implications for the future of HIV therapy and for attempts to try to cure HIV infection. In this CATIE News bulletin, we first give a brief explanation of the immune system and some terms that we will use before detailing the recent U.S. findings.

Cells and systems

One group of cells that helps to fight infections is called lymphocytes. These cells can be divided into two groups as follows:

- T-cells (or T-lymphocytes) – these can help organize the immune response to infection; some T-cells can directly attack infected cells and tumours
- B-cells (or B-lymphocytes) – these make antibodies that can help fight some infections

Some T-cells have a receptor called CD4 and we commonly call these cells CD4+ cells. Other T-cells have another receptor called CD8 and we commonly call these cells CD8+ cells.

Deep within the body

The immune system is distributed throughout the body and only a small fraction of its cells (between 2% and 5%) are in the blood at any time. Most T-cells and most of the immune system’s cells are found in places such as the following:

- lymph nodes – locations include the neck, under the arms, in the groin
- lymphatic (or lymphoid) tissue – small extensions of the immune system found around the gut, lungs, mouth, throat (tonsils), sinuses in the nasal passages and ano-genital tract. Germs enter the body through these parts of the body and can be intercepted by cells of the immune system.
- organs – for example, the bone marrow makes all of the immune system’s cells, the thymus gland makes hormones and helps to turn immature cells into T-cells, the spleen filters the blood for germs

Where things happen

Lymph nodes and lymphatic tissue are busy sites of activity. Cells of the immune system bring germs there to be broken down and analysed. Lymph nodes and tissues contain many immune cells; when alerted to the presence of germs, these cells multiply and travel throughout the body to contain infections.
A virus in hiding

Clinics commonly use blood tests such as viral load to assess the effect of ART. However, most HIV is not in the blood but is lying deep within the body where most lymphocytes are located—in lymph nodes and lymphatic tissues. Extracting samples of lymph nodes or lymphatic tissue is usually only done as part of studies.

Undetectable in the blood but detectable and active in lymph nodes

A team of researchers at universities in Nebraska, Minnesota and elsewhere in the U.S. has been studying the concentration of some commonly used anti-HIV medicines, comparing the concentrations in the blood and lymph nodes and lymphatic tissue. They found that the concentration of drugs was generally greater in the blood and in cells of the immune system in the blood compared to in lymph nodes and lymphatic tissues. This happened even though levels of HIV in the blood were “undetectable” (less than 48 copies/ml—the lower limit of detection according to the tests they generally used). Levels of HIV in the lymph nodes and tissues prior to treatment were between 100- and 10,000-fold greater than found in blood. Even after treatment, the amount of HIV in the lymph nodes and tissues was still greater than that in the blood. At a minimum this means that “undetectable” in the blood does not mean that there is zero virus in the body or that any residual HIV is somehow defective. Instead, the researchers found evidence that HIV was infecting cells of the immune system in the lymph nodes and lymphatic tissues while tests of the blood found that HIV was undetectable.

The scientists stated that their findings underscore the need to develop and test new anti-HIV therapies that can penetrate and build up in lymphatic tissues. Potentially, such therapies could further reduce the amount of HIV in the body so that patients on these new drugs could realize additional benefits. These and other implications from the present study’s findings are discussed later in this CATIE News bulletin.

Study details

Researchers recruited 12 HIV-positive participants between June 2009 and July 2012. They had small samples (biopsies) of lymph nodes and lymphatic tissue removed as well as blood collected prior to taking ART.

Further tissue and blood samples were collected at months 1, 3 and 6 after starting ART. During the third month of the study, participants were kept overnight in a hospital so that intensive blood collecting could be done over a 24-hour period.

Tissues were analysed with very precise laboratory techniques and technologies to assess them for the presence of medicines and HIV.

Before entering this study, 10 of the 12 participants had never used ART while the remaining two had, according to the researchers, “previously been treated but had stopped taking ART [for undisclosed reasons].” The average profile of participants was as follows:

- age at HIV diagnosis – 27 years
- CD4+ count at the start of the study – 467 cells
- HIV viral load – 35,000 copies/ml

Participants had their HIV assessed prior to treatment to find out which drugs it was sensitive to so that any prescribed treatment would have a high likelihood of working. The medicines used were standard doses of the following:

- Atripla – a fixed-dose combination of efavirenz + tenofovir + FTC
- atazanavir (Reyataz) + ritonavir (Norvir) + Truvada (a fixed-dose combination of tenofovir + FTC)
- darunavir (Prezista) + ritonavir + Truvada

Technicians performed hundreds of analyses of many blood and tissue samples.

Results—blood vs. lymph nodes and lymphatic tissues

The concentrations of drugs in the blood were within expected ranges in all participants. Furthermore, the concentration of drugs within lymphocytes and other cells in the blood were also within expected ranges.
However, the concentration of all anti-HIV drugs within lymphocytes taken from lymph nodes and lymphatic tissues was markedly less than technicians found when they assessed lymphocytes and other cells of the immune system taken from the blood.

Here are some differences that generally show how much lower the concentrations of drugs were within immune system cells taken from lymph nodes and lymphatic tissues compared to cells taken from the blood:

- tenofovir – 80% less drug in lymph nodes and lymphatic tissues
- FTC – 66% less drug in lymph nodes and lymphatic tissues
- atazanavir – 100% less drug in lymph nodes and lymphatic tissues
- darunavir – 99% less drug in lymph nodes and lymphatic tissues
- efavirenz – 94% less drug in lymph nodes and lymphatic tissues

**Distant parts of the immune system**

In previous unrelated studies where HIV-negative volunteers took tenofovir to reduce their risk of becoming HIV positive, researchers found relatively high concentrations of tenofovir in cells of the immune system taken from the rectum and nearby parts of the intestinal tract. In the present study, researchers found similar results. Levels of atazanavir were higher in cells of the immune system from the rectum than they were in such cells taken from the blood.

**Role of adherence**

The ability to take ART every day, exactly as directed, is called adherence. Relatively high levels of adherence are needed for ART’s effects to be sustained over the long-term. It is possible that some critics of the findings in the present study may point the finger at poor adherence as a cause of the discordance between drug levels in the blood vs. lymphatic tissue. However, because of frequent sampling of blood and tissues, and analysis of lymphatic tissue and the levels of HIV and amount of infected cells in such tissues, it is highly likely that all participants had good adherence.

**Impact on HIV**

All participants had HIV viral load in their blood fall to “undetectable” levels; that is, generally less than 48 copies/ml.

Despite this apparent suppression of HIV in the blood, researchers stated that they found “striking visual and quantitative evidence of continued virus production in [the lymph nodes] in four [of the participants].”

Assessment of lymph nodes and lymphatic tissues from the gut showed a massive decrease in HIV and HIV-infected cells in all participants in the first month of the study. Researchers did not have tissue samples from all participants at all points in time. However, they did state: “In four of nine [participants] from whom we had sufficient samples for analysis [to the end of the sixth month, the rate of disappearance of HIV and reduction of infected cells] then either slowed, or increased in one [participant].”

**Of major importance**

The major finding from this study, according to researchers, has been that the concentration of “many commonly used drugs was lower in lymphatic tissue than observed in blood cells.” Furthermore, researchers found robust evidence of “continued virus production during ART” in cells from lymphatic tissues. They also stated: “Measures of virus replication in the blood do not necessarily reflect the impact of [ART] on virus production at its principal source in lymphatic [tissues].”

**Why the differences?**

The cells in the blood represent only a fraction of CD4+ cells that could move to and be inside lymph nodes. So assessing cells in the blood does not give an accurate picture of what is happening in lymph nodes and lymphatic tissues. Also, one previous study found that the concentration of the anti-HIV drug indinavir (Crixivan; this is no longer commonly used in high-income countries) diminishes faster in lymphatic fluids compared to blood.
The researchers think that the reasons for the different concentrations of drugs in different parts of the body may depend on at least the following factors:

- the molecular size of the drugs; bigger molecules seem to have better penetration into lymphatic tissue
- the ability to dissolve in fat (lipids); being able to dissolve in lipids seems to give drugs better access to lymphatic tissue

**Integrase inhibitors and other drugs**

The newest class of anti-HIV drugs is called integrase inhibitors. Examples of integrase inhibitors (in order of regulatory approval) are as follows:

- raltegravir (Isentress)
- elvitegravir (in Stribild)
- dolutegravir (Tivicay)

When used as part of combination therapy, in general, these drugs quickly reduce the amount of HIV in the blood.

Studies need to be done to assess the effectiveness of integrase inhibitors in the lymph nodes and tissues and to assess the effectiveness of all recommended HIV therapies used today for their impact on lymphatic tissue.

**Inflammation**

Although ART greatly reduces the level of HIV-related inflammation, such inflammation persists despite many years of treatment. Likely, continuing production of HIV deep within the body helps inflammation linger. It is possible that this lingering inflammation may play a role in accelerating the pace of certain complications, including the following:

- thinner-than-usual bones
- significant increased risk for cardiovascular disease
- decline of major organ-systems, including the brain, kidneys, lungs and so on

As a first step toward further reductions of the burden of HIV in the body and related inflammation, the following steps could be implemented:

- create and test new formulations of existing drugs that can penetrate and remain in lymphatic tissues
- create and test new anti-HIV drugs that are better at impairing the production of new copies of HIV from infected cells located in lymphatic tissues

**Why time is needed**

There has always been a lag between scientific discoveries and the implementation of such discoveries in research and clinical networks. It will therefore take time to translate the results from the U.S. research into something meaningful. Here are several additional steps that need to be taken:

- new lipid-soluble formulations of existing anti-HIV drugs or entirely new anti-HIV drugs need to be created
- the anti-HIV activity and safety of such new formulations and drugs has to be assessed in lab experiments with lymphocytes and other cells, particularly within samples of lymphatic tissues

Later, these drugs need to be tested in animal models of HIV infection, either mice with transplanted human immune systems or monkeys susceptible to simian immunodeficiency virus (SIV). This latter virus is closely related to HIV and causes an AIDS-like condition in some monkeys.

If the new medicines pass muster in the previously mentioned tests, they then need to be tested in people, particularly for their safety and effectiveness in Phase I, II and III trials. Throughout this research in people, there will need to be a substantial proportion of volunteers, particularly in the early phases of studies, willing to undergo biopsies of lymph nodes and tissues on at least several occasions.

**Multiple effects, caution and timelines**

The quest for more potent therapy has the potential to reenergize HIV research efforts. Scientists will likely intensify
their study of parts of the immune system deep within the body—specifically lymph nodes and lymphatic tissues—and how they change over time in response to HIV and other germs. This has the potential to help better understand immunity or susceptibility to HIV and other germs, as well as tumours.

New drugs that can better penetrate into lymph nodes and lymphatic tissues are likely to be able to penetrate other tissues where HIV is known to hide, such as the brain and spinal cord. Scientists sometimes call these parts of the body “reservoirs” or “sanctuaries” for HIV. With new and more effective therapies that will be developed in the future, researchers have the potential to greatly reduce the amount of HIV deep within reservoirs. This may, in theory, hasten the day that HIV can be cured.

However, we caution readers to keep in mind that the timeline for drug development is usually a long one and multiple drugs will be needed to help suppress HIV in lymphatic tissues. Along the development path to new drugs (or new formulations of existing drugs) there is potential for inadvertent mistakes and setbacks. These are a normal part of the drug development process for any condition. The newer, more powerful lipid-soluble anti-HIV drugs may not become available for between five and 10 years.

Also, there is this issue—no one knows what the new anti-HIV therapies might cost. Exploring an example of another infection, hepatitis C virus (HCV), may be useful. Indeed, while there are emerging therapies for HCV that are simple and have reduced toxicity and can cure this infection, there is a great deal of concern about the cost of these new therapies.

The initial wave of anti-HIV drugs tested in the 1980s and early 1990s had more side effects and was less effective than subsequently developed drugs, particularly compared to drugs that are recommended for initial HIV therapy today. This has been the case with therapy for HCV, and likely other conditions. Furthermore, it may also be the case with new anti-HIV drugs designed to penetrate and accumulate in lymphatic tissue.

**No changes recommended at this time**

The U.S. team that made the discovery about the discordance between lymphatic tissue and blood cautions that “it would not be prudent at this time to alter clinical management [of HIV-positive patients] based on our results,” as currently approved regimens are good at controlling HIV, preventing the development of AIDS and greatly extending survival.

**Back to the future**

About once a decade in HIV research the phrase “everything old is new again” seems to apply as old discoveries are revisited and reinterpreted and theories are refined with more precise techniques and technologies yielding new data and adding to scientists’ understanding of how HIV damages the immune system.

Ironically, when the HIV epidemic first became apparent in the early 1980s, scientists studied lymph node biopsies and found evidence of a large concentration of HIV there as well as active infection of cells in that compartment. However, work later became focused on blood and cells of the immune system extracted from blood because this was easier to obtain and simpler to investigate. Furthermore, with assays to assess viral load and the life-extending effect of treatment available for blood samples, it seemed that assaying lymph nodes and lymphatic tissue was unnecessary. Now, 31 years after the discovery of HIV, investigating the lymph nodes of HIV-positive people may yield new clues as to how HIV disables the immune system and new ways for doctors to treat this infection.

—Sean R. Hosein

**REFERENCES:**


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