GMHC GAY MEN'S HEALTH CRISIS

treatment ISSUES

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Looking Where the Light Is

By Bob Huff

There is an old science joke about a drunk who is searching for his car keys under a streetlight. A cop happens along, and after appraising the situation, asks the drunk where he had last seen the keys. The drunk points toward a dark alley.

"Then why aren't you looking over there?" asks the exasperated cop. The drunk looks up and replies, "Because the light is better over here."

We know what we do about AIDS from two basic kinds of experience. Soon after the syndrome was first reported, clinical observation quickly revealed that people with the disease tended to waste away or were stricken with any of a number of unusual infections. Most eventually died. At the same time research scientists started looking for abnormalities in blood and tissue samples from AIDS patients, searching for a cause. But the kinds of tests available at that time were limited and the conclusions researchers could draw from their laboratory experiments were general and few. They were simply looking where they could shine a light. After twenty years, new assays and experiments, producing high quality streams of data, continue to illuminate the underlying causes of this disease. Nevertheless, the workings of immunity are so complex and so difficult to observe in living persons that darkness still shrouds many crucial aspects of what goes wrong when HIV interacts with the human immune system.

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History Lesson

From the dim beginnings of the AIDS crisis, researchers tried to find a laboratory assay that would predict what was in store for an individual at risk for acquiring the immune deficiency. In particular, clinical researchers needed some kind of surrogate for disease progression to spare patients having to get sick or die before determining if an experimental treatment was efficacious. Surrogate markers are stand-ins for other kinds of data that are either inconvenient, take too long, or are otherwise impractical to collect. Ideally, a surrogate marker should be minimally invasive—such as a blood test—and be cheap, quick and simple to perform. It should provide an unambiguous numerical read-out to indicate with a high degree of certainty, exactly how sick someone is, then tell whether they are getting better or worse. An ideal marker should warn if symptoms are about to occur and report promptly if a course of treatment is going to be successful or not. Finally, this fantastic marker should give the same reliable results for everyone, no matter what stage of disease or genetic makeup they have.

An ideal surrogate marker still does not exist for HIV/AIDS. In fact, after two decades of looking, only two assays, CD4+ T-lymphocyte count and HIV RNA viral load, have been widely adopted as

imperfect surrogates for monitoring and predicting the course of disease in people with HIV. These markers have been fairly well correlated with the natural history of HIV infection and progression to AIDS, but each has limitations. For example, routine viral load testing does a good job of reporting what is happening to virus levels in the blood, but not in lymphoid tissue where HIV interacts most significantly with the immune system. And it's our ability to assess and analyze the effects of HIV on immunity that is most critically in need of improvement. We still lack reliable assays that can report on immune reconstitution under the effects of HIV treatment or that can tell when the body is capable of controlling HIV with its own immune resources.

It's possible to imagine that, one day, a new approach to treating HIV could be developed, perhaps using a drug that interrupts a crucial, very specific step in HIV-mediated immune dysregulation.

Since the first recognized AIDS cases involved very sick people, early clinical reports detailed mostly non-specific markers of infection and impaired immunity such as skin antigen recall tests, erythrocyte sedimentation rates, or increased white blood cell counts. A few curious researchers working at the frontiers of intercellular signaling reported elevated levels of interferon and tumor necrosis factor in people with AIDS. Others, specializing in virology and antigen recognition, reported increased levels of cytomegalovirus and herpes virus particles in their patients' blood. These observations were made using the best tools available at the time and the data obtained trickled into the sketchy literature that was slowly accumulating about the mysterious syndrome.

When first described in 1981, it was recognized that the opportunistic diseases attacking a growing number of young, homosexual men, were also those that afflicted people with genetic or chemotherapy-induced deficits in a wing of the immune system called cellular immunity. This branch of immunity depends on T-cells—white blood cells that earned their name because they were observed to develop in the thymus—and is responsible for eliminating diseased cells that have been invaded by outside pathogens.

In the seventies, someone noticed that T-cells could be identified and separated from other white blood cells by mixing them with red blood cells (erythrocytes) from sheep. The sheep erythrocytes would then cluster around the T-cells and form rosettes (E-rosettes) that could be viewed and counted under a microscope. An assumption eventually emerged that the sheep cells attached themselves to certain cell-surface proteins that were characteristic of a subtype of T-cells called the T-helper cell. The various receptors and coreceptors we now know as CD4, CD8, etc., were then simply thought of as cell surface antigens—as late as the mid eighties some papers still referred to the newly identified CD4 receptor as the E-rosette receptor. But even this rudimentary technique revealed that, in AIDS, T-helper cells were disappearing.

The need for a quicker and more reliable measurement of disease progression was urgent. Fatefully, AIDS started to appear just as a new wave of technology for marking and automatically counting immune cells was becoming more available. CD4 cell staining with fluorescent antibodies and flow cytometry machines that could rapidly and accurately count the tagged cells soon led to the establishment of the CD4 cell count as a standard marker for the severity of HIV disease. The CD4 count eventually proved to be useful for predicting disease progression, warning of risk for opportunistic infections (OI), serving as a diagnostic milestone for the onset of AIDS, and, after an initial debate, as a surrogate for demonstrating that an experimental antiretroviral therapy was likely to have clinical benefit. Today, most guidelines for making decisions about starting and stopping treatment and initiating OI prophylaxis continue to rely heavily on CD4 counts.

However, absolute CD4 counts (along with the ratio of the number of CD4 cells to CD8 cells, another important immune system metric, still preferred by some clinicians) are at best somewhat crude measurements of the effects of HIV on an individual's immune system. Within the broad category of CD4 cells, there are many subtypes, each with particular characteristics and roles to play in the orchestra of immunity. And while simple CD4 counts are invaluable for making treatment decisions, they cannot describe which subsets of cells are affected by HIV infection or how well they are functioning. Despite the ability of HAART to raise CD4 counts for many people, concerns about drug toxicities, resistance and the difficulties of sustaining adherence to HAART have brought new urgency to improving the ways we have of measuring and understanding the complexities of the immune system and for finding new ways of treating it.

Immune Therapies

Although an enormous amount of attention has been paid to antiviral drugs—well deserved given the success they've brought—most people who think about the future of HIV realize that finding some way of enabling the body's own resources to fight, disable or ignore the harmful effects of HIV would be a step for-

ward as great as that achieved by HAART. Usually, this hoped-for breakthrough is imagined to be a vaccine. Theoretically, a vaccine would work by priming the immune system to react in multiple ways against multiple physical features of HIV, thereby overwhelming an infection before it could run out of control. A dream vaccine would prevent everyone who took it from ever becoming infected. But as the difficulties of HIV vaccine development have become painfully clear, many researchers are now aiming for a vaccine that would simply help keep an established infection under control by enhancing the body's HIV-specific immune capacity. For now, the outlook for vaccines remains gloomy.

Who's Story Is It?

Theories about the mysteries of nature are essentially stories that take the observations, experimental evidence, and conventional wisdom of the day and stitch them together into a coherent narrative. In biology, perhaps faster than in other fields, these explanations are revised as new evidence is gathered. But the force of an appealing pathogenesis narrative can shape research agendas (as well as political agendas) for years. The danger is that valuable time and resources may be squandered pursuing compelling but misguided strategies built on insufficient data.

For example, observations about the density of E-rosettes fueled some of the first speculation about the possibility of immune-based treatments for AIDS. A 1982 paper reporting on in vitro findings announced, "Drugs which are able to modulate T-cell functions, such as thymosin, transfer factor, isoprinosine ..., also increase the percentage of active T-rosettes." (Wybran J) These agents and other purported immune modulators were experimentally used and periodically tested throughout the rest of the decade, although they failed to show any consistent clinical value. Interest in IL-2, another immune regulatory messenger recognized during the first years of AIDS, has never gone out of favor and large trials continue to this day. Nevertheless, some have argued that the early, vigorous, focus on antiviral therapy for HIV may have slowed work on immune-based studies.

In the early eighties the basic narrative of immunity went something like this: During a cellular immune response, T-helper cells sound the alarm for antigen-specific immune cells to start multiplying. When the attack has gone far enough, other T-cells, called T-suppressor cells, sound the retreat and start eliminating the now superfluous antigen-specific attackers. Although some early researchers hypothesized that HIV-mediated autoimmune processes were responsible for the resulting immune dysregulation, by mid-decade, conventional wisdom held that AIDS was directly caused by the virus, which, in the words of one famous researcher, "kills T-cells like a Mack truck."

In recent years, we've learned that most depleted T-cells are never actually infected with HIV, and a more sophisticated view of pathogenesis is emerging. There is growing understanding that immunity is a system of give and take, which, when regulated, works very well. We know that over five to ten years or more, T-cell counts decline at a slow but steady pace. Yet the picture of what happens to the turnover of the body's immune cell inventory is still developing. Although it's obvious that something is seriously wrong with the system of immune regulation in AIDS, surprisingly little is known about what actually causes T-cell stores to dwindle. One theory says that T-cells are destroyed at a consistently high rate but are also replaced at a similar rate, thus maintaining a rough balance. Over time, the theory goes, the body's capacity to replace T-cells starts to wear down, leading to their eventual depletion. But which part of the T-cell replacement mechanism is wearing out and what is responsible for wiping out the T-cells that disappear? New research tools are slowly getting at these questions.

In this issue, Daniel Raymond catalogs some of the latest assays that immune system scientists are using to pick apart the mysteries of T-cell depletion. He also describes what their experiments are telling us about how HIV might be doing its damage. Although these studies are still in fairly early stages, it's possible to imagine that, one day, a new approach to treating HIV could be developed, perhaps using a drug that interrupts a crucial, very specific step in HIV-mediated immune dysregulation.

Yet despite accelerating progress, new assays are not born and accepted overnight. It may take years to validate initial observations in competing labs. Then standards must be established before test results can be used diagnostically or correlated with other assays. So, although new assays may be casting new light onto the workings of T-cell creation, destruction and replenishment, many of the explanations constructed from this emerging evidence remain tentative and sometimes contradictory. In 2002 we continue to look where the light is best and make up stories for what we find that fit with what we already know. Hopefully this dawning generation of new immune assays will help us locate the sorely needed key to immune control of HIV.

Stalking HIV Immune Dysregulation

By Daniel Raymond

Immune assays are laboratory tests that attempt to measure various aspects of the immune system such as function, quantity and stage of maturation. Researchers are seeking correlations between these markers and health in a desire to test and describe the potential benefits of immune-based HIV therapies such as IL-2 and other immune regulators, as well as vaccines that might have therapeutic potential. Newer assays are also starting to shed light on the mechanisms behind the immune depletion that occurs during HIV progression and those involved with immune reconstitution after therapy. While these new generation assays are still used exclusively in research settings, the findings that they've made possible have already influenced clinical practice by justifying experimental treatment strategies such as Structured Treatment Interruptions (STIs) and antiretroviral (ARV) therapy during acute HIV infection. Several of the assays expanding our knowledge of HIV pathogenesis and HIV-specific immunity are also contributing to important research on the role of immune function in other diseases such as cancer. Because of these broader implications, it is important to understand what these assays measure and what they can tell us—as well as their limitations. While immune assays have provided a wealth of data, the interpretations of these data often remain contested.

What does the cell do? Is it in a resting or activated state? Is it proliferating or dying? Is it still functional—can it respond to pathogen? Or is it a useless imposter masquerading as a viable T-cell?

Types of Immune Assays

The emergence of AIDS in the early 1980s created a number of challenges for researchers and doctors. The first of these was to identify the cause of AIDS, which eventually led to the isolation of HIV. Subsequently a screening method to detect HIV infection was developed, and the HIV antibody test entered into widespread use. But long before there was any agreement that a virus was responsible for the new syndrome, doctors had recognized that patients were losing immunity to common pathogens because one of the crucial components of a healthy immune systems was being selectively depleted: the T-helper cell, now known as the CD4 cell.

One of the first and still most important tools for studying cells was the fluorescence activated cell counter or sorter (FACS). These devices let scientists count and separate populations of immune cells by tagging them with special antibodies. These machines are now in routine use in diagnostic labs around the world producing thousands of CD4 counts for doctors and patients daily. But the elements of FACS technology remain at the forefront of nearly every immunologist's research toolkit.

Newer immune assays—some of which are not new at all, but are only recently coming into use for HIV research—are allowing scientists to probe deeper into the number and function of T-cells and ask increasingly incisive questions. For example:

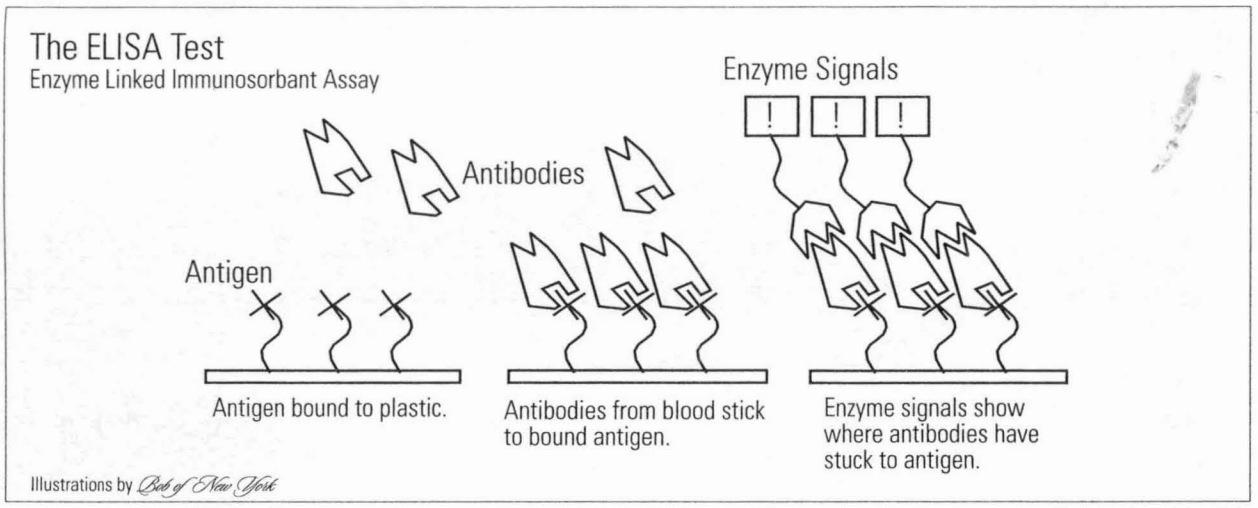
What kind of T-cell is it? Is it a naïve or a memory cell? Can the T-cell recognize and respond to HIV? How many of each of these kinds of cells is present at any particular time?

What does the cell do? Is it in a resting or activated state? Is it proliferating or dying? Is it functional—can it respond to pathogen? Or is it a useless imposter masquerading as a viable T-cell?

These newer immune assays are built on the key technologies that made FACS technology possible in the late 1970s: monoclonal antibodies and flow cytometry. Monoclonal antibodies were first developed in 1975 by fusing an antibody-producing B-cell with a long-lived cancerous cell called a myeloma. The fusion created a hybridoma, an immortal cell that could continually reproduce a single antibody specific for a particular antigen. These antibodies could then be used to detect the presence of a target antigen through various methods, including the enzyme-linked immunosorbent assay, or ELISA test.

The standard HIV test uses ELISA to detect HIV antibodies in blood or saliva. A tiny plastic well is coated with bits of antigen—fragments of common HIV proteins that HIV antibodies will stick to. Then the sample (e.g., blood) being studied is added; if HIV antibodies are present in the sample, they will bind to the antigen. Next, a monoclonal antibody, custom made to bind to HIV antibodies and linked to a special enzyme, is added. Then the well is washed out leaving only the enzyme-linked antibodies that have attached to the patient's HIV antibodies. Finally, the enzyme's substrate is added to the mix and wherever the enzyme remains, a color change occurs, thereby revealing the presence of HIV antibodies. This is a positive result. If the sample does not contain HIV antibodies, the secondary antibodies (the monoclonal antibodies) will have nothing to bind to, and the color won't change.

Monoclonal antibodies have a myriad of diagnostic and therapeutic applications, includ-



ing the ability to characterize cell populations by sticking to cell surface proteins that are unique to certain classes of cells. For example, some monoclonal antibodies can tag CD4 proteins while others identify CD8 cell surface markers. CD stands for cluster of differentiation, in that the CD4 molecule differentiates T-helper cells from cytotoxic (cell-killing) T-cells, marked with CD8. An array of multiple types of antibodies used in combination can further identify a cell as having naïve or memory markers, resting or activated markers and so on.

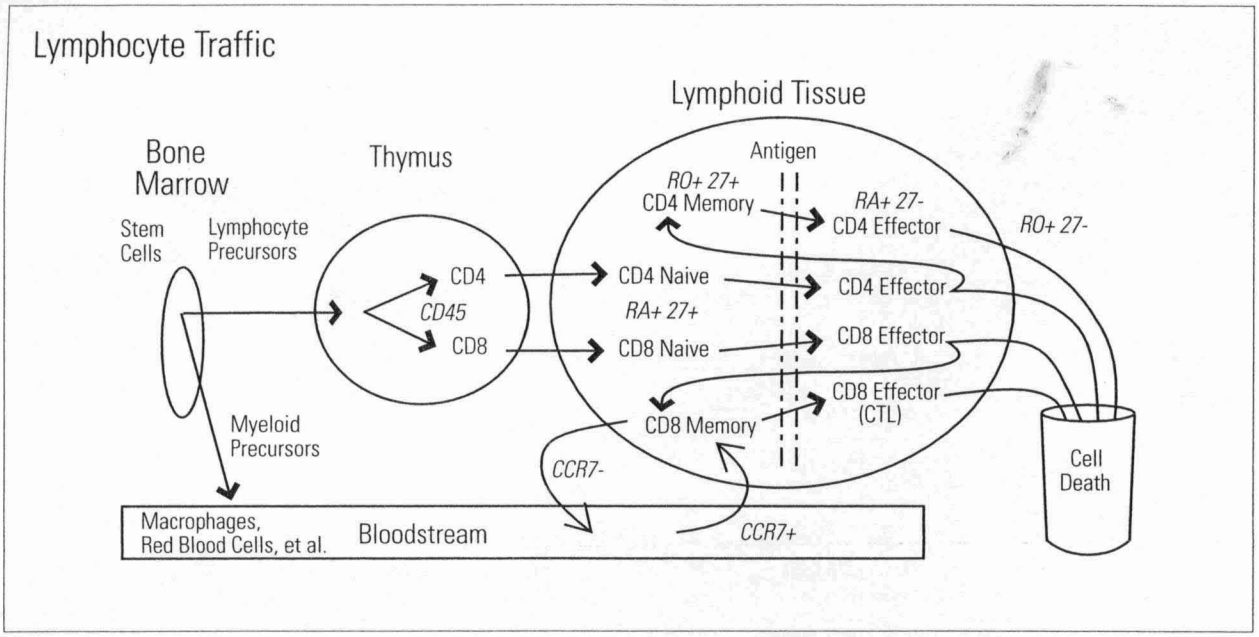
Both the absolute and relative frequency of different cell types in a blood sample can be measured by various elaborations of the basic flow cytometry techniques developed in the 1970s. Antibodies labeled with a fluorescent dye are mixed with a blood sample containing all kinds of cells. But only the kind of cell that is being counted is tagged with the fluorescent antibody. The fluid of the sample is passed down a very narrow channel that is only wide enough for one cell to pass through at a time. A laser beam briefly illuminates each cell in the column. When the laser strikes a fluorescing antibody stuck to a cell, light will be emitted at a signature wavelength that can be recorded by a detector and counted as one cell. More advanced versions of this technology actually allow the counted cells to be shunted into a separate channel for collection. This is called cell sorting and is an extremely powerful function of FACS technology. In HIV research, FACS analysis can be used to isolate particular subsets of cells for further analysis and manipulation. The newest generation of cell counters and sorters use more than one colored laser and can detect several subtypes of cells all in one pass by using multiple kinds of antibodies and special fluorescent dyes. Monoclonal antibodies and flow cytometry have revolutionized molecular biology and over the years increasingly detailed portraits of cell subsets in HIV infection have been produced using variations on these methods.

The next revolution, now underway, will come from the development of extensive matrices of tiny DNA detectors called microarrays that can reveal which genes are active in cells and when they become activated. (See *Microarrays on the Horizon*) This promises to bring previously unimagined resolution to what actually happens inside cells as they mature and adopt new functions. While the full potential of this technology is years away, in the meantime, researchers have a variety of methods at their disposal to measure the dynamics of immunity.

When a naïve T-cell encounters an epitope that matches its receptor, a process of activation and proliferation takes place.

Characterizing T-cell Populations

As we've seen, T-cells can be separated and counted based on cell surface markers. Besides binding to the manmade antibodies in the assay systems, these molecules on the surface of T-cells have functions in the normal life of the cell. Cell surface proteins may bind to messenger proteins produced by other cells, which in turn sends a series of signals that may activate a gene in the cell's nucleus. Take the CD4 and CD8 cell surface markers. As a normal part of immune function, both of these CDs work as coreceptors helping the main T-cell receptor to recognize and respond to foreign antigens that ought to be eliminated. In HIV infection, the typical ratio of CD4 to CD8 cells in a healthy person (about 2:1) becomes inverted as CD4 cells are depleted. And as CD4 cells dwindle, the risk of falling victim to one of the opportunistic infections of AIDS is increased. While CD4 and CD8 are two of the best-known cell surface markers, currently, over 200 different



CDs have been described—with certainly more to come. The job of figuring out what all of these proteins do will take a great deal of additional research with new techniques and assays.

Naïve, Effector, and Memory Cells

T-cells, like all blood cells, start life in the bone marrow as progenitor stem cells. The T-cell line spins off and migrates to a specialized collection of cells called the thymus, a kind of finishing school for immune cells. In the thymus, each individual T-cell develops a receptor with a highly specific affinity for the size and shape of a particular protein fragment. The look and feel of this fragment is called its epitope. After leaving the thymus, before the cell has encountered its antigenic mate, the T-cell remains naïve.

When a naïve T-cell encounters an epitope that matches its receptor, a process of activation and proliferation takes place. The cells produced by proliferation are called effector cells. CD4 effector cells are cells that help guide the immune response; CD8 effector cells are called cytotoxic T-lymphocytes (CTLs) and are responsible for killing infected cells. After an infection has been controlled, most effector cells die, but a small number of memory T-cells are left behind. The memory cells now have hair triggers for when they next encounter their antigen and are able to react with a stronger and more rapid response than their naïve predecessors. Naïve, effector and memory T-cells are distinguished in the laboratory by the particular subtype (called isoform) of CD45 cell marker on its surface. Cells with the CD45RA isoform are naïve; effector and memory cells express the CD45RO isoform. Immunologists use a kind of shorthand to refer to the two types of cells. Naïve cells are described as CD45RA+ (+ as in positive), or alternatively as CD45RO-, or lacking the RO isoform. This ability to distinguish between naïve and effector or memory cells has opened the door to observations that HIV infection causes a significant depletion of the pool of naïve CD4 cells, while at the same time, most of the HIV-infected but surviving cells are memory cells. This surprising finding has raised an intriguing question: If HIV is not present in substantial numbers of naïve cells, then how and why is the naïve pool being depleted? The classic assumption that T-cells die as a direct result of HIV infection—with depletion occurring as either HIV or the immune system kill off the infected cells—can't explain the deficit in naïve cells. This observation has opened up much research and speculation about the role of immune activation and thymic output

But the picture becomes even more complicated as some features of some memory cells confound this interpretation. Under certain conditions, memory cells seem to revert to the naïve type (CD45RA+), though there is other evidence this reversion may be illusory. Furthermore, a different cell surface protein called CD27, a costimulatory molecule, has been proposed as a possible means of differentiating between naïve, effector, and memory cells. At this point we need a map:

Naïve: CD45RA+ CD27+
Effector: CD45RA+ CD27-
Memory: CD45RO+ CD27+

The CD45RO+ CD27- cells are terminally differentiated and unable to proliferate; increased numbers of these dead-end cells are associated with certain immune disorders as well as with aging. Another complication is the discovery of two types of memory cells—central memory cells, which can travel through lymphoid tissue and are marked by the chemokine receptor CCR7, and effector memory cells, which do not enter the lymph nodes and are CCR7-. However, it remains unclear whether these represent two distinct subsets of memory cells or two different phases, since memory cells may acquire and shed cell surface markers as needed.

Activation Markers

Activated T-cells express various markers, which play different roles in the activation process; for instance, some markers provide a costimulatory signal when the T-cell receptor binds to antigen, while others help the cell to bind more strongly to another cell, as in the case of CTLs binding to a cell targeted for destruction. T-cell activation can be studied by looking for activation markers, though again there is not always a direct correlation between activation and the presence of a marker. Some markers are expressed during different stages of activation, so that an assay measuring a marker expressed late in activation may underestimate the amount of activation. Other markers are also expressed in low levels on resting cells; during activation their expression increases and they appear on the cell surface in higher concentrations

Given the variety of activation markers, some studies use more than one marker to get a fuller picture of activation rates. A growing body of research is demonstrating that in HIV infection, overall activation levels are higher than those seen in HIV- controls. This has led to the hypothesis that activation-induced cell death rather than direct cell killing of HIV-infected cells drives T-cell depletion. Activation-induced cell death (AICD) occurs following rapid proliferation; most of the effectors cells are short-lived, surviving only for the time it takes to perform their role in bringing an infection under control. In turn, the AICD hypothesis has prompted speculation that drugs that suppress immune activation, such as cyclosporin A, may paradoxically slow T-cell depletion. Studies are underway to explore this possible therapeutic strategy.

Recent Thymic Emigrants

Another open question is whether T-cell depletion is solely due to death of T-cells, or might also reflect a decrease in the production of new T-cells. Recall that T-cells are produced in bone marrow and mature in the thymus. All T-cell progenitors, or thymocytes, start with the same set of genes for the T-cell receptor. These genes contain all of the possible varieties of T-cell receptors that the individual can produce; the potential tremendous variety means that a huge number of antigens can be recognized and dealt with. While in the thymus, T-cell progenitors undergo a random shuffling of genes to select a specific T-cell receptor for each cell, a process that results in an enormous diversity of T-cell receptors. As a byproduct of this shuffling, the unused T-cell receptor genes assemble into loops of waste DNA called TRECs, or T-cell receptor rearrangement excision circles. TRECs remain inside the naïve cells after they leave the thymus and begin circulating through the blood and lymphatic tissue. Since TRECs are not reproduced during cell division, their presence can help distinguish between new cells straight from the thymus and cells subsequently reproduced through proliferation.

Because the size of the thymus was observed to shrink with age, it had long been believed the thymus no longer actively produced new T-cells after adolescence. But tracking TRECs has helped show that the thymus can continue to produce new cells throughout adulthood, though output slowly decreases with age. Still, analysis of TRECs in HIV infection has yielded conflicting results, with some studies finding a decline in thymic production over the course of HIV infection that is reversed with HAART, and others observing no change other than the normal slowdown that comes with age. Some researchers have raised the possibility that the thymus compen-

Other common activation markers:

CD25 (high levels): the alpha chain of the IL-2 receptor, which stimulates proliferation

CD38 (high): regulates activation and proliferation

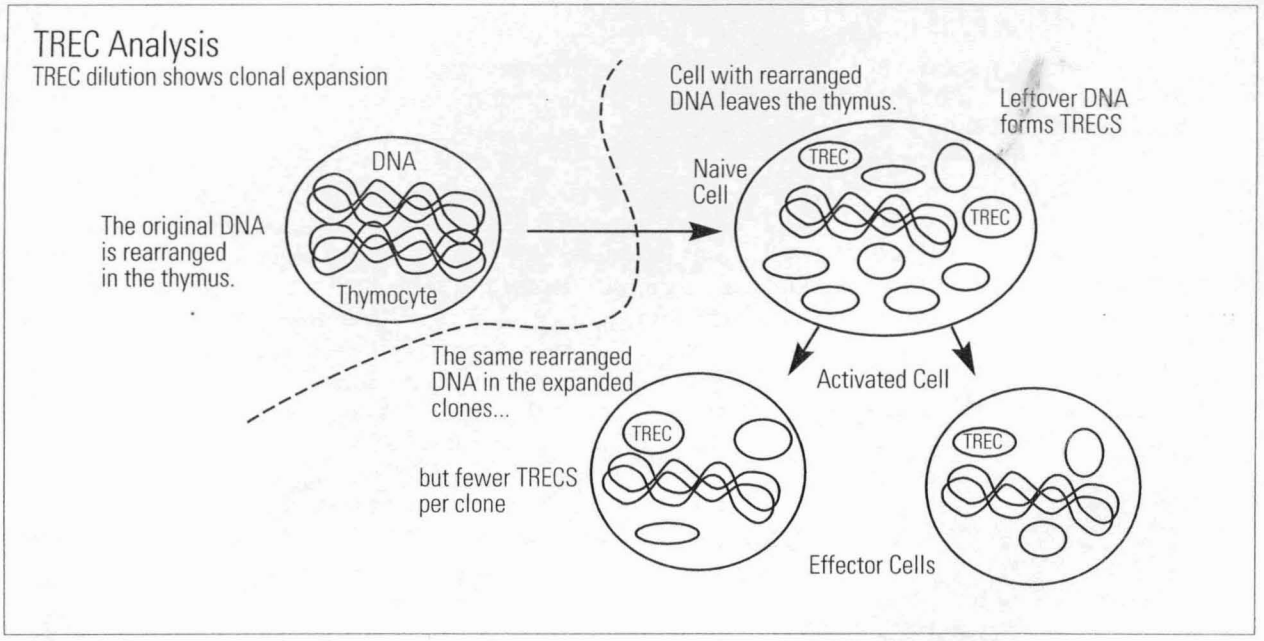
CD69: early activation marker

CD70: binds to CD27; may have costimulatory role

CD95: also known as Fas; mediates cell death signals

CTLA-4: Cytotoxic T-lymphocyte-associated protein 4, also classified as CD152; downregulates activation and proliferation; competes with CD28, a costimulatory molecule—both bind to B7 (CD80, CD86)

HLA-DR: Major histocompatibility complex molecule; used in antigen presentation



sates for T-cell loss by increasing its production of replacement cells, although this theory remains controversial.

Part of the difficulty lays in interpreting TREC levels. Since TRECs are measured as the proportion of T-cells containing the excision circles, their ratio relative to the overall number of cells becomes diluted as existing cells proliferate, thereby reducing the relative number of TRECs found. If cells are proliferating rapidly, then declining TREC levels would not necessarily reflect a decrease in thymic output. This means TREC results have to be analyzed in the context of immune activation. TREC levels may also be affected by cell death; in untreated HIV infection T-cell half-lives are shorter, and TREC levels in HIV-positive populations may underestimate thymic output. TREC data, suggestive as it is, has not yet provided conclusive evidence for an impairment of thymic production in HIV infection. Many questions concerning the role of thymic output in HIV disease remain unresolved despite evidence for direct HIV infection of and replication in the thymus. Enlarged thymic tissue has been observed in some (but not all) people with HIV—although very often those who experience stronger CD4 rebounds following HAART. This supports the idea that, in at least some cases, there may be a mechanism that allows the thymus to increase production in response to T-cell depletion. The potential benefits of treatments proposed to increase thymic production, such as IL-7 and recombinant human growth hormone may be evaluable by determining whether these treatments increase TREC levels.

Examining T-cell Dynamics

When T-cells encounter antigen, they become activated and begin proliferating. Ultimately, most of the newly produced cells will be removed by programmed cell death, or apoptosis. Assays that measure proliferation and apoptosis are also yielding new insights into pathogenesis.

Proliferation

When a cell divides it begins a cycle with several distinct phases as the cell's DNA is unwound, copied, separated into two nuclei and ultimately into two new cells. These stages are referred to as G1, S (the DNA synthesis phase), G2, and M (the mitosis, or cell division, phase). Ki-67 is a nuclear protein expressed during late G1, S, G2, and M phases of the cell cycle; following proliferation, the cell ultimately returns to a resting, or G0, state, where Ki-67 is not detectable. Detection of the Ki-67 antigen by monoclonal antibodies has therefore been used as a generalized marker of proliferation. One limitation of this marker is that cells arrested during the cell cycle will also contain Ki-67 even though they are not actively proliferating. HIV has been observed to cause cell cycle arrest in infected cells; its effect on the validity of Ki-67 as a marker for proliferation is unknown.

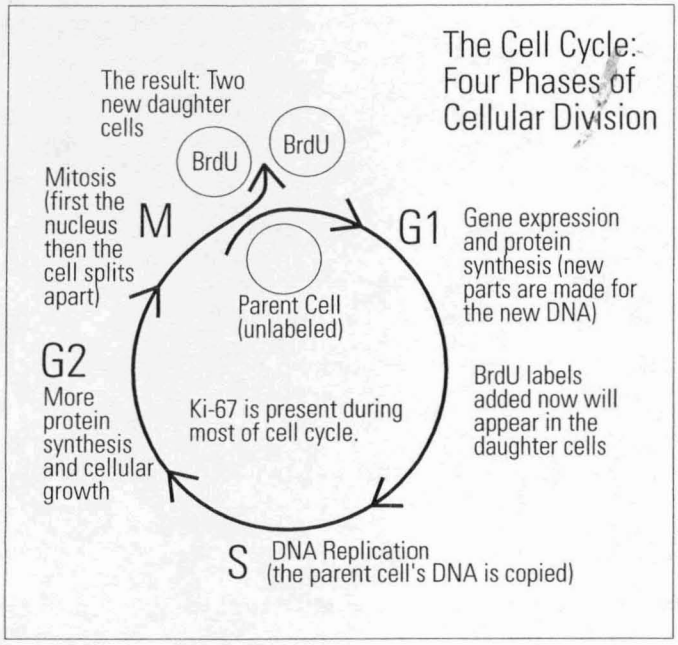
Ki-67 staining has demonstrated that proliferation rates increase during HIV infection, and decrease upon treatment with HAART. These studies shed light on an important question in HIV pathogenesis: Is increased proliferation an attempt to compensate for cell death and maintain a homeostatic balance of cells?

Or does proliferation result from increased activation, sending cells into the cell cycle? Homeostasis describes the normal dynamics of T-cells: cell death and the creation of new cells (by the thymus or through proliferation) balance each other out, so that the overall number of T-cells remains stable. Even in HIV infection, the immune system remains in a relative state of homeostasis and T-cell decline is generally slow—it takes, on average, 10 years following infection to completely lose the balance between influx and destruction and progress to AIDS. Therefore, at any given time, losses due to cell death are roughly offset by gains in new cells.

Ki-67 levels increase as CD4 counts decrease, indicating that as CD4 cells are removed, proliferation speeds up. While this could suggest that cells are proliferating as a result of depletion (arguing for a homeostatic response), when people with low CD4 counts begin therapy with HAART, the Ki-67 signal, and therefore proliferation, immediately begins to decrease, even before substantial gains in CD4 counts are observed. This would argue for HIV-induced activation as the cause of increased proliferation.

Improved techniques have been developed to directly label cells produced during proliferation. Bromodeoxyuridine (BrdU) and deuterated glucose assays were developed as alternatives to the classic [3H]thymidine test, a radioactive assay long considered the "gold standard" for measuring proliferation. The newer assays can be used with flow cytometry techniques that allow for sorting and quantifying the proliferating cell types. BrdU and deuterated glucose are typically administered for a set period of time, during which all new cells undergoing synthesis incorporate the labeled molecules into their DNA. Thus labeled, proliferation can be quantified (using monoclonal antibodies or mass spectrometry) and the longevity of marked cells can be measured by sampling over time. BrdU labeling correlates well, though not perfectly, with Ki-67 levels. Recent studies have demonstrated that proliferation rates are elevated in people with HIV, but begin to normalize upon treatment with HAART. Decay rates (the disappearance of labeled cells, implying cell death) are also increased for CD4 cells associated with HIV infection, suggesting rapid turnover, at least among a subset of proliferating cells.

The extent of proliferation can also be measured indirectly by examining telomere lengths to gauge the replicative history of cells. Telomeres are DNA sequences that cap the ends of chromosomes to preserve their physical integrity during cell division. Telomere lengths tend to shorten after each cycle of DNA synthesis. This can ultimately lead to replicative senescence—when the telomeres become too short to guarantee that the cell can successfully divide. Once again, this picture is complicated by the ability of an enzyme called telomerase to extend telomere length during DNA synthesis and offset the losses during replication.



Telomeres can be measured to determine an average length, thus providing an estimate of how many replication cycles a cell has undergone—the shorter the average telomere length, the more replication it has seen. Telomerase activity can also be measured in cells, to determine whether there is compensation for telomere shortening. Assays have compared telomere lengths in people with and without HIV, finding that while telomeres are shortened in CD8 cells during HIV infection, no significant shortening occurs in CD4 cells, with other assays ruling out increased, compensatory telomerase activity as an explanation. This contradicts one proposed model explaining CD4 cell depletion as a consequence of exhaustion due to rapid turnover in HIV infection; if that were the case, telomere lengths should be significantly shortened. Another factor that would influence average telomere length is thymic production of naïve cells. Since naïve cells haven't undergone proliferation, they will have the longest telomere lengths. Therefore, a change in thymic output could affect average telomere length—decreased output would lead to a shorter average length, while increased output would extend the average

Overview of Immune Assays

T-cell Subsets

Assay	What it measures	Technical notes
Naïve, Effector, and Memory Cells		
Naïve	CD45RA+CD27+	Uses monoclonal antibodies and flow cytometry; measures absolute number but not function
Effector	CD45RA+CD27-	
Memory	CD45RO+CD27+	
Activation Markers	CD25 (high levels); CD38 (high); CD69; CD70; CD95; CTLA-4; HLA-DR	Uses monoclonal antibodies and flow cytometry; tagging multiple markers increases sensitivity
TRECs	Recent thymic emigrants	Uses PCR; numbers may be diluted against a background of increased cell proliferation

T-cell Dynamics

Assay	What it measures	Technical notes
Ki-67	Proliferating cells	Uses monoclonal antibodies; provides a snapshot of levels of proliferation; also stains non-proliferating cells arrested in cell cycle
BrdU; deuterated glucose, deuterated water	Cell proliferation	Directly measures cells produced through proliferation; requires multiple visits and blood draws
Telomere length	Replicative history	Indirect measure of the extent of prior cell proliferation; interpretation complicated by potential telomerase activity, or changes in thymic output
TUNEL; Annexin V	Apoptosis	Can be used with flow cytometry to characterize apoptotic cells

T-cell Function

Assay	What it measures	Technical notes
Delayed-type hypersensitivity (DTH)	T-cell memory response to recall antigen	In vivo assay; requires two visits 2–3 days apart; measurement somewhat subjective, and therefore hard to standardize; does not necessarily predict effectiveness of immune response
Lymphoproliferative response (LPR)	Proliferative capacity of antigen-specific T-cells	Storage and treatment of samples can negatively affect results, so samples should ideally be analyzed at an on-site lab; requires a control to determine non-antigen-specific proliferation (background proliferation); time-consuming
Tetramers	Antigen-specific CD8 T-cells	Highly sensitive; measures both functioning and non-functioning cells; requires knowledge of HLA alleles and matching epitopes; may not pick up responses restricted through other alleles
ELISpot; Intracellular Cytokine (ICC) staining	Antigen-specific T-cell response	Measures only functioning antigen-specific CD4 and CD8 cells; does not detect anergic cells; may detect partially functional cells; ELISpot may be more sensitive, though ICC staining can be used with flow cytometry.

length. This suggests the importance of using multiple assays—i.e., combining telomere assays with TRECs—in order to guide the interpretation of results.

Apoptosis

Apoptosis (programmed cell death) can be triggered in multiple ways but follows a tightly scripted series of events once induced. These events include chromatin condensation, changes in membrane permeability, release of cytochrome-c from the mitochondria, caspase activation, and DNA degradation. All of these can be measured with varying degrees of sensitivity and specificity. Again, using two or more assays can increase the reliability of results.

TUNEL (Terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labeling) measures fragmented nuclear DNA, which is a late-stage event in apoptosis following cell surface and nuclear morphological changes—meaning that this assay may not detect cells in early stages of apoptosis. However, it is considered very sensitive. DNA fragmentation is not unique to apoptosis; it also occurs in necrotic cells (necrosis is "accidental" cell death resulting from injury), so TUNEL assays used alone may not be highly specific; specificity is increased when used in combination with another apoptosis-specific assay, such as immunohistochemical labeling of active caspase-3 (Cytosolic Aspartate-Specific Protease 3)—an enzyme involved in the disassembly of apoptotic cells. One review of various apoptosis assays found that TUNEL was more reliable based on specificity and its correlation with microscopic observation of cells. Another common assay uses Annexin V, a protein that binds with high affinity to phosphatidyl serine, a molecule that moves from the inner cell membrane to the outer membrane during cell death. Both sensitive and specific, Annexin V can detect cells in early stages of apoptosis.

Microarrays on the horizon

The sequencing of the human genome has opened up vast new areas of scientific inquiry. Microarray assays are one of the pioneering methods for using genomics data for new research opportunities. When a gene is expressed, cellular machinery transcribes messenger RNA (mRNA) from the DNA sequence encoding the gene. This mRNA is then translated into a specific protein or enzyme, according to the function of the gene. All cellular functions begin with DNA transcription. By looking at which mRNAs are present in a cell, you can determine what the cell is doing, assuming that you know the function of the gene encoded. This is the concept behind microarrays, sometimes referred to as gene chips or DNA chips by analogy to the fabrication process for computer chips. There are different methods for constructing a microarray and different techniques for applying genetic information to the chip, or surface, but they all follow similar principles. In one version, small samples of DNA representing the coding regions of various genes in question are bound to a glass microscope slide to create a microarray. Thousands of genes can be represented on a single slide in this manner. Then, mRNA is extracted from the cells being studied and reverse-transcribed into complementary DNA (cDNA), which is labeled with a fluorescent dye. The cDNA preparation is hybridized with the slide, with each cDNA binding to its respective gene and forming fluorescent spots. Gene expression is then measured with a scanning laser microscope and analyzed by computers; the intensity of fluorescence indicates the level of gene expression.

HIV research is only beginning to incorporate microarray assays with early work focusing on the role HIV plays in regulating gene expression. One study found that in infected cells, HIV progressively takes over the transcription machinery, leading to increased production of HIV mRNA while host-cell mRNA production declines. Simultaneously, genes that promote apoptosis and activate cell death associated-proteins called caspases are also increased. Another study found that the viral protein Nef could induce virtually the same transcriptional processes as occur when a T-cell is activated (at least artificially, or in vitro, by anti-CD3 antibodies), with differences only in factors regulating HIV transcription (Nef upregulates transcription, while anti-CD3 antibody activation favored factors downregulating HIV transcription). These results should be interpreted with caution, given that they may not fully reflect in vivo processes; for instance, apoptosis may be more easily triggered under in vitro conditions. But these findings do lend support to other research suggesting a critical role for Nef, and for studying its value as a potential target for drug and vaccine development.

Other studies underway will help elaborate both the effects of HIV infection on cells as well as on more general immune processes. Microarrays can clarify differential gene expression by comparing two conditions or states of cells, such as treated vs. untreated, resting vs. activated, naïve vs. memory, or cells at different points in time during proliferation. An obvious limitation is the need to understand as fully as possible the role and function of the different genes being observed. Despite the successful sequencing of the human genome, many questions persist about the functions of the many proteins encoded. Microarray assays also produce a huge volume of data, demanding an increased role for the techniques of bioinformatics to manage the flood of information produced by these studies. While other techniques for measuring differential gene expression exist, in some cases predating microarrays, these new assays will allow for rapid, simple, simultaneous comparison of an enormous number of genes. The trick will lay in figuring how to best use these tests—and what to do with the results.

Researchers generally agree that the majority of apoptosis in HIV infection happens in uninfected cells, with a variety of potential mechanisms proposed. Viral proteins vpr, tat and nef have been implicated (though vpr may also inhibit apoptosis), as has the binding of gp120 to the CD4 receptors of uninfected cells. HIV protease may play a role in apoptosis of infected cells, perhaps by increasing degradation of Bcl-2 (a key apoptosis inhibitor). HIV infection can also increase Fas expression, Fas susceptibility, and Fas ligand expression (Fas, or CD95, is a major pathway inducing apoptosis). HAART reduces apoptosis levels, with some question as to whether protease inhibitors in particular play a direct role (perhaps by inhibiting caspases). Despite the increasing body of knowledge about the role of apoptosis in HIV infection, many questions remain.

Defective HIV-specific immunity—the flawed ability of T-cells to react to HIV—may be the main reason that humans ultimately fail to control HIV infection.

Measuring Immune Response

Many of the assays discussed so far can distinguish between different kinds of T-cells, but they don't measure T-cell function—whether the cell can mount an effective response to antigen. When T-cells respond to antigen, they proliferate and produce various chemical messengers called cytokines and chemokines, several of which can be measured under laboratory conditions. In HIV research, these lab assays are of particular interest for evaluating the activity of immune cells that would normally be expected to attack and eliminate infected cells. Defective HIV-specific immunity—the flawed ability of T-cells to react to HIV—may be the main reason that humans ultimately fail to control HIV infection. Assays of HIV-specific immunity are also expected to become useful for the evaluation of anti-HIV vaccines and other immune-based therapies. Nonetheless, these tests can only provide an indirect picture of immune function, and conclusions about their meaning must be approached with caution.

Delayed-Type Hypersensitivity

Delayed-type hypersensitivity, or DTH, was one of the first tests developed to assess immunological memory. The DTH response was discovered over 100 years ago in an attempt to develop a tuberculosis vaccine. Robert Koch observed that about two days after injecting a killed tuberculin preparation under the skin, an inflammation in the form of a small swelling, or hard bump called an induration appeared in people who had previously been exposed to the tuberculin bacillus. Later it was determined that indurations were caused by T-cells and other white blood cells rushing to the injection site to attack the invader. The DTH test in current use measures the diameter of the induration to gauge the presence and extent of immune memory. The absence of an immune response, indicated by little or no induration, is referred to as anergy (a state of immune system nonresponsiveness).

DTH tests can measure general immune responsiveness as well as responses to particular antigens. General immune responsiveness evaluates the ability in vivo to mount a reaction to several common antigens that virtually everyone has been exposed to sometime in their past. Theoretically, anyone with a healthy set of memory T-cells will trigger a recall response. Tetanus toxoid, candida albicans, and mumps are commonly used as recall antigens in a general test; one version, the Multitest CMI, probes for responses to seven different antigens. The progression of HIV disease can sometimes be predicted by DTH response, with diminished responses, or anergy, signaling an increased risk for experiencing an opportunistic infection. By the same token, the reappearance of DTH response has also been used to demonstrate immune reconstitution following antiretroviral therapy. In theory, DTH tests using MAC and CMV antigens could help determine when it is safe to discontinue OI prophylaxis following a HAART associated rise in T-cells, as long as a recall response indicated that the immune system has regained the ability to keep these infections under control. This approach remains hypothetical, however; DTH tests for these pathogens are not routinely available, and no potential for guiding clinical practice has been established.

DTH tests may also prove useful for examining responses to HIV vaccine candidates. If a vaccine has properly primed an individual's memory T-cells with its antigens, injecting a protein fragment under the skin similar to that expressed in the vaccine should elicit an immune response; the appearance of an induration at the injection site would demonstrate that the vaccine has had some effect. However, while DTH testing might be useful in eliminating vaccines that fail to generate immune responses, the simple presence of a DTH reaction can not demonstrate that a vaccine will be protective—just because a few T-cells respond to one isolated protein fragment doesn't mean that the entire system can successfully fight off an HIV infection.

Lymphoproliferative Response

Lymphoproliferative response (LPR) is another way of measuring the ability of CD4 cells to respond to antigen. In this assay, an individual's T-cells are cultured in the lab then mixed with an antigen plus a radioactively labeled building block of DNA called 3H-thymidine. If the antigen stimulates the cells to proliferate, the new cells will incorporate the radioactive thymidine into their chromosomes. The amount of labeled thymidine detected in the cells gives a quick indication of the power of the antigen to stimulate a proliferative response. This measurement is reported as a stimulation index. LPR can assess the general function of one's immune response by culturing cells with multiple antigens. A decrease in LPR generally correlates with HIV disease progression while immune reconstitution following HAART is associated with an increase in LPR. Improved LPR after immune reconstitution supports the view that CD4 cell recovery represents both a quantitative and qualitative restoration of immune function; in other words, not only are more T-cells available than before treatment, but they also do a better job of responding to antigen. This observation underlies the rationale for discontinuing prophylaxis for OIs such as PCP, MAC, and CMV following sustained improvement of CD4 counts.

LPR assays can also be used to assess HIV-specific immune responses. To do this, CD4 cells are cultured with one or more viral proteins and the ability of the cells to respond and proliferate is measured. It is generally thought that HIV-specific immune response is lost during early infection, perhaps due to HIV preferentially infecting and killing HIV-specific CD4 cells. The rationale for initiating ARV treatment during acute HIV infection gained support after research showed that early treatment might help preserve a strong HIV-specific CD4 response. It is hoped that if this response can be preserved, then progression to disease may be delayed. Several studies have measured whether HIV-specific immune responses can be influenced by certain treatment strategies, including periodic treatment interruption. Although some research has indicated increased HIV-specific LPR in people with chronic HIV infection after undergoing structured treatment interruptions (STIs), these responses tended to quickly disappear, apparently providing only limited control of viral replication. Treatment with HAART during chronic infection also fails to restore HIV-specific immunity as measured by LPR; in fact, LP responses to HIV antigens tend to decrease to the point of disappearance as long as HAART mediated viral suppression is sustained. This finding suggests that the survival of HIV-specific memory cells requires at least some antigenic exposure to HIV and that during effective treatment, there may not be enough circulating HIV to keep the HIV-specific cells in play. Research currently underway will explore whether therapeutic vaccines or repeated cycles of treatment interruption that allow periodic, controlled viral rebound might possibly stimulate more durable HIV-specific CD4 cell responses.

Tetramers

Tetramer staining assays are producing a revolution in our understanding of cellular immune responses. This technique allows antigen-specific CD8 cells to be directly detected and quantified. Introduced in 1996, the tetramers assay used a new method to bind antigen to CD8 T-cell receptors. Major Histocompatibility Complex (MHC) is a cell surface molecule that displays antigen and is required for binding with T-cell receptors in vivo. Under laboratory conditions CD8 cells bind only weakly to antigen complexed with MHC. However, a specially constructed four-part complex of the antigen with MHC — the tetramer — allowed the antigen complexes to bind avidly with CD8 cell receptors, which can then be tagged and counted by flow cytometry. The technique is now widely used to identify and count HIV-specific cytotoxic T-lymphocytes (CTLs), especially in studies of HIV progression. Although tetramers able to bind to CD4 cells have been synthesized, their ability to effectively measure T-helper response has not yet been established.

Tetramer assays were crucial in establishing the role that HIV-specific CTLs play in the body's immune control of HIV. Studies in HIV-infected long-term nonprogressors and in monkeys suggest correlations between greater numbers of HIV-specific CTLs, slower disease progression, and lower viral loads. Over the course of infection, CTL numbers tend to decline as viral load rises. According to one study, although CTLs specific for HIV and Epstein-Barr virus persisted during chronic infection and could be detected by tetramer staining, the lymphocytes lacked the ability to proliferate and kill infected cells, possibly due to a lack of sufficient numbers of functional CD4 cells to trigger them. Yet, as we've seen, HIV-specific CTLs virtually disappear during successful HAART treatment, suggesting that HIV-specific CD8 populations are not maintained in the absence of circulating virus. Interrupting HAART has been shown to increase the number

of HIV-specific CTLs in circulation, which some have hoped can bolster at least partial immune control of the virus.

ELISPOT and Intracellular Cytokine Staining

ELISpots tests are derived from ELISA techniques and are used in HIV research to quantify antigen-specific CD4 and CD8 responses by measuring the amounts of various cytokines produced. When a T-cell is stimulated after encountering its antigen, it begins to produce several messenger proteins. In an ELISPOT test, a plastic well is coated with antibodies to the cytokine of interest (for instance, interferon-gamma, or IFN-g, which is secreted by CD4 and CD8 cells). A layer of cells that have been exposed to the antigen in question are placed in the wells and incubated. If any IFN-g is produced in response to the antigen, it will bind to the antibodies attached to the plastic well. After washing the well to remove excess cells, a chemical is added that binds to the IFN-g forming colored spots that highlight the areas in which cytokines have been secreted. The number of spots counted gives an indication of the number of antigen-specific T-cells found in the original sample.

Intracellular Cytokine (ICC) staining goes one step further by measuring cytokine production on the inside of CD4 and CD8 cells. Cells are chemically frozen and then treated with a detergent that makes their membranes permeable to fluorescent monoclonal antibodies. Once inside the cell, the antibodies tag the cytokines the researcher is looking for. Cells are exposed to antigen then processed to determine which have produced cytokines in response. As an additional step, the internally tagged cells can be sorted and counted by flow cytometry. This allows responding cells to be characterized in multiple, highly specific ways.

Unlike tetramer assays that can distinguish between CD8 cells that may or may not be able to kill their targets, the ELISpots and ICC staining assays only identify functional cells. For this reason, ELISpots and ICC assays are used in conjunction with tetramers to develop a more complete picture of HIV-specific CTLs. Tetramers identify a cell's specific antigenic match and the ELISpot and ICC tests report on the strength of the cell's response to that antigen. Although ELISpot and ICC assays are most commonly performed on blood samples, a recent study found that the majority of HIV-specific CTLs reside in the lymph nodes, and that the number of those cells declined less than those in the blood during treatment with HAART. ELISpot and ICC staining are revealing much about the role of cell-mediated immunity in HIV infection. According to one study that measured IFN-g production in response to p55 antigen (gag), on average, about 0.12% of circulating memory CD4 cells from HIV infected individuals were HIV-specific. This proportion was about one-tenth the frequency of CMV-specific CD4 cells, implying a significant deficit in the HIV-specific CD4 subset. While nonprogressing individuals tended to have a somewhat higher average proportion of HIV-specific CD4 cells (0.40%) than did progressors, the correlation was neither absolute nor exclusive. This suggests that cell counts alone cannot fully account for individual differences in resistance to disease progression or immune control of the virus. Another study measuring IFN-g production found that a population of HIV-specific CD4 cells persisted during chronic infection but that they were unable to proliferate in an LPR assay. This inability to proliferate was correlated with disease progression. In contrast, HIV-specific CD4 cells from nonprogressors retained the ability to proliferate in response to antigen.

Fred Gormley 1951–2002

Early on the beautiful spring morning of May 19, a few hours before 42,000 people gathered in Central Park for New York's annual AIDS Walk, Fred Gormley, a longtime friend of GMHC's *Treatment Issues*, passed away.

Fred died from a cascade of complications beginning with weight loss and persistent thrush which led to ports for amphotericin infusions and parenteral feeding, culminating in a staph infection, hospitalization, and kidney failure. Fred died from AIDS.

Fred was responsible for managing *TI*'s mailing list and monthly production, but better known for his darkly humorous articles about one gay New Yorker's odd life with AIDS. He also took delight in crafting a sassy table of contents and in finding piquant pull quotes for each issue. Our readers will miss him.

In Central Park, Tyne Daly quoted Edna St. Vincent Millay: "Quietly they go, the intelligent, the witty, the brave." Fred was an admirer of Ms. Daly and all things theatrical; he would have enjoyed pronouncing the poet's name. We will miss him.

Further evidence of functionally impaired HIV-specific CD4s came from a study that examined production of IFN-g (through ELISPOT) and IL-10 (through ICC staining) in response to gag antigen. IL-10 production was higher in people with progressive HIV disease compared to non-progressors and seronegatives, and high levels of IL-10 production were associated with little or no production of IFN-g in response to gag. IL-10 has been shown to induce T-cell anergy, downregulating IFN-g production and the proliferation of activated cells. In this study, the IL-10 producing cells were not necessarily HIV-specific, which leaves open the possibility that the gag antigen itself may induce IL-10 production. Treatment with HAART reversed this effect, restoring IFN-g production and decreasing IL-10 levels.

ICC staining in combination with tetramer analysis has also identified functional defects in HIV-specific CTLs. When CTLs are activated by antigen, they secrete IFN-g, tumor necrosis factor a (TNF-a), macrophage inflammatory protein b (MIP-b), and perforin, a protein that punches holes in the membranes of infected cells targeted for CTL-mediated killing. One study compared expression of these substances in CMV-specific and HIV-specific CD8 cells, finding that while cytokine secretions were similar, HIV-specific CTLs expressed much lower levels of perforin. A cell-killing assay was then used to measure the cytotoxic function of the CTLs. In this lab assay, target cells are labeled with 51Chromium (51Cr) then mixed with antigen (HIV or CMV) to infect the target cells. CD8 cells are then added and their ability to kill the infected cells is assessed. As target cells are killed, they release 51Cr, which can be measured to determine how many infected cells were killed by CTLs. In this study, HIV-specific CTLs were less effective at killing infected cells than the CMV-specific cells were, a finding consistent with reduced perforin expression. These HIV-specific cells also carried high levels of a cell surface protein called CD27, a marker indicating that these CD8 cells had not become fully mature effector cells capable of killing their target cells.

Limitations of Functional Assays

Assays such as tetramers, ELISpots, and ICC staining have been widely used in research aimed at strengthening or restoring HIV-specific immune function, particularly in STI and vaccine studies. However, these assays do not measure immune response in the body, and there is significant controversy about how relevant these in vitro models are to the dynamics of immune function in vivo. For instance, in light of the possible defect in perforin expression, studies measuring IFN-g production by CD8 cells in response to stimulation with HIV antigen may overestimate their functionality. Similarly, tetramer analysis requires identification of well-characterized human leukocyte antigen (HLA) types that will produce MHC molecules capable of presenting specific HIV antigen fragments. This means that the value of tetramer assays is currently limited to studying populations of individuals with HLA types that are clearly identified and relatively homogenous. This also requires a clear understanding of which MHC molecules are able to present specific antigenic fragments. To complicate matters, the ability of HIV to develop mutations able to escape CTL response has been observed. Finally, all of these assays use laboratory standard, wild-type viral antigens, which may not be representative of the viral strains existing in study patients. This too could possibly result in an overestimation of functional immune response.

Dr. Allan Landay of Rush Medical College, chair of the Federal AIDS Clinical Trials Group's (ACTG) immunology committee, has suggested that an ideal measure of HIV-specific immunity might be an integrated assay of immune function that examines all of the cellular players—antigen-presenting cells, CD4 cells, and CD8 cells—simultaneously. Dr. Landay also emphasizes the desirability of culturing the viral populations actually present in a patient's body to determine whether individuals can mount an effective immune response to their own HIV infections. While work toward this ideal is underway, such a comprehensive assay does not currently exist and may not appear in clinical use for many years.

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The Perils of Interpretation

By Daniel Raymond

Immune assays have generated several key findings about HIV pathogenesis: The selective depletion of naïve CD4 T-cells is a hallmark of progressive HIV disease. Chronic immune activation results in increased proliferation and apoptosis rates, which may play a major role, perhaps overshadowing the contribution of the direct cytopathic effects of HIV and/or cell-mediated destruction of HIV-infected cells. The possibility of impaired thymic production, and the prospects for stimulating the thymus to compensate for CD4 loss, represent important but contested areas of research. HIV-specific CD4 and CD8 T-cell responses, which are significantly impaired in untreated progressive HIV disease, continue to decline during the course of antiretroviral therapy. Studies of interrupted suppressive therapy suggest that periodic antigenic stimulation may temporarily awaken these responses.

Research indicates an association between HIV infection and multiple forms of immune dysfunction, processes that may operate independently or synergistically. Still other research has focused on the innate immune response and a perceived shift in the course of HIV infection from a predominantly Th1-type response (where effector CD4 cells prime cell-mediated immunity, including CTLs) to a Th2-type response (in which CD4 cells direct their support to humoral immunity, stimulating B-cell and antibody production). As of yet, however, there is no "smoking gun" revealing the precise mechanism(s) of HIV pathogenesis. Pools of consensus ebb and flow around various hypotheses, and while research has led to a range of proposals for therapeutic strategies, no new agents have been approved to treat the immune system rather than the virus.

Immunity research has had its most direct impact on clinical practice by justifying and guiding the treatment of acute HIV infection with a combination of HAART and treatment interruption. This approach has yielded promising data, which in turn has influenced thinking on vaccine development, but a demonstration of long-term clinical benefit is years away. Moreover, such early-stage treatment could only benefit the relative few who are diagnosed very soon after infection, and are willing and able to begin treatment immediately.

Ultimately, the applicability of in vitro assays to in vivo immune system dynamics and virus-host cell interactions must be questioned. For instance, the extensive research into the mechanisms of HIV-mediated apoptosis has generally been conducted in vitro, yet there is ample evidence that in vitro cell cultures can have different requirements for activation, signal transduction, and transcription.

Furthermore, it may be questioned if demonstrating that HIV affects the induction of apoptosis would necessarily mean that this mechanism plays a role in disease progression. Would anti-apoptotic agents in development to treat other diseases have any benefit in HIV treatment? Perhaps questions about how and why cells are dying in HIV disease are less important than looking at which cells are dying—too many of the useful cells and not enough of the infected or functionally impaired cells. Or perhaps lowering activation rates or increasing thymic output would be more effective in countering T-cell depletion.

Immune assays and the researchers who use them have become increasingly sophisticated at answering certain questions about the immune system and T-cell dynamics: What kind of cells? How many? What are they doing? Are they functional? However, these assays are only valuable for the kinds of questions they know how to answer; the whys and hows of pathogenesis and immune reconstitution can only be addressed through the vicissitudes of interpretation and speculation. The models or assumptions already in place at any given time tend to guide the research, and preconceived assumptions have certainly been overturned more than once in the history of HIV research. Yet the gradual accumulation of data inevitably shapes the scientific consensus on what we can say we know about HIV; certain theories are judged more or less consistent with the evidence, as other hypotheses and lines of inquiry gain support or are ruled out. Finally, as our investigational tools continue to evolve, perhaps the advent of research informed by genomics will afford us the luxury of worrying that our ability to ask questions will increase faster than our capacity to make sense of the answers.