

# AIDS and the Immune System

In order to understand *AIDS* (acquired immunodeficiency syndrome), we need to discuss two major biological phenomena: the virus that causes AIDS, known as human immunodeficiency virus, *HIV*, and the cells in the immune system that are by HIV. Only when both are described does the disease begin to make sense. We begin by extending our earlier description of how the immune system works and then examining the details of the life cycle of .

In our earlier discussion of immune cells, we listed two major classes of lymphocytes that together carry out the bulk of the functions needed for effective immunity - the B lymphocytes, whose descendant make antibodies, and the T lymphocytes. The disease of AIDS is ultimately about T lymphocytes, or rather their loss.

The T lymphocytes (so named because they begin their development and diversification in the *thymus*, the gland near the thyroid gland in the neck) can be further divided into two subclasses: the cytotoxic or killer lymphocytes ( $T_C$  cells) and the helper T cells ( $T_H$  cells). The  $T_C$  cells are specialized to recognize and kill infected or otherwise defective cells throughout the body. By killing a cell recently infected by a virus, for example, the  $T_C$  cells can abort the viral infectious cycle that may have started inside the cell before the virus has a chance to multiply significantly. This is a highly effective way by which the immune system prevents the extensive spread of viruses throughout the body.

## B cell activation

The other class of T lymphocytes, the  $T_H$  cells, are the central targets of attack by HIV, the causal agent of AIDS. For that reason, we will now elaborate in more detail  $T_H$  cell function.  $T_H$  cells are not responsible for directly making antibody (the job of the B cells) or for killing infected or defective cells (the job of the  $T_C$  cells). Their role is instead largely regulatory: the  $T_H$  cells tell the B cells when they should begin clonal expansion and antibody diversification. Thus, they control the B cells. Such control is vital to a well-functioning immune system that might otherwise make inadequate or excess antibody in response to antigenic challenge. An outline of how this occurs is pictured schematically in part (a) of Fig. 19.18 in Purves.

In our previous discussion of antibody production, the implication was that foreign protein molecules serve as the antigenic stimulus to provoke B cells. In fact, the provoking antigenic stimuli in the immune system are never entire protein molecules, but only fragments thereof. These oligopeptide fragments are generated by and displayed on the surface of macrophages and the B cells. The macrophages and the B cells can ingest foreign antigenic proteins and cleave them into small pieces. The resulting protein fragments are positioned on the external surface of the cell where they serve as the immediate antigenic stimuli that cause B cell clonal expansion and antibody production. A macrophage cell itself has no immunological specificity. In the case of the poliovirus particles discussed in a previous lecture, the macrophage will ingest poliovirus, break down the capsid protein molecules into a number of small oligopeptide fragments and then export these to its plasma membrane, displaying them on its extracellular surface

Now many T<sub>H</sub> cells come by and look over the oligopeptides that the macrophage is displaying. Each of the T helper cells that passes by and examines the macrophage's oligopeptide "wares" (as would a shopper in a store) has a very narrow, circumscribed interest in only one particular antigen. When, by rare coincidence, an antigenic fragment displayed by the macrophage is recognized by the T helper, the T<sub>H</sub> cell becomes physiologically activated, and begins a program of rapid clonal expansion. Of course, in the great majority of cases, encounters between the helper T cell and macrophages will not result in clonal expansion because the T<sub>H</sub> cell will not happen across the antigenic fragment in which it has a special interest.

Once the T<sub>H</sub> cell has undergone physiologic activation and clonal expansion, it will go on to its next phase of life, its next job. It will now seek out B lymphocytes that happen to be interested in the very same antigenic fragment. The B cell's "specialized interest" in one or another antigen arises, as described in the immunology lectures, through the unique antibody on the B cell surface. The T<sub>H</sub> cell, having found a B cell which shares interest in the same oligopeptide antigen, will then bind to the B cell and send a signal (via growth factors termed *cytokines*) to the B cell that induces the B cell to become activated and undergo clonal expansion. The B cell will then proliferate in response and produce descendant plasma cells that eventually begin to secrete antibody.

In effect, the helper T<sub>H</sub> cell is saying to the B cell "I have recognized an oligopeptide antigen fragment displayed by a macrophage. I see that you're interested in the same oligopeptide antigen. The fact that we have both encountered this antigen on separate occasions suggests that this is an important antigen against which you must mount a response. For that reason, I am now urging you proliferate and make antibody against this antigenic fragment."

Without this command coming from the T<sub>H</sub> cell, the B cell will not begin its program of clonal expansion and antibody production. Only now do we begin to sense the vital (albeit indirect) role played by the T<sub>H</sub> cell in antibody production.

## AIDS

We have all read about the discovery of the AIDS virus, both in France and in the United States in 1981. In the U.S., a cluster of 5 young, homosexual men were discovered who suffered from *Pneumocystis carinii* pneumonia infections, a disease that was otherwise only known in severely immuno-suppressed individuals. Soon thereafter, it was realized that their immuno-suppression was more far-reaching, involving mucosal yeast infections, widespread, disseminated cytomegalovirus infections, and chronic peri-anal herpes simplex viral infections. In addition to these debilitating infections, these individuals showed swollen lymph glands and night sweats. They also frequently contracted an unusual malignancy - Kaposi's sarcoma (a proliferation of cells lining the small blood vessels) - which until then had been encountered in the U.S. only on rare occasion and then in old men of Mediterranean (Italian, Greek, Jewish) extraction.

These young men were clearly immuno-deficient, in that they suffered from a variety of *opportunistic infections*, that is, infections by pathogens that surround us constantly, waiting for the opportunity to overwhelm the normally very effective immune defense that our bodies have erected. Moreover, the frequency and geographic occurrence of the disease, specifically its

clustering in a small, relatively homogeneous population having a shared lifestyle, suggested that the disease was triggered by some type of infectious agent.

Within a two years period of time, the agent responsible for causing the disease, initially called HTLV-III or LAV, was isolated in two laboratories - that of Robert Gallo of the U.S. National Cancer Institute (HTLV-III) and that of Luc Montagnier (LAV) in Paris. The present term, HIV, for human immunodeficiency virus was chosen as a compromise in nomenclature between the two warring camps, each of whom claimed priority in the discovery of the virus and thus the right to name it.

## Earlier Retrovirus Research

How did we come to discover the agent for AIDS so quickly? The lag between the description of a disease and the elucidation of its causative agent encompassed only two short years, almost unprecedented in the history of biomedical research. Usually decades have intervened between these two events.

By 1981, we were already well-poised to understand the nature of the infectious agent for AIDS for two important reasons: In 1971, President Nixon signed the War on Cancer Bill, which authorized the National Cancer Institute (NCI) to step up its attempts to find the causes of cancer - thought then to be retroviruses. The result was an intensive, decade-long study of retroviruses by many research groups, the result of which was a large corpus of information on retrovirus replication and a convincing proof that retroviruses were not the cause of most human malignancies, as many had anticipated.

The second factor was the continuing obsession of Robert Gallo of the NCI that retroviruses were common in humans and were responsible for a wide variety of malignancies. Even after their role as causative agents in human cancers had been disproven, he persisted in his attempts to find retroviruses in all types of human pathologic tissues. Thus, when the AIDS syndrome was first discovered, he responded with his usual conviction that it was a disease having, as its causative agent, a retrovirus.

## Retroviruses and their Replicative Cycle

Retroviruses have a most unusual life cycle that we review here in some detail, having discussed it briefly earlier in the semester. Recall that all viruses are really gene packets that move from cell to cell, entering a host cell and making more copies of their genes in the infected host cell. Dozens, hundreds, or thousands of progeny particles may result from such multiplication. The progeny virus particles then leave the infected host and proceed to infect other host cells nearby, continuing the infectious cycle.

The viral genome, either RNA or DNA, controls the ability of the virus to enter cells and multiply within them. In the case of retroviruses, a large class of structurally (and evolutionarily) related viruses, the viral genomes are single-stranded RNA molecules, which have plus-stranded polarity. Being (+) stranded, the viral RNA molecules can serve as mRNAs when associated with ribosomes.

Recall as well that virus particles, also known as *virions*, are composed of the viral genomes surrounded by a coat. The virion coat has two functions: to protect the viral nucleic acid genome from destruction while the virus particle passes from cell to cell, and to introduce the viral genome into an infected host cell, much as a hypodermic needle injects fluid into tissue. Introduction of the virion into cells involves several steps, including the initial tethering of the virion to the surface of the host cell, and the subsequent physical insertion of the viral genome into the host cytoplasm, a process that requires a penetration through the plasma membrane of the cell. In the case of retroviruses, the virion coat, like many other viruses, is composed of a lipid bilayer. Protruding from the lipid bilayer are a series of glycoprotein spikes. The latter are used by the virion for tethering to host cells.

The initial tethering (*adsorption*) of the virus to an infectable target cell occurs through the ability of the viral glycoprotein spikes to bind to specific glycoproteins displayed on the surface of the target cell. Such a target cell protein is termed a *receptor* for the virus. The cell receptor is always a normal cellular protein that the cell uses routinely for its own biological purposes. Viruses take advantage of these pre-existing cell surface proteins and evolve an affinity for them, exploiting them as convenient docking sites on the cell surface.

After the virus tethers itself to the surface of the target cell via the receptor molecules, the virion must insert its internal contents into the cytoplasm of the target cell. In fact, the innards of the retrovirus virion are composed of more than the single-stranded viral genomic RNA. In addition, a series of viral proteins is assembled in a quasi-geometric icosahedron (much like a Buckminster Fuller construction). The RNA together with the surrounding icosahedral protein layer is called the viral *nucleocapsid*.

Once tethered to the surface, the virus must fuse its own lipid bilayer with the lipid bilayer forming the plasma membrane of the target cell. Only then can the nucleocapsid be injected into the cell cytoplasm. This fusion is also mediated by the viral glycoprotein spikes that were involved in the initial tethering. Once the fusion has occurred, the viral nucleocapsid now is firmly ensconced in the cytoplasm of the host cell.

In the case of retroviruses, the nucleocapsid actually carries more than the viral genomic RNA. It also contains a copying enzyme, the viral *reverse transcriptase (RT)* enzyme. Once the nucleocapsid is in the cytoplasm, deoxyribonucleoside triphosphates made by the cell percolate into it. This allows the RT enzyme to be activated. It now begins to copy the viral genomic RNA.

The process of reverse transcription, as carried out by the RT is more complicated than when the enzyme is used by researchers as a reagent to make cDNAs from an mRNA template. To begin, a (-) stranded cDNA copy of the (+) stranded viral RNA is made. Then, the RT enzyme degrades the RNA portion of the DNA:RNA hybrid formed by the initial reverse transcription. Finally, the RT enzyme makes a (+) stranded DNA using the initially formed (-) stranded DNA as template. The end result is a DNA double-stranded helix that contains all the viral genetic information.

This viral DNA is then released from the nucleocapsid, transported into the nucleus, and covalently inserted into the host cell chromosomal DNA. Such insertion, the process of

*integration*, involves cutting and pasting by another virus-encoded enzyme. This enzyme makes a cut at a randomly chosen site in the host cell chromosome and then links the ends of the linear viral DNA (recently synthesized by reverse transcription) to the cut ends of the chromosomal DNA. The resulting integrated viral genome, termed the *provirus*, now assumes a molecular configuration equivalent to that of a cellular gene. The provirus can be transcribed by host cell RNA polymerase II, the resulting viral RNA transcripts can be polyadenylated, spliced where needed, and exported into the cytoplasm.

These newly synthesized viral transcripts in the cytoplasm can have two alternative fates. If they are unspliced, genomic length RNA, they may be packaged in progeny nucleocapsids. These will then be pushed through the host cell plasma membrane, during which process the lipid bilayer and glycoprotein spikes are acquired. This allows the newly formed virus particle to leave the infected cell and go on to infect other cells. Alternatively, the cytoplasmic viral RNA may serve as mRNA templates for the synthesis of progeny virus proteins. Some of these cytoplasmic RNAs may be spliced prior to export from the nucleus to the cytoplasm. This splicing allows a number of distinct reading frames in the viral genomic RNA to be fused to the 5' end of the viral transcript, resulting in a number of distinct mRNAs and hence distinct viral proteins.

*An aside: The translation of the viral proteins takes place at two sites in the cytoplasm. The (spliced subgenomic) mRNA encoding the viral glycoprotein spikes is translated on ribosomes associated with the rough endoplasmic reticulum, and these proteins are inserted into the ER, glycosylated, and exported via the Golgi apparatus to the cell surface. The other viral mRNAs, which make soluble proteins (which form the nucleoprotein core and the reverse transcriptase) are associated with free polyribosomes in the cytoplasm. The viral gene encoding the glycoprotein spikes is often termed *env* (the virion envelope), that encoding the nucleocapsid core is termed *gag* (for group-specific antigens), while that encoding the reverse transcriptase is called *pol* (for polymerase).*

## Consequences of the Retrovirus Replication Cycle

The unusual replication cycle of the virus places it in an especially intimate relationship with the cell that it infects, particularly with the cell genome. Here are some of the consequences of this peculiar life cycle:

### **Persistent infections**

If the viral infection does not rapidly kill the cell, which is indeed the case with many types of retrovirus infectious cycles, then the virus can persist, in the initially infected cell for an indefinite period of time. For example, the infected cell may produce progeny virus particles for an indefinite period of time at a slow, steady rate. Also, since the viral genome, its provirus, is integrated into the host cell chromosome, the provirus can be replicated and transmitted like all other cellular genes to progeny cells. Hence, the descendants of an initially infected cell can inherit the provirus and they may continue to produce virus particles like the initially infected cell did.

### **Latency**

The provirus, once integrated into the host cell chromosome, may be immediately transcribed, resulting in the release of progeny virus particles, or it may be transcriptionally repressed. This will cause the provirus to participate in a latent infection in which its presence will be inapparent

(since no viral proteins, RNA or virus particles will be produced). Some time later, a physiologic signal may awake the provirus from its dormant, latent state, causing it to be transcribed after months or years of silence, resulting suddenly in the expression of viral proteins and release of virus particles from a cell that seemed, until that moment, to be uninfected.

### **Germline transmission**

Some retroviruses may infect germ cells (in the ovaries and testes). As a consequence, the resulting proviruses may be transmitted via sperm or egg to the next generation of organisms, and thereafter to all lineal descendants. In effect, the provirus has become a Mendelian gene. Such endogenous proviruses may then be activated in an animal that has inherited them, resulting in the release of virus particles which may then spread through its tissues via normal infectious cycles. Mice, chickens and cats have many such endogenous proviruses. For reasons that are unclear, no new proviruses have been inserted into the human germline for the past 5-10 million years (although they were before then into the germlines of our ape-like ancestors). Thus, HIV is spread, as far as we know, exclusively through horizontal transmission, that is, from one person to another rather than genetically (vertically) from parent to offspring.

### HIV infection of T lymphocytes

With all this in mind, how does the retrovirus growth cycle help us to understand the process of AIDS pathogenesis (the process of causing disease)? To begin, we note that HIV-1 (one of the strains of HIV), like many other viruses, has a narrow *host range*. That is, it infects only a limited range of cells in the body; moreover, it infects only certain Old World monkeys as well as ourselves. In this sense, it is a highly specialized virus.

The cells that HIV-1 infects include largely the helper T cells, certain cells in the monocyte-macrophage lineage, and certain other cell types. We will focus here on its effects on T<sub>H</sub> cells that were described earlier. They are the main targets of HIV attack. Soon after the discovery of AIDS, it became apparent that AIDS patients are immunodeficient in large part because they have low and sometimes non-existent helper T cells in their circulation. This suggested that T<sub>H</sub> cells were eliminated in some way by the AIDS agent.

Some years later, it became apparent why and how HIV specifically targets the helper T cells: the receptor that it uses to tether to target cells is the CD4 protein. As described above, T cells associate with both macrophages and B cells. The CD4 protein normally helps the T cell to dock to these two classes of cells. Therefore, HIV virus exploits a normal T<sub>H</sub> lymphocyte protein as a receptor for binding itself to its target cell.

The CD4 protein is present largely, but not exclusively on T<sub>H</sub> cells. Infection of T<sub>H</sub> cells by HIV can be blocked if monoclonal antibody made against CD4 is added to these lymphocytes prior to attack by HIV. The anti-CD4 antibody, by covering up the CD4 molecule, prevents HIV particles from accessing the CD4 and tethering themselves to the T<sub>H</sub> cell surface. Moreover, certain human cells that are usually not infectable by HIV-1 can be converted to infectable cells by introducing a cloned CD4 gene into these cells, thereby forcing them to express CD4 on their surface. This might suggest a useful anti-HIV therapy, in which the CD4 proteins of infected T cells are blocked with some protective antibody. But that would not be a useful strategy, since it

would prevent these T<sub>H</sub> cells from having normal interactions with macrophages and B cells that are essential for maintenance of the normal immune response.

HIV may enter into a T lymphocyte and become latent by being transcriptionally silent. Thus, the HIV provirus contains at its left (5' end) a transcription promoter termed LTR. The viral LTR promoter contains within it a DNA sequence that serves as a binding site for the cellular transcription factor NF-κB. In resting T lymphocytes, this cellular transcription factor is unavailable. However, if the T lymphocytes becomes functionally activated (as described, for example, earlier), then the T lymphocytes begin to make active NF-κB transcription factor as part of their normal activation response. This transcription factor then binds to the viral RNA promoter (along with binding to other cellular gene promoters), helps to turn on transcription of the provirus, thereby activating and revealing the hitherto latent viral infection.

The consequences of this latency are important for the viral infectious cycle. The ability of the immune system to eliminate infecting viral pathogens like HIV depends in large part on the ability of the immune system to recognize and kill virus-infected cells. But if many helper T<sub>H</sub> cells contain a latent, unexpressed HIV proviruses, then these cells will escape killing by the immune system because they are phenotypically normal and outwardly have the appearance of being uninfected. These latently infected cells may harbor the viral infection for years and there is no way of knowing where they are in the body and there is no means of selectively eliminating these cells. Months or years later, when a physiologic stimulus (e.g. antigenic stimulation) should activate such a latently infected T cell, the virus may then pop out suddenly and trigger rounds of infection. This explains the difficulty of totally ridding the body of HIV once it has established itself.

## Antigenic Variability

The easiest way for the immune system to recognize either an HIV particle or an HIV-infected cell is through the presence of the viral glycoprotein spikes that protrude from the HIV virion and from the plasma membrane of infected cells.

Recall that:

- 1) the infected cell membrane displays these viral glycoproteins;
- 2) the viral nucleocapsid in the cytoplasm forces its way through the plasma membrane; and
- 3) in the course of pushing its way out of the cell, the nucleocapsid coats itself with a lipid bilayer containing these viral glycoprotein spikes.)

The viral glycoprotein contains several regions that are highly antigenic, that is, attract most of the attention of the immune system by eliciting antibody responses. Once the immune system makes antibodies against the viral glycoprotein antigenic regions, these antibodies can either bind to virus particles and neutralize/inactivate them or they can bind to the surface of HIV-infected cells, facilitating the elimination of these infected cells by other immune cells (such as macrophages). This explains why the production of anti-virion antibody is useful in establishing an anti-viral response.

However, the virus makes itself into a moving target that is continually eluding the immune system. In particular, HIV is capable of changing the amino acid sequences of these antigenic regions of the glycoprotein spikes from month to month and year to year - a form of constant microbial guerrilla warfare. This continually results in the appearance of new variant strains of

HIV in an infected individual that are no longer recognized by the existing repertoire of antibodies that this person's immune system has mustered until then. As soon as his/her immune system has a chance to make antibody against one variant of the glycoprotein, the virus pops up with a novel glycoprotein version. The new version is not recognized by the existing antibody and as a consequence, escapes antibody attack until the immune system catches up with it days or weeks later. Poliovirus, by contrast, is not antigenically variable and immunity established against one form of poliovirus results in lifelong protection against reinfection.

This changeability of the viral glycoprotein antigen is made possible by the mutability of the viral genome. The reverse transcriptase enzyme is rather sloppy, creating frequent copying errors during reverse transcription. This leads in turn to a high frequency of mutant glycoprotein genes, some of which specify novel antigenic variants of HIV that escape existing immune defenses and multiply unhindered for a period of time until the immune system catches up with them.

For this reason, the progression of an HIV infection over many years is marked by a series of episodes in which the virus bursts out, replicates, is brought under control by the immune response, only to have a new round of infection start up by an antigenic variant months later. Eventually the immune system's defenses are worn down to the point that HIV replication is unimpeded. By this time, the helper T cells have become so depleted (see below) that the body is now wide open for attack by a variety of viral, bacterial, and fungal agents that create the devastating infections seen in AIDS patients.

## Cell Killing

Unexplained by all this is the mechanism by which HIV actually kills helper T cells that it has infected. The very process of making progeny virus particles is lethal for many types of virus-infected cells. In the case of poliovirus, the process of making hundreds of thousands of viral progeny particles depletes the cell of many of its normally required macromolecules. But in the case of HIV infection, the mechanisms of the viral cytopathic effects are more varied and subtle.

One explanation of HIV's cytopathic effects comes from a reexamination of the functions of the viral glycoprotein. Recall that in addition to its ability to adsorb to a cell surface by tethering to the CD4 protein, the glycoprotein also has an ability to fuse the lipid membrane of the virion with the plasma membrane of the infectable target cell. In the case of a helper T cell that is actively producing progeny virus particles, its plasma membrane is studded with HIV glycoprotein spikes, many of which will eventually be picked up by progeny HIV particles on their way out of the cell.

These viral spikes may already be functionally active while they are on the surface of the virus-infected cell, even before they have become incorporated into progeny virus particles. The consequences of this can be devastating for the cell. First, they will cause the surface of the virus-infected cell to adsorb to that of other, still uninfected helper T cells displaying CD4 protein on their surface. Second, the fusogenic activity of the viral glycoprotein spike will cause this cell to fuse with such uninfected helper T cells. Indeed, this cycle can be repeated over and over, resulting in the fusion of the infected cell with dozens if not hundreds of uninfected helper T cells. The results will be a multi-nucleated syncytium which is non-viable. The cells in such a syncytium will die after several days.



## The History of an HIV infection

We can now assemble and integrate all of the above information into a reasonably coherent picture of how HIV begins its infectious cycle in an individual. It is usually transmitted from person to person through blood or semen. In both cases, the bulk of the transmission seems to occur by passage of infected cells rather than by free-floating virus particles.

Once inside the body, virus is released from the introduced infected cells, largely infected  $T_H$  cells. HIV begins rounds of infection, largely in the  $T_H$  cells of the lymphoid organs (e.g. lymph glands, spleen) of its newfound host. This results in an *acute phase infection*, in which high viral titers (high concentration of free virus in the blood) can be detected in the recently infected individual several weeks post-infection. After several more weeks, the viral titer drops precipitously. This drop is due to an immune response that the still largely intact immune system of the host individual mounts. Two immunologic mechanisms are used to reduce the viral titer: cytotoxic T lymphocytes ( $T_C$  cells) are produced which kill off the great majority of HIV-infected  $T_H$  cells. Later on, the B cells of the host produce high titers of anti-HIV antibody.

Now the HIV infection goes underground, or at least seems to. Large numbers of latently infected  $T_H$  cells can be found for a number of years. While the viral titer in the blood is reduced, actively replication of HIV continues however in a number of lymphoid organs such as lymph glands. In such individuals, as many as  $10^7$  to  $10^9$  virions may be produced per day and eliminated (perhaps by antibody neutralization) almost as soon as they are produced. Occasional flare-ups of high viral titers (*viremia*) may occur as HIV variants evolve new glycoprotein antigens and temporarily evade the existing immune response. All the while, as many as  $2 \times 10^9$   $T_H$  may be killed throughout the body each day!

For a number of years (highly variable from one infected individual to another), this enormous death rate of  $T_H$  cells is compensated by an equally large proliferation of  $T_H$  cells, resulting in a semblance of a stable, steady-state infection. Sooner or later, however, the ability of the immune system to replenish the ranks of the  $T_H$  cells is exhausted, often irreversibly. Then the number of  $T_H$  cells plummets and the overall functioning of the immune system collapses, for reasons described earlier. One of the first consequences is the inability of the immune system to continue to suppress the HIV infection, leading the virus to break through the immune defense barriers that have held it in check for years.

Soon opportunistic infections by other viral, bacterial, and fungal pathogens strike various sites throughout the body. The Kaposi's sarcoma frequently observed in homosexuals but rarely in those infected by blood transfusions may be caused by sexual transmission of a recently discovered herpes virus-like agent termed HHV-8. Lymphomas may also occur at high rates, a common occurrence in individuals with a compromised immune system. These days, lifespan and a reasonable quality of existence can be extended for a number of years using antibiotics, antifungal agents, and chemotherapy, converting AIDS into a chronic disease for this period. But in the great majority of cases (>99%), the end is clear, sad and inescapable.