Chapter 4
of

Contrary Life and Technical Fixes
from
malaria vaccine
to
hormone contraceptive

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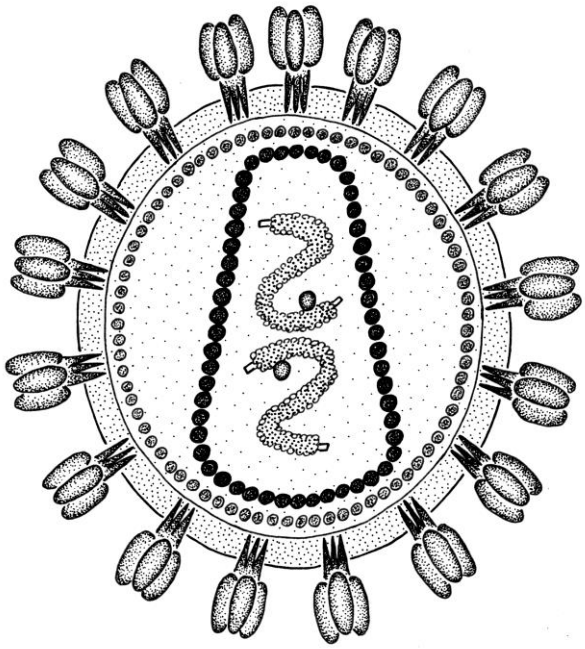
HIV and Retrovir, the first drug against AIDS



Where and when the infection started he never knew. He could hardly remember how many partners he had enjoyed the company of, let alone those he had failed to use a condom with. Probably his troubles started one night at a party in Cuidad del Carmen. Onshore leave from an oil-rig in the Gulf of Mexico was a time to lose memories of the heat, noise and smells of work in the canteen kitchen. Back on land he would enjoy a game of soccer then laze on the beach dreaming of becoming a professional in the game, of saying goodbye to the rigs forever. As the sun was setting he would start asking around for where the best party might be. What was the point of earning good money on the rigs without having plenty of fun when back on dry land – a time to forget the claustrophobia of the kitchen, a time to lose yourself?

The virus penetrated his body at his penis. All that the virus needed was close contact with a mucus membrane of the person it was already infecting and a minute rawness at the opening of the other person's urethra. The individual particles of the virus, the virions, were only 100 nanometers across, sixty times smaller than a red

blood cell. They slipped easily between the loosely bound cells at the outer layers of his skin.

This particular region and type of skin, urinogenital mucosal epithelium, contains many cells of the immune system that are specialized for detecting the varied microbes that might be a threat at this vulnerable site. Macrophages and dendritic cells reside in the submucosa layer of this skin. They bear long tentacle-like extensions of their surface reaching between structural cells of the skin to act as sentinels that will warn the rest of the immune system of an invasion.

A few virions, of the human immunodeficiency virus type 1, attached to the outside surface of a dendritic cell. The cell's outer surface, its plasma membrane, was studded with large molecules of proteinaceous lectin that acted to detect invading microbes. This particular lectin, called DC-SIGN, acts as a detector or as a receptor, depending on how you look at the process. The structure of DC-SIGN gives it a strong affinity for mannose sugar components of a molecule that protrudes from the outer surface of the HIV virions. This viral molecule is a large densely convoluted mass of glycoprotein of molecular weight 120 kilodaltons, called gp120. The virions were covered with these molecules as knobby studs protruding from the surface. They bound tight to the DC-SIGN molecules. The dendritic cell responded to this binding by maturing into a cell that could then migrate out of the skin, push into a fine duct of the lymphatic circulation and thence travel inwards toward the man's groin to reach a lymph node several days later.

The healthy purpose of such a journey would be to present the virus, in processed and non-infective form, to the immune system so that a full defense could be deployed against further HIV virions. But these virions avoided engulfment into the interior of the dendritic cell. They were not endocytosed for digestion into protein fragments, peptides. So the cell could not present any peptides as foreign, non-self material, to the immune system. Any foreign material that the immune system can respond to is called antigen; it stimulates the production of antibody and activity of certain defensive cells.

These dendritic cells normally present any antigen in urgent alarm to any of the millions of cells in a lymph node that are adapted to respond. But these virions arrived in his lymph node whilst still out on the surface of the dendritic cell, still potent and infectious.

In the node there were many millions of white blood cells of the lymphocyte type – small, with a thin rim of cytoplasm around their spherical nucleus and without the charge of active granules of typical bacteria-destroying cells like neutrophils. The lymphocytes comprised two main types: B-cells and T-cells. They are the workforce of

that part of the immune system that can rapidly adapt to attack with high specificity any possible new threat from outside.

As the dendritic cell, with its infectious passenger, migrated through the softly loose mass of cells in the lymph node it pushed up against many of these lymphocytes by chance. Normally it would have bonded with a particular type of T-cell. It would have formed an intimate connection of intermolecular forces called an immunological synapse where it could pass on its message to cells capable of killing invaders. The dendritic cell needed a T-cell with surface adhesion receptors that would match its own similar receptors. Once the two cells were closely bound with these intermolecular attractions the virions shuffled over the surface of the dendritic cell and squeezed into the narrow space between the two cells. This T-cell was a type equipped with many large molecules, embedded in its plasma membrane, of a protein called CD4. The molecules protrude out like hairs and have a crucial role in activating other immune cells. The gp120 glycoprotein of the HIV virions has a strong affinity for CD4 protein; the two molecules stick tightly together, binding the virion to the T-cell.

The gp120 molecule is intertwined with a complex rod-shaped molecule called gp41, like a stalk through its center. In response to binding with the gp120 the CD4 molecule was induced to bend down toward the plasma membrane of the T-cell. This pulled the gp120 closer to the T-cell where it came in contact with another protein molecule called CCR5 that was embedded in the plasma membrane next to the CD4. The force of bonding between these two molecules thrust the rod of gp41into the plasma membrane of the T-cell. Then contact with the T-cell triggered the gp41 molecule to fold down sharply. These series of reactions were repeated hundreds of times in a spreading zone of contact. The virion was pulled so close to the T-cell that the plasma membranes of both the cell and the virion met. Moreover, these membranes were of the same origin. The virion had stolen its outer coat from another T-cell – the cell in which the virion had replicated when in the man's partner. Like to like, these two bilipid layers smoothly ran into each other as a single layer and as they flattened out they pushed the genetic core of the virion into the cytoplasm of the T-cell.

The genetic material of the virion infected the man with the information needed to produce more virions of HIV-1. The virions had subverted the healthy immunological synapse between the antigen-presenting dendritic cell and the T-lymphocyte into an infectious synapse, a route of trans-infection. Once inside the T-cell the core of the virion was hidden from all those parts of the immune system that rely on open and direct contact with microbes.

The virion's genetic material lay in a columnar box, a capsid made of protein, and it contained two molecules of ribonucleic acid, RNA, and associated enzymes. The

capsid opened to release the RNA into the cytoplasm of the T-cell, reducing the virion to nothing more than its essentials of genetic material and enzymes.

Bound to each single strand of RNA was one molecule of an enzyme called reverse transcriptase. This molecule started working its way along the strand, simultaneously chopping off complete subunits of the nucleic acid from the end of the strand and synthesizing a new strand of deoxyribonucleic acid, DNA, to replace the original RNA template. The subunits of the RNA were the nucleotides corresponding to the nucleobases cytosine, guanine, adenine and uracil.

The necessary supply of metabolites was supplemented from the cytoplasm of the host cell, including swapping the nucleobase uracil with thymine for the assembly of DNA. This new DNA was complementary to the RNA: its nucleobases paired exactly with their different but matching nucleobases in the RNA sequence, as a key fits in a lock. The reverse transcriptase then doubled back on its direction of travel, along the first DNA strand whilst using that as template for another strand. Finally these two strands matured with a cap and tail at either end as a functional double stranded DNA of HIV. The other molecule of reverse transcriptase repeated the process with the paired original RNA strand producing a second full DNA molecule. They held the genome of infective HIV, containing all the information needed to synthesize viral proteins and RNA for assembly into new virions.

Using pathways within the physical sub-structure of the T-cell the viral genome migrated into the nucleus of the T-cell. Finally, to complete its capture of the essence of this cell the viral DNA used its associated integrase enzyme to embed itself into the human strands of DNA within the cell. This ultimate parasite, a material object reduced to information as a sequence of nucleotides, became a provirus. Lurking in the nucleus, it was capable of either rapid replication into more virions or a long wait for the best time to replicate.

The infected T-cell became naturally activated by the normal components of its synapse with the dendritic cell. The provirus responded to this activation by producing transcripts of messenger RNA out into the machinery of protein synthesis of the cell – its Golgi body and ribosomes. As the nine different proteins of HIV gathered in the cytoplasm of the cell a stepwise self-assembly of new virion cores began. Thousands of separate capsids assembled, replete with their new RNA strands and accessory enzymes. The molecular pair of gp120 and gp41 separately migrated into the plasma membrane of the cell. They embedded there, with the gp120 molecules exposed to the exterior. Each full capsid and assembly of viral proteins then pushed outward into the cell's plasma membrane, finally budding off with an outer coat of the plasma membrane of the T-cell. They matured into virions capable of infecting other

cells, with a strong affinity for lymphocytes bearing CD4, but also capable of infecting macrophages and the glial cells of the nervous system.

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Most of the group of virions that invaded his skin soon succumbed to basic immune defenses. They were killed by a branch of innate immunity called the complement system. It works through a series of chemical reactions between protein molecules that permeate the blood and lymph at low dilution. One of these proteins, mannose binding lectin, has an intense affinity for the specific molecular pattern of the glycoprotein gp120 of HIV. This binding lectin circulates as an oligomer of nine molecules, looking like a bunch of tulips. Attached to that bunch is a serine protease that can split other proteins. When this lectin bound to the viral gp120 of the virions in the man's skin the protease splitting action on a particular molecule of the complement system left one part of the complement molecule attached to the virion and the other part left free to trigger the next step in a cascade of splitting, binding and triggering. The virions became coated with a growing mass of complement in a process called opsonization. Some of the opsonized virions were ruptured by the physical forces exerted by the coating alone. Other virions were bound by receptors for complement on the surface of macrophages which promptly engulfed them into intracellular endosomes. There the acidic conditions, combined with reactive oxygen species of molecules, killed the virions.

These macrophages were activated by the successful viral killing. In response they secreted a chemical messenger that alerted surrounding cells to the threat and stimulated a local inflammation. This induced the minute capillaries in the skin to become more permeable, allowing more lymph to the site of infection and bearing lectins, complement, macrophages and natural killer cells. These natural killers have an innate attraction to the interferon chemical messenger released by cells infected by the HIV virions. The killer cells aligned close to the infected cells to blast from their cytoplasm a shot of toxic granules at the infected cell. Any infected cell assaulted by the toxins died, along with the virions and provirus it had been burdened with.

He was unaware of this microscopic turmoil, the skirmishes between the invading virus and his innate immune system. He was young, strong, fit, and well nourished. His immune system had easily fought off many types of infection and acquired comprehensive resources. At the site of infection were a few dendritic cells that were able to perform as they should, to engage with and endocytose virions into internal compartments with acid conditions. Here the virions split into sub-components, peptides from the long chain polypeptides that constituted proteins of

HIV. These peptides were foreign to his body thus his adaptive system would respond to them as antigens.

First the system's dendritic cells needed to present the antigens to other types of immune cells. They had to hold out the peptides for open display so that these other cells could detect and respond. Moreover, the peptides had to be coupled with large glycoprotein molecules within the dendritic cells. These molecules were precisely defined by many parts of the cell's genetic code comprising a whole known as the major histocompatibility complex. They occur in two functional types, MHC-I and MHC-II. When out on the cell's surface the MHC molecules were anchored into the plasma membrane by one or two stalks and the antigenic peptide was presented outward in a cleft formed by two short arms.

In his infected lymph node the man's lymphocytes jostled and squeezed between the structural tissues. All the cells of the two main groups, the B-cells and the T-cells, were equipped at their origin, in the bone marrow or thymus gland respectively, with receptors on their plasma membranes. Receptors each possessed a single specificity and affinity for a unique molecular form of antigen. These properties were generated by random shuffling of many genes to provide billions of specificities to the immune system at any moment, able to respond to a vast variety of potential threats from invaders from the outside world. The specific lymphocytes existed in small clones, every cell identical; one or two of the billions of receptors available were certain to match the antigens of HIV.

His lymphocytes came in three types that could fight against the virus. There were CD4 T-lymphocytes equipped with the co-receptor molecule CD4. These would mature into helper cells whose work was to activate and mature other types of lymphocyte with ability to kill directly the virus. There were B-lymphocytes that would secrete antibodies against the virus. There were CD8 T-lymphocytes with their CD8 co-receptor molecules that would mature into cytotoxic T-cells capable of killing cells infected with the virus. These CD4 and CD8 co-receptors acted alongside the main T-cell receptor molecules – they protruded like antennae above the squat T-cell receptor molecule on the surface of the cell. When one of these receptors came in contact with a peptide of HIV presented in the arms of an MHC molecule it bound to the peptide. Then the CD co-receptor linked onto the side of the MHC molecule to reinforce and confirm the binding.

A few of his dendritic cells presenting HIV peptide on MHC-II molecules contacted CD4 T-cells that happened to have receptors specific for HIV. Normal immunological synapses were formed and a sequence of recognition and verification signals passed between the cells. This ensured that the CD4 T-cells activated correctly, specifically. These several CD4 T-cells then started to secrete the chemical messenger

interleukin-2. This stimulated the same type of cells to expand individually into a lymphoblast state. The nucleus prepared for division as the cytoplasm expanded into a broad irregular halo. The lymphoblasts divided repeatedly, expanding the clone. All the while they secreted more interleukin in a self-stimulating cycle, accelerating the clonal expansion to spread through his lymphoid tissues. Soon these fully matured CD4 T-cells became available in their billions as helpers in service of cells secreting antibody, and cytotoxic cells.

His B-cells came similarly equipped with receptors in the form of immunoglobulin molecules embedded in the cell's plasma membrane and amongst them was a small clone whose receptor was specific for the gp120 glycoprotein of HIV. When these cells first met the antigenic gp120 directly on the few virions briefly free in the lymph and blood the receptors bound to the antigen in its native, raw, state. The B-cells internalized antigens of the virion, split them apart enzymatically then displayed their antigenic peptides in the arms of MHC-II molecules. Mature and active CD4 T-cells with receptors specific for HIV interacted with antigen presented by the B-cells. This interaction provided the B-cells with a second signal, a verification, that they should mature into plasma cells. Such cells became capable of synthesizing and secreting antibody out into the lymph and blood. The activated B-cells divided repeatedly and rapidly as an expanding clone of antibody secreting cells and also a smaller population of memory B-cells.

The memory cells were there to form a very long-lived reserve capacity of cells specifically primed in advance. They could expand rapidly into plasma cells capable of secreting the same antibody in response to renewed infection with the same virus. The plasma cells fattened with large dense areas of Golgi bodies and ribosomes as antibody factories capable of secreting two thousand molecules per second. Some of the antibody bound to the gp120 and gp41 on free virions. Complement bound onto the top of those pairings to build up an opsonization coating. Macrophages were able to engulf these coated virions to kill them.

Despite the vigorous response of his immune system to activate all these antibody secreting cells there was insufficient killing of the virions. Too many of them escaped opsonization and phagocytosis because of a contrariness in the method of replication of HIV. The reverse transcription method of making DNA from RNA, to go from virion to provirus then back to RNA for new virions, is error prone. Far more prone in copying the sequence of bases than is the usual DNA to DNA copying. The enzyme is poor at proof-reading, but this seeming inefficiency was to the advantage of the virions surrounded by antibody.

The errors in proof-reading, as mutations, generated variation at a high rate. Meanwhile the virus replicated at a rate of more than a billion new virions daily.

Variants of HIV were produced in numbers beyond the limits of the spontaneous ability of lymphocytes to recognize new antigens. The variations included continuous changes in the conformation of the surface glycoproteins of the virus. Antibody synthesized to match and bind to an early variant failed to bind strongly to later variants during the infection. At best the binding was weak because of the high level of glycosylation of the gp120, shielding the virions behind an armor of glycans.

The man's population of CD8 T-cells also contained a clone bearing receptors specific for HIV. In contact with a mature dendritic cell that was presenting HIV antigen on MHC-I molecules, the T-cells bound to the dendritic cell. The CD8 co-receptor molecules joined forces with the HIV-specific T-cell receptor to doubly bind with the MHC and the antigenic peptide held up in its arms. An immunological synapse formed and a recognition signal passed to the T-cell, prompting it to mature. These T-cells were destined to become charged and ready with potent toxins. His immune system needed to aim this weapon accurately or risk collateral damage. A second verification signal was provided by helper CD4 T-cells already sensitized to HIV, acting along with the dendritic cell presenting the antigen. The helper cells secreted interleukin-2 to stimulate the dendritic cell, which in turn secreted more interleukin-2. This local flood of chemical messenger prompted the CD8 T-cells to mature and divide rapidly as two expanding clones. The bigger clone comprised cytotoxic T-cells and the smaller clone comprised memory cells with the latent capacity to divide rapidly in response to renewed infection.

Any of his cells infected with virus reacted by presenting antigenic peptides on MHC molecules. The cytotoxic T-cells now circulating throughout his body contacted infected cells by chance and bound to these signals of infection. In the cytotoxic cells, granules of toxins in the form of perforin and granzyme migrated through the cell's cytoplasm toward the area where the two cells were bound, to align with the infected cell. By exocytosis these toxins shot over the infected cell, perforating its plasma membrane. Once inside the infected cell the granzyme triggered a cascade of reactions that led to a program of dissolution and clearance of cellular debris, so that the infected cell disappeared. The cell committed suicide, or apoptosis, and along with its remains were the dead remains of virus it could no longer support. The cytotoxic cell retracted to re-organize itself and hunt for more infected cells.

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Five days after becoming infected he began to feel ill – a vague malaise. Was it something to do with the filthy smoke pouring densely from the flare-stacks he wondered? On windless stinking hot days in the Gulf smoke shrouded the rigs,

darkened the sun. Maybe it was a bout of influenza or one of those odd virus infections from all those mosquitoes breeding amongst the mangroves to the south of his town?

His capacity for generating new lymphocytes was running as fast as his body could supply the materials and energy for several billion new lymphocytes every day. Their individual reactions with the virus were releasing tiny amounts of potent cytokine messengers between cells, and toxic by-products of inflammation and cytotoxicity. As the days dragged by he developed vague aches and persistent nausea. His manager noticed his lethargy and sent him to the company clinic in Cuidad del Carmen on the next spare seat in the service helicopter. The physician, puzzled by the odd mix of symptoms, played safe and recommended vitamin tablets and two weeks of onshore sick-leave.

Later, back on the rig, he struggled with this acute phase of the infection for several more weeks, whilst gradually recovering some ability to get through another day in the kitchens. He craved fresh cool air and clear sunshine; every week not just during the routine leave. Within his body a microscopic battle raged. The virus was not producing pustules like chicken-pox or cold-sores; it was not making him feverish. Instead his infection was almost imperceptibly gaining in numbers of virions in circulation. It spread in lymph and blood, it infected the glial cells of his nervous system and CD4 memory cells of his immune system. There lurked the provirus, deep within these cells, manipulating his own DNA. Long-lived cells, a reservoir of infection that with repeated topping up could outlive him.

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Two years went by and the numbers of his helper cells, the CD4 T-cells, began to decline. The battle of attrition between his immune system and the prodigious replication of the virus was gradually being lost. The CD4 co-receptor on these helper cells was the prime target for the gp120 and gp41 molecules on the surface of the virions. The virus had evolved to latch onto precisely this one molecular species. The receptor was conveniently available on lymphocytes that were common in areas of skin susceptible to invasion by microbes: penis, vagina, cervix, mouth and anus. Tissues that were compromised by contradictions between their physiological role and their exposure to the outside world. Reproduction in mammals requires risky exposure of at least one sex of gamete cells, and sensitively tactile sex organs.

CD4 was available on some other types of cell as well, but a healthy body was always teeming with T-lymphocytes of this type. And the CD4 T-helper cells were managing and organizing the adaptive immune system. As more of them became infected, rendered non-functional, then killed by cytotoxic cells, fewer of the CD8 cells

were correctly activated and matured. The balance was fine, just the slightest tilt toward the virus, but the shift was inexorable. The capacities of the machinery for generating lymphocytes were powerful and widespread, but they battled against many new virions replicated daily.

He did not feel distinctly ill neither did he enjoy his former vitality. He noticed he was losing weight despite eating well. His mind held no understanding of the battle deep within his body. There was little virus freely circulating in his blood. Little of it was reaching his semen so his now regular partner back onshore was scarcely at risk of catching the virus from him. The vast metal box he worked in, noisy, stifling, endlessly busy, depressed him. With his due turn for onshore leave approaching and his enthusiasm for days at the small beach resort on the north shore of town waning he booked a proper holiday. His tour visit to see the Mayan temples and museums in Guatemala and Belize was enjoyable, except for the last celebratory meal of barbequed bushmeat in a specialty restaurant that the tour included. He preferred beefsteak.

Back on the rig he suddenly came down with diarrhea and vomiting; his boss promptly dispatched him to the clinic onshore. The physician took a detailed medical history and a stool sample from her patient. She also included a sample of his blood, and sent them to the main state hospital in Mérida, with a copy of the patient's medical notes and a request for tests to diagnose the cause of this gastroenteritis. A norovirus outbreak starting in the rig canteen was her worry.

The diagnostic technicians, whilst testing the stool, passed over the blood sample to a separate research project that was estimating the local prevalence of infection with HIV-1. For this the researchers ran two serological tests in series: enzyme linked immunosorbent assay followed by Western blotting. They requested a second blood sample to repeat the tests. At the second checkup visit to the clinic he became worried by the physician's reluctance to explain why more blood was needed, especially since his gut had settled back to normal.

The news that he was HIV positive shocked him into a state of disbelief. He was only twenty six and planning a new life for himself. Surely all I need is a new job and place to live to make me feel better he thought? The only comfort his physician could offer was that the research project in Mérida was evaluating a new drug that had recently been registered for use in the USA. How might it fit into medical services elsewhere? She knew little about this drug, regarding virus infections as untreatable except by supportive therapy. She warned him that whatever this drug might do, it was unlikely to provide a quick cure.

He resigned from his job with the oil company and cashed in his savings. He packed two cases of possessions and caught the bus back home to Mérida. As he stared blankly at the countryside flashing past the window he fretted with anxiety about how

to break the news to his family. He was ready to try anything, to accept all the help he could find. Within a week he was at the city hospital. The clinicians put him on a course of zidovudine, or AZT as it was then often known. He obediently swallowed all the pills and endured their side effects. Within one month he was cheered by the news that the counts of his CD4 cells were stabilizing. By then he know about CD4 T-cells.

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Zidovudine, also known as azidothymidide, and by brand name Retrovir, is a nucleoside analogue. It stops specifically the replication of viruses that use the reverse transcription process for copying their genetic material. Although patented as a drug to treat HIV infection in 1988, decades before then nucleosides and nucleotides had been subject to intense research.

They were of interest to biochemists such as Phoebus Levene of the Rockefeller Institute of Medical Research, New York, and Alexander Todd in the University of Cambridge, from the 1930s to the 1950s. Levene invented the terms nucleoside and nucleotide. Nucleosides are glycosylamines consisting of a nucleobase and a sugar (ribose or deoxyribose). Adenosine is a nucleoside, as are thymidine and uridine and so on. A nucleotide consists of a nucleoside with one or more phosphate groups added. Nucleotides, of various types according to the nucleobases, constitute the nucleic acid of living cells.

These acids in turn were considered in those days to be some kind of internal scaffold to support the proteins for their putative role as the carriers of genetic information. Some researchers realized that protein must be impossible for this task and asked deeper questions. By the early 1950s researchers had accumulated the wealth of knowledge about nucleic acids that discoverers of the structure and function of DNA drew on for their breakthrough in 1953. This discovery opened a surge of research, much of it on the deeper problem of how proteins are synthesized.

Some biochemists, however, saw a route to manipulating DNA in cancerous cells to stop DNA replication and thus cell division. If pure preparations of nucleosides could be altered slightly in the laboratory then they could possibly work as drugs that would be incorporated into the machinery of synthesis of new nucleic acids. That would then halt the process by blocking the completion of the reactions for lack of exactly the natural chemical conformation. A similar rationale for research was applied to searches for anti-viral drugs, but at that time considered a forlorn hope compared to the growing ease of finding antibiotics to cure bacterial infections.

At Yale University Medical School in 1959 William H. Prusoff and colleagues invented a molecule and called it idoxuridine. This was a nucleoside analogue with an

iodine atom inserted into it. They intended this to stop the replication of viruses by blocking base pairing. They found it effective against herpes simplex viruses. Although during testing they proved it too toxic for internal administration, they demonstrated its efficacy against herpes simplex causing keratitis, or inflammation of the cornea. It could be administered topically as eye-drops. They had invented the first anti-viral drug to be registered by the Food and Drugs Administration of the USA. Idoxuridine remained in use into the 2000s.

In the 1960s researchers such as Gertrude B. Elion, George H. Hitchings and Howard Schaeffer, working at the Burroughs Wellcome laboratories in North Carolina, sought the prizes of anti-viral drugs and better anti-cancer drugs. Their rationale was for nucleoside analogues to block copying of nucleic acids and so treat or cure viral infections and cancers. They discovered and developed the anti-viral drug acyclovir in the 1970s and this remains in use against herpes virus infections, including those causing shingles.

Between 1963 and 1964 simultaneous but independent research by two teams produced the nucleoside analogue azidothymidine (3'-azido-3'-deoxythymidine). Naishun Miller and Jack Fox worked in the Sloan Kettering Institute for Cancer Research at Cornell University Medical School, whilst Jerome H. Horwitz and colleagues worked at the Detroit Institute of Cancer Research. Both teams described their findings in an implied context of potential anti-cancer drugs, whilst publicizing sufficient methods for the laboratory synthesis of azidothymidine.

A decade passed before anyone combined the clues emerging from research papers about the potential for azidothymidine as a nucleoside analogue that might block copying of nucleic acids of viruses. First was Wolfram Ostertag, working in 1974 at the Max Planck Institute for Experimental Medicine in Göttingen. He used a virus of mice that causes a murine leukemia; Friend virus it is called. Azidothymidine blocks replication of Friend virus. Then Prusoff's team, with lead author Tai-Shan Li from the Chinese Academy of Medical Science, Beijing, published in 1978 their method of synthesis of azidothymidine as an anti-cancer and anti-viral drug.

A fearful shock hit anti-viral researchers with the news from Luc Montagnier at the Pasteur Institute, Paris, in 1983, closely followed by that of Robert Gallo, at the National Cancer Institute, Maryland. The newly emerging acquired immunodeficiency syndrome was being caused by a virus. Could anything be found as soon as possible for researchers to test against this new plague? At the same institute William Broder and colleagues first tried suramin against HIV grown in cultured human cells. This drug was invented long ago. It is still used to treat livestock infected with *Trypanosoma* protozoa, and also to treat humans where these protozoans cause the chronic wasting disease called sleeping sickness. Suramin works, but poorly in

comparison with modern drugs. It is toxic at normal dosage and only remains in use against trypanosomiasis because there are few alternatives for treating that neglected disease of poor people living far away. A measure of the urgency of the AIDS crisis was that this old drug, with its severe side-effects, was taken down from dusty shelves to be tested against HIV.

Next, Hiroaki Mitsuya in 1985 in Broder's lab, synthesized azidothymidine using Lin and Prusoff's method. He then tested it against HIV in cultured cells: it worked. Meanwhile, at the Burroughs Wellcome labs Janet Rideout applied in 1985 for American and British patents on azidothymidine as a drug specifically to treat infections with HIV-1. The patents were granted in 1988.

At that time Burroughs Wellcome were in early stages of building laboratories and training researchers for development of anti-HIV drugs. The dramatically publicized urgency of the growing epidemic prompted the firm to seek a partnership. The National Cancer Institute was already involved in search for such an anti-viral drug. An unconventional field for them, but they had well established clinical research laboratories with staff intimately collaborating with physicians who were daily attending to patients in the associated hospital. The collaboration, also including Duke University, was formally agreed in 1985.

By 1986 a member of Broder's team, Robert Yarchoan, successfully tested azidothymidine in a small number of people infected with HIV. They showed few signs of toxic reactions and the drug reduced their burdens of virus. Again the urgency of the problem justified such an early test in humans. Carrying the code name BWA509X the drug went back to the formal procedures for testing for toxicity and other side effects in mice and rats. The extraordinary push toward full clinical trials led to approval by the FDA for azidothymidine for use in humans to treat HIV infection by 1987 – an unprecedented short span.

The biochemical basis of this nucleoside analogue is that when it diffuses into cells the normal cell metabolism phosphorylates the nucleoside to its active form, the nucleotide azidothymidine triphosphate. This active form is closely similar to the natural thymidine triphosphate normally used by DNA in its replication. When readily incorporated by DNA azidothymidine triphosphate acts against the reverse transcriptase enzyme by competitive inhibition and chain termination. Azidothymidine was given the formal chemical name zidovudine, and was brought to market under the proprietary name Retrovir. It was described as a nucleoside reverse transcriptase inhibitor (NRTI). Unfortunately, because it only interfered with replicating virus, it had no effect on provirus latently infected cells. Thus it was not promoted as a curative drug.

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When the drug became available to clinicians, as tablets to be taken orally, on a dosage regime and cost that required expenditure for one patient for one year of approximately US$10,000, there was dismay. When it dawned on people that this drug was not curative, that treatment would have to continue, probably lifelong, there was outcry. Who could afford this? Why was a private company patenting this drug then charging so much for it when the development and testing was performed in an institute funded by taxpayers? Why was it taking so long to find something, anything, to treat this disease, to stem this epidemic? Why are so many of us dying?

The short time to registration and release for use of zidovudine was in belated and reluctant response to the political and social pressure exerted by gay rights activists. Centered in New York, people organized themselves as Treatment Action Group, and Act Up, to stage mass demonstrations and rallies full of angry and determined people. They demanded rapid access to drugs, fast tracking of clinical testing and registration. For too many of them, those whose infection had progressed to AIDS, they knew they had little to lose, but were desperate for a few more months of life, or at least regain some dignity despite their fate.

Meanwhile a legal dispute developed between NCI and Burroughs Wellcome over the patent rights, but the judge ruled in favor of the private company. Pharmaceutical companies need to make large profits on as many as possible of the drugs they sell. Otherwise they go out of business trying to pay for their costs of research, development, testing, registration and marketing of all the potential drugs they have. Such costs are counted, for each drug whether a success or failure, in many hundreds of millions of dollars.

Alternative routes to the invention, development and sale of pharmaceutical drugs are less defined. Universities and other taxpayer funded institutes do much inventing and some patenting, but then usually make agreements with private companies to manufacture and sell the drugs. Private enterprise and publicly funded institutions generate between themselves a dynamic tension: attract and repel, collaborate and compete. The patent, typically lasting eighteen years, allows the commercial firm time to recover their costs then start to make a profit. In the case of the patent on azidothymidine as a drug against HIV what mattered was who was first to be granted the right to exclusivity for that use, not necessarily any of those who contributed to invention of the drug.

This chemical was synthesized at small scale in a biochemistry lab to use for experiments on blocking cell division by known chemical interference with the mechanisms of nucleic acids. It has an intellectual history branching far back.

In the context of researches by chemists such as Phoebus Levene and Alexander Todd, it was physicist William Astbury in 1937 who first revealed something of the ultrastructure of the substance comprising the nuclei of cells. He made the first X-ray crystallograph of it, on the hunch that the image might provide some clue as to how it might carry genetic information. Erwin Chargaff, a chemist, continued in the 1940s with studies on the purine and pyrimidine bases of nucleic acids, demonstrating their fixed ratios of bases when examined in bulk. Then the finer X-ray crystallographs of chemist Rosalind Franklin and physicist Maurice Wilkins provided the theoretician physicist and biologist team of Francis Crick and James Watson with the information finally to decipher the structure of what they were to call DNA.

From that discovery arose molecular biology: the ability to examine how cells work using the tools and concepts of chemists. If DNA is the molecule that self-replicates in a dividing cell, and it consists of simple sub-units of sugar–base–phosphate, then surely it can be manipulated with other chemicals to interrupt its replication? That was the question some cancer researches asked themselves: hence the nucleoside analogues of William Prusoff, Naishun Miller, Jack Fox, Jerome Horwitz and other colleagues. What person invented the anti-HIV drug azidothymidine may have been a significance question for a patent attorney. For scientists the question has little value.

At 2012 the wholesale price in America of Retrovir recommended by the National Institutes of Health generated a cost of treatment per patient per year of $6,600. After the patent expired this same zidovudine, as a generic molecular copy, had a yearly cost of $4,300. But anti-retrovirals are prescribed in combinations of two or three drugs, multiplying the costs. There are thirty different chemicals now available as anti-HIV drugs for a clinician to choose from. The cost of developing and bringing them to market has been borne to meet the demand for multiple chemical classes remaining effective against the virus as it acquires resistance to the earlier drugs prescribed to a patient. The cost of second and third line drugs escalates. The ceaseless genetic variation of this reverse transcription virus provided the strains that might survive better than the rest when under the natural selection pressure of an anti-retroviral. For a patient whose infection has progressed to AIDS the prognosis is bleak. Costly drugs with noxious side-effects, other opportunistic infections, and then vitality terminally drains away long before natural life expectancy.

Despite this pessimism there is a better story to tell; it is still developing. In many countries anti-retroviral combinations against HIV are available at less than $200 per patient per year. Zidovudine remains one of the main drugs of these first line combinations available to those recently diagnosed with the virus. When testing is

offered and promoted and the subsequent treatment reaches eighty percent of those positive, there follows a decline in the rate of transmission of the virus from one person to another. Thus the numbers of new cases per defined population per year, the incidence of disease, declines. The disease is pushed back. It declines not just because of the anti-retrovirals of course: public health medicine, safe-sex, and messages on safe needle use are crucial. Nevertheless, the glint of an idea shining in the minds of cancer researchers in the 1960s has led to a battery of drugs that are crucial in this trio of interventions slowly reducing this plague to something we can learn to live with.

♦

His family in Mérida was large. Some of them coped poorly with idea of one of their own suffering from AIDS, but as the publicity and knowledge of the disease grew most of them came to a philosophical acceptance of his problem. Secure in the knowledge that his family was strong enough to house and feed him, he borrowed money to buy a secondhand delivery truck and set up in business supplying restaurants with fresh vegetables direct from the wholesale markets. No more partying and soccer for him. He felt frail but recovered sufficient vitality to gain acceptance as a volunteer referee for the matches held between youth clubs in his area.

Sources and notes for Chapter 4

General biology of HIV and AIDS.

Anonymous, 2005. HIV and its transmission. *Centers for Disease Control*. https://web.archive.org/web/20050204141148/http://www.cdc.gov/HIV/pubs/facts/transmission.htm

Anonymous, 2006. The impact of AIDS on people and societies. *Joint United Nations Programme on HIV/AIDS*. http://data.unaids.org/pub/GlobalReport/2006/2006_GR_CH04_en.pdf

Chaisson, R.E., Gallant, J.E., et al., 1998. Impact of opportunistic disease on survival in patients with HIV infection. *Aids*, **12**: 29-33.

Crawford, D.H., 2000. *The Invisible Enemy: a natural history of viruses*. Oxford University Press. [An accessible introduction to biology of viruses. Chapters 2 and 6 with material on HIV and AIDS.]

Merigan, T.C., Bartlett, J.G. & Bolognesi, D., (Eds.) 1999. *Textbook of AIDS Medicine*. Williams and Wilkins, Baltimore.

Richman, D.D., Morton, S.C., et al., 2004. The prevalence of antiretroviral drug resistance in the United States. *Aids*, **18**: 1393-1401.

UN-Aids, 2013. UNAIDS report on the global AIDS epidemic. http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf

[See the UN-Aids website for more background information about work of the United Nations in this field.]

Weeks, B.S. & Shors, T., 2013. *AIDS: the biological basis.* Jones & Bartlett Learning, Burlington. [A comprehensive textbook on HIV and AIDS written for non-specialists, including much on prevention and treatment.]

HIV-1 invasion and replication

Cameron, P.U., Freudenthal, P.S., et al., 1992. Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to CD4+ T cells. *Science*, **257**: 383-387.

Igakura, T., Stinchcombe, J.C., et al., 2003. Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. *Science*, **299**: 1713-1716.

McDonald, D., Wu, L., et al., 2003. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. *Science*, **300**: 295-1297.

Sattentau, Q., 2008. Avoiding the void: cell-to-cell spread of human viruses. *Nature Reviews Microbiology*, **6**: 815-826.

Wiley, R.D. & Gummuluru, S., 2006. Immature dendritic cell derived exosomes can mediate HIV-1 trans infection. *Proceedings of the National Academy of Sciences of the United States of America*, **103**: 738-743.

Dendritic cells and antigen presentation

Burleigh, L., Lozach, P-Y. et al., 2006. Infection of dendritic cells (DC), not DCSIGN-mediated internalization of human immunodeficiency virus, is required for long-term transfer of virus to T cells. *Journal of Virology*, **80**: 2949-2957.

Nobile, C., Petit, C., et al., 2005. Covert human immunodeficiency virus replication in dendritic cells and in DC-SIGN-expressing cells promotes long-term transmission to lymphocytes. *Journal of Virology*, **79**: 5386-5399.

Fackler, O.T., Alcover, A. & Swartz, O., 2007. Modulation of the immunological synapse: a key to HIV-1 pathogenesis? *Nature Reviews Immunology*, **7**: 310-317.

Innate and adaptive immunity against HIV

Levy, J.A. 2001., The importance of the innate immune system in controlling HIV infection and disease. *Trends in Immunology*, **22**: 312.

Medzhitov, R. & Janeway, C. 2000. Innate immunity. *New England Journal of Medicine*, **343**: 338-344.

Murphy, K. 2012., *Janeway's Immunobiology*, Garland Science, Taylor Francis group, New York. [Chapters 2 to 10 for detailed information on immunology and Chapter 13, sections 13.24 to 36 for HIV and AIDS infection and immunity.]

Evasion of immunity by HIV

Chen, L., Kwon, Y.D., et al., 2009. Structural basis of immune evasion at the site of CD4 attachment on HIV-1 gp120. *Science*, **326**: 1123-1127.

Collins, K.L. & Baltimore D., 1999. HIV's evasion of the cellular immune response. *Immunological Reviews*, **168**: 65-74.

Johnson, W.E. & Desrosiers, R.C., 2002. Viral persistence: HIV's strategies of immune system evasion. *Annual Review of Medicine*, **53**: 499-518.

Kirchhof, F., 2010. Immune evasion and counteraction of restriction factors by HIV-1and other primate lentiviruses. *Cell, Host & Microbe*, **8**: 55-67.

Klenerman, P., Wu, Y. & Philips, R., 2002. HIV: current opinion in escapology. *Current Opinion in Microbiology*, **5**: 408-413.

Malim, M.H. & Emerman, M. 2008., HIV-1 accessory proteins – ensuring viral survival in a hostile environment. *Cell Host & Microbe*, **3**: 388-398.

Nucleoside analogues and other anti-retroviral drugs

Badri, M., Maartens, G., et al., 2006. Cost-effectiveness of highly active antiretroviral therapy in South Africa. *PloS Medicine*, **3**: e4.

Broder, S., Yarchoan, R., et al., 1985. Effects of suramin on HTLV-III/LAV infection presenting as Kaposi's sarcoma or AIDS-related complex: clinical pharmacology and suppression of virus replication in vivo. *Lancet*, **ii**: 627-630.

Cheng, Y.C., Neenan, J.P. et al., 1975. Selective inhibition of herpes simplex virus by 5'-Amino-2 ',5 '-dideoxy-5-iodouridine. *Journal of Virology*, **15**: 1284-1285.

Horwitz, J.R., Chua, J. & Noel, M., 1964. Nucleosides V. The monomesylates of 1-(2'-deoxy-beta-D-lyxofuranosyl) thymine. *Journal of Organic Chemistry*, **29**: 2076-2078.

Lin, T.-S. & Prusoff W.H., 1978. Synthesis and biological activity of several amino analogues of thymidine. *Journal of Medicinal Chemistry*, **21**: 109- 112.

Miller, N. & Fox J.L., 1964. Nucleosides. XXI. Synthesis of some 3'-Substituted 2',3'-dideoxyribonucleosides of thymine and 5-methylcytosine. *Journal of Organic Chemistry*, **29**: 1772-1776.

Mitsuya, H., Weinhold, K.J. et al., 1985. 3' azido-3'-deoxythymidine (BWA509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type 111/lymphadenopathy- associated virus in vitro. *Proceedings of the National Academy of Sciences of the United States of America*, **82**: 7096-100.

Mitsuya, H. & Erickson, J., 1999. Discovery and development of antiretroviral therapeutics for HIV infection. In: Merigan, T.C., Bartlett, J.G. & Bolognesi, D. (Eds.) *Textbook of AIDS medicine*. Chapter 45a, 751-780. Williams and Wilkins, Baltimore.

National Institutes for Health, 2013. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Appendix B, Table 8: Monthly suggested wholesale price of antiretroviral Drugs.
http://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf

Ostertag, W., Roesler, G., et al., 1974. Induction of endogenous virus and of thymidine kinase by bromodeoxyuridine in cell cultures transformed by Friend virus. *Proceedings of the National Academy of Sciences of the United States of America*, **71**: 4980-4985.

Prusoff, W.H., 1959. Synthesis and biological activities of iododeoxyuridine, an analog of thymidine. *Biochimica et Biophysica Acta*, **32**: 295-296.

Rideout, J.L., 1988. Treatment of human viral infections. *United States Patent* 4,724,232. http://patft.uspto.gov/netahtml/PTO/srchnum.htm

RxList, 2015. Retrovir (zidovudine). http://www.rxlist.com/retrovir-drug.htm [User information about Retrovir.]

Skowron, G. & Ogden, R. (Eds). 2006. *Reverse Transcriptase Inhibitors in HIV/AIDS Therapy*. Humana Press, Totowa. [17 chapters by 34 authors. See the forward by J.M.A.Lange for an account of the huge impact the introduction of anti-retroviral therapy in the mid 1990s has had on the morbidity and mortality associated with HIV in the developed world. In addition, because the earlier NRTI and non-nucleoside reverse transcriptase inhibitors can be now manufactured at low cost and combined for simple therapuetic regimes there has been a similar impact in developing countries.]

Economics and politics of AIDS

Anonymous, 2014. The new drug war: hard pills to swallow. *The Economist*, January 4-10, pgs 46-47. [An account from a business economics

perspective of the costs, profits and international legal provisions for patented pharmaceuticals.]

Chirac, P., von Schoen-Angerer, T., et al., 2000. AIDS: patent rights versus patient's rights. *The Lancet*, **356**: 502. [Last paragraph reads*: 'The gap between the prospects for people with AIDS living in the western world and those living in less developed countries makes the problem of access to AIDS treatments much more than a medical concern. This gap is also a social, economic, moral, and political issue. The question of AIDS treatment leads to a wider reflection of the balance between public and private interests, between patent rights and the rights of patients. Access to health care and to medical progress as a human right is a challenge that AIDS poses to humanity. It is no longer morally acceptable to debate if antiretrovirals should be provided. We should now concentrate on how quickly they can be provided*.']

Cochrane, J.M.T., 2000. Zidovudine's patent history. *The Lancet*, **356**: 1611-1612. [A reply to the letter by Chirac et al., 2000, by an employee of GlaxoWellcome plc. Last two sentences read: '*Nevertheless the cost of medicine needs to be addressed as part of a concerted effort to tackle all the factors inhibiting access to care and treatment for AIDS. That is precisely the approach of the UN/Industry joint endeavour. Multisectoral partnerships led by committed national governments and involving international agencies,donor countries, the private sector and non-governmental organisations, offer the best prospect for a sustainable expansion of treatment for HIV/AIDS in less-developed countries.*']

Collins, H. & Pinch, T. 2014. *The Golem at Large: what you should know about technology*. Cambridge University Press, England. [See Ch. 7 'ACTing up: AIDS cures and lay expertise.' for an analysis of the Act Up movement.]

France, D., 2012. *How to survive a plague*. (Film: produced by Dan Cogan, distributed by Sundance Selects.) [A documentary / drama portraying the campaigns for early access to anti-retrovirals.]

Freeburg, K. A., Losina, E., et al., 2001. The cost effectiveness of combination antiretroviral therapy for HIV disease. *New England Journal of Medicine*, **344**: 824-831.

Gallo, R.C., 2002. The early years of HIV/AIDS. *Science*, **298**: 1728-1730.

Montagnier, L.A., 2002. A history of HIV discovery. *Science*, **298**: 1727-1728.

Oddone, E. Z. Cowper, E., et al., 1993. Cost effectiveness analysis of early zidovudine treatment of HIV infected patients. *British Medical Journal*, **307**: 1322-1324.

Pisani, E. 2008. *The Wisdom of Whores: bureaucrats, brothels and the business of AIDS*. Granta, London. [A journalistic account of the social epidemiology and politics of AIDS, mainly in South East Asia and Africa.]

Sanders, G. D., Ahmed, M., et al., 2005. Cost-effectiveness of screening for HIV in the era of highly active antiretroviral therapy. *New England Journal of Medicine*, **352**: 570-585.

Vallée, J-M., 2013. *Dallas Buyers Club*. A film produced by Robbie Brenner, distributed by Focus Features. [A highly dramatized portrayal of the desperation of AIDS sufferers in the late 1980s for early access to antiretroviral drugs and their self-help solution of forming buyer's clubs to smuggle and distribute early supplies of AZT. The film develps a positive and informative message. Worth viewing just for the lead performances by Matthew McConaughey and Jared Leto.]

History of anti-retroviral drugs

De Clercq, E., 2009. The history of antiretrovirals: key discoveries over the past 25 years. *Reviews in Medical Virology*, **19**: 287-299.

Broder, S., 2010. The development of antiretroviral therapy and its impact on the HIV-1/AIDS pandemic. *Antiviral Research*, **85**: 1-2.

Elion, G.B., 1988. *The purine path to chemotherapy*. (Nobel Prize Lecture)
http://www.nobelprize.org/nobel_prizes/medicine/laureates/1988/elion-lecture.pdf

Hitchings, G.H., 1988. *Selective inhibitors of dihydrofolate reductase* (Nobel Prize Lecture)
http://www.nobelprize.org/nobel_prizes/medicine/laureates/1988/hitchings-lecture.pdf

Levene, P.A. & Bass L.B., 1931. *Nucleic Acids.* The Chemical Catalog Company Inc. New York.

Todd, A.R., 1957. *Synthesis in the study of nucleotides*. (Nobel Prize Lecture)
http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1957/todd-lecture.pdf