

CPD - Caring for Patients and their Disorders

This edition was originally meant to focus mainly on an understanding of antiretroviral agents. However, I have taken a rather round about route and in the 'back to basics' section have concentrated on retroviruses themselves, including HIV-1 and HIV-2. I am sure some purists will find this an oversimplification, and the reader is asked to be aware that there are complexities which I have simply ignored for the sake of brevity and hopefully, clarity.

The mechanism of action of some of the antiretrovirals is touched on, and a very broad approach is suggested in terms of their use. I have listed only those which are presently registered in South Africa.

It must be emphasised that antiretroviral agents are at least equivalent to anti-cancer chemotherapeutic agents in their toxicity (and expense!). At present they are not available in the public sector. As a result of intense lobbying, at the time of writing, there seems to have been a breakthrough in terms of providing antiretrovirals at low cost to the government. How this will develop into a policy and/or guidelines for the use of these agents remains to be seen.

Extensive research on different agents continues, and new drugs are being developed all the time.

One major problematic side effect has recently surfaced in trials across the world. The combination of stavudine and didanosine has resulted in a fatal lactic acidosis in pregnant women. These drugs are both nucleoside reverse transcriptase inhibitors (NTRIs).

A major side effect of protease inhibitors (PIs) is the development of lipodystrophy.

At the time of writing, the presidential AIDS advisory panel has apparently completed its report and will submit it to cabinet. By the time you read this, the report may be public and the response of cabinet already known. It remains to be seen whether or not it has been a worthwhile exercise, and more importantly, whether or not it will have resulted in decisive action which will make direct inroads into the epidemic at multiple levels of intervention.

Finally, I would encourage anyone to access and download my major reference for this edition's CPD: **PATHOLOGY OF AIDS. Version 10, by Edward C. Klatt, MD. (Department of Pathology, University of Utah, February 7, 2001 Copyright ©Edward C. Klatt, MD. All rights reserved worldwide.)**

It is available as a pdf file by following the link on:

<http://medstat.med.utah.edu/WebPath/TUTORIAL/AIDS/AIDS.html>

The excellent figure of the life-cycle of the retrovirus is used with grateful thanks to Garland publishing:

<http://www.essentialcellbiology.com/>

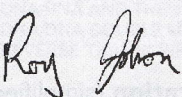
The original figure can be found at:

http://www.accessexcellence.org/AB/GG/retro_Life.html

A rather fun link, which has animated graphics of retroviral processes and the mechanisms of action of antiretrovirals is:

<http://www.cellsalive.com/hiv0.htm>

Enjoy the article and don't forget to complete and submit the questions on the flyleaf!



Roy Jobson
(CPD sub-editor)

Retroviruses and Anti-retroviral agents

Our 31 year old male patient, Johannes, is HIV-infected. You are surprised to discover that he has been using 'virodene' – which he obtains from a friend via the 'black market'. You notice that he has developed marked palmar erythema. He has however slightly reduced his smoking following your encouragement to quit at the last visit, and is still trying to stop altogether. (See Feb-March 2001 edition of CPD.)

The structure of this CPD exercise is as follows:

1. Back to basics

- i) Anatomy and functioning of retroviruses
- ii) Finer details of the Human Immunodeficiency Virus
- iii) Sites of action of antiretroviral drugs

2) HIV/AIDS precautions for doctors doing surgery or autopsies

3) Evidence-based conclusions about the use of antiretroviral agents.

4) Questions for CPD points (to be found on the flysheet in the envelope)

Part One: Back to Basics

i) Anatomy and functioning of retroviruses

Types of retroviruses

Retroviruses are a large group of RNA viruses, most of which infect animals. Seven genera have been identified.

Table 1: 7 genera of retroviruses

- | |
|--|
| 1. Genus 'Mammalian type B retroviruses' |
| 2. Genus 'Mammalian type C retroviruses' |
| 3. Genus 'Avian type C retroviruses' |
| 4. Genus 'Type D Retrovirus group' |
| 5. Genus 'BLV-HTLV retroviruses' |
| bovine leukaemia virus (BLV) |
| human T-lymphotropic virus 1 (HTLV-1) |
| human T-lymphotropic virus 2 (HTLV-2) |
| 6. Genus Lentivirus |
| Subgenus: Primate lentivirus group |
| human immunodeficiency virus 1 (HIV-1) |
| human immunodeficiency virus 2 (HIV-2) |
| simian immunodeficiency virus (SIV) |
| 7. Genus Spumavirus |

They're called retroviruses because the usual process – through which DNA is 'read' by the enzyme transcriptase to manufacture RNA – is reversed. In other words, the viral RNA of retroviruses produces DNA. The enzyme involved in this is called 'reverse transcriptase'.

Retroviruses were also formerly known as the 'RNA tumor virus' group.

HTLV-1 infection is associated with adult T-cell leukaemia/lymphoma (ATLL) and with a form of chronic progressive neurologic disease known as HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP).

HTLV-1 infection has endemic foci in Japan and Okinawa, central Africa, parts of the southeastern United States, Central and South America, the Middle East, and the Caribbean. The rate of infection varies from 1 to 2% of the population. The lifetime risk for ATLL or HAM/TSP in infected persons is only about 5%. There is an additional 5% risk for less serious complications that can include infectious dermatitis, uveitis, polymyositis, and arthropathy.

HTLV-2 has been identified primarily in native populations of North and South America as well as in parts of Africa. Testing in the United States has demonstrated HTLV-2 seropositivity mainly among intravenous drug users, particularly those with increasing age and those living within large metropolitan areas. Although postulated, there is no clear association between HTLV-2 and 'hairy T-cell leukaemia'. Some forms of chronic neurologic disease may however be linked to HTLV-2 infection.

Structure of retroviruses (refer back to CPD Feb-March 2001 for general viral terminology.)

Retroviruses have a host-cell-derived lipid envelope into which glycoproteins gp41 and gp120 are incorporated. The nucleocapsid contains structural proteins (for example: p24) as well as enzymes. These enzymes are 'reverse transcriptase', 'integrase' and 'protease'. Finally there is the viral genome itself which is single-stranded RNA. The genetic code includes at least nine different genes. The three major genes, gag, pol, and env, are common to all retroviruses. The group specific antigen (gag) gene contains the code for the structural proteins. The polymerase (pol) gene contains the code for the enzymes, and the envelope (env) gene contains the code for the proteins in the lipid envelope.

Mechanism of infection and replication (simplified)

When a retrovirus infects a cell, a receptor-attachment mechanism involving the envelope glycoproteins (gp41 and gp120) ensures that the virus fuses to the cell membrane.

The viral envelope is incorporated into the cell membrane as part of endocytosis, and the nucleocapsid enters the host cell. The structural proteins are removed, leaving the RNA in the cell cytoplasm.

A strand of DNA is then synthesized using reverse transcriptase, to create an RNA:DNA compound. The RNA component is then degraded and a complementary strand of DNA (cDNA) is synthesised ending up with a DNA:DNA double helix. The integrase enzyme assists in splicing ('pasting') this double helix into the host cell's chromosomes inside the nucleus. This spliced section of the chromosome is then known as a provirus, and is a permanent part of the cell genome, permanently producing viral components. The provirus is inherited by all subsequent offspring of the host cell and they too continue to produce virus particles.

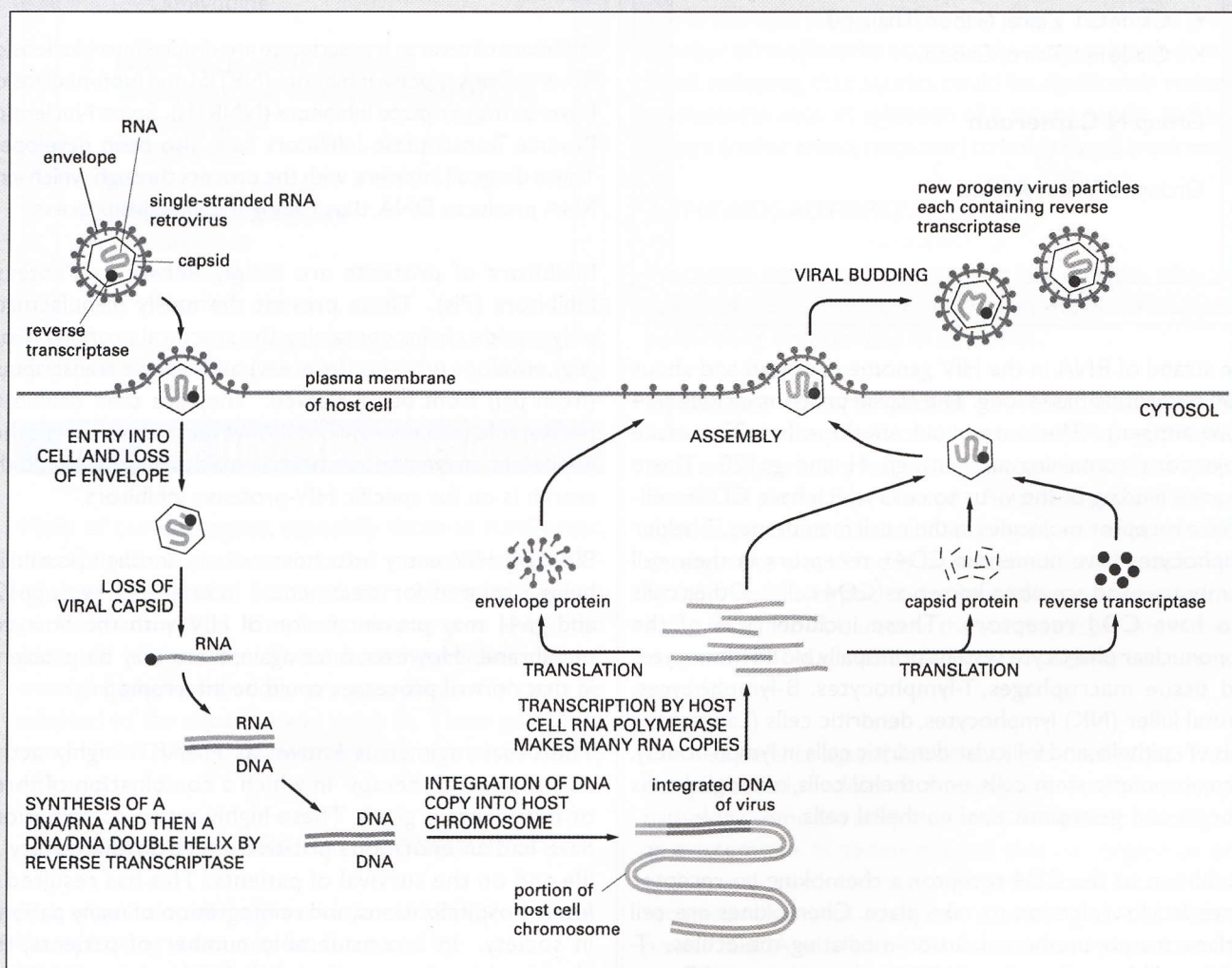
Within an infected cell, the cell's own 'RNA polymerase' (transcriptase) blindly makes multiple copies of the viral

RNA (containing the gag, pol, and env genes) along with the cell's messenger RNA and other proteins. Translation of the viral RNA produces polypeptides containing the structural proteins of the capsid; the enzymes; and envelope proteins. These are cleaved by the protease enzyme into their separate components, reassembled into their retroviral morphology, and eventually disgorged from the host cell through a process of 'budding'.

Unfortunately reverse transcriptase may make 'mistakes' reading the RNA sequence. The result is that not all viruses produced in a single infected cell are necessarily alike. Instead, they may end up with subtle molecular differences in their surface coats and enzymes.

Some retroviruses reproduce themselves indefinitely within their hosts without killing them. One cell is capable of producing hundreds of new virus particles. The technical term for this is 'burst size'. Other retroviruses permanently alter the host cells and may lead to cancers and other disorders.

Figure 1: The Life Cycle of a Retrovirus



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ii) *Finer details of the Human Immunodeficiency Virus*

The Human Immunodeficiency Virus (HIV) was initially known as human T-lymphotropic virus III (HTLV-III), and then lymphadenopathy associated virus (LAV) before being christened 'HIV'. Three major groups of HIV-1 exist: M, N and O groups. Group N appears to have originated from interaction between a group M and a group O virus. Within Group M several major subtypes of HIV-1 have also been described. They may also be referred to as 'clades'. Clade C is the subtype prevalent in South Africa.

Table II: Subgroups of HIV

Group M

- Clade A : Central Africa
- Clade B : South America (including Brazil), United States, Europe, Thailand
- Clade C : Brazil, India, Southern Africa
- Clade D : Central Africa
- Clade E : Thailand, Central African Republic
- Clade F : Brazil, Romania, Zaire
- Clade G : Zaire, Gabon, Thailand
- Clade H : Zaire, Gabon

Group N **Cameroon**

Group O **West Africa**

The strand of RNA in the HIV genome is diploid and about 9200 nucleotide bases long. The capsid proteins include p24 (core antigen). The outer lipid envelope has 72 surface projections containing antigens, gp 41 and gp120. These enhance binding of the virus to cells which have CD4+ cell-surface receptor molecules in their cell membranes. T-helper lymphocytes have numerous CD4+ receptors in their cell membranes and are often known as 'CD4 cells'. Other cells also have CD4 receptors. These include cells of the mononuclear phagocyte system, principally blood monocytes and tissue macrophages, T-lymphocytes, B-lymphocytes, natural killer (NK) lymphocytes, dendritic cells (Langerhans cells of epithelia, and follicular dendritic cells in lymph nodes), haematopoietic stem cells, endothelial cells, microglial cells in brain, and gastrointestinal epithelial cells.

In addition to the CD4 receptor, a chemokine co-receptor is needed for infection to take place. Chemokines are cell surface membrane-bound fusion-mediating molecules. T-tropic and M-tropic strains of HIV are dependent on different co-receptors. Dual tropic HIV strains have also been

described that can use more than one chemokine co-receptor. Chemokines play a role in the inflammatory process, and many areas of inflammation contain increased numbers of mononuclear cells containing the co-receptor to which M-tropic strains of HIV are bound.

HIV variants, possibly arising from reverse transcriptase induced 'mistakes', can lead to differences in viral pathogenetic effects. Three main variants are described: (1) non-syncytium-inducing (NSI) variants that have a low replicative capacity; (2) non-syncytium-inducing variants with a high replicative capacity; and (3) syncytium-inducing (SI) variants. Thirty to sixty percent of HIV-infected persons may eventually develop such variants.

As with all retroviruses, the key enzymes in HIV are reverse transcriptase, integrase and protease. Finding inhibitors of these enzymes has formed the basis of most of the antiretroviral drugs now available. However, to date, no clinically useful inhibitors of the integrase enzyme have been found.

iii) *Sites of action of antiretroviral drugs*

Inhibitors of reverse transcriptase are divided into Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs). Some Nucleotide Reverse Transcriptase Inhibitors have also been developed. These drugs all interfere with the process through which viral RNA produces DNA, thus halting the infective process.

Inhibitors of protease are simply known as Protease Inhibitors (PIs). These prevent the newly manufactured polypeptide chains containing the structural proteins (from gag), envelope proteins (from env), and reverse transcriptase (from pol) from being cleaved. They are then unable to reassemble into new virions. However protease has other important enzymatic functions in maintaining health, so the search is on for specific HIV-protease inhibitors.

Blocking HIV entry into host cells is another possibility being explored for treatment. Interference with gp120 and gp41 may prevent fusion of HIV with the host cell membrane. However, once again, there may be problems in that normal processes could be interrupted.

The usual regimen is known as HAART: 'highly active anti-retroviral therapy' in which a combination of three or four drugs is given. These highly active combinations have had an enormous positive effect on the quality of life and on the survival of patients. This has resulted in fewer hospitalizations, and reintegration of many patients in society. In a considerable number of patients, the viral load has been reduced to below detection limits for prolonged periods.

Table III: Standard HAART Regimens

- **NRTI + NRTI + PI**
- **NRTI + NRTI + NNRTI**

Most 'immunomodulation' therapies have met with limited success, although they may be useful as adjunctive therapy.

Table IV: Anti-Retroviral Drugs

1. Nucleoside Reverse Transcriptase Inhibitors (NRTI's) [Nucleoside analogues]

Zidovudine
Zalcitabine
Didanosine
Stavudine
Lamivudine

2. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI's)

Nevirapine
Efavirenz

3. Protease Inhibitors

Saquinavir
Indinavir
Ritonavir

3. Use of instruments and not fingers to hold or retract tissues
4. Not picking up dropped or broken sharps with fingers
5. Keeping needle use to a minimum
6. Keeping track of sharp instruments in use
7. Use of blunt instruments where applicable
8. Use of fluid-resistant gowns (or a plastic apron) when blood splashing to the body may occur
9. Use of face protection when blood splashing to the face may occur
10. Requiring non-operating room personnel to wear gloves and gowns while in the operating theatre

Surgical procedures in the operating theatre are associated with a 1.7 to 5% risk for blood exposure for personnel in that setting. The majority of these exposures are to skin and eye, and the majority could be avoided by use of gloves, face protection, and fluid-resistant gowns.

Surgeons and scrub staff have the highest risk for percutaneous exposures, about 1 incident per 100 procedures. Blood contacts are more frequent when performing emergency procedures, when patient blood loss exceeds 0.25 L, and when personnel are in the operating room longer than 1 hour. The greatest number of needlesticks occur on the surgeon's non-dominant hand, indicating that injuries could be significantly reduced if manoeuvres such as palpation of a suture needle, and use of fingers (rather than a retractor) to hold tissues, were avoided.

2. THE AIDS AUTOPSY

Protective masks and eyewear (or face shields), laboratory gowns, gloves, and waterproof aprons should be worn when performing or attending all autopsies.

All autopsy material should be considered potentially infectious for both HIV and Hepatitis B Virus. Onlookers with an opportunity for exposure to blood or fluid splashes should be similarly protected. Routine standard infection control practices should be employed for all cases. Use a detergent to clean bloody or soiled work surfaces, followed by 0.5% sodium hypochlorite (normal household bleach) as a disinfectant in ALL areas of the autopsy department, except on sensitive equipment. Alternative disinfectants may be used for this.

The best defense against accidents is good technique. Though many dissection skills in autopsy are similar to surgical pathology, some are unique and require specific training, such as evisceration of abdominal and thoracic organs or brain and spinal cord removal. A protective face shield is recommended with use of an oscillating saw, as with any power tool. Also available for use are hand protectors such as steel mesh gloves or 'fishing' gloves of tough fabric which may provide additional security with evisceration of organs.

Part Two: HIV/AIDS precautions

Many of our colleagues, especially those in rural areas, are obliged to perform invasive procedures in HIV-infected individuals. Some of our colleagues have to perform autopsies. They may or may not know the patient's HIV status. Some broad guidelines about managing these situations are provided, which can be adapted to the situation you work in. These guidelines come from 'The AIDS Handbook' mentioned in the editorial.

1. INVASIVE AND SURGICAL PROCEDURES

Risk of infection through reduction in blood contacts in the operating room may be decreased by:

1. Use of double gloves
2. Use of cut-resistant gloves if these are available

Although disposable latex or vinyl gloves are quite reliable, leakage can occur, so double gloving is recommended. If cuts or abrasions on potentially exposed skin surfaces are present, they should be taped or covered before protective gear is put on.

Fixed tissues or fluids may be disposed of in a routine fashion through a tissue grinder into a sanitary sewer or through incineration. Fresh tissues, blood, and body fluids can be autoclaved or placed in fixatives prior to disposal in accordance with local statutes. Formalin is the most cost-effective and efficacious fixative. Other contaminated wastes can be collected into marked, leak-proof plastic bags and incinerated. Housekeeping personnel handling this material should use protective gear. Needles should never be recapped, and all needles or other sharp objects such as scalpel blades should be discarded into specifically designated containers.

Disposable paper scrub suits and gowns are often easier to work with and more cost-effective than cloth materials. If linen or other cloth scrub suits, gowns, or aprons are used they may be collected into bags that can be directly laundered without removal of the contents (bag dissolves in water).

The experience of the past decade in public hospitals and other centers performing large numbers of AIDS autopsies has shown that AIDS is not a threat to pathologists or other laboratory workers. There is no such thing as a 'high risk' autopsy because the autopsy room environment can be well-controlled. It is also unlikely that requirements for unusual, extraordinary, or unwieldy procedures will add a definable margin of safety, but such procedures may lead to accidents or failure of compliance. A system of standard, routine procedures should be followed at all times.

Part Three: Some of the evidence

The Cochrane Collaboration abstracts have three systematic reviews relevant to this CPD exercise.

Zidovudine (AZT) versus AZT plus didanosine (ddI) versus AZT plus zalcitabine (ddC) in HIV infected adults

(Last amended on 06 March 2000)

Reviewers' conclusions: The use of ddI and, to a lesser extent, ddC delayed both HIV disease progression and death, at least when added to AZT.

[Darbyshire J, Foulkes M, Peto R, Duncan W, Babiker A, Collins R, Hughes M, Peto T, Walker A. Zidovudine (AZT) versus AZT plus didanosine (ddI) versus AZT plus zalcitabine (ddC) in HIV infected adults (Cochrane Review). In: The Cochrane Library, 1, 2001. Oxford: Update Software.]

Three- or four- versus two-drug antiretroviral maintenance regimens for HIV infection

(Last amended 22 February, 2000)

Reviewers' conclusions: Although it is desirable to reduce the number of antiretroviral drugs given in combination therapy for reasons of compliance and toxicity, maintenance regimens with fewer drugs are associated with significantly increased risk of loss of viral suppression. Successful induction therapy, as evidenced by suppression of viral load, should not be modified in the maintenance phase unless clinically necessary.

[Rutherford GW, Feldman KA, Kennedy, GE. Three- or four-versus two-drug antiretroviral maintenance regimens for HIV infection (Cochrane Review). In: The Cochrane Library, 1, 2001. Oxford: Update Software.]

Immediate versus deferred zidovudine (AZT) in asymptomatic or mildly symptomatic HIV infected adults

(Last amended on 06 March 2000)

Reviewers' conclusions: Although immediate use of AZT halved disease progression during the first year, this effect was not sustained, and there was no improvement in survival in the short or long term.

[Darbyshire J, Foulkes M, Peto R, Duncan W, Babiker A, Collins R, Hughes M, Peto T, Walker A. Immediate versus deferred zidovudine (AZT) in asymptomatic or mildly symptomatic HIV infected adults (Cochrane Review). In: The Cochrane Library, 1, 2001. Oxford: Update Software.]

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