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The Threat of Epidemics

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The Biochemical Challenges of HIV

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THE BIOCHEMICAL CHALLENGES OF HIV

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ABSTRACT

The biochemical interactions of HIV and the susceptible cells are so profound that we are yet to find a way of Eliminating the viruses from cells. In this paper the virion and genomic structures are presented. The structure of HIV-1 Env. gene product and the biochemical processes involved in virus entry into cells are discussed. The biology and molecular biology of HIV and the changes brought about after HIV cellular entry and the long-term effects are discussed. The current treatments and associated problems of treatment are discussed.

Introduction

Despite the advances in knowledge and treatment of AIDS in recent years, the human race stand unprotected from the ravages of HIV and other viral diseases. The HIV host infection is much wider and the ramifications of HIV disease so extensive that WHO will have to rethink some strategies.

Let me remind you that when 5 young American men were diagnosed as having a "new severe disease" in 1982, America alone at the time had an estimated 200,000 infected persons as part of a world wide epidemic which has continued to spread. When the disease was first recognised as an epidemic, wrong notions were formed and are still being formed that the disease

is confined to certain segment of the human race. The disease is transmitted primarily though sexual activities. Nobody should be stigmatised for carrying the disease. Millionaires doctors actors academicians priests government officials rich and poor have been victims of HIV disease alike. To date, nobody has found a way of eliminating the virus from the body system once it enters.

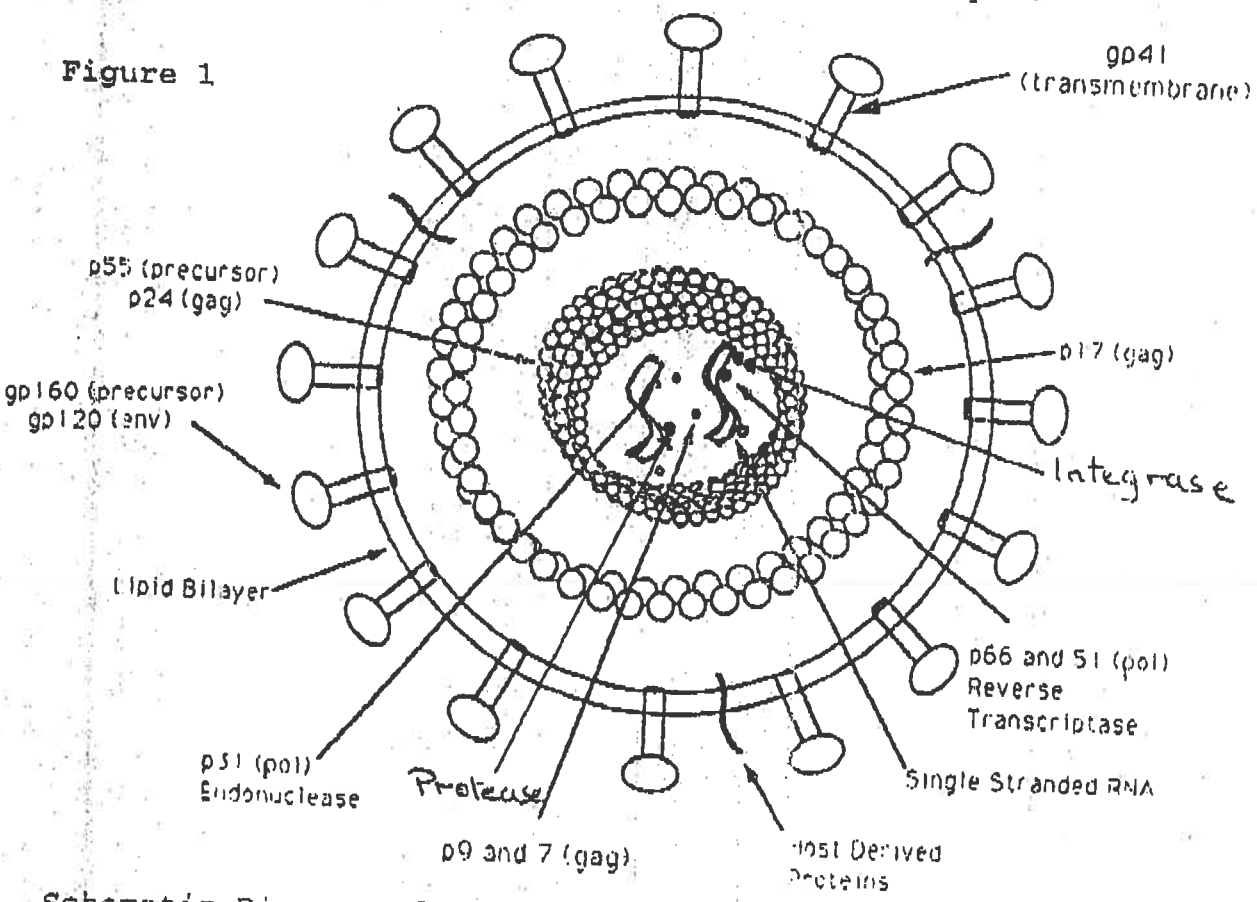
The Virion and Genomic structure of HIV-1.

The HIV-1 virus is icosahedral, enveloped RNA virus of the Lentivirinae subfamily of retroviruses that primarily infect human white blood cells. They are retroviruses because their genetic material is RNA which must be transcribed to DNA before the virus can complete its replicative cycle. The lentiviruses characteristically cause indolent infection in their animal hosts. The infections are notable for involvement of the nervous system, long periods of clinical latency and weak humoral immune responses complicated by persistent viremia. The lentiviruses have complex viral genomes. In addition to the three usual genes gag, pol and env found in many retroviruses, HIV has at least six additional genes vif, vpu vpr tat rev and nef. The gag and env encode the core nucleocapsid polypeptides and surface court proteins of the virus respectively, whereas the pol gene gives rise to the viral reverse transcriptase and other enzymes.

As shown in schematic (Fig.1), the virion has 72 external spikes formed by two major viral envelope proteins glycoprotein gp 120 and glycoprotein gp 41. The HIV-1 lipid bilayer is studded with various host proteins including Class I and Class II histocompatibility antigens acquired during virion budding. The core of HIV-1 contains four nucleocapsid proteins P 24, P

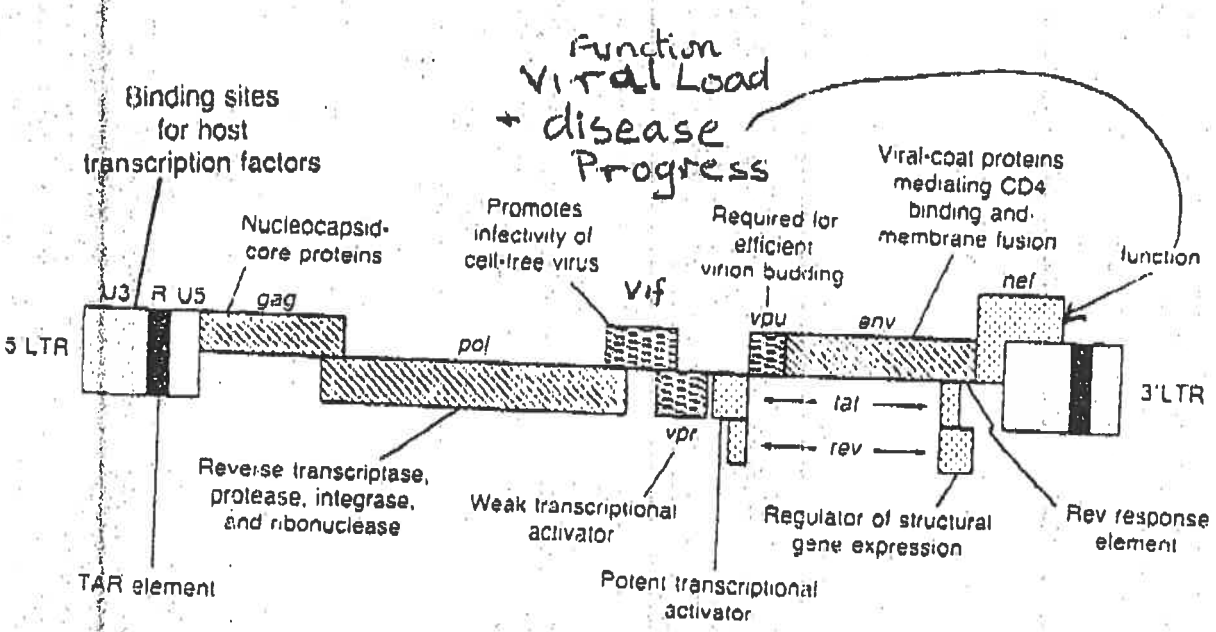
17, P9 and P7 each of which is proteolytically cleaved from a 53-kd Gag precursor by the HIV-1 protease. The phosphorylated P24 polypeptide forms the chief component of the inner shelf of the nucleocapsid (Fig 1) whereas the P17 protein is associated with the inner surface of the lipid bilayer and possibly stabalises the exterior and interior components of the virion. The P7 protein binds directly to the genomic RNA through some structural motif and together with P9 protein, form the nucleoid core. Importantly the retroviral core also contains two copies of the single-stranded HIV-1 genomic RNA that is associated with the various preformed viral enzymes

Figure 1



Schematic Diagram of HIV-1 Virion.
There are two proteins gp 120 env and gp 41 env contained in the membrane and four in the nuclear region with two single-stranded RNA genome. Also the key enzyme are found in the nuclear region.

Figure 2: Genomic Structure of HIV-1.



Each of the nine known genes of HIV-1 are shown, and their recognized primary functions summarized. The 5' and 3' long terminal repeats (LTRs) containing regulatory sequences recognized by various host transcription factors arealso depicted, and the positions of the Tat and Rev RNA response elements (TAR [transactivation response] element and Rev response element) are indicated.

including the **Reverse Transcriptase Integrase and protease** (Fig 1). The linear genomic structure of HIV- is shown in (Figure 2) (Barre-Sinoussi, F, et al 1983, Varmus H, 1988, Fauci AS 1988, Gelderblom H.R. et al 1987,

Biosynthesis and processing of envelope precursors.

As in other retroviruses, the envelope glycoprotein of HIV - I is synthesised as a polyprotein precursor which is fragmented by host proteases into the surface and transmembrane subunits of the mature envelope glycoprotein complex.

The polyprotein envelope precursor has been designated based on its apparent molecular mass of gp 160. The mature surface subunit and transmembrane envelope glycoprotein subunit are designated gp 120 and gp 41 respectively. Sequence comparison of a number of HIV - I isolates indicate that (a) gp 120 is highly variable between virus isolates and (b) this variability is non uniform leading to the designation of conserved (c) and hypervariable (V) domains within gp 120 (Fig 3) (Starcich et al 1986).

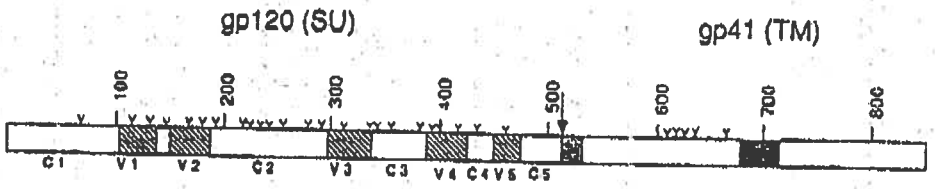


Fig.3 Linear representation of the structure of HIV-1 Env. Hypervariable regions (V1-V5) are indicated as cross-hatched boxes (▨), conserved domains (C1-C5) are shown as open boxes. The amino acid positions are shown above the bar; the arrow indicates the site of gp 160 cleavage to gp 120 and gp 41. Sites of glycosylation are indicated as Y, the stippled box (▩) denotes the location of the gp 41 fusion peptide, and the black bar represents the gp41 transmembrane domain.

A series of highly conserved Cys residues, which are involved in intramolecular disulphide bonding crucial for achieving and maintaining envelope protein tertiary structure, are found throughout gp 120 and gp 41 (Leonard et al 1990).

Like other glycoproteins destined for the plasma membranes, gp 120 is synthesised on the rough endoplasmic reticulum (ER) and is glycosylated and inserted into the lumen

of the ER. A single stop-transfer, membrane spanning sequence is located in the central region of the gp 41 domain (Fig 3 Berman et al 1988). Soon after synthesis, gp 160 monomers form oligomers (Schwaller et al 1989 and Earl et al 1991) a process which is thought to be necessary for transportation from ER to Golgi complex, (Willey et al 1991). Once in the Golgi, N-linked oligosaccharide side chains are modified to more complex forms and gp 160 is proteolytically cleaved to gp 120 and gp 41. The HIV-1 envelope protein is extensively glycosylated, nearly half of the molecular mass of gp 120 is made up of oligosaccharides, (Allan et al 1985).

Incorporation of Envelope into virus particles.

The HIV-1 envelope plays a major role in receptor binding and membrane fusion and the virion incorporation of the envelope is essential for the formation of infections virus particles. In certain virus systems - the alphaviruses-an interaction between the envelope protein intracytoplasmic tail and the viral capsid has been demonstrated directly, and this interaction is required for virus release (Suomalainen et al 1992 and Paredes et al 1993).

CD Binding

The initial step in HIV-1 infection involves the binding of virion-associated gp 120 to the cell surface molecule CD4, which serves as the major receptor for HIV-1 and the related HIV-2 and simian immuno deficiency viruses (SIVs) (Dalgleish et al 1984 and Maddon et al 1986). The envelope determinants of CD4 binding map to gp 120, CD4 binding to gp 120 induces conformation changes in both gp 120, and gp 41 that result in exposure of envelope domains that are thought be involved

directly in the membrane fusion reactions, (Sattentau et al 1991, Kang et al 1993 and Thali et al 1993, Pellchen-Matthews et al 1995).

Following the identification of CD4 as the primary receptor for HIV, it was shown that soluble CD4 could neutralise virus infection ability (Smith et al 1987, Fisher et al 1988). This neutralisation was shown to be mainly a result of enhanced shedding of gp 120 from virions following treatment with soluble CD4 (Hart et al 1991 and Moore et al 1990).

In addition to binding CD4 on the cell surface during the early stages of virus infection, HIV-1 envelope associates with CD4 intracellular soon after gp 160 synthesis in the ER. Transport inhibition studies indicate that within 30 minutes of synthesis, gp 160 adopts conformation suitable for CD4 binding (Fennie et al 1989, Littman 1995). The association of envelope and CD4 early in transport pathway leads to the down-regulation of CD4 expression from the surface of envelope-expressing cells (Hoxie et al 1986, Kawamura et al 1989 and Jabbar et al 1990). This decrease in the level of cell surface CD4 may reduce the ability of envelope-expressing cells to become infected with additional virions (Stevenson et al 1988) a phenomenon known as super infection interference found in other retroviruses (Rubin, H. 1960).

Fusion in membranes.

The ability to induce fusion between the lipid bilayer of the viral envelope and host cell membranes is a central feature of the envelope glycoprotein function. Envelope expression in an infected cell can also lead to cell-to-cell fusion with neighbouring CD4 cells a process that contributes to HIV cytopathogenicity in culture (Lodson et al 1986 and Tersmette

Cytopathogenicity in culture (Lodson et al 1986 and Tersmette et al 1988). In addition to domains required for gp 160 proteolytic cleavage and CD4 binding, a number of determinants in both gp 120 and gp 41 have been suggested to play a role in membrane fusion.

The first domain recognised as being directly involved in HIV-1 envelope induced membrane fusion was the highly hydrophobic sequence at the amino terminus of gp 41. A number of studies involving hemagglutinin protein of orthomyxoviruses and the F protein of paramyxoviruses had suggested analogous domain (fusion peptide) plays a role in membrane fusion function of these proteins (Wiley et al 1987 White J.M. 1992 and Lamb R.A. 1993 Gaundin et al, 1995). Analysis of Lentiviral envelope glycoproteins indicated that single amino acid changes in the highly hydrophobic amino termini of the HIV-1, HIV-2 and SIV transmembrane subunit glycoproteins blocked Envelope induced syncytium formation (Felser et al 1989, Freed et al 1990, Freed et al 1992). The HIV-1 envelope glycoprotein also undergoes a series of conformational changes following CD4 binding leading to the exposure of the gp 41 fusion peptide (Sattentau et al 1991). Many fusion peptides may act in concert to destabilise the lipid bilayer of the target membrane by forming "fusion pores" between the two bilalyers (While J.M., 1992). The hypothesis that envelope glycoproteins behave cooperatively to promote membrane fusion is supported by the finding that substitution of polar amino acids in the fusion peptide of HIV-1 gp 41 elicits a negative effect on syncytium formation and virus infectivity (Freed et al 1992 and Buchschacher et al 1995 Layne et al 1990).

It has been suggested for sometime that molecules other than CD4 may be necessary for membrane fusion induced by HIV-1

envelope glycoprotein. Factors provided by the human cell may be necessary: (a) Expression of human CD4 in murine cells does not confer upon them the ability to support HIV-1 infection. (b) In a cell-fusion reaction the target cell must be of human origin whereas the envelope expressing cell can be of non human origin (Ashorn et al 1990). (c) The formation of some somatic cell hybrids between human cells and CD4 expressing non human cells can overcome the fusion defect observed in human CD4-expressing non human cells (Weiner et al 1991 and Broder et al 1991).

Tropism in Tissue.

Additional function of the HIV-1 Envelope glycoprotein is to determine the cell type specificity-tissue tropism-of the virus infection. In culture, HIV-1 infects either cells of the monocyte/macrophage lineage, or immortalised T-cell lines but rarely both. Primary virus isolates obtained from infected individuals during the early asymptomatic phase of infection are frequently non-syncytium-inducing and macrophage-tropic, and cells of the monocyte/macrophage lineage are thought to be important targets for virus infection invivo (Meltzer et al 1990). HIV-1 isolates which are syncytium inducing and capable of productively infecting T-cell lines tend to arise late in infection after the onset of AIDs-defining symptoms (Schuitemaker et al 1992). It has been suggested that the evolution in vivo of syncytium-inducing, T-cell line tropic, variants may play a causal role in disease development (Tersmette et al, 1988). **The block to infection in nonpermissive cells appear to be primarily at the level of entry probably resulting from a defect in membrane fusion** (Cann et al, 1990, Stefano et al, 1993).

Interactions of envelope glycoproteins.

We have largely focused on the functions of discrete domains within the HIV-1 Envelope glycoprotein. However it is increasingly clear that interactions between nonadjacent sequences within gp 120 or between gp 120 and gp 41 are essential for most envelope functions. Debilitating mutation in C2 affecting infectivity could be reversed by changes in C1 an V3 suggesting functional interactions between these domains of gp 120 (Willey et al 1989 and Freed et al 1994). These studies are consistent with the concept that while distinct domains within HIV-1 envelope are involved in specific functions, complex interactions between and within these domains are essential for the full range of biological activities required for productive infection (Pinter et al 1993, Moore et al 1994, McKeating et al 1992).

The Biology and molecular Biology of HIV

After infective entry into the cell, the genome is released from coating in the cytoplasm. Viral replication begins with the generation of a first strand DNA copy of viral RNA mediated by HIV-1 encoded **Reverse Transcriptase**. The Second Strand DNA synthesis is also controlled by **Reverse Transcriptase** but proceeds only after **pol gene product ribonuclease H** which partially degrades the original viral RNA template. When completed **Reverse Transcriptase** yields a double stranded DNA replica of the original RNA genome containing LTR (long terminal repeats) at each end of the DNA. After transfer to the nucleus, the viral DNA duplex is inserted into the host DNA by the **integrase, another product of the pol gene** (Fig. 4)

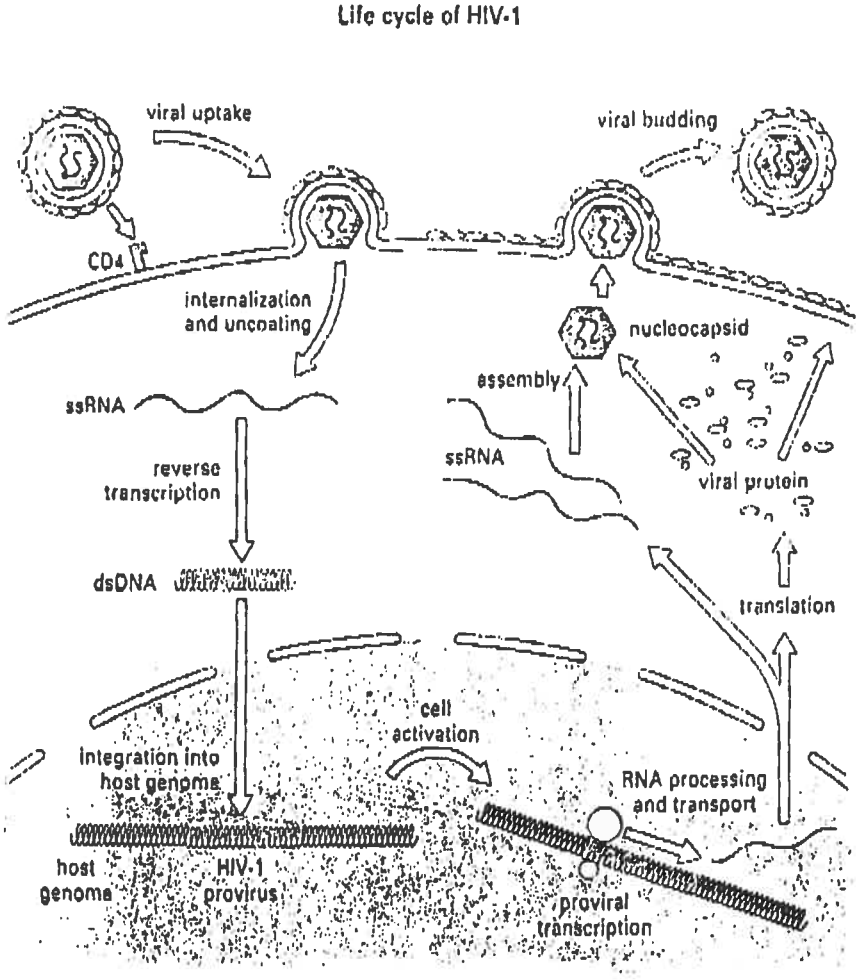


Figure 4: *After the interaction of gp 120 env with the CD4 membrane receptor, gp 41-mediated membrane fusion occurs, leading to the entry of HIV-1 into the cell. After uncoating, reverse transcription of viral RNA begins, and this results in the production of the double-stranded DNA form of the viral genome. In turn, the HIV-1 integrase promotes the insertion of this viral DNA duplex into the host genome, giving rise to the HIV-1 provirus. The free HVI-1 virions produced by viral budding from the host cell can then reinitiate the retroviral life cycle by infecting other CD4⁺ target cells.*

The significance of this integration reaction extends beyond the perpetuation of the virus: **(a) the life cycle of the virus involves an obligatory stage in which double-stranded DNA is inserted into the host genome; (b) a retroviral sequence that is integrated remains in the cellular genome as a provirus; (c) Cellular sequences occasionally recombine with retroviral sequence and can be transposed with it to new locations; and (d) Cellular sequences that are transposed by a retrovirus may change the properties of a cell that becomes infected with the virus,** (Varmus H. 1988, Levy J.A. 1985, Goff S.P. 1992, Lai M.M.C. 1992, Katz Shalka 1994, Hu and Temiu 1990).

The provirus is generated by directly inserting a linear DNA into a target site. In addition to linear DNA there are circular forms of the viral genome sequence which may persist in cells (Levy J.A. 1985). Integrase acts on both the retroviral linear DNA and the target DNA. The ends of viral DNA are important. The integrase brings the ends of linear DNA together in a ribonucleoprotein complex and converts the blunt ends into recessed ends by removing some bases. Target sites are chosen at random with respect to genome sequence. The viral DNA integrates into the host genome at randomly selected sites. A successfully infected cell gains 1-10 copies of the provirus. Retroviruses can replicate most favourably in proliferating cells because entry into the nucleus requires the cell to pass through mitosis when the viral genome gains access to nuclear material.

The U3 of each LTR carries a promotor responsible for initiating transcription of the provirus. Sometimes the promotor in U5 LTR sponsors transcription of host sequences that are adjacent to the site of viral integration. It also

carries an enhancer that can act on cellular as well as viral sequences. Integration of a retrovirus can be responsible for converting a host cell into a tumorigenic state when certain types of genes are activated in this way. (Finnegan D.J. 1985).

When viral DNA integrates in a germline cell it becomes an inherited endogenous provirus of the organism. Endogenous viruses are usually not expressed but can be activated at times by external events such infection with another virus.

Two copies of RNA genome are packaged into each virion making the individual virus particle effectively diploid. When a cell is invaded by different but related viruses, it is possible to have heterozygous virus particles. The production of a retroviral particle involves packaging the RNA into a core surrounding it with capsid proteins and pinching off a segment of membrane from the host cell.

The enzyme Reverse Transcriptase has an error rate of about one wrong nucleotide added per 20,000 nucleotides. This is because they do not have 3'-5' proof reading exonucleases. A likely consequence is appearance of new strains of disease causing viruses. (Boeke J.D. 1990, Varmus H. 1987). Thus HIV has inability to repair mutations that occur in the genome. As many as 28 different. HIV variants have been found in a single infected individual some of which must have come from internal mutations due to reverse transcriptase errors (Sancor A. 1996 and Wood R.D. 1996).

The HIV Disease Progress

Not every person exposed to the virus becomes infected. There are a few cases who inspite of repeated exposure to the virus remain virus free (Fernandez-Cruz E, et al 1996). Then

there are those who are infected but show no signs of the disease for a very long time (Cao, Y. et al 1995).

Exposure to the virus has four types of outcomes: (a) a tiny minority who do not get infection inspite of repeated exposures to the virus (b) a small population who seem to have an indefinite period of latency, (c) a big portion (may be the majority) who have a 5-10 year period of latency and (d), a small number who sucumb to the ravages of the disease very quickly and may die within less than 5 years after getting infected (Embreltson J. et al 1995).

For those cells with a provirus in their genome, it is not clear what triggers off the transcription processes leading to viral multiplication. Also it is not clear why relatively few infected T cell are activated. For every T cell actively producing virus nine other T cells contain latent virus (Schnittman S.M. et al 1989, Cullen B.R. and Green W.C. 1989, Rosenberg Z.F. Fauci A.S. 1990). Then there are very many immunogenic and viral factor other than HIV which activate HIV infected T cell into replication processes. Many of these activating agents induce expression of select host transcription factors notably some enhances binding proteins (Nabel G, Boltimore 1987, Jones K.A. et al 1986). However **nef gene product** can be considered essential regulatory protein critical for achieving high viral load and disease progression (Harris M. 1995). Nef expression has been shown by many groups to down regulate cell surface expression of the viral receptor glycoprotein CD4. (Guy B. et al 1987, Garcia J. & Miller A.D. 1991, Garcia J. et al 1993, Aiken C. et al 1994).

Nef has also been demonstrated to enhance infectivity of HIV-1 in primary lymphocyte culture by increasing the capacity of viral particles to infect these cells productively.

(Chowers, M.Y. et al 1994, Miller M.D. et al 1994, Saksela K. et al 1995, Miller M.D. et al 1995, Harris M. Coates K. 1993, Kim S. et al 1989).

HIV-1 expression after infection is a multistage process with specific co-factor requirements of specific viral **tat, rev and nef gene products**. In absence or viral tat protein very little full length viral RNA is made, and that which is made is in short fragments. The viral tat protein permits rapid accumulation of full length viral RNA. Full length RNA must accumulate to allow synthesis of viral rev protein. In absence of rev protein, the full length RNA is fragmented by host splicing enzymes to form short fragments of viral RNA (Miller W.E.B. et al 1990, Taylor J.B.C. et al 1992, Gunnery, s. et al 1997). These groups have demonstrated that HIV-1 tat product can transactivate expression of cellular matrix genes in astrocytic cell lines. This added the mounting evidence that HIV-1 tat gene products can affect a variety of non viral gene promotors (Jones K.A. et al 1986).

For the assembly of infections HIV-1 virions, the retroviral structural and enzymic proteins must be produced. These proteins are uniquely encoded by incompletely processed viral transcripts including the unspliced **gag-pol MRNA and singly spliced env MRNA.** The transition between the synthesis of early regulatory gene and late structural gene products appear vitally dependent on the **HIV-1 Rev protein.** The Rev protein appear to exert its regulatory activity at a post-transcriptional level by activating the cytoplasmic expression of unspliced and singly spliced forms of HIV-RNA that codes for the products of **gag,pol and env genes.** (Kims, S. et al 1989).

There are conflicting reports of the functions of other viral genes. The **vpr gene product** is packaged within the virus particle itself. The vpr protein is made late in virus replication and is present in multiple copies in the virus particle (Wongstaal F., et al 1987, Cohen E.A. et al 1990, Ogawa K. et al 1989, Yu, X.F., et al 1990). The vpr protein acts to increase the rate of transcription of a variety of promotors including those of HIV-1.

Two viral proteins made late in the infections cycle are **vif and vpu gene products** which seem to affect viral assembly (Strebel K. et al 1988, Terwilliger E.F. et al 1989, Klimkait T, et al 1990, Strebel K. et al 1987, Guy B. et al 1991).

Virus particle maturation and release depends upon the activity of a virus specified protease. The protease is made and incorporated into the assembling virus particle as part of the precursor of the replicative enzyme. The viral protease cleaves both the capsid precursor and replicative enzyme precursor. In the absence of the protease activity immature non-infectious viral particles are released from infected cells. (Gottlinger H.G. et al 1989, Kohl, N.E., et al 1988, Peng, C. et al 1989, Debouck c. et al 1987, Mous J. et al 1988, Gottlinger H.G. 1991).

Cellular Pathogenesis of HIV

The ability of HIV-1 to produce cytopathic effects within $CD4^+$ subset of human T lymphocytes and other cells is an important contributor to the state of profound immunodeficiency the virus induces. HIV-1 induced killing in vitro involves cell fusion and formation of syncytium mediated by the gp 41 env protein after gp 120 env protein interacts with CD4. This fusion is facilitated by mere presence of HIV-1 env proteins on

the surface of CD4 expressing multinucleated cells, infected and non-infected. (Leonard, R. et al 1988, Levy J.A. et al 1985, Brenzle D. et al 1996). The formation of syncytia ultimately leads to cell death. Cells with low levels of CD4 such as monocytes and macrophages are not affected as much.

The peripheral blood stream CD4⁺ lymphocytes are heavily involved in the pathogenesis and progress of HIV disease. As a result many immune system associated defects do appear as the disease advances. However it seems the virus involved in latter stages of the disease is different from the earlier stages. The virus in chronic stage of the disease replicates faster and has enhanced cellular host-range, seems more destructive of CD4⁺ population and appears to resist neutralisation. The CD4⁺ lymphocyte count drops and the helper T4 cells are depleted and can no longer **(a), activate B lymphocytes that are responsible for antibody production, (b), influence cytotoxic cells and natural killer cells in the cell mediated immune response, (c) influence macrophages whose products modulate the activity of many cell types** (Schnittman S.M. 1989, Science 1989, Werner A et al 1990, Maddon P.J., et al 1986, Takeuchi Y. et al 1991, Gehri R. et al 1996, Bienzle D. Et al, AIDS 1996, Lyn W., et al 1988, Walker B.D., et al 1987).

The early phase of HIV infection is immunologically characterised by type 2 cytokine secretion and alteration in the expression of phenotypic markers and closely resemble the more advanced phase of HIV infection. These immunologic alterations are temporarily limited by the successive return to a normal profile, thus HIV infection is an immunological complex process even in the earliest phases (Meroni L. et al 1996).

It is increasingly recognised that infection with HIV-1 is

a disease characterised by **immune dysregulation** and not simply immune deficiency. HIV-1 infection disturbs the immune system in several ways **(a), direct killing of CD4 T helper (H) lymphocytes, (b), destruction of virally infected cells by cytotoxic T lymphocytes and (c), by generation of aberrant signalling via cytokine and cell-to-cell contact.** Other mechanisms of immune dysregulation may involve preferential deletion of memory (TH) lymphocytes in the initial phase infection, antigen-independent deletion of activated T Cell clones and depletion TH! lymphocytes (Weis R.A. 1993, Schattner A. Ent, Z. 1993, Kazi S. et al 1995, Salvaggio, A. et al 1996, Clorici M., et al 1996).

So HIV disease is a profoundly complex. This complexity extends to wide spread viral infection of other types of cells and organs.

HIV-1 infected macrophages and microglia have sustained productive viral infections. Microglia undergo cytopathic changes including formation of multinucleated gland cells and mononucleated stellate cells with altered morphology. In human brain they produce large amounts of progeny virions, thus the brain serves as important reservoir for the virus. (Lipton S.A. & Gendelman H. 1995, Wiley C.A., et al 1996).

Several cell types in the central nervous system in addition to microglia or macrophages may become infected in vivo with variable levels of HIV-1 MRNA expression. The diverse cellular reservoir for HIV-1 in CNS may be linked to the molecular mechanisms involved in HIV-1 neuropathogenesis (Bagasra O., et al 1996) which leads to dementia associated with AIDS.

The number of cell types latently infected in the

pathogenesis of HIV disease is increasingly important (Knight S. 1996). One of these cell system is lymphoid tissue (Corne P. et al 1999). Splenic lymphoid tissue has a much higher frequency of HIV-1 producing $CD4^+$ T lymphocytes in comparison with peripheral blood $CD4^+$ T lymphocytes (Corne P, 1999). Other workers have indicated the presence of gp 120 on plasma membranes of apoptotic CD4 cells from lymphnodes of HIV-1 infected individuals (Sunila I et al 1997, Pantaleo G. et al 1993). Blood and lymphodes are major reservoirs and major sites for replication (Embretson J. 1993).

In cells, Mitochondria are energy transducing organelles. These are significantly altered and have a tendency to undergo apoptosis in patients with acute HIV syndrome (Cossarizza, A. et al 1997).

Apart from the fatal consequences of severe immunodepression resulting in extensive opportunistic infection, it is becoming clear that HIV infection increases chances of various types of cancers some of which may appear opportunistic. Among homosexuals there is a high incidence anal infection with human papilloma virus in HIV infected individuals, leading to anal squamous intra epithelial lesions (Palefsky J.M. 1999).

Cervical abnormalities have been observed in HIV-Ceropositive women (Olaitan A. et al 1997). Other workers have noted increased frequency of cervical dysplasia in women infected with HIV and having a high degree of immonosuppression (Schafer A. et al 1991).

Also gynecological complications are common among HIV Seropositive women with $CD4^+$ lymphocyte count below 500 cells/μl and more severe with advanced immunosuppression (Watts D.H. 1999).

Treatment of HIV Disease

Inspite of the tremendous amount of resources used in terms of time money and the advances so far made in knowledge of HIV, **AIDS has No cure yet.**

There are two basic rationales in the treatment of HIV disease: (a), development of vaccines against the virus, and (b), the development of effective enzyme inhibitors to inhibit or stop metabolic activities of the virus. Both of these have proved problematic and have not succeeded in devising a cure for AIDS.

(a) Vaccines

The development of vaccines has proved difficult because of the **complexity of the envelope glycoprotein, and the life cycle of the virus.** As indicated in Fig 3, the Env gene product has two important areas which seen vital for the infection process, namely V3 and V1/V2 region. Antibodies to V3 were capable of neutralising virus infectivity without affecting CD4 binding (Linsley P.S. et al 1988, Skinner M.A., et al 1988). Antibodies for V1/V2 region are also capable of neutralising virus infectivity. (Fund, M.S. et al 1992, McKeating et al 1993). These are **Hypervariable regions** which are subject to high rates of mutational modifications but in addition these regions seem to act in concert with other regions in the env protein.

While distinct domains within HIV-1 are involved in specific functions, complex interactions between and within these domains are essential for a full range of biological activities needed for productive infection. **It would be of great benefit if one could pinpoint the elements contained within the virus which can give rise to whole protective immune response** (Crange M.P., et al 1991).

The other complexity of HIV vaccine is associated with the way virus DNA is formed. The Reverse Transcriptase has high error element in that in the course of its functioning it produces some mutants, which makes it difficult to produce effective vaccine against so many varying types.

It seems that by the time the viral particles reach the peripheral blood stream, for antibodies to attack them, they are already established in lymphnodes and other cellular hiding places to make the vaccine effective, (Van Cott T.C., et al 1995, Pope M. et al 1995, Ramarli D. 1995).

Between 1985 and 1989 extensive vaccine-related studies were conducted in chimpanzees. The immunogens used derived principally from HIV-1 envelope included killed virus, subunits peptides and vaccinia recombinants. None of these approaches protected against infection but importantly, low or no neutralising antibodies and very weak cellular immunity were measurable at the time of challenge (Girard, M.P., et al 1989, Eichberg, J.W. 1990).

Because of the poor immunogenicity of these preparations the prime/boost approach was employed. Several combinations of whole killed virus, vaccinia recombinant vectors bearing the HIV-1 envelope, envelope and gag viral subunits, and non structural viral proteins were tried. Overall none of these combinations produced protective immunity (Girard M. et al 1989). However when animals were boosted with petides representing the principal neutralising determinant (PND) of the virus substantial neutralising antibodies appeared (Javaherian K. et al 1989) and the animals were protected on being challenged with the virus. This study used immunogeus formulated with aluminum hydroxide which seem to give protection in vitro and in vivo in experimental animals even

when challenged with the virus itself (Emin E.A. et al, 1990, Emini E.A. et al 1991, Rusche J.R. et al 1988, Palker, T.J. et al 1988, Kenealy W.R. et al 1989).

The PND is known to be situated within V3 of the exterior gp 120 glycoprotein and represents a uniquely hypervariable region. The variability is apparently responsible for predominantly isolate-specific neutralising antibodies which result upon immunisation or in response to natural infection. The variation in this region is extensive among naturally occurring HIV-1 isolates and significant variability can be detected within the population of viruses in a single individual. This extensive variation coupled with the apparent immuno dominant nature of the variable domains presents a major obstacle for vaccine development as far as this epitome is concerned (Leonard C.K. et al 1990, Putnet S.D. McKeating J.A. 1990, LaRosa G.J. et 1990, Takahashi H. et al 1988). It is believed that this variability is the means by which the virus escapes from host immune defences (Graham B.S. & Wright P.F. 1995).

The effort to develop vaccines should continue. Various types of vaccine may have to be developed, the most desirable of which are vaccines to prevent infection and offer cross-protective immunity effective against existing swarm of HIV-1 isolates under natural modes of transmission. Equally important is to develop new insights into vaccinology principles that would deal with unprecedented variability of HIV-1 and how to elicit secretory immunity within the setting of comprehensive systematic immunity to this end there have been attempts to develop gene therapy (Fig5-6).

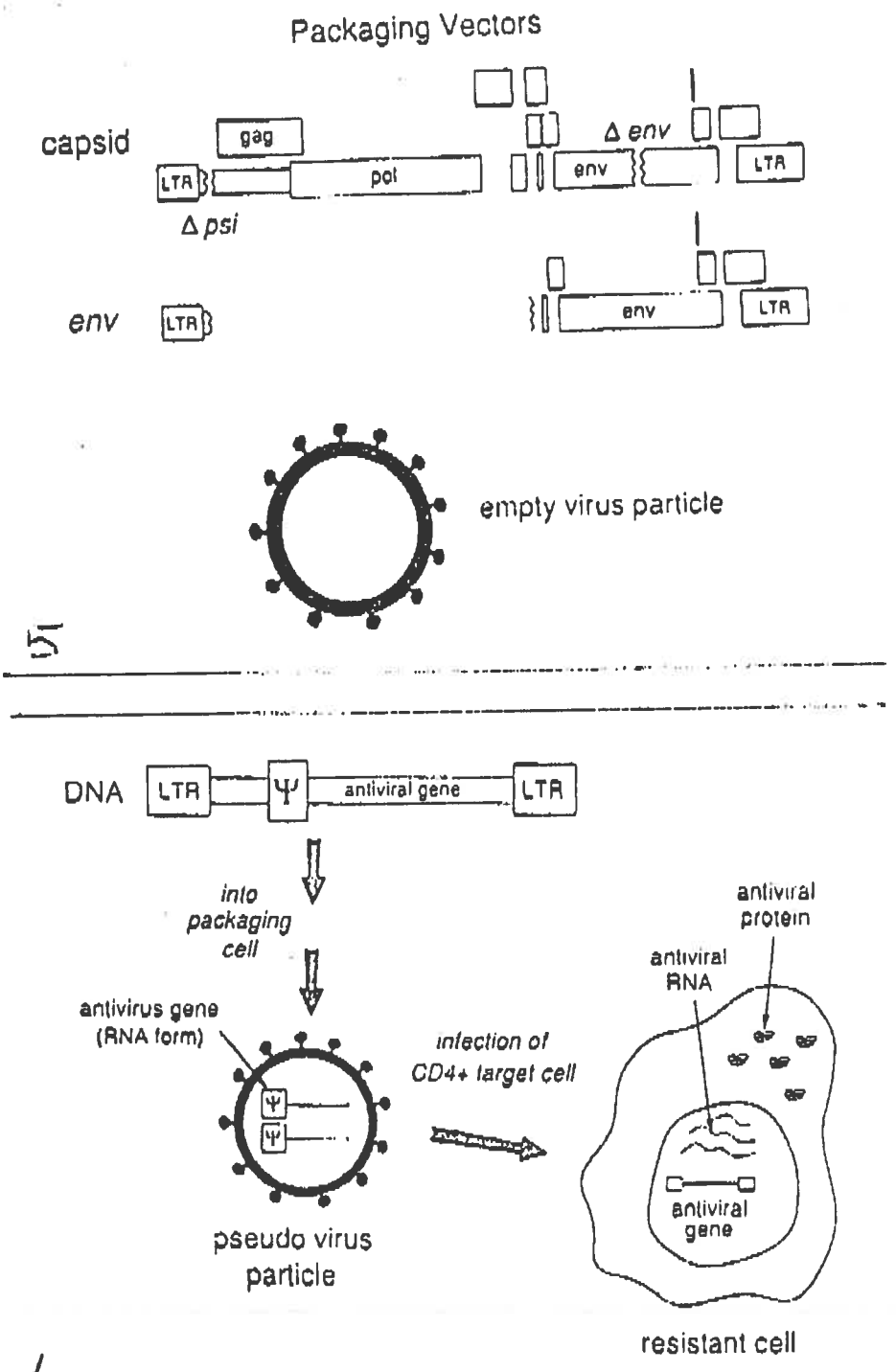


Fig.5 **Diagram or HIV-1 vector designed for gene transfer.**
Fig.6 **Mechanism for using the HIV-1 vector for antiviral gene therapy.**

(b) Enzymes Inhibitors as Drugs

The rationale for drugs used as enzyme inhibitors is based on the fact that HIV virus has a few key enzymes in its life cycle

namely Reverse Transcriptase involved in formation of viral DNA, Integrase involved in integrating viral DNA into host DNA and Proteases involved in fragmenting, products of viral MRNA into appropriate components for viral propagation. If these various enzymic activities are effectively interfered with then viral replication and propagation will not take place.

The enzyme inhibitors used as antiretroviral drugs fall into two categories. (a), Purine and Pyrimidine analogues which are pseudo nucleotide bases when incorporated they produce abnormal and malfunctioning products. (b), Protease inhibitors which block the cleavage of HIV-encoded precursor polypeptide forming gag and pol gene products thus suppressing maturation of viral particles to infections virions. (Fig.7)

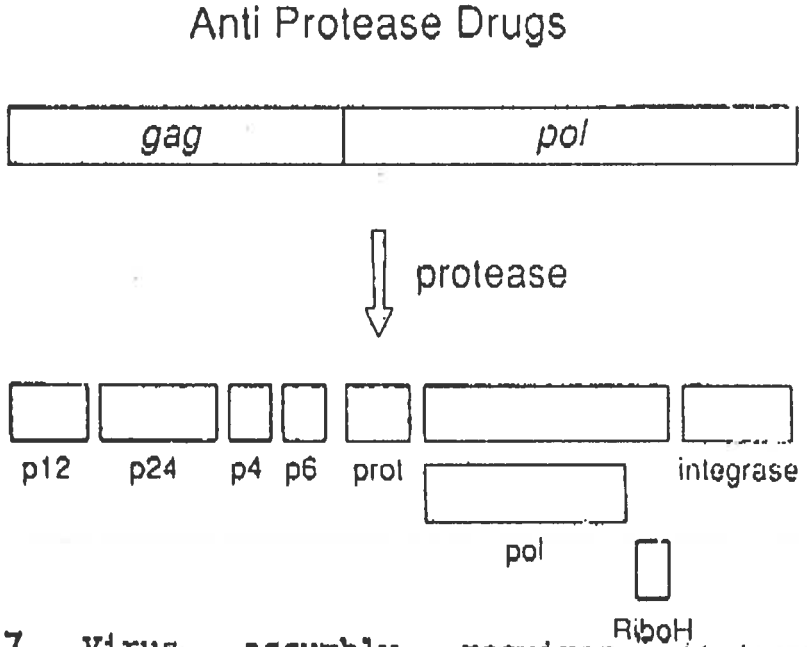


Fig 7 Virus assumbly requires protease activity. Antiprotease drugs are being analyzed to inhibit the formation of infectious particles.

The first effective retroviral enzyme inhibitor was AZT or ZDV which was first synthesised in 1964 as anticancer agent. AZT is a structural analogue of deoxy thymidine which is 3 Azido-2,3 dideoxythymidine. The other widely used drug is 2,3,

dideoxy inosine (DDI) a purine derivative. The drugs are taken up by the T lymphocytes and converted to triphosphate derivatives. The HIV reverse transcriptase has a higher affinity for AZT triphosphate. Binding of AZT triphosphate to the enzyme competitively inhibits the binding of normal nucleotides. In addition AZT can be added at 3 end of the growing polynucleotide, but because AZT has no 3′ hydroxyl, the polynucleotide chain is prematurely terminated and the viral synthesis of polynucleotide polymers are quickly terminated. Unfortunately AZT is toxic to bone marrow cells that give rise to erythrocytes leading to severe anaemia.

Although extensive use of combinations therapy for HIV infection in several studies have indicated a consequent decrease in mortality and morbidity (Paletta F.J. et al 1998, Egger M. et al 1997, Mocroft A. et al 1998) no extensive evaluation has been made as to whether the risk of developing specific AIDS defining illness has been reduced with combination treatment. The comparative studies done so far indicate that combination treatment has no effect on AIDS related neoplasms so far (Pezzotti, P. et al 1999).

Other workers have reported high incidence of malignant lymphomas and cervical cancers in patients with progressive HIV disease (Ridolf, A.L. 1996, Maiman M. et al 1993, Zanetta G. et al 1995). The other problems associated with prolonged combination therapy are changes in body habitus due to abnormal fat distribution with associated anaemia (Lipsky J.J. 1998, Dong K.L, et al 1999, Lo J.C. et al 1998). Also care must be exercised to avoid patients with renal insufficiency or liver disease (Jayasekara D. et al 1999, Moore K.P.H., et al 1995).

The biggest problem associated with both R.T. and protease inhibitors is the growing drug resistance. (Durant J. et al

199, Deeks S.G. et al 1997).

Inhibitors of retroviral enzymes have **dramatic effects initially lowering the blood viral load and improving the clinical state of patients** (Collier, A.C. et al, 1996, Richman D.D. et al 1994, Ho D.D. et al 1995, Kakuda T.N. et al 1998, Gibbons S., Mukahy F.B., 1997, Margolis, D. et al 1999, Bartlet, J.G. et al, 1998).

However, **virus resistance to the inhibitory effects of drugs develops rapidly causing continued progression of disease symptoms.** This is true in R.T. inhibitors and protease inhibitors (Condra J.H. et al 1995, Ridky T., Leis J. 1995, Iversen A.K.N. et al 1996, Durant J. et al 1999, Rusconi, S. et al 1997, Milazzo L, et al 1999, Sondergaard S.R. et al 1999, Pollard R.B.et al 1999).

Concluding Discussion

The life cycle of the virus is such that it involves conversion of its RNA to DNA which is then integrated into host DNA. The virus replicates by copying the viral DNA into RNA then using RNA, it makes new protein and new virions. **Once infected, one is infected for life.**

Although highly active antiretroviral therapy including HIV-1 protease inhibitors represents important advances in the management of HIV disease, many patients suffer adverse side effects. These include diarrhea, gastrointestinal discomfort, gastroesopagel reflux (Deeks S.G. et al 1997). Other abnormalities include elevated plasma concentrations of hepatic enzymes such as creative kinase, bilirubin, glucose, triglycerides abnormal fat distribution (Deeks, S.G. et al 1997, Parlett J.G. et al 1998). Further more prolonged period of treatment with combination therapy leads to repopulation

with less activated T cells with increased proliferation capacity (Dussaux L. et al 1993).

Many of the drugs being used for AIDS treatment are prescribed with very limited long term data and without knowledge of long term safely of these agents (Deeks S.G. et al 1997).

There is need for more fundamental knowledge about the behaviour of HIV under various types of cellular environment. There are reports of altered host-range of HIV-1 after passage through various human cell types (Cheng-Mayer C., et al 1991). The effects of HIV passage through many cell types to infectivity is not known. (Knight S. 1996).

The role of epithelial cells in AIDS pathogenesis need more investigation. (Tomolo A. et al 1995, Lafeuillate A. et al, 1996).

Thank you for the opportunity to share some ideas and problems with you all.

THE BIOCHEMICAL CHALLENGES OF HIV

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