

## CHAPTER II

### LITERATURE REVIEW

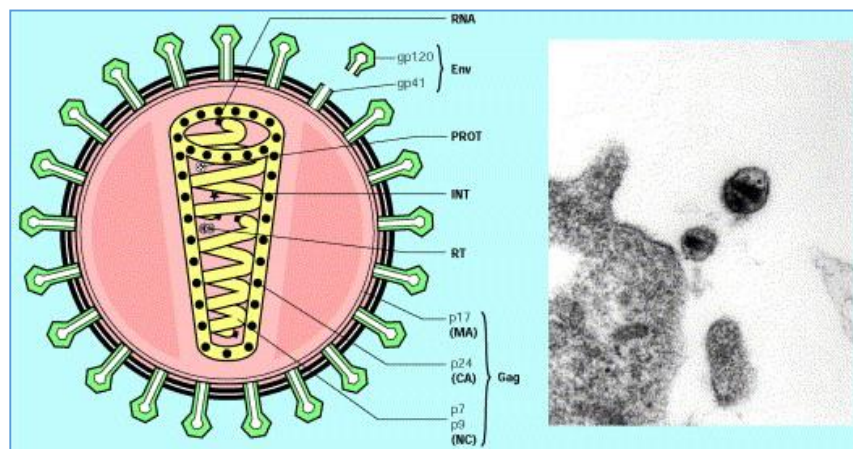
#### 1.1 Human Immunodeficiency Virus (HIV)

Two types of HIV can be distinguished genetically and antigenically. HIV-1 is the cause of the current worldwide pandemic while HIV-2 is found in west Africa but rarely elsewhere. HIV-2, which is transmitted in the same ways as HIV-1, causes AIDS much more slowly than HIV-1 but otherwise clinically the diseases are very similar.<sup>44</sup>

Human immunodeficiency virus (HIV) is a blood-borne virus typically transmitted via sexual intercourse, shared intravenous drug paraphernalia, and mother-to-child transmission (MTCT), which can occur during the birth process or during breastfeeding.<sup>45</sup> HIV belongs to a family of viruses known as retroviruses.<sup>46,47</sup> Like the other retroviruses, HIV virion (figure 2.1) contains a virus capsid, which consists of (a) the major capsid protein, p24; (b) the nucleocapsid protein, p7/p9; (c) the diploid single-stranded RNA genome; and (d) the three viral enzymes, protease, reverse transcriptase, and integrase.<sup>47</sup> The viral capsid is surrounded by a matrix protein (p17; figure 2.1), which is located underneath the virion envelope. The matrix protein is involved in the early stages of the viral replication cycle and plays a part in the formation and transport of the preintegration DNA complex into the nucleus of the host cell.<sup>47</sup>

The initial interaction of the viral gp120 with the CD4 binding site on the cell surface which leads to conformational changes in the envelope, allowing a second interaction of the virus envelope with chemokine coreceptors. This latter event produces a further change in gp41 before it fuses and penetrates the cell surface.<sup>48</sup> HIV attaches itself to target cell using a coreceptor (CCR5 or CXCR4). It then gains entry into the target cell and uses its machinery to complete its life cycle however destroys them in the process. Target cells include macrophages and T cells.<sup>49</sup>

The diagnosis of HIV infection is typically made by detection of antibodies to the virus in the blood.<sup>46</sup>

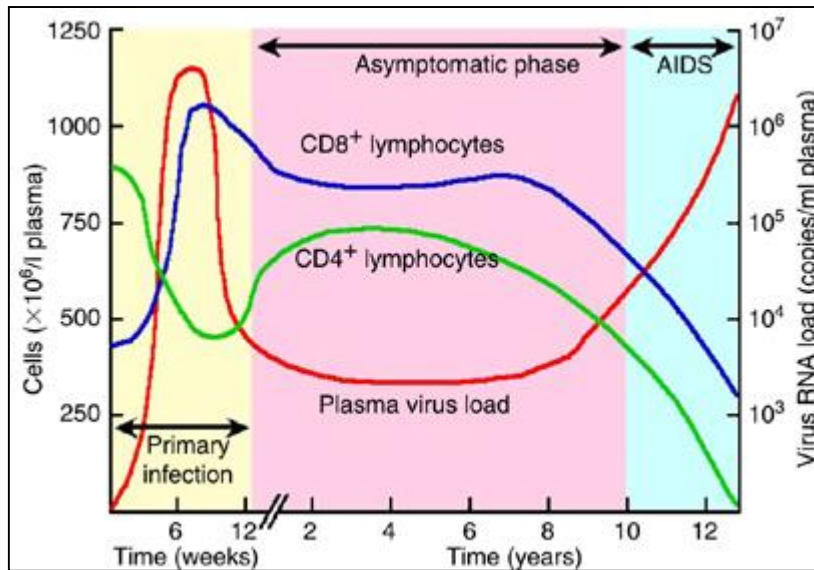


*Fig.2.1 Schematic diagram of an HIV virion and electronmicrograph Location of the structural proteins (see text) is indicated. Electronmicrograph by Dr P Gounon. Departement of Electron Microscopy. Institut Pateur.*

## 1.2 Pathogenesis of HIV

Natural history of HIV-1 infection encompasses an acute/primary phase that lasts for months, followed by a clinically latent phase that typically lasts for

few years and ultimately by the collapse of immune system that characterized AIDS.<sup>14, 50</sup> Pattern of commonly detected HIV-specific immune responses and plasma viral level is shown in Fig. 2.2<sup>51</sup>



**Fig.2.2 Immunological and virological events in natural course of HIV infection**

### **Acute phase of HIV-1 infection**

From the time one gets infected with HIV-1, it may take three to six weeks for anti-HIV antibodies appearing in peripheral circulation. This period is called “window period”. The diagnostic tests that detect anti-HIV antibodies are negative during this period. However, this is a very important stage in HIV pathogenesis. There is surge of viraemia with plasma viral load reaching peak in 2-3 weeks and loss of T helper cells during this period causing a transient drop circulating CD4<sup>+</sup> T lymphocytes. The host generates immune response during this period that helps in controlling the virus multiplication leading to sharp decline in plasma viraemia. About 4-6 months post-

infection, steady state of viraemia (virologic set point) is achieved in each patient. The plasma viral load set point is prognostic for the future course of the disease.<sup>14</sup>

The resolution of the clinical symptoms and the subsequent decrease in plasma viremia are associated with the emergence of HIV-1 specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses. The remarkable early inhibition of viremia by CD8<sup>+</sup> T cells reduced plasma viremia to a “set point” level. Over time, CD8<sup>+</sup> T cells responses increase, but without a change in the control of viral replication or further reduction in the viral set point. The early viral set point, consequent on the first CD8<sup>+</sup> T cells responses, is highly predictive of the later course of disease progression, the preservation of these early responses has been associated with slower disease progression.<sup>11</sup> The end of acute infection is associated with the emergence of specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses and the establishment of a chronic phase of infection.<sup>10</sup> The end of this period coincides with the first signs of a CD8 immune response against HIV.<sup>49</sup>

### **Chronic phase of HIV-1 infection**

Chronic phase characterized by an immune activation with a massive production of proinflammatory cytokines, which in turn is responsible for clonal deletion and gradual loss peripheral CD4<sup>+</sup> T cells over time.<sup>10</sup> During chronic untreated HIV infection, practically every arm of the immune system that has been investigated has been shown to be in hyperactive state : high T cell turnover, nonspecific T cell activation and proliferation, polyclonal activation of B cells and elevated proinflammatory cytokines. The assessment of immune activation can be made through high frequency of T cells

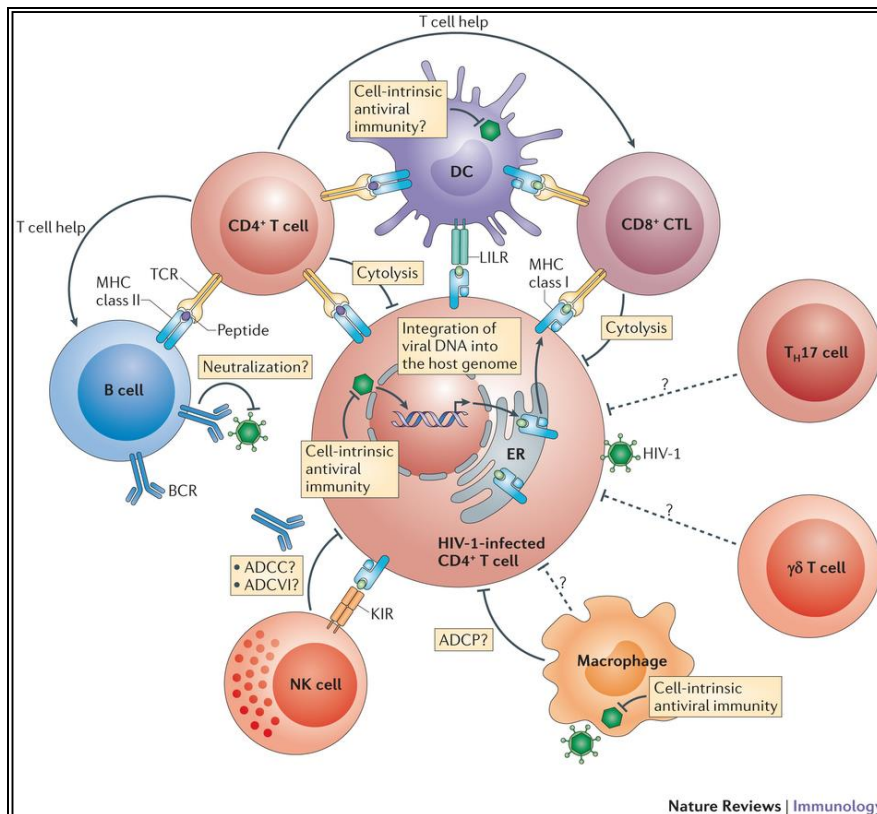
expressing markers of activation and proliferation, high levels of activation-induced apoptosis of uninfected T cells and high level of T-cells proliferation.<sup>52</sup>

The plasma virus load level remains stable for several years post plasma virus load set point. The infected person remains by and large asymptomatic during this period. It is important to stress that, although patients are without symptoms during the second phase of the infection, the virus replicates continuously during this period that leading to the destruction of CD4<sup>+</sup> T lymphocytes. As a results there is gradual drop of CD4<sup>+</sup> T lymphocytes in peripheral circulation.<sup>46</sup> Plasma virus load and CD4<sup>+</sup> T lymphocytes counts are hence two important parameters of HIV disease progression.<sup>14</sup> The CD8 responses are thought to control the virus to low levels but the CD4 cell numbers continue to decline. The length of this phase may range from a few months to 15 or more years.<sup>49</sup>

### **1.3 Immune activation**

Immune activation plays an important role in the pathogenesis of HIV disease.<sup>53</sup> It has been shown that immune system can provide a control mechanism against HIV progression, and that the immune response to HIV can be boosted in vivo to increase effectiveness.<sup>21</sup> HIV infection is characterized by chronic immune activation and CD4 T cell depletion leading to dysfunction of the immune system. While a direct infection of CD4 T cells by HIV partially explains the CD4 T cell depletion, it is clear that the overall disruption of immune function in patients with HIV infection is the sum of multiple factors. Immune activation is a major contributor to the pathogenesis of HIV disease and is manifested in many ways varying from increased T cell proliferation. Despite the

presence of immunodeficiency, virtually all cellular components of the immune system, B cells, NK cells, T cells and macrophages show evidence of immune activation.<sup>54</sup>



**Fig. 2.3 Immune activation**

#### 1.4 CD4<sup>+</sup> T Lymphocyte and HIV

Untreated HIV disease is characterized by a gradual deterioration of immune function. Most prominently, the CD4<sup>+</sup> T-cells are disrupted and destroyed during the typical course of HIV infection. It is known that the CD4<sup>+</sup> T-cells (T-helper cells) play a major role in the immune response, signaling other cells in the immune system to perform their specific functions.<sup>55</sup>

Basically, a healthy, HIV-negative person usually has 800 to 1,200 CD4<sup>+</sup> T-cells per mm<sup>3</sup> of blood. During untreated HIV infection, the number of these cells in a person's blood progressively declines. When the CD4<sup>+</sup> T cell count falls below 200/mm<sup>3</sup>, a person becomes particularly vulnerable to the opportunistic infections that are commonly associated with AIDS (the end stage of HIV disease).<sup>55</sup>

HIV produces cellular immune deficiency characterized by the depletion of helper T lymphocytes (CD4<sup>+</sup> T cells).<sup>9, 10, 19, 46, 55, 56</sup> The concentration of CD4<sup>+</sup> T cells in the blood (the 'CD4 count') provides a very good indication of the progression of the disease.<sup>14, 23, 46</sup>

Based on experimental studies, different mechanisms combined to provide explanations for HIV mediated depletion of CD4<sup>+</sup> T-cells. These mechanisms range from accelerated destruction of matured CD4<sup>+</sup> T-cells, chronic activation, oxidative stress to impaired production of CD4<sup>+</sup> T-cells. The altered immune systems lead to failure in signal network, followed by failure in the immune system to respond to invading organisms (imbalance between production and destruction of CD4<sup>+</sup> T-cells) enabling HIV-infected persons to become vulnerable to opportunistic infections that represent AIDS.<sup>55</sup>

Within hours of exposure to HIV, CD4<sup>+</sup> T lymphocytes are found to be infected showing active viral replication. The infected CD4<sup>+</sup> cells release virions by budding through the cell membrane or by lysis of the infected cells. The released virus particles then infect uninfected CD4<sup>+</sup> T lymphocytes. CD4<sup>+</sup> T lymphocytes also serve as important reservoirs of HIV: a small proportion of these cells carry HIV provirus integrated in the host DNA without active virus multiplication.

During the primary HIV infection, the number of CD4<sup>+</sup> T lymphocytes in the bloodstream decreases by 20% to 40%. HIV brings about the lysis of HIV infected cells as well as bystander uninfected cells using various mechanism such as lysis of the cells infected with HIV. Billions of CD4<sup>+</sup> T lymphocytes may be destroyed every day, eventually overwhelming the immune system's regenerative capacity. In acute HIV-1 infection, in addition to the decline in CD4<sup>+</sup> T lymphocyte counts, qualitative impairments of CD4<sup>+</sup> T lymphocytes function are detected. The impairment of HIV-1 specific CD4<sup>+</sup> T lymphocytes function occurs very early in acute infection. Following acute primary HIV infection, one may remain free of HIV-related illness, often for years, despite ongoing replication of HIV in the lymphoid organs and relentless destruction of the immune system. However, during the period, the immune system remains sufficiently competent to provide immune surveillance and to prevent most infections. Although the decrease in the total number of T lymphocyte marks the decrease in immune competence, sometimes the quantitative loss of CD4<sup>+</sup> T lymphocytes may not be matched by the qualitative function. A number of assay such as cytokine induction, antigen induced proliferation, measurement of activation markers etc, can assess the functions of lymphocytes. However, the total CD4<sup>+</sup> T lymphocytes number still remains the most robust marker of immune competence.

The progressive loss of CD4<sup>+</sup> T lymphocytes eventually results in the loss of an ability to mount desirable immune response to any pathogen and vulnerability to opportunistic pathogens characteristic of AIDS. The estimation of peripheral CD4<sup>+</sup> T lymphocytes counts is relied upon for taking a decision on initiation of ART.



The estimation of peripheral CD4<sup>+</sup> T lymphocytes counts has also been used as a tool for monitoring disease progression and the effectiveness of antiretroviral treatment (ART). The changes in the CD4<sup>+</sup> T lymphocytes counts are important indicators of the response to ART. HIV plasma virus load is a sensitive indicator of the progression of HIV disease. However, due to the relatively high cost of virus load estimation, the CD4<sup>+</sup> T lymphocytes count remains the most important key indicator for initiation and monitoring of ART and a measure of the effectiveness of the treatment in clinical trial evaluations.<sup>14</sup>

### **1.5 CD8<sup>+</sup> T lymphocyte and HIV**

CD8<sup>+</sup> responses can be divided into (i) *The lytic response (Cytotoxic T Lymphocytes, CTLs)* which make use of proteins in their cytoplasm such as perforin and granzymes for cell lysis. This is also known as a direct killing response. (ii) *Non-lytic responses (Chemokines)* are soluble substances secreted by CD8<sup>+</sup> cells, for example is cytokines. These work by either inhibiting HIV replication or inhibiting viral entry into target cells.<sup>49</sup>

The dynamic of CD4<sup>+</sup> and CD8<sup>+</sup> T cells are altered in many ways during HIV infection. Distinct pathways differentially influence proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in patients with HIV infection. Proliferation of CD4<sup>+</sup> T cells is driven by a combination of the homeostatic response to CD4<sup>+</sup> T cell depletion (CD4<sup>+</sup> T cell counts) and viral load (HIV RNA levels). In contrast, CD8<sup>+</sup> T cell proliferation is driven mainly by HIV RNA levels.<sup>53</sup>

CD4<sup>+</sup> T-cells initiate production of CD8<sup>+</sup> T-cells. Two pathways by which this occurs were defined. Traditionally, the role of CD4<sup>+</sup> cells was to produce a cytokine, interleukin (IL-12). This cytokine trigger dendritic cells to produce CD8 cells. This is

called the classical pathway. On the other hand, some viruses can directly stimulate the dendritic cells. Whether HIV also does this remains to be clarified. Alternatively CD4 cells activate antigen-presenting cells *APC* which then trigger the production of CD8 cells. This is called the CD4-APC-CD8 pathway.<sup>49</sup>

CD8<sup>+</sup> T-cells play some role in HIV infection, by the following: (1) CD8<sup>+</sup> T-cells responses can reduce the viral set point value. (2) Persistent CD8<sup>+</sup> T-cells responses are required for successful viral control. (3) CD4 helper cells are efficient in establishing a persistent CD8 responses. (4) Lytic CD8<sup>+</sup> responses (CTLs) can be detrimental to the host hence a cooperation with non-lytic CD8<sup>+</sup> responses results in a more beneficial outcome. (5) HIV can escape CTL responses through variations with in the virus for instance mutations in epitopes. (6) HIV makes use of different viral tropisms to adapt to its environment. (7) Switching of viral tropics can be a mechanism for disease progression.<sup>49</sup>

During chronic phase of infection, HIV-specific CD8 T cells are functionally impaired and clonally exhausted. They show a skewed maturation phenotype and express high levels of inhibitory molecules, a reduced proliferative capacity and an increased sensitivity to Fas-induced apoptosis. The functional effector impairment includes loss of perforin expression, inability to secrete cytokines in response to antigen restimulation, and a reduced capacity to lyse target cells in vitro. The global loss of function of HIV-specific CD8 T cells in the chronic phase of infection has been well characterized in contrast to events occurring in the acute phase of infection.<sup>57</sup>

The CD8<sup>+</sup> T cell response is central to the control and eventual elimination of persistent virus infections. Although it might be expected that CD8<sup>+</sup> T cell activation would be associated with a better clinical outcome during virus infections, in chronic human

immunodeficiency virus type 1 (HIV-1)-infection, high levels of CD8<sup>+</sup> T cell activation are instead associated with faster disease progression. Cell surface expression of CD38, a flow cytometric marker of T cell activation of CD8<sup>+</sup> T cells, had predictive value for HIV-1 disease progression that was in part independent of the predictive value of plasma virus burden and CD4<sup>+</sup> T cell number. Measurements of CD38 antigen expression on CD8<sup>+</sup> T cells in HIV-1-infected patients may be of value for assessing prognosis and the impact of therapeutic interventions. The pathogenetic reason why CD8<sup>+</sup> T cell activation is associated with poor outcome in HIV-1 disease is unknown. Possibly CD8<sup>+</sup> T cell activation contributes to immunologic exhaustion, hyporesponsiveness of T cells to their cognate antigens, or perturbations in the T cell receptor repertoire.<sup>58</sup>

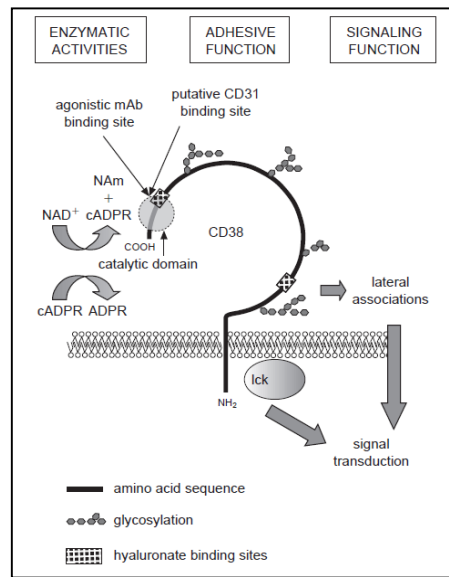
## **1.6 CD38 expression and HIV**

The analysis of CD38 expression on lymphocytes has become an important tool for monitoring patients during HIV-1 infection and has recently been proposed for use in the follow-up of highly active antiretroviral therapy (HAART).<sup>25</sup> Several studies have shown that such increased CD38 expression is a strong predictive marker for disease progression in HIV-1 infection.<sup>59</sup>

The CD38 molecule is a 45kDa single-chain transmembrane glycoprotein with a short amino-terminal intracytoplasmic tail and long extracellular carboxi-terminal domain contains four potential glycosylation sites and two hyaluronate-binding motifs (fig.3).<sup>25</sup> For some membrane-associated antigens, the number of molecules expressed per cell carries information about the cell's differentiation and activation state. Quantitating antigen expression by flow cytometry has immediate application in

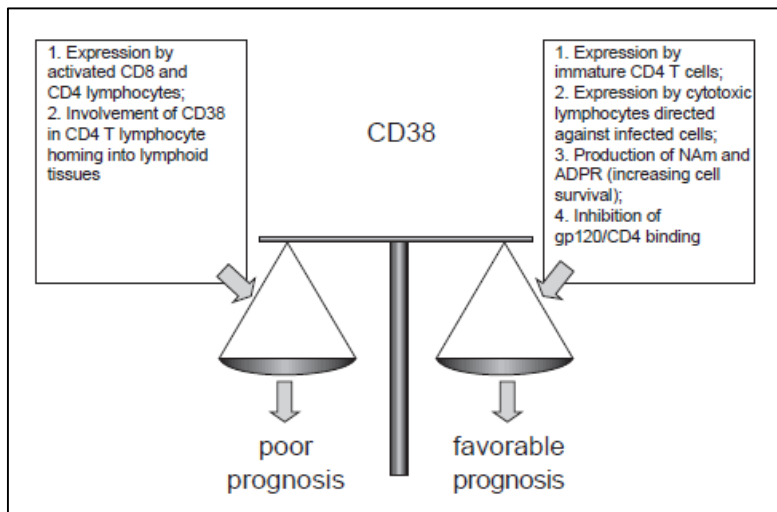
monitoring CD38 expression on CD8<sup>+</sup> T cells in HIV-1 disease, where elevated CD38 antigen expression is a marker of CD8<sup>+</sup> T cell activation and a poor prognostic indicator.<sup>60</sup> CD38 has been considered as an activation-related antigen and it has been extensively used for the immunophenotypic characterization of leukemic cells from different T, B and myeloid lineage hematological malignancies. At present, it is well known that CD38 is expressed on the majority of Peripheral Blood (PB) cells, including T, B, and NK-cells, as well as on monocytes and to a lesser extent on platelets and erythrocytes.<sup>25</sup> Accumulating evidence exists that increased levels of CD38 expression on PB CD8<sup>+</sup> T lymphocytes strongly correlates with a poor prognosis in HIV-1 infected individuals.<sup>59</sup>

CD38<sup>+</sup> displays complex lateral interactions on the cell membrane, docking at several surface receptors including CD4. The study suggested that gp120 play a role in the pathogenesis of infection by increasing lymphocyte homing into lymphoid tissues associated with mucosae and contributed to depletion of uninfected peripheral CD4<sup>+</sup> T cells, by affecting lymphocyte recirculation, it would decrease the probability of encounters between antigens and lymphocytes into peripheral tissues, and contribute to immune deficiency.<sup>25</sup>



**Fig.2.4 Hypothetical model of human surface CD38**

There are two lines of observation suggest that expression of CD38 may be a useful tool for monitoring HAART. The first showed that HIV-infected subject displaying high proportions of CD8<sup>+</sup>CD38<sup>+</sup> cells will respond to antiviral therapy more rapidly than individual with low levels. This evidence suggest that these cells may contribute to viral clearance and become effective when HAART decrease viral load.<sup>61</sup> The second line showed that decreased CD38 expression on CD8<sup>+</sup> T cells is a marker of effective response to HAART, probably because it follows the decreased viral load induced by therapy.<sup>25</sup> The observation showed that the decreased proportions of CD8<sup>+</sup>CD38 T cells marking positive response to antiviral therapy can already be detected 1 month after the start of therapy.<sup>25</sup>



**Fig.2.5 Analysis of the factors influencing the prognostic role of CD38 expression in HIV-1 infection.**

## **2.7 Antiretroviral Therapy**

Antiretroviral therapy (ART) is treatment of people infected with human immunodeficiency virus (HIV) using anti-HIV drugs. The standard treatment consists of a combination of at least three drugs (often called “highly active antiretroviral therapy” or HAART) that suppress HIV replication. Three drugs are used in order to reduce the likelihood of the virus developing resistance.<sup>62</sup> The widespread use of HAART has substantially improved the prognosis of patients infected with HIV-1. Shortly after the initiation of HAART, there is a rapid decline in the incidence of opportunistic infections related to a sharp rise in the peripheral CD4<sup>+</sup> T-cell count.<sup>63</sup> HAART has the potential both to reduce mortality and morbidity rates among HIV-infected people, and to improve their quality of life.<sup>62</sup>

Antiretroviral drugs have developed rapidly in the last few years, as has clinicians’ ability to manage HIV-infected patients. The drugs are more potent, easy to take, and have a fewer and less serious toxicities than those that made up original HAART

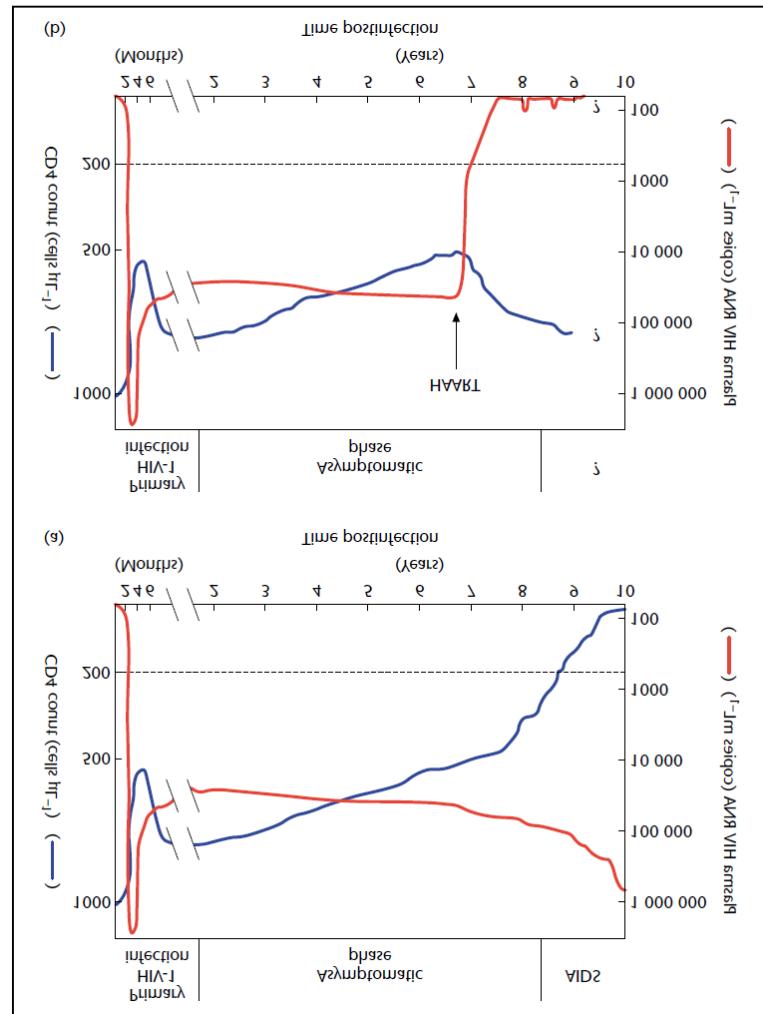
regimens. Antiretroviral medicines that are often used to treat HIV include : 1) Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), also called nucleoside analogs, such as [abacavir](#), [emtricitabine](#), and [tenofovir](#). 2) Nonnucleoside reverse transcriptase inhibitors (NNRTIs), such as [efavirenz](#), [etravirine](#), and [nevirapine](#). 3) Protease inhibitors (PIs), such as [atazanavir](#), [darunavir](#), and [ritonavir](#). 4) Entry inhibitors, such as [enfuvirtide](#) and [maraviroc](#). 5) Integrase inhibitors, such as [raltegravir](#). Some medicines are available combined together in one pill. This reduces the number of pills to be taken each day.<sup>64</sup>

First-line ART should consist of two nucleoside reverse-transcriptase inhibitors (NRTIs) plus a non-nucleoside reverse-transcriptase inhibitors (NNRTIs). Second-line ART for adult should consist of two nucleoside reverse-transcriptase inhibitors (NRTIs) plus a ritonavir-boosted protease inhibitor (PI).<sup>65</sup>

When patients are started on an effective regimen of multiple antiretroviral drugs, plasma virus levels drop rapidly in the first 2 weeks of treatment, reflecting the short plasma half-life of the virus and the short half-life of most productively infected cells. The decline in plasma virus shows a second, slower phase that is due to turnover of a second population of cells with a longer half-life. However, within 1–4 months, plasma virus levels fall to below the limit of detection of current RT-PCR assays. CD4 counts increase and opportunistic infections case (fig.5).<sup>46</sup>

Antiretroviral therapy (ART) results in decreased T-cell activation, including diminished expression of CD38 on CD8 T cells. There is conflicting evidence regarding levels of T-cell activation and the degree of CD4 T-cell recovery after effective ART, with some evidence supporting a role of immune activation limiting CD4 T-cell

recovery.<sup>66</sup> Elevated levels of CD8<sup>+</sup>CD38<sup>+</sup> T cells were found in HIV-1-infected patients who failed to suppress viral replication with ART.<sup>67</sup>



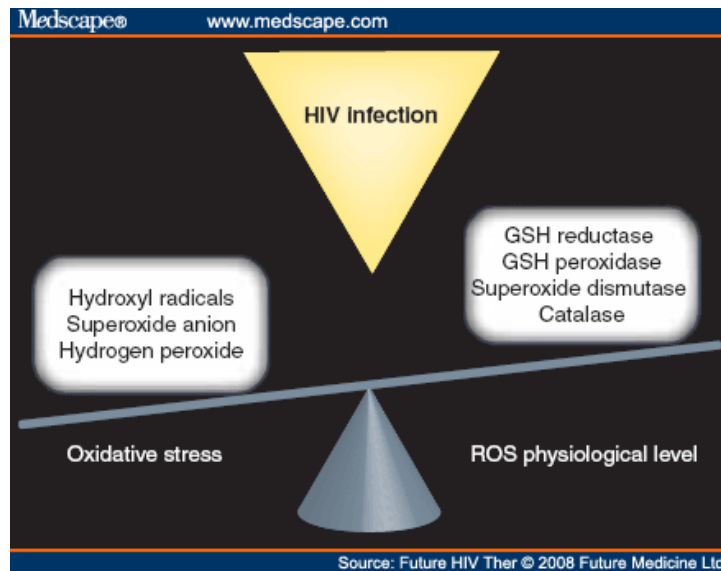
**Fig.2.6 Natural history of HIV-1 infection. (a) Natural history in the absence of effective treatment. (b) The effect of treatment with highly active antiretroviral therapy (HAART).**

## 2.8 Oxidative Stress

Oxidative stress can be defined as an imbalance between the oxidant and antioxidant system, with an advantage towards the oxidant system: this effect is subsequent to depletion of endogenous antioxidant moieties and an increased production

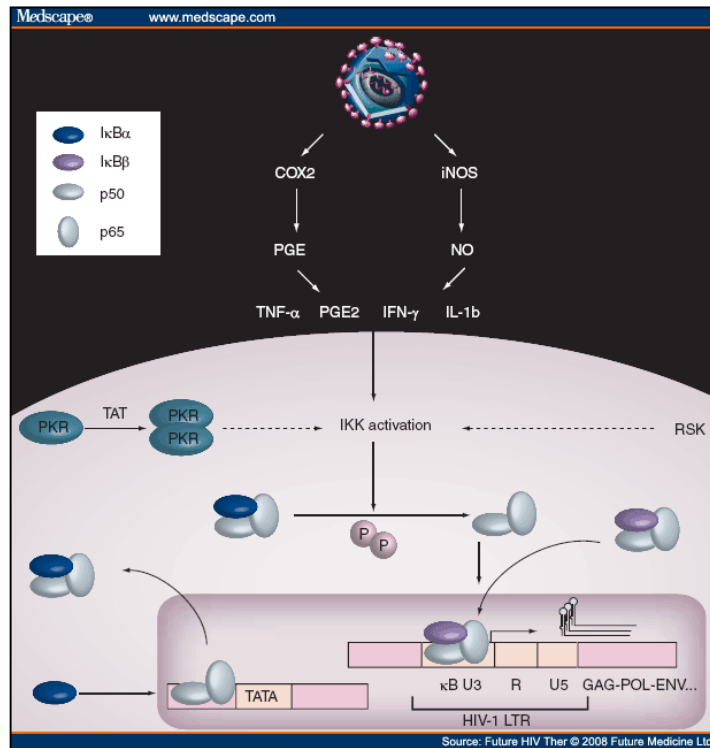


of reactive oxygen species (ROS).<sup>68</sup> The role of oxidative stress in the destruction of immune cells has been described.<sup>69-72</sup>



**Fig 2.7. Oxidative redox imbalance. Redox imbalance between the oxidant and antioxidant system during HIV infection is due to an increased production of reactive oxygen species and a decrease in antioxidant defense systems.<sup>73</sup>**

Oxidative stress (OS) is thought to play an important role in the progression of HIV infection. It has been observed that perturbations in antioxidant defense systems, and consequently redox imbalance, are present in many tissues of HIV-infected patients. Existing evidences suggest that oxidative stress may contribute to different stages of viral life cycle including viral replication and its consequences such as inflammatory response and decreased immune cell proliferation.<sup>68</sup> Specifically, the severe depletion of total antioxidant status in the AIDS stage of HIV supports that indicated increase reactive oxygen species production correlated with increased viral load and decreased CD4+ T-cell count.<sup>71</sup>



**Fig.2.8 Simplified flow diagram showing the generation of nuclear factor-κB-formation during HIV infection and the main mechanisms counteracting them. The increased production of reactive oxygen species during HIV infection starts a cascade of transduction; HIV in particular increases the production of nuclear factor-κB that controls the transcription of genes for HIV replication.<sup>73</sup>**

Laboratory evidenced report shows that HIV-1 tat protein amplified the activity of tumor necrosis factor (TNF), a cytokine that stimulates HIV-1 replication through activation of NF-κB. Therefore, tat-mediated effects on the cellular redox state were analyzed increased the apoptotic index by increasing production of intracellular reactive oxygen species.<sup>74</sup> Antioxidants that are naturally endowed to protect the immune defence system are consumed and hence depleted in the process of protecting the cells against reactive oxygen species-induced oxidative damage. The depletion of these antioxidants which includes antioxidant enzymes lead to the generation of more reactive oxygen species which consequently leads to immune depression and in turn further enhances HIV replication and further suppression of the immune system/destruction of immune cells,

followed by opportunistic infections as a result of depressed immune system and development of AIDS.<sup>75</sup>

Previous study showed that oxidative stress in HIV-1 patients were increase, mainly depends on the infection and/or antiretroviral therapy. Serum oxidant levels were significantly higher in an HIV-1 treated with HAART compared to untreated and control groups. HIV-1 infection increases oxidative status, and that it is further increased by HAART. Consequently they suggest that HAART plays an important role in determining oxidative stress in these patients. Supporting this, patients with optimal adherence show higher oxidative stress than those with poor adherence.<sup>32</sup> Therefore , it was thought that the exogenous application of some natural plant product, antioxidant supplementation or synthetic antioxidants might suppressed the effects of oxidative stress and slow disease progression.<sup>34, 68</sup>

## **2.9    *Garcinia mangostana***

Mangosteen (*Garcinia mangostana*) Linn belongs to the family of Guttiferae and is named “the queen of fruits”. A type of fruit that cultivated in the tropical rainforest of some Southeast Asian nations like Indonesia, Malaysia, Myanmar, Thailand, Philippines, Sri Lanka and India. The fruit is dark purple or reddish in colour and contains soft and juicy edible white pulps inside. The flavor is slight acidic and sweet and it has a delightful smell.<sup>38, 39</sup> The pericarps of this fruit have been used for many years as traditional medicine in treating sicknesses such as trauma, skin infection, abdominal pain, diarrhea, dysentery and infected wounds. Experimental studies have demonstrated that extracts of *Garcinia mangostana* contain prenylated and oxygenated xanthenes that has been proven

to have antioxidant, antitumoral, antiallergic, anti-inflammatory, antibacterial, and antiviral activities.<sup>39</sup>



*Fig.2.9 Mangosteen fruit*

Secondary metabolites, known as xanthenes, have been isolated from the pericarp of mangosteen and are attributed to the medicinal properties of the fruit. Xanthenes have a unique chemical structure composed of a tricyclic aromatic system. At least 68 distinct xanthenes have been identified in different parts of the *G. mangostana* plant with 50 being present in the fruit's pericarp at higher concentrations than in the aril or edible portion of the fruit.<sup>76</sup> The most abundant xanthenes in the pericarp of mangosteen fruit are  $\alpha$ - and  $\gamma$ -mangostin.<sup>77</sup>

The assay that has been used to measure the antioxidant activity of foods is Oxygen Radical Absorbance Capacity (ORAC) which measures the degree of inhibition of peroxy-radical-induced oxidation by the compounds of interest in a chemical milieu. It measures the value as Trolox equivalents (TE) and includes both inhibition time and the extent of inhibition of oxidation. The antioxidant value of Mangosteen raw described

in ORAC units is: 2,510  $\mu$  mol TE/100g.<sup>78</sup> Chemical properties and bioactivities of mangosteen can be seen in Table 2.1<sup>79</sup>

**Table 2.1 Available reviews on chemical properties and bioactivities of xanthenes in mangosteen**

Chemical properties	Biological activities
natural and synthetic derivatives of xanthone	enzyme modulation, anti-tumor activity, anti-microbial, central nervous system(CNS) depressants, CNS stimulants, neurological disorders, anti-convulsant, analgesic, anti-arrhythmic, anti-hypertensive, anti-inflammatory, anti-allergic and immunomodulatory activities
xanthenes isolated from pericarp, whole fruit, trunk, leaves and branches	anti-oxidant, anti-tumor, anti-inflammatory, anti-allergic, anti-bacterial, anti-fungal, anti-viral and anti-malarial activities
structural characterization of mangosteen xanthenes in whole fruit, stem, aril, seeds, heartwood, leaves	anti-oxidant, anti-bacterial, anti-fungal, anti-malarial, anti-HIV, cytotoxic, aromatase inhibitory, anti-cancer and anti-inflammatory activities
chemical constituents and methods of isolation from pericarp, whole fruit, stem, aril, seeds, heartwood, leaves	anti-oxidant, anti-fungal, anti-bacterial, cytotoxic, anti-histamine, anti-HIV, CNS-depressant, cardiovascular, anti-inflammatory and anti-ulcerative activities
xanthenes from mangosteen extracts	anti-cancer, anti-inflammatory, pro-apoptotic, cell cycle arresting, anti-invasive and anti-metastatic activities

### ***Pharmacokinetic and bioavailability***

Healthy subjects consumed 59 mL of a xanthone-rich mangosteen juice product containing 94.2 mg xanthenes. The maximum plasma concentration of  $\alpha$ -mangosteen ( $3.12 \pm 1.47$  ng/mL) was reached within 1 h. The antioxidant capacity measured with the oxygen radical absorbance capacity (ORAC) assay was increased with a maximum effect of 18% after 2 h, and the increased antioxidant level lasted at least 4 h.<sup>37</sup>

In a more recent human study, xanthenes from 100% mangosteen juice (containing both liquid and pericarp particles) were found to be absorbed and partially conjugated by healthy adults ingesting a single dose (60 mL) of the mangosteen juice

(containing 130 mg of xanthenes) with a high fat Western-style breakfast. Both free and glucuronidated/sulfated xanthenes ( $\alpha$ - and  $\gamma$ -mangosteen, garcinones D and E, 8-deoxygatanin and gartanin) were detected in serum and urine. Variability in maximum concentration of  $\alpha$ -mangosteen in serum ( $113 \pm 107$  nmol/L), as well as in time to maximum concentration ( $3.7 \pm 2.4$  h), was noted for the 10 subjects. Urinary excretion of xanthenes accounted for 2% of the ingested dose.<sup>80</sup>

Another study was conducted to compare the pharmacokinetic characteristics of  $\alpha$ -mangostin and  $\gamma$ -mangostin in rats if administered as either a pure compound or as a component of a mangosteen fruit extract. The absolute bioavailability of  $\gamma$ -mangostin when administered as a pure compound was determined by giving male Sprague Dawley rats 2 mg/kg  $\gamma$ -mangostin intravenously or 20 mg/kg orally. A 160 mg/kg aliquot of mangosteen fruit extract was administered, containing  $\alpha$ - and  $\gamma$ -mangostin doses equal to 20 mg/kg and 4.5 mg/kg of each pure compound, respectively. The half-life of the distribution phase was 2.40 min, and that of the elimination phase was 1.52 h. After oral administration, food supplements containing mangosteen fruit extracts should be preferred over the administration of pure xanthenes.<sup>81</sup>

Therefore to study the pharmacokinetic profile of mangosteen, the mangosteen at the dosage of 40 mg/kg was administered to male Sprague-Dawley rats by oral gavage. The main pharmacokinetic parameter were :  $t_{1/2}$  7.24 h;  $t_{max}$  62.99 min;  $C_{max}$  4.79  $\mu$ g/mL; AUC 702.45  $\mu$ g min/mL, respectively.<sup>82</sup>

### ***Toxicity tests***

Oral toxicity studies of *Garcinia Mangostana* methanolic extract powder was carried out in rat model. *Garcinia mangostana* extract (1,2 and 3 g/kg) was given orally

for 14 days to Wistar rats, the study showed that there was neither death nor alteration in the body weight, relative organ weight, cytoarchitecture of organs, clinical biochemistry, serum marker enzymes and hematological parameters in treated groups compared to the control groups.<sup>83</sup>

The oral administration of ethanolic extracts from fruit pericarp of mangosteen was performed in Wistar rats, receiving the extract at doses of 10, 100, 500, 1000 and 1000 mg/kg/day for six months respectively. The extract at any tested doses did not affect the animals behavior, health status and nor did produce any abnormality of clinical manifestations and hematological values. Histopathological results of visceral organs revealed no significant lesion related to the extract.<sup>84</sup>