Chapter 2 – Review of Literature

2.1 HIV Discovery

HIV is thought to have originated in non-human primates in sub-Saharan Africa and was transferred to humans late in the 19th or early in the 20th century (Figure 1) (Korber et al., 2000; Salemi et al., 2001; Worobey et al., 2008).

![Figure 1: Zoonotic transmission of Simian immunodeficiency virus (SIV) from monkeys into humans. The HIV-1 pandemic is believed to have arisen as a result of zoonotic transmission of SIVcpz which infects chimpanzees following the butchering and consumption of infected bush meat. SIVcpz is also believed to have arisen as a result of the predation of greater spot-nosed and red-capped mangabey monkeys (found naturally infected in the wild) (Gao et al., 1999; Hahn et al., 2000; Heeney et al., 2006).](image)

In 1981, the New York Times reported an outbreak of a rare form of cancer among gay men in New York and California, first referred to as the "gay cancer"; but medically known as Kaposi Sarcoma. About the same time, Emergency Rooms in New York City began to see a rush of seemingly healthy young men presenting with fevers, flu-like symptoms, and a pneumonia called Pneumocystis. Later, a new disease syndrome appeared in human populations in the United States and elsewhere characterized by a deficiency in the immune system (Friedman-Kien, 1981). This termed as acquired immune deficiency syndrome (AIDS), consisted of a marked reduction in CD4+ cell numbers, enhanced B-cell proliferation and
hypergammaglobulinemia. These findings most likely reflect immune activation, which have recently been re-appreciated as a major cause of the pathogenic pathway. In this regard, chronic inflammation has received better attention as a cause of cancer, cardiovascular diseases, and other co-morbidities appearing in long-term HIV infected people. Two years after the recognition of AIDS, in 1983, scientists at the Pasteur Institute in France isolated the virus that was responsible for AIDS. They called it as lymphadenopathy-associated virus (LAV) (Barre-Sinoussi et al., 1983; Gallo et al., 1984; Levy et al., 1984). Early observations had indicated that virus was spreading through intimate sexual contact (e.g., genital fluids), blood and blood products, and through mother-to-child transmission (Jaffe et al., 1983).

2.2 The state of the AIDS Epidemic

At the end of 2010, an estimated 34 million people (31.6 million to 35.2 million) were living with HIV worldwide (Figure 2, UNAIDS).

![Figure 2: Graph showing year wise, the estimated people living with HIV-1 infection worldwide, 1990-2010 (UNAIDS: World AIDS day report, 2011).](image)

In 2010, there were 2.7 million (2.4 million to 2.9 million) new HIV infections and 1.8 million (1.6 million to 1.9 million) people died of AIDS-related causes (Figure 3). Till date, AIDS is one of the most destructive pandemic recorded in the history.
Figure 3: Graph showing the difference between newly HIV infected and AIDS related deaths between the year span of 20 years. (UNAIDS: World AIDS day report, 2011).

Figure 4: Global scenario of HIV infection by the end of 2011. The largest epidemics in sub-Saharan Africa—Ethiopia, Nigeria, South Africa, Zambia, and Zimbabwe.
2.3 Indian Scenario

India’s first few cases of HIV were diagnosed among sex workers in Chennai and Tamil Nadu (Simoes et al., 1987). In 1986, a National AIDS Control Program (NACP) was launched and by the end of year 135 more cases came to light (Kakar D.N., 2001). At present, as per United Nation Development report (UNDP) of the year 2010, India had 2.39 million (2.1 million to 2.8 million) people living with HIV at the end of 2009 with prevalence of 0.3% (0.3% to 0.4%) (www.unaids.org). While the overall prevalence is low, mainly because of India’s large population size, it is third in the world in terms of greatest number of people living with HIV (Figure 4). With a population of around 1.2 billion, a mere 0.1% increase in HIV prevalence would increase the estimated number of people living with HIV by over half a million.

The vast majority of infections occurs through heterosexual transmission (87%), and is concentrated among high risk groups including commercial sex workers (CSW), men who have sex with men (MSM), and injecting drug users (IDU) as well as truck drivers and migrant workers (Figure 5).

![Figure 5: Routes of transmission of HIV in India, 2009-10 (Country Progress Report, India 2010).](image)
Estimation of HIV infection using globally comparable methods and findings from the independent Impact Assessment Study shows that the National AIDS Control Programme is progressing steadily towards the objective of halting and reversing the HIV epidemic in India over the period 2007-2012. Available evidence on HIV prevalence and future statistical projections show signs of stabilization of HIV epidemic in India at national level.

Figure 6: Map with district categories to highlight the geographical diversity of HIV in 2007. (UN General Assembly Special Session on HIV/AIDS (UNGASS) (2010, March 31st) India - Country Progress Report). ANC (Antenatal clinic); HRG (High risk group).
Figure 7: State wise estimated adult HIV prevalence, 2006 and 2007 (Source: HIV sentinel surveillance and HIV estimation in India, 2007).

Data is obtained from public prenatal clinics and the National AIDS Control Organization (NACO), Ministry of Health and Family Welfare, Government of India. The state prevalence is the average prevalence for all sites in each district (Figure 6 and Figure 7).

- High HIV prevalence (Andhra Pradesh, Karnataka, Maharashtra, Manipur, Nagaland, and Tamil Nadu) was defined by a rate of HIV positivity of more than 1% among women visiting antenatal clinics (ANC) and a rate of more than 5% among patients visiting clinics for sexually transmitted diseases (STD).
- Moderate prevalence (found in Gujarat, Goa, and the Union Territory of Pondicherry) was defined by a rate of HIV positivity of less than 1% among women visiting ANC and a rate of more than 5% among patients visiting clinics for STD.

Poor/no data was available for few districts in the northern half of India.
2.4 HIV and AIDS

2.4.1 Human immunodeficiency virus (HIV-1)

HIV-1 is a *lentivirus* (a member of the retrovirus family) that causes acquired immunodeficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections. Previous names for the virus include human T-lymphotropic virus-III (HTLV-III), lymphadenopathy-associated virus (LAV), and AIDS-associated retrovirus (ARV) (Weiss, 1993; Douek et al., 2009).

2.4.2 HIV structure

The viral genome consists of two plus single-stranded RNA molecules of approximately 9.8 kB (Figure 8A and Figure 8B). The RNA strands are capped in the 5’ ends and polyadenylated in the 3’ ends, and thus resembles eukaryotic mRNAs. The single-stranded RNA is tightly bound to nucleocapsid proteins, p7 and enzymes needed for the development of the virion such as reverse transcriptase (RT), protease (PR), ribonuclease (RN) and integrase (IN) (Figure 9). The capsid is surrounded by a matrix layer that in turn is enclosed by a lipid bilayer, the envelope. The lipid bilayer is acquired from the host cell but is equipped with viral glycoproteins that protrude from the membrane (Figure 8B).

Figure 8A: Thin-section transmission electron micrograph (TEM) depicts the ultra structural details of a number of HIV-1 virus particles, or virions. B: Diagrammatic representation of structure of HIV.
2.4.3 HIV genome

The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS) and nine genes (\textit{gag}, \textit{pol}, and \textit{env}, \textit{tat}, \textit{rev}, \textit{nef}, \textit{vif}, \textit{vpr}, \textit{vpu}, and sometimes a tenth \textit{tev}, which is a fusion of \textit{tat} \textit{env} and \textit{rev}) encoding 19 proteins (Figure 9). Three of these genes, \textit{gag}, \textit{pol}, and \textit{env}, contain information needed to make the structural proteins for new virus particles. The six remaining genes, \textit{tat}, \textit{rev}, \textit{nef}, \textit{vif}, \textit{vpr}, and \textit{vpu} (or \textit{vpx} in the case of HIV-2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease (HIV\_Sequence\_Compendium, 2009).

![Figure 9: Schematic diagram of the genes of HIV-1.](image)

2.4.4 HIV transmission

The transmission of HIV occurs through direct contact with a body fluid that contains the virus or infected cells. HIV can appear in nearly all body fluids, but transmission occurs mainly through blood, semen, vaginal secretions, and breast milk (Table 1). Although tears, urine, and saliva may contain low concentration of HIV, transmission through these fluids is extremely rare, if it occurs at all. Here is a list of ways in which HIV can be transmitted:

- **Sexual contact**

  HIV infection is mainly acquired through unprotected sexual relations. The risk of female-to-male transmission is 0.04% per act and male-to-female transmission is 0.08% per act.
• **Blood products**

In general, if infected blood comes in contact with an open wound, HIV may be transmitted. This transmission route can account for infections in intravenous drug users, hemophiliacs, recipients of blood transfusions and blood products. A health care worker who is accidentally pricked with a HIV-contaminated needle has about a 1 in 300 chance of contracting HIV. The risk increases if the needle penetrates deeply or if the needle contains HIV-contaminated blood (as with a hollow-bore needle used to draw blood) rather than simply being coated with blood (as with a needle used to inject a drug or stitch a cut). Infected fluid splashing into the mouth or eyes has less than 1 in 1,000 chance of causing infection.

• **Mother to Child**

HIV is transmitted to the fetus through the placenta or at birth during passage through the birth canal or through breast milk (Table 1).

**Table 1:** Estimated risk and timing of mother to child transmission (MTCT) in the absence of interventions.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Risk (%)</th>
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<tr>
<td>During pregnancy</td>
<td>5–10%</td>
</tr>
<tr>
<td>During labour and delivery</td>
<td>10–15%</td>
</tr>
<tr>
<td>During breast-feeding</td>
<td>5–20%</td>
</tr>
<tr>
<td>Overall without breast-feeding</td>
<td>15–25%</td>
</tr>
<tr>
<td>Overall with breast-feeding to 6 months</td>
<td>20–35%</td>
</tr>
<tr>
<td>Overall with breast-feeding to 18 to 24 months</td>
<td>30–45%</td>
</tr>
</tbody>
</table>

2.4.5 **Cells that are infected by HIV**

HIV specifically infects CD4+ cells in humans. Some cells that harbor (Figure 10) and allow replication of this virus are:

- CD4+ T-Helper cells
- Macrophages
- Monocytes
Chapter 2 – Review of Literature

- Natural killer cells
- CD8 T-cells
- Certain endothelial cells
- Central nervous system:
  - Microglia of the nervous system
  - Astrocytes
  - Oligodendrocytes
  - Glial cells
  - Brain macrophages
  - Neurones – indirectly by the action of cytokines and gp-120
- Dendritic cells (DCs) (Cunningham et al.)

![Figure 10: Cells that are infected by HIV](image)

2.4.6 Natural Life cycle of HIV

Although HIV can infect a number of cells in the body, the main target is an immune cell called a lymphocyte, more specifically a CD4+ helper T-cell. T-cells are an important part of the immune system because they help in facilitating the body's
response to many common but potentially fatal infections. Without enough T-cells, the body's immune system is unable to defend it against many infections.

Dendritic cells (DCs) are one of the first cells encountered by the virus during sexual transmission. They are currently thought to play an important role by transmitting HIV to T-cells when the virus is captured in the mucosa by DCs using mannose-specific C-type lectin receptors such as Dendritic cell-specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN) (Pope and Haase, 2003).

HIV enters target cells by attaching its gp120 molecule (the envelope glycoprotein) to the CD4+ molecule on the surface of T cells (Rubbert et al., 1998; Blanco et al., 2002) along with a chemokine receptor, either CCR5 (R5 tropic virus) or CXCR4 (T4 tropic virus) (Figure 11). Gp120 primarily binds to certain epitopes of CD4 and induces conformational changes in gp120 that promote a more efficient interaction of the V3 loop of gp120 with its respective co-receptor. Binding to the co-receptor in turn triggers a rearrangement of gp41 to extend the α-helical fusion domain, which then interacts with the cell membrane. Penetration of some components of the viral particle into the host cell is preceded by uncoating, i.e. when only the viral core enters the cytoplasm of the target cell. Viral RNA is then converted to proviral DNA in the cytoplasm of the target cell. This conversion is mediated by the viral enzyme reverse transcriptase (RT) and it is the most important step in viral replication. RT inhibitors are currently widely used to inhibit viral replication (nucleoside (NRTI) and non-nucleoside (NNRTI) analogues) and are utilized for effective treatment in HIV-1 infected individuals. The proviral DNA then translocates to the nucleus and integrates into the host chromosome with the help of the viral enzyme, integrase. The pro-viral DNA remains in the cell for as long as the cell survives, and is the continuous source of new viral progeny. The RNA transcript is formed from pro-viral DNA, processed to yield a complex pattern of sub-fragments of the initial transcript that serve as messenger RNA for generation of viral proteins. Viral proteins are then expressed in the form of long peptide chain which is processed into appropriate size with viral protease enzyme and then assembled and packaged into a mature virus particle that buds from the cell as a free virion released into the extracellular space.
Chapter 2 – Review of Literature

Figure 11: Showing the seven steps of HIV replication. 1. HIV attachment and fusion to the host cells via CD4 receptor and CCR5 or CxCR4 co-receptors. 2. Uncoating and release of contents of the virion into the host cell. 3. Reverse transcription. 4. Integration of the viral genomic DNA into the host genome. 5. Production of spliced viral mRNAs and full-length RNA genomes and production of viral proteins. 6. Budding of new immature virion. 7. Viral maturation. (Credit: National institute of Allergy and Infectious diseases).

2.4.7 HIV pathogenesis

HIV-1 infection targets the immune system with progressive impairment of cell mediated immune response resulting in AIDS, which is characterized by severe immune deficiency state, conducive for opportunistic infections. The pathogenesis of HIV infection is characterized by massive depletion of memory CD4⁺ T cells in acute HIV infection followed by gradual loss of CD4⁺ T cells due to persistent immune
hyperactivation coupled with insufficient regeneration and replenishment of the lost cells in chronic HIV infection (Picker and Watkins, 2005) (Figure 12).

The natural history of HIV infection comprises of an acute phase lasting 4-6 weeks (acute or primary HIV infection, by definition prior to seroconversion), a chronic (often) clinically asymptomatic phase lasting 2-10 years (chronic HIV infection) and a late symptomatic phase (advanced HIV disease) characterized by immune failure and AIDS, with ultimate immune collapse and death (Figure 12) (Pantaleo et al., 1993).

Figure 12: A generalized graph showing the relationship between HIV copies (viral load) and CD4 counts over the average course of untreated HIV infection; any particular individual's disease course may vary considerably. ([http://en.wikipedia.org/wiki/HIV-timecourse.png](http://en.wikipedia.org/wiki/HIV-timecourse.png))

- **CD4⁺ T cell count (cells per µL)**
- **HIV RNA copies per mL of plasma**

### 2.4.8 Acquired immunodeficiency syndrome (AIDS).

The hallmarks of HIV infection include chronic activation of the immune system and loss of CD4 T-cells, which ultimately leave affected individuals mortally susceptible to opportunistic infections. Clinical AIDS is diagnosed when peripheral CD4⁺ T cell numbers decline to less than 200 cells/µL of blood. AIDS patients can
present with atypical infections such as *Pneumocystis carinii*, *Candida albicans*, cytomegalovirus, *Mycobacterium tuberculosis* and some cancers like Kaposi’s sarcoma, B cell lymphomas etc. The annual number of people dying from AIDS-related causes worldwide is estimated 1.8 million (1.6 million to 1.9 million) in 2010 (Figure 13). Without treatment and prophylaxis, people living with HIV have a 20-30 times higher lifetime risk of developing active tuberculosis, compared with people without HIV (WHO, 2011c). In 2010, people living with HIV accounted for about 13% of all new tuberculosis cases worldwide, and about 360,000 people died from HIV-related tuberculosis (WHO, 2011b).

![Figure 13: Number of people died from AIDS-related causes globally, 1990–2010.](image)

2.5 CD4 cells depletion

HIV infection initiates a series of complex events culminating in profound immunosuppression caused by functional abnormalities and quantitative depletion of CD4+ T lymphocytes. Despite decades of intensive research, the mechanism(s) underlying CD4+ T-cell depletion remains widely debated (Veazey *et al.*, 1998; Siliciano and Siliciano, 2000; Veazey *et al.*, 2000; McCune, 2001; Brenchley *et al.*, 2004; Deeks *et al.*, 2004; Dandekar, 2007; Bandera *et al.*, 2010). David Ho and colleagues initially proposed ‘tap and drain’ hypothesis. The CD4 T-cells disappear by viral infection and subsequent cytolytic effects, and/or by removal of infected CD4+ T-cells by the immune response (Ho *et al.*, 1995). To account for the slow time scale of CD4+ T-cell deletion, CD4+ T-cell production (a wide open tap) is ultimately
exhausted by the homeostatic response that is almost perfectly compensating for the large daily deletion (the drain) of CD4+ T-cells due to HIV infection.

Current observations suggest that direct infection of target cells is only partially responsible for T-cell depletion. Rather a more complex model which also includes alterations in immune activation, T-cell turnover and homeostatic regulation, is favored now (Appay and Rowland-Jones, 2002; Appay et al., 2002; Betts et al., 2006; Brenchley et al., 2006b; Appay and Sauce, 2008; Bandera et al., 2010). HIV infection leads to sustained immune activation and causes major alterations in T cell homeostasis. In particular, naive cells, both CD4 and CD8 alike, are progressively depleted, possibly as a consequence of their frequent activation and differentiation into memory cells caused by chronic and high antigenic stimulation (Silvestri et al., 2003; Brenchley et al., 2006a; Anselmi et al., 2007).

To date, the potential factors which are thought to be involved in HIV-induced loss of CD4+ lymphocyte number and function are as under:

- Direct cytopathic effects of HIV and its proteins on CD4+ cells and progenitor cells.
- Effect of HIV on cell membrane permeability; enhanced fragility of CD4+ cells.
- Induction of apoptosis via immune activation.
- Destruction of bone marrow (e.g., stem cells, stromal cells).
- Cytokine cytotoxicity.
- Destruction of lymphoid tissue (e.g., thymus) and reduced production of new cells.
- Anti-CD4+ cell cytotoxic activity (CD8+ and CD4+ cells; NK cells).
- Anti-CD4+ cell autoantibodies.

### 2.6 HIV-1 co-receptor CCR5 and CxCR4

The initial stage in infection with HIV-1 involves the interaction of the viral envelope (Env) with CD4 as well as a specific chemokine receptor on the surface of
target cells which promote conformational changes that allow fusion of the viral
envelope with the plasma membrane of CD4 cell (Wyatt and Sodroski, 1998; Berger
et al., 1999; Clapham et al., 1999). The major coreceptors used by HIV type 1 (HIV-
1) are CXCR4 and CCR5, members of the superfamily of seven-transmembrane
domain G-protein-coupled receptors (Deng et al., 1996; Doranz et al., 1996; Feng et
al., 1996; Rossi and Zlotnik, 2000).

The CCR5 receptor (also referred to as CD195), a natural receptor for
RANTES (Regulated on Activation, Normal T-cell Expressed and Secreted), MIP
(Macrophage inflammatory protein)-1α and MIP-1β, is responsible for infection of
CD4 positive cells by virus that can use this co-receptor to enter the cell (Cocchi et
al., 1995; Douek et al., 2003). This co-receptor is used by almost all primary HIV-1
isolates regardless of viral genetic subtype. Viruses that use CCR5 co-receptor do not
induce syncytia and are also known as nonsyncytium inducing or NSI viruses or R5-
tropic viruses. (Rossi and Zlotnik, 2000; Coakley et al., 2005) The CXCR4
chemokine receptor (also referred to as CD184 or Fusin) is the natural receptor for
SDF-1 (Stromal cell-derived factor 1) or CXCL12. It is present on the surface of T-
lymphocytes and is the principle co-receptor for virus that infects these cells. The
viruses that can use CXCR4 as a co-receptor are known as X4-tropic viruses. They do
cause syncytium formation or cell fusion in lymphocytes in culture and so they are
also known as syncytium inducing or SI viruses (Deng et al., 1996; Feng et al., 1996;
Coakley et al., 2005).

2.7 HIV-1 co-receptor switching

One of the critical factors involved in HIV infection is the chemokine receptor
(CxCR4 and CCR5) that is utilized by the virus for gaining entry into the cell.
Polymorphism in the gene and promoter region of CCR5 has been associated with
disease susceptibility as well as disease progression (Gorry and Ancuta, 2011). The
natural course of HIV infection almost always starts with robust replication of the
CCR5-utilizing M-tropic viruses (Veazey et al., 1998; Brenchley et al., 2004;
Mehandru et al., 2004). The R5 viruses can quickly infect, replicate and kill a large
number of target cells. In about 50% of the infected patients (mostly subtype B
infection), there is a viral switch in the co-receptor usage, from CCR5 to CxCR4, in the advanced stage of the disease.

It is generally believed that relatively late appearance of X4 viral variants is driven by evolution of virus population (Holmes, 2001). This assumption is in conflict with viral property of rapid viral turnover of about $10^{10}$ to $10^{12}$ virions every two days combined with the high mutation rate of HIV (De Jong et al., 1992; Fouchier et al., 1992; Mansky and Temin, 1995; Holmes, 2001; Perelson, 2002; Jensen et al., 2003; Pastore et al., 2004; Bozek et al., 2009; Bunnik et al., 2011). This should result in emergence of X4 variants fairly early during infection, especially because, in some cases, only two mutations are thought to be necessary for co-receptor switch. Hence factors that influence this evolution of the virus remain largely unknown.

### 2.8 CD4 T-regulatory cells in HIV-1 infection

#### 2.8.1 History

Subpopulation of CD4$^+$ T Lymphocytes called Regulatory T-cells (Treg) has attracted a significant attention due to their ability to negatively regulate immune responses. Although the existence of these cells was proposed 35 years ago, it was only in the year 2000 that a distinct lineage of CD4$^+$CD25$^+$ T regulatory cell population was identified (Suri-Payer et al., 1998; Takahashi et al., 1998; Baecher-Allan et al., 2001; Dieckmann et al., 2001; Jonuleit et al., 2001; Levings et al., 2001; Ng et al., 2001; Taams et al., 2002). Subsequent studies lead to the discovery of more rational Treg specific marker, Forkhead box P3 (FoxP3) which has been described to master control genes for development and optimal functioning of Treg cells (Roncador et al., 2005).

Over the past 15 years (Figure 14), research has clearly established that a specialized population of CD4$^+$ regulatory T cells expressing the transcription factor FoxP3, play a critical role in preventing autoimmunity and limiting immune-mediated inflammation (Piccirillo, 2008; Sakaguchi et al., 2008; Barnes and Powrie, 2009; Feuerer et al., 2009; Chinen et al., 2010; Mueller, 2010; von Boehmer and Melchers, 2010; Wing and Sakaguchi, 2010; Campbell and Koch, 2011; Wang et al., 2011).
Additionally, Treg cell-mediated immune modulation can block tumor eradication and suppress T cell responses during infection with bacterial, viral, and parasitic pathogens (Baecher-Allan and Hafler, 2006; Sakaguchi et al., 2008; Belkaid and Tarbell, 2009; Lanteri et al., 2009; Chinen et al., 2010; Campbell and Koch, 2011).

**Figure 14:** Timeline of FoxP3 T-regulatory cell discovery (Sakaguchi et al., 2010).

Regulatory T cells are characterised by their ability to suppress proliferation of various cell types, including CD4\(^+\) and CD8\(^+\) T cells, and inhibit cytokine release. In humans, this population which is CD25\(^+\), comprises 5 to 10% of normal CD4\(^+\) T lymphocytes (Mills, 2004; Roncarolo and Battaglia, 2007; Curotto de Lafaille and Lafaille, 2009). Broadly speaking, Treg cells can be divided into two groups; ‘natural’ Treg and ‘adaptive’ or ‘induced’ Treg cells (Figure 15).

Thymic origin of natural Treg cells: In humans most of the T cell developmental event takes place in utero. The human thymus produces mature T cells as early as the thirteenth week of gestation (Stites and Pavia, 1979), and by the fourteenth week, ~7% of mature CD4\(^+\) thymocytes express high levels of CD25, a phenotypic marker associated with Treg cell function in adult peripheral blood (Darrasse-Jeze et al., 2005). These thymic-derived Treg cells also constitutively
express FoxP3 (termed nTreg) and are functionally suppressive when co-cultured with effector T cells.

In contrast, adaptive CD4^+CD25^+ cells are induced from CD25^- precursors in the peripheral lymphoid organs (termed iTregs) (Seddon and Mason, 1999). Another category of Treg cells, the Type 1 regulatory T (termed Tr1) cells arise in the periphery when naive CD4^+ T cells are activated by tolerogenic antigen-presenting cells (APCs) in the presence of IL-10 (Roncarolo et al., 2006). The resulting Tr1 cells regulate immune responses by secreting IL-10 and transforming growth factor (TGF)-β and have the capacity to suppress both naive and memory T-cell response \textit{in vivo} and \textit{in vitro} (Groux et al., 1996; Allan et al., 2008; Fujio et al., 2010; Ernerudh et al., 2011). Currently, there are yet no known specific cell-surface markers of Tr1 cells, and it is therefore not possible to readily track or purify these cells \textit{ex vivo}.

Figure 15: A schematic and simplified view of T helper cell development and differentiation to different cell subsets.

In the late stages of thymocyte development, antigen recognition can result in cell death associated with negative selection or it can result in differentiation to
natural Treg (nTreg) cells (Hsieh et al., 2006). In peripheral lymphoid organs, CD4⁺ T cells that recognize antigen differentiate into one of four distinct, though not necessarily stable, phenotypes characterized by signature cytokine secretion: T helper 1 (Th1 producing interferon-gamma (IFN-γ)); Th2 (interleukin-4 (IL-4)), Th17 (IL-17); or induced Treg (iTreg) cells (TGF-β) (Wan and Flavell, 2009; Zhu and Paul, 2010). For some not fully established T helper subsets (Th3 and T regulatory subset 1, Tr1), no specific transcription factor has yet been defined. Regulatory T cells, expressing the transcription factor FOXP3, emigrate from thymus (natural regulatory T cells), but they can also be induced in the periphery (induced regulatory T cells). Depicted are also chemokine receptors representative of each subset. Treg cells probably secrete IL-10, TGF-beta and IL-35 (Ernerudh et al., 2011).

2.8.2 Mechanism of suppression by T regulatory cells
The precise molecular mechanisms of suppression by human Treg cells remains to be determined, although in vitro and in vivo mouse studies have implicated several mechanisms (Table 2).

<table>
<thead>
<tr>
<th>Key molecules</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTLA4</td>
<td>Downregulation of APC co-stimulatory function</td>
<td>(Wing et al., 2008)</td>
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<tr>
<td></td>
<td>Interaction with CD80 and CD86 on conventional T cells</td>
<td>(Paust et al., 2004)</td>
</tr>
<tr>
<td>CD39–CD73</td>
<td>Ectonucleotidase. Hydrolysis of inflammatory extracellular ATP to AMP, which may be subsequently catabolised to immunosuppressive adenosine.</td>
<td>(Deaglio et al., 2007)</td>
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<td>LAG3</td>
<td>Induction of inhibitory signalling through MHC class II molecules</td>
<td>(Huang et al., 2004)</td>
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<tr>
<th>Key molecules</th>
<th>Function</th>
<th>References</th>
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<tr>
<td>Granzyme B (mouse) and granzyme A (human)</td>
<td>Lysis of conventional T cells</td>
<td>(Gondek et al., 2005; Cao et al., 2007) (Grossman et al., 2004)</td>
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<td>CD95 (Fas)–CD95 ligand (FasL)</td>
<td>Induction of apoptosis in conventional T cells</td>
<td>(Strauss et al., 2009)</td>
</tr>
<tr>
<td>TGF-β and LAP</td>
<td>Induction of FOXP3 in conventional T cells</td>
<td>(Chen et al., 2003; Andersson et al., 2008)</td>
</tr>
<tr>
<td>IL-10</td>
<td>Attentuation of DC function</td>
<td>(Ito et al., 2008)</td>
</tr>
<tr>
<td>Galectin 1</td>
<td>Cell cycle arrest and apoptosis in conventional T cells</td>
<td>(Garin et al., 2007)</td>
</tr>
<tr>
<td>CD25</td>
<td>Adsorption of IL-2</td>
<td>(Pandiyan et al., 2007)</td>
</tr>
<tr>
<td>IL-35</td>
<td>Induction of conventional T cell expression of IL-35 by Treg cells enhances suppression (IL-35 is not expressed by human Treg cells)</td>
<td>(Collison et al., 2007) (Bardel et al., 2008)</td>
</tr>
</tbody>
</table>

Based on their molecular characterization and function, effector molecules of Treg cells can be classified into four groups:
2.8.2.1 **Immunosuppressive cytokines**

Among these inhibitory molecules, TGF-β and IL-10 have been reported as key mediators of Treg cell suppression. However, the inhibitory role of TGF-β and IL-10 in vivo has been demonstrated in various experimental models, including inflammatory bowel disease (IBD), Leishmania skin infection, type-1 diabetes, transplantation, and tumor models. The inhibitory cytokine IL-35, a member of the IL-12 family, has recently been described as a potent suppressive molecule of Treg cells. IL-35 is preferentially produced by Treg cells and its transcript levels are markedly up-regulated in Treg cells that are actively suppressing. The important contribution of IL-35 to Treg cell function has been demonstrated both in vitro and in vivo in an animal model of IBD.

![Figure 16](image)

**Figure 16:** Secretion of inhibitory cytokines have been proposed as a major pathway by which Treg cells exert their function.

2.8.2.2 **Molecules involved in metabolic signaling**

The molecules involved in the metabolic signalling of Treg cell suppressive functions are as follows:

A. These molecules include the inhibitory second messenger cAMP, which can be transferred directly by Treg cells into target cells through membrane gap junctions. Upon entrance to the target cell, cAMP inhibits IL-2 production and proliferation (Bopp *et al.*, 2007).
**B.** Concordant expression of ectoenzymes CD39 and CD73 is shown to generate pericellular adenosine, which suppressed effector T cell function through activation of the adenosine receptor A2A (Borsellino et al., 2007; Deaglio et al., 2007; Vignali et al., 2008; Shalev et al., 2011).

**C.** In addition to the role of CD25 in the maintenance and generation of Treg cells, it has been suggested that high level expression of the receptor is used by Treg cells to absorb local IL-2 required for non-Treg cell survival. The deprivation of essential growth factor IL-2 leads to apoptosis of naïve and effector T cells (Collison et al., 2007).

![Diagram](image)

**Figure 17:** Treg effector molecules that are involved in the homeostatic disruption of the effector cell-targets and directly perturb metabolic pathways and signaling.

### 2.8.2.3 Cytolytic molecules

Granzyme molecules are highly expressed in Treg cells and in addition to regulation by suppression, Treg cells can also mediate their activity by killing effector cells. This process involves the release of cytolytic molecules, such as granzyme A/B, perforin or through the TRAIL-DR5 (tumor-necrosis-factor-related apoptosis inducing ligand-death receptor 5) pathway (Nguyen et al., 2008; Vignali et al., 2008).
Figure 18: Treg cells causing apoptosis via cytolytic molecules.

2.8.2.4 Membrane-associated molecules that down-modulate the activity of effector cells.

Treg cells suppressive mechanisms involving membrane associated molecules for the down modulating effector cell activity are as follows:

A. Lymphocyte-activation gene 3 (LAG3) interaction with MHC II in mice induces a negative signal that inhibits DC maturation and function (Huang et al., 2004).

B. CTLA4 ligation of CD80 and CD86 on DCs can induce expression of the immune-suppressive enzyme indolamine 2,3-dioxygenase (Sgarbanti et al.) (Onodera et al., 2009).

C. Treg cells can also induce perforin-dependent cytolyisis in DCs in tumour-draining lymph nodes (Boissonnas et al., 2010).
2.8.3 Role in HIV infection

It has been well established over the years that HIV-1 infection results in progressive loss of CD4 T-cells together with the dysfunction of antigen-specific T cell responses in the context of chronic immune activation (Koup et al., 1994; Trautmann et al., 2006). One of the mechanisms to counteract excessive tissue damage following virus infection is the induction, activation or expansion of several types of Treg cells. Regulatory T cells may play an important role due to their potent suppressive activity on both T cell activation and effector function. However, whether these cells have deleterious or beneficial effects in the HIV pathogenesis remains controversial, as these cells show divergent functions. Some authors have reported that in vitro removal of Treg cells from HIV-infected humans and SIV-infected macaques enhances antiviral immune responses (Aandahl et al., 2004; Kinter et al., 2007). It has been proposed that excessive Treg reactivity suppresses the function of multiple cell types and leads to faster progression of HIV pathogenesis (Kinter et al.,...
Chapter 2 – Review of Literature

2007). On the other hand, Treg cells may protect individuals from the deleterious effects of immune activation that are typically observed in chronic infection (Fazekas de St Groth and Landay, 2008).

The imbalance between effector T cells and Treg cells can create a predominantly Treg compartment, which hampers efficient effector T cell responses, that have been observed in both cancer and chronic viral infections (Bluestone, 2011). Treg frequency but not the absolute number increases in lymphoid tissues as well as peripheral blood during chronic HIV infection (Eppe et al., 2006; Nilsson et al., 2006; Kared et al., 2008; Bi et al., 2009; Bandera et al., 2010; Nikolova et al., 2011; Presicce et al., 2011; Schulze Zur Wiesch et al., 2011; Moreno-Fernandez et al., 2012), but the underlying mechanisms have not yet been characterized. Treg expansion could be the consequence of exaggerated immune activation (Kared et al., 2008; Shaw et al., 2011) but other mechanisms have also been proposed to explain this relative increase in Treg frequency:

- Increased CD4\(^+\)CD25\(^+\)Foxp3 cells in HIV infected thymus (Bandera et al., 2010).
- Low TCR re-stimulation as compared to CD4\(^+\)CD25\(^{negative}\) T cells, suggesting a relative resistance to activation induced cell death (Fritzsching et al., 2005).
- Inactivated HIV up-regulated FoxP3 mRNA levels (Nilsson et al., 2006).
- CD4-gp120 interaction results in inhibition of Treg apoptosis leading to increased survival of pre-existing Treg cells via up-regulation of the anti-apoptotic protein Bcl-2 (Ji and Cloyd, 2009).
- Tat-DCs interaction increase CTLA4 expression on autologous Treg cells (Hsieh et al., 2007).

Treg cells can suppress HIV-specific CD4 and CD8 T-cell mediated immune responses by inhibiting cytokine production and cell proliferation in vitro (Aandahl et al., 2004; Kinter et al., 2004). The in vivo augmentation of Treg cell frequency is accompanied by a diminution of HIV-specific T cell responses (Mendez-Lagarés et al., 2012). It has recently been reported that CD39\(^{+}\) Treg cells are expanded in HIV infection and these cells are involved in HIV pathogenesis and AIDS progression by
inhibiting HIV-specific responses (inhibiting IL-2 production via CD39/adenosine/cAMP pathway) (Nikolova et al., 2011; Schulze Zur Wiesch et al., 2011) suggesting a deleterious role of Treg cells in HIV-pathogenesis by diminishing HIV immunity.

However, other reports have suggested that Treg cells play a largely beneficial role, either by suppressing HIV-replication in conventional CD4 cells through direct transfer of cAMP to conventional T cells via gap junction (Moreno-Fernandez et al., 2011) or by limiting the deleterious effect of HIV-induced chronic immune activation (Liu et al., 1997; Giorgi et al., 1999; Sousa et al., 2002; Deeks et al., 2004; Belkaid and Rouse, 2005; Chase et al., 2008; Fazekas de St Groth and Landay, 2008; Jiao et al., 2009). Indeed, because immune activation closely correlates with disease progression, it is possible that in the context of chronic infection, the beneficial aspects of the Treg cells response outweigh the disadvantages. Recent studies have found that frequency of Treg cells in HIV elite controllers and long-term non-progressors is similar to that in uninfected healthy subjects (Brandt et al., 2011; Li et al., 2011; Schulze Zur Wiesch et al., 2011). Hence assessing the significance of Treg cells in HIV infection requires a systematic analysis of both Treg cell function and number.

Though not consistently found (Ndhlovu et al., 2008), most studies showed an inverse relationship between Treg frequency and CD4 cell counts (Eggena et al., 2005; Tsunemi et al., 2005; Lim et al., 2007; Jiao et al., 2009; Suchard et al., 2010; Hunt et al., 2011; Nikolova et al., 2011; Schulze Zur Wiesch et al., 2011; Angin et al., 2012) suggesting increase in Treg cell frequency is directly related to the degree of CD4 depletion. In contradiction to the studies showing increased Treg cell frequency in HIV infected patients, other reports suggest decreased levels of Treg cells (Andersson et al., 2005; Apoil et al., 2005; Chase et al., 2008). Interestingly, in the later studies, Treg cell levels were measured by quantitative RT-PCR for FoxP3 mRNA in Peripheral blood mononuclear cells (PBMCs) (Apoil et al., 2005), purified T cells (Andersson et al., 2005), or CD4 T cells (Chase et al., 2008). There is no obvious explanation for this paradox, but decreased FoxP3 mRNA could be because of following reasons:
Chapter 2 – Review of Literature

- Lower level of FoxP3 transcripts per-cell basis (Oswald-Richter et al., 2004).
- Measured the peripheral CD4 T cell depletion when Foxp3 is measured in unfractionated PBMCs or T cells
- Expansion of inducible Treg (iTreg) cells that do not express FoxP3 as a result of antigen persistence (Vieira et al., 2004).

2.9 Impact of tuberculosis

Tuberculosis continues to be the most successful pathogen around the world, and its interaction with HIV fuels both epidemics. Of the approximated 39 million people living with HIV, about one third are estimated to have concomitant latent infection with M. tuberculosis. In 2010, of 8.8 million incident TB cases worldwide, 1.1 million were among people living with HIV, with estimated 350,000 deaths [320,000-390,000], making it a leading cause of death in people living with HIV/AIDS. India and China account for 40% of the world’s notified cases of TB (WHO, 2011a).

Even though tuberculosis is a disease of antiquity, several aspects of the disease remain poorly or incompletely known. The most intriguing aspect, despite repeated exposures, some individuals remain undiseased while others manifest with clinical forms. Studies carried out so far indicate that cell mediated immune response to the pathogen plays a key role. Therefore, it is not surprising that HIV-TB co-infected patients who become severely immunodeficient face the brunt of both the pathogens (Abdool Karim et al., 2009) TB accelerates the course of HIV disease by activating viral replication and increased depletion rate of CD4+ T cells (Manoff et al., 1996).

Besides HIV infection, CD4+Foxp3+ cells were found to play a role in M. tuberculosis. An increase in the number of circulating regulatory T cells in tuberculosis patients was first described in 2006 (Guyot-Revol et al., 2006; Ribeiro-Rodrigues et al., 2006). The increase in the frequency of these cells was directly associated with the inhibition of Interferon-γ (IFN-γ) secretion. The treatment of patients infected with M. tuberculosis resulted in a decrease in the number of
regulatory T cells and restored the production of IFN-γ (Ribeiro-Rodrigues et al., 2006; Chen et al., 2007). In experimental tuberculosis model, the depletion of Foxp3+ cells in infected C57BL/6 mice resulted in fewer bacteria (measured as colony-forming units) in the lungs compared with mice with Foxp3+ cells (Scott-Browne et al., 2007). While C57BL/6 mice exhibited an increased magnitude of their Th1 response and a lower effector function of their regulatory T cells, BALB/c mice had a lower magnitude of Th1 response and effector function of their regulatory T cells that suppressed IL-2 and IFN-γ secretion. These findings suggested that regulatory T cells may potentially represent a susceptibility factor in tuberculosis (Paula et al., 2011).

2.10 Factors influencing synergetic affect

2.10.1 Heme oxygenase (HO)-1

In this study, we focused on HO-1, the rate-limiting enzyme that initiates heme degradation and maintains cellular homeostasis during stress through its depletion of pro-oxidant heme, through generation of cytoprotective carbon monoxide and biliverdin, and through induction of ferritin by Fe^{2+} release. Biliverdin is then rapidly converted to bilirubin by biliverdin reductase. Research over the last two decades has made it apparent that the precursors and catabolic products of the heme oxygenase system are capable of antimicrobial and antiviral activities (Figure 20). HO-1 induction or overexpression promotes a wide range of antiviral activities in HIV (Devadas and Dhawan, 2006). Notably, HO-1 confers protection against HIV-1 via inhibition of HIV-1 protease (McPhee et al., 1996). It may be emphasized that the inhibitory activity of biliverdin and bilirubin were noted to take place at near physiological concentrations (17 μM is 1.0 mg/dl) (DeCamp et al., 1992).

Previous studies have shown suppressive effect of HO-1 on T-cell proliferation which is similar to suppressive activity by T-regulatory cells (Brusko et al., 2005; Chabannes et al., 2007). Beyond its direct suppressive functions, HO-1 has also been implicated in activation as well as the induction and/or expansion of Treg cells (Lee et al., 2007; Xia et al., 2007; George et al., 2008; English et al., 2009; Burt et al., 2010; Carrion et al., 2010; Mougiakakos et al., 2011). HO-1 has been shown to
accumulate during glioma progression and to play a critical role in FoxP3 mediated immune suppression. There are evidences suggesting that HO-1 mRNA expression is linked to the induction of FoxP3 in CD4⁺CD25⁺ glioma infiltrating Tregs, indicating its suppressive role in growth of malignant brain tumors. The exact functional role of HO-1 expression is however not fully understood in T-regulatory cells.

**Figure 20:** Heme oxygenase and biliverdin reductase enzymes and the HIV-1 inhibition activity.

A similar scenario may be present in case of HIV patients resulting in over expression of HO-1 in Tregs, inhibiting T-cell proliferation (inhibiting IL-2 production) and function (interferon (IFN)-γ production). The down regulation of monocyte/macrophage functions will thus increase the risk in the individual of acquiring opportunistic *M.tuberculosis* infections.

### 2.10.2 Nuclear factor-κB (NF-κB)

NF-κB transcription factor plays a pivotal role in the host innate and adaptive immune responses underlying protection against viruses. Conversely, immune evasion by viruses often involves interception of the NF-κB signaling cascade or interference with the action of downstream NF-κB inducible gene targets (Tato and Hunter, 2002). HIV-1 utilizes the host transcriptional machinery to achieve high level of RNA
genome production leading to budding of infectious virions. Its transcription can be initiated by prototypical NK-κB p50/RelA heterodimers and components of canonical NF-κB pathway (Perkins et al., 1993; West et al., 2001; Brass et al., 2008; Zhou et al., 2008; Chan and Greene, 2012). The enhancer region, located at -104 to -80 position from the transcriptional start site within the 5’ long terminal repeats (Lopes et al.), harbors two identical κB sites that are highly conserved among various HIV-1 subtypes (Burnett et al., 2010). HIV-1 viruses engineered to contain mutated LTR-κB enhancer sequences infect cells but fail to produce de novo transcripts (Alcami et al., 1995). The p50/RelA heterdimer mediates LTR transcriptional initiation and elongation (Barboric et al., 2001; West et al., 2001). In HIV-1 chronic stage, super-induction of NF-κB by Tat improves HIV-1 replication and creates an inflamed, activated state in the host, which further encourages HIV-1 replication.

**Missing Gaps in the knowledge**

There is a scarcity of knowledge over the characteristic and functional nature of T-regulatory cells within HIV-1 individuals at different stages of the disease as well as during *Mycobacterium tuberculosis* co-infection. Though controversial, there are reports of increase in Treg cells frequency in HIV-1 (Schulze Zur Wiesch et al., 2011; Angin et al., 2012) and *M.tuberculosis* (Guyot-Revol et al., 2006; Ribeiro-Rodrigues et al., 2006) infected individuals. More knowledge on the pathogenesis of HIV-1 infection in terms of Treg cells in the presence of opportunistic infection like *M.tuberculosis* needs to be explored.

This study examines the expression of HIV-1 co-receptors (CCR5 and CxCR4) on T-regulatory cells for their possible role in changing the Treg cell frequency with disease progression. Over the course of infection, the co-receptor usage by HIV-1 changes from a preferential CCR5 to CxCR4 in 50% of the infected individuals (Fenyo et al., 1988; Tersmette et al., 1989; Schuitemaker et al., 1992; Berger et al., 1999; Blaak et al., 2000; Karlsson et al., 2005). Rac1, known to be specifically involved in regulating the conformation of CXCR4 making it available for HIV-1 binding (Zoughlami et al., 2012), may play an important role in controlling
this co-receptor switching. Hence, change in Rac-1 expression/viral co-receptor switching could play an important role in regulating the Treg cell frequency.

During active tuberculosis, infected macrophages induce stimulation of T\textsubscript{H}1 cells and increased CCR5 expression on CD4 T cells (Murphy \textit{et al.}, 2008; Stenger and Modlin, 1999; Boom \textit{et al.}, 2003; Schlager and Rom, 1998; Almeida \textit{et al.}, 2009). Consequently, during HIV-\textit{M.tuberculosis} disease, there are more activated CD4 T cells available because of the amplification of immune activation generated by the two infections (Wolday \textit{et al.}, 2005). R5 viral variants were preferentially recovered from these patients (Morris \textit{et al.}, 2003). Therefore it would be interesting to study the gene expression axis and correlation between CCR5, CXCR4 and Rac1 in PTB and HIV-PTB co-infected individuals.

Research over the last two decades has made it apparent that the precursors and catabolic products of the heme oxygenase (HO-1) system are capable of antimicrobial and antiviral activities (Devadas and Dhawan, 2006; McPhee \textit{et al.}, 1996). Previous studies have shown suppressive effect of HO-1 on T-cell proliferation which is similar to suppressive activity by T-regulatory cells (Brusko \textit{et al.}, 2005; Chabannes \textit{et al.}, 2007). Beyond its direct suppressive functions, HO-1 has also been implicated in activation as well as the induction and/or expansion of Treg cells (Lee \textit{et al.}, 2007; Xia \textit{et al.}, 2007; George \textit{et al.}, 2008; English \textit{et al.}, 2009; Burt \textit{et al.}, 2010; Carrion \textit{et al.}, 2010; Mougiakakos \textit{et al.}, 2011). The findings of these studies suggest that similar scenario may be present in case of HIV patients making them more vulnerable to tuberculosis.

HIV-1 transcription involves NK-\kappa B p50/RelA heterodimers and components of canonical NF-\kappa B pathway (Perkins \textit{et al.}, 1993; West \textit{et al.}, 2001; Brass \textit{et al.}, 2008; Zhou \textit{et al.}, 2008; Chan and Greene, 2012) creates an inflamed, activated state in the host by modulation of downstream NF-\kappa B inducible gene targets, further encouraging HIV-1 replication. How PTB infection modulates its expression in HIV-1 infected individuals needs to be addressed. More knowledge of the molecular mechanisms resulting in synergistic effect in HIV-PTB co-infection needs to be explored.