Chapter 1

hiv/aids

Introduction
Chapter-1

Introduction

1.1 Background

1.2 Isolation of HIV

1.3 Epidemiology

1.4 Course of HIV infection

1.4.1 Typical Progressors

1.4.2 Rapid Progressors

1.4.3 Long-term Nonprogressors

1.5 Clinical Staging

1.6 Structure of HIV

1.7 Life cycle of HIV

1.8 Why study Nef

1.8.1 Functional Aspects

1.8.2 Evolutionary Aspects

1.8.3 Immunological Aspects
1.1 Background

In 1981, Acquired Immunodeficiency Syndrome (AIDS) was first described, as a distinct clinical disease among young men having sex with men (MSM), who presented either with Pneumocystis carinii pneumonia and/or a rare type of cancer of skin, Kaposi’s sarcoma, seen only in immunocompromised persons. They showed unique pattern of underlying immunosuppression without any identifiable cause (Gottlieb et al., 1981). Similar cases were subsequently reported in intravenous drug users (IDUs) who shared needles and syringes (Hardy et al., 1985) and hemophilia patients who had received blood products (Davis et al., 1983). The epidemiological evidence suggested that it may be an infection that is transmitted through blood much same as Hepatitis B virus.

It was also postulated that a variant of Human T lymphotropic retrovirus (HTLV) might be the etiological agent of AIDS, with similar cell tropism and transmission route (Broder and Gallo, 1984). After the recognition of the syndrome, within 2 years the causative agent, Human immunodeficiency virus (HIV) was isolated from the patients of AIDS.
1.2 Isolation of HIV

The virus responsible for AIDS was first reported from Dr. Luc Montagnier’s Laboratory at the Institute Pasteur, Paris in May 1983. The virus was isolated from a lymph node of a person with Lymphadenopathy. The virus was designated as Lymphadenopathy Associated virus (LAV) (Barre-Sinoussi et al., 1983). Subsequently in 1984, Gallo and his colleagues demonstrated that the virus was unequivocally the cause of AIDS and related conditions and named it HTLV-III i.e. Human T lymphotropic virus type III (Gallo et al., 1984). Almost during the same period Levy et al isolated AIDS related virus (ARV) (Levy and Shimabukuro, 1985). It was later concluded that all these viral isolates are genetic variants of the same virus. In 1986, The International Committee of Nomenclature named it as the Human immunodeficiency Virus (HIV) (Coffin et al., 1986a; Coffin et al., 1986b).

Molecular genetics data suggest that HIV type 1, the most common specie of HIV that infects humans, has been derived from the Simian Immunodeficiency Virus, SIVcpz, of the Pan Troglodytes; troglodytes subspecies of chimpanzee, and hence shares high sequence homology with it.
1.3 Epidemiology

No disease in modern times has had quite the impact on the civilized world as AIDS has. According to UNAIDS estimates, till December 2004, about 39.4 million individuals were infected with HIV, of whom about 70 percent live in sub-Saharan Africa and Asia.

Based on the phylogenetic analysis of numerous isolates obtained from diverse geographical regions, HIV is divided into types, groups, subtypes, genotypes, circulating recombinant forms (CRFs) and unique recombinant forms (URFs). HIV-1 can be divided into 3 major Groups; M (major), N and O (Outlier). Group M is further subcategorized into many subtypes; A-D, F-H, J and K. Group O is distinctly different and genetically more closely related to simian immunodeficiency virus (SIV) and HIV-2. Group N appears to have arisen from interaction between a group M and a group O virus.

Geographical distributions of HIV-1 subtypes (Fig 1.1)

Subgroups of HIV-1

Group M

⇒ Subtype A West and Central Africa
Fig. 1.1 Geographical distributions of HIV-1 subtypes
Subtype B South America (including Brazil and Argentina), United States, Europe, Thailand, Russia

Subtype C India, Sudan, Southern and Eastern Africa, China

Subtype D East and Central Africa

Subtype F Brazil, Argentina, Eastern Europe, Central Africa

Subtype G Western and Eastern Africa, Central Europe

Subtype H Central Africa

Subtype J Central America

Subtype K Democratic Republic of Congo, Cameroon

Group N Cameroon

Group O West Africa

During the later part of the epidemic recombination between co-infecting subtypes have given rise to recombinant viruses, some of which have established as CRFs and are transmitted horizontally as well as vertically. At least 16 different CRFs for Group M have been identified to date (Las almos database). The majority of the CRF genomes are highly complex intersubtype recombinants. For instance,
CRF01_AE is clearly E in env, U/A/E in the regulatory region and A in gag and pol, while the CRF04_cpx has an extremely complex genome structure including subtypes A, G, H, K and U. To date CRFs have been identified in nearly every region of the world where two or more subtypes co-circulate and may account for 10 per cent of new HIV-1 infections.

In some cases, unique recombinant form (URF) viruses seem to have originated by secondary recombination of a CRF (Lal et al., 2005). Mosaics involving CRF02_AG have been observed in various African countries, and some of the established CRFs, like CRF11 and CRF13, contain sequences that are derived from CRF01_AE. Recombinations between two CRFs have been described in studies from China and Niger (Lal et al., 2005).

In India, the first case of HIV infection was detected in 1986 from Tamil Nadu. Since then, the spread of HIV epidemic in India is a major public health concern. According to National AIDS Control Organization (NACO), India, 5.28 million Indians were estimated to be HIV-1 positive as of December 2004 and prevalence among various states of India are indicated in Fig.1.2 Genotypically more than 80% of the strains tested from India belonged to subtype C although some B/C
Fig. 1.2. Intensity of prevalence for HIV epidemic in various states of India.
and A/C recombinants have also been identified (Lole et al., 1999; Tripathy et al., 2005). Sequence analysis for most subtype C isolates has revealed an extra or third transcription element NF-κB in the long terminal repeat (LTR). The presence of extra transcription element may augment in HIV-1 transcription thus influencing viral fitness.

1.4 Course of HIV infection

The most consistent feature of HIV infection is the gradual diminution of CD4+T cell, both qualitatively and quantitatively as HIV primarily infects CD4+ lymphocytes. Based on the duration of HIV infection and kinetics of virological and immunological events, 3 dominant patterns of disease progression can be observed (1) 80% to 90% of HIV-infected persons are “typical progressors” and experience a course of disease with median survival time of approximately 7-10 years. (2) Approximately 5% to 10% of HIV-infected persons are “rapid progressors” and experience an unusually rapid (3 to 4 years) disease course. (3) Less than 5% of HIV-infected persons do not experience disease progression for an extended period of time (10 years or more) and are termed “long-term non-progressors” (LTNP).
1.4.1 Typical Progressors

The course of HIV infection for a typical progressors includes acute primary HIV infection which leads to period of clinical latency (median duration ~10 years) followed by clinically apparent disease or AIDS-defining illness, and finally death from AIDS as schematically indicated in Fig. 1.3. The duration of clinical latency following primary HIV infection varies widely among individuals depending upon many factors such as; host’s immunological status, viral pathogenesis and fitness, nutrition and co-infections.

1.4.1.1 Acute retroviral syndrome

During this early stage, peak plasma viremia is frequently observed (Fig 1.3). The peripheral blood CD4+ T-lymphocyte count and the CD4/CD8 ratio also decline. Within 2-5 weeks post exposure to HIV-1, mononucleosis-like syndrome develops in 40-70% of subjects and is associated with seroconversion for HIV-1 antibodies. This syndrome may include fever, erythematous rash, aseptic meningitis, generalized lymphadenopathy etc. But, these acute illnesses usually resolve spontaneously within 2-3 weeks. An HIV-specific antibody and cellular immune response follows the primary infection which reduces
Acute Retroviral Syndrome Phase of Clinical Latency AIDS

High p24 antigenemia & viremia CD4 counts stable, low viremia, symptomatic High viremia, low CD4 count, OIs

Opportunistic Infections (OIs) in HIV-1 infected Indians
Mycobacterium Tuberculosis
Salmonella typhimurium
Pneumocystic carinii
Entamoeba histolytica
Hepatitis viruses
Herpes Zoster
Candida albicans

Fig. 1.3 Schematic representation of course of HIV infection in infected individuals
viremia to a considerable extent. This acute infection is followed by long period of clinical latency.

1.4.1.2 Clinical latency

The generation of HIV-specific immune response is associated with a marked decline in plasma viremia. Although plasma virus load may stabilize at lower level, HIV replication continues in lymph nodes and other tissue compartments/organs. This is the phase of clinical latency and may last for 7-10 years. During this phase, the clinical presentations are infrequent and the infected individual may lead a normal life although there is ongoing virus multiplication and loss of CD4 cells (Fig.1.3). The plasma level of HIV viremia tends to reach a steady state (termed 'viral set point') at the end of the acute phase following primary infection. Viral set point is an important prognostic indicator for the rate of HIV disease progression. High viral set point correlates with faster, and low viral set point with slower, rates of disease progression (Mellors et al., 1996; Perelson et al., 1996).

1.4.1.3 AIDS

This clinically apparent, symptomatic stage is a consequence (secondary manifestation) of the progressive and profound deterioration of the immune system that occurs over time in most patients with HIV
infection. This stage is characterized by CD4 counts less than 200/cubic mm, high viral load and p24 antigenemia. Opportunistic infections are very frequent and recurrent. Some of the constitutional symptoms, opportunistic infections, and other manifestations of advanced symptomatic HIV disease commonly found in India are with following pathogens:

Bacterial:

- Mycobacterium tuberculosis
- Salmonella typhimurium

Protozoa:

- Pneumocystic carinii
- Entamoeba histolytica
- Giardia lambia
- Cryptosporidium

Viruses

- Epstein-Barr virus
- Hepatitis viruses
- Herpes viruses (Herpes Zoster)

Fungi

- Candida albicans
Cryptococcus neoformans

1.4.2 Rapid Progressors

In a minor percentage (5% to 10%) of HIV infected persons, rapid progression of HIV infection is observed. AIDS occurs within 2 to 3 years after seroconversion in them. There is very high rate of CD4+ decline and rapid increase of viral load. Although, it is unclear whether HIV-specific Cytotoxic T Lymphocyte (CTLs) response is defective in rapid progressors, it has been shown that the CD8+ T cell mediated suppression of HIV replication is severely impaired.

1.4.3 Long term Non-progressors

Small percentage of infected persons (5%) do not experience clinical progression of HIV infection and have stable CD4 T cell count for many years, in absence of therapy. The criteria used for non progression include documented HIV infection for more than 7-10 years, stable high CD4+ counts, low or undetectable viral load, absence of clinical symptoms.

From an immunologic stand point, immune functions are conserved in LTNPs and HIV specific cellular (CTLs) and humoral immune response is very strong. In addition to normal CD4+ count, the
absolute number of CD8+ T lymphocytes is consistently higher in most LTNPs. The drop in CD4 cell number is very slow.

From a virologic standpoint, level of viral load, including frequency of HIV DNA-containing cells and virus replication in both peripheral blood and lymph node mononuclear cells as well as plasma viremia are 4-folds to 20-folds lower in LTNPs than in typical progressors (Dunn and Newell, 1992; Dunn et al., 1992; Pizzo and Butler, 1991). Despite very low viral load, virus replication persists in LTNPs indicating that it is replication-competent as well as infectious; however very limited information is available concerning the pathogenicity of HIV in LTNPs. In this regard, a nef-deleted HIV has been isolated from a LTNP. This, together with the lack of pathogenicity of nef-deleted SIV in rhesus monkeys, has led to speculations that LTNPs may be infected with less pathogenic or non-pathogenic viruses.

1.5 Clinical Staging

In 1991, The Centers for Disease Control (CDC), USA, revised the case definition of AIDS to include all patients with CD4 count of less than 200 cells/mm$^3$ or CD4 percentage of less than 14%, regardless
of whether the patients had developed true AIDS-defining conditions as indicated in table 1.1

Table 1.1: CDC categorization of HIV infection

<table>
<thead>
<tr>
<th>CD4 count (cells/mm³)</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;500</td>
<td>A1, B1, C1</td>
</tr>
<tr>
<td>200-500</td>
<td>A2, B2, C2</td>
</tr>
<tr>
<td>&lt;200</td>
<td>A3, B3, C3</td>
</tr>
</tbody>
</table>

Symptom Category

A: Acute retroviral syndrome, Generalized lymphadenopathy, Asymptomatic disease

B: Symptoms of AIDS-related complex
Candidiasis, mucosal, cervical dysplasia, herpes zoster, idiopathic thrombocytopenic purpura, listeriosis, oral hairy leukoplakia, pelvic inflammatory disease, peripheral neuropathy

C: AIDS-defining conditions
CD4 count < 200 cells/mm³, Candidiasis, Cervical cancer, Cryptosporidiosis, Cryptococcal meningitis, Cytomegalovirus infection, Herpes Zoster, HIV encephalopathy, Kaposi’s sarcoma,
Mycobacterial infection, Pneumocystis carinii infection, Progressive multifocal leukoencephalopathy, Salmonellosis.

1.6 Structure of HIV

HIV are lentiviruses belonging to family retroviridae. They are enveloped, positive-strand (i.e. having as the genome the same strand as the mRNA) RNA viruses, 80 to 140 nm in size that rely on a unique enzyme, reverse transcriptase to convert their RNA genome into a DNA “provirus”, which is integrated into cellular genome.

HIV is a biologically simple virus. The viral core is bounded by a protein coat consisting of two proteins p24 and p15 (so designated to correspond to their molecular weight). HIV-1 genome is an 9.8 kb RNA and two identical copies are packed in each particles along with the viral enzymes, reverse transcriptase, integrase and protease. Like all retroviruses, HIV has lipid membrane or ‘envelope’ which surrounds the protein core. Embedded within the HIV envelope are discrete viral glycoproteins which are described as ‘knob’ or ‘spikes’ giving the viral particle its distinctive appearance. Each knob consists of an outer glycoprotein (gp120), which is anchored to a transmembrane protein (gp41). The core protein, p17 lies between the protein core and
envelope and is embedded in the envelope at discrete points. The basic macromolecular structure of HIV is schematically shown in Fig 1.4.

Fig. 1.4. Macromolecular structure of HIV-1.
**HIV-1 Genome**

The genome of HIV, like other retroviruses in general, contains three major genes *gag*, *pol*, and *env*. The *env* codes for envelope glycoproteins (outer glycoprotein gp120 and transmembrane gp41 derived from glycoprotein precursor gp160). The genomic organization of HIV-1 is schematically shown in Fig.1.5. The *gag* codes for nucleocapsid proteins (capsid, or "core" antigen p17, matrix nucleocapsid p24); while *pol* codes for enzymes (protease, reverse transcriptase and integrase). Regulatory and accessory genes carried by HIV include *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu* (for HIV-1) or *vpx* (for HIV-2). Table-1 indicates functions currently attributed various HIV-1 genes.
Table 1.2. HIV and SIV proteins, their function along with location within virions/host cell.

<table>
<thead>
<tr>
<th>Name</th>
<th>Size</th>
<th>Function</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural Genes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gag MA</td>
<td>P17</td>
<td>Nuclear transport of viral proteins (core), anchoring to membrane,</td>
<td>Virion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interaction with Env</td>
<td></td>
</tr>
<tr>
<td>Gag CA</td>
<td>P24</td>
<td>Viral core capsid protein</td>
<td>Virion</td>
</tr>
<tr>
<td>Gag NC</td>
<td>P7</td>
<td>Nucleocapsid, binds with RNAs</td>
<td>Virion</td>
</tr>
<tr>
<td></td>
<td>p6</td>
<td>Binds with accessory protein Vpr</td>
<td></td>
</tr>
<tr>
<td>Protease</td>
<td>P15</td>
<td>Cleavage and maturation of gag-pol precursor</td>
<td>Virion</td>
</tr>
<tr>
<td>Reverse Transcriptase, RNase H</td>
<td>P66</td>
<td>Reverse transcription of viral RNA, RNase H activity</td>
<td>Virion</td>
</tr>
<tr>
<td></td>
<td>p51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Integrase</td>
<td></td>
<td>DNA provirus integration in Host cellular DNA</td>
<td>Virion</td>
</tr>
<tr>
<td>Env</td>
<td>gp120/gp41</td>
<td>External viral glycoprotein which binds with CD4 and co-receptor of CD4+ T cells</td>
<td>Virion envelope, Plasma membrane,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Regulatory Genes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tat</td>
<td>P16/p14</td>
<td>Transactivator protein which influence viral transcription</td>
<td>Host cell nucleus</td>
</tr>
<tr>
<td>Rev</td>
<td></td>
<td>Influence viral RNA transport, stability and functionality</td>
<td>Host cell nucleus/nucleolus</td>
</tr>
<tr>
<td>Nef</td>
<td>P27/p25</td>
<td>Down-regulation of CD4, MHC-I on CD4+ cells</td>
<td>Plasma membrane, cytoplasm,</td>
</tr>
</tbody>
</table>
### Accessory Genes

<table>
<thead>
<tr>
<th>Accessory Genes</th>
<th>P23</th>
<th>Role in viral maturation and counter Host antiviral proteins Apobec3G</th>
<th>Cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vif</td>
<td></td>
<td>Inhibit host cell division at G2/M stage, Promotes nuclear localization of preintegration complex</td>
<td>Virion, host cell nucleus</td>
</tr>
<tr>
<td>Vpr</td>
<td>P10</td>
<td>Regulate and influence ion channels of host cells, degrade CD4 receptors</td>
<td>Integral membrane protein</td>
</tr>
<tr>
<td>Vpu</td>
<td></td>
<td>Only present in HIV-2 and SIV. It is an homologue of Vpr of HIV-1</td>
<td>Integral membrane protein</td>
</tr>
</tbody>
</table>

### 1.7 Life cycle of HIV:

HIV-1 primarily infects cells that have CD4 molecules on their surface and may include cells of the mononuclear phagocyte system, principally blood monocytes and tissue macrophages, T lymphocytes, natural killer (NK) lymphocytes, dendritic cells (Langerhans cells of epithelia and follicular dendritic cells in lymph nodes), hematopoietic stem cells, endothelial cells and microglial cells in brain. After entering the body, the viral particle is attracted to a cell with the appropriate CD4 receptor molecules. They enter cell by either fusion with susceptible cell
membrane or by process of endocytosis. In addition to the CD4 receptor HIV requires co-receptors (chemokine receptors), CXCR4 or CCR5 to infect cells. It may utilize more than one receptors of the CXC or CCR family.

Fig. 1.6. Schematic representation for interaction of viral protein gp120 and gp41 with cell surface molecules like CD4 and chemokine receptors.

Gp120 initially binds with CD4 receptors which induces a conformational change in transmembrane gp41 which subsequently interacts with chemokine receptors (Fig 1.6).

Chemokine receptors usage by virus may cause different strains of HIV infect selectively different cells. T-tropic strains selectively interact with the CXCR4 chemokine receptor to infect lymphocytes.
while M-tropic strains interact with the CCR5 chemokine co-receptor to infect macrophages and dendritic cells. CCR8 has been identified as a cofactor to permit infection by either T-cell tropic or by M-tropic strains of HIV. Dual tropic HIV strains have also been identified that can use more than one chemokine coreceptor. The life cycle of HIV infection is schematically indicated in Fig.1.7

**Once HIV** is internalized, the protein coat is shed off and the exposed viral RNA genome is reverse transcribed to double stranded DNA with the help of viral reverse transcriptase. This proviral DNA then migrates to nucleus and gets integrated into the host cell genome using another viral enzyme; integrase. On appropriate stimulus (primarily T cell activation, co-infection), the virus is triggered out of latency and makes use of host cell machinery. The integrated proviral DNA is transcribed into mRNA which is then translated into precursor polypeptide chain. These remain inactive unless cleaved by viral enzyme protease into their biologically active form. Finally, new virions are assembled and are released from infected cells by surface budding, or lysis and infect new uninfected cells.
Fig. 1.7 Life cycle of HIV
1.8 Why study Nef?

Regulatory protein Nef (Negative factor) of Human Immunodeficiency Virus type 1 (HIV-1) is supposed to be crucial for viral replication. Its critical role in AIDS pathogenesis was first emphasized with an observation that some subjects infected with virus carrying naturally occurring Nef deletions became long-term nonprogressors (Dyer et al., 1997; Mendila et al., 1999). Nef is a 25-30 kD myristoylated, early phase viral protein with multiple functions. Extensive characterization of Nef is essential for:

1.8.1 Functional Aspects

Multiple and distinct functions have now been attributed to Nef which include down-regulation of cell surface CD4 receptors (Craig et al., 1998) and MHC-1 expression (Cohen et al., 1999; Mangasarian et al., 1999), modulation of cellular activation, influencing signal transduction pathways and enhancement of viral infectivity (Arold and Baur, 2001; Fackler et al., 1999). It is also known to delay the apoptosis of infected cells (Geleziunas et al., 2001). More than 30 putative Nef targets and associated functions have already been identified but the molecular mechanisms and their contribution to pathogenesis are not fully elucidated (Geyer et al., 2001).
Many *in vitro* studies have already shown the enhancing effect of Nef on viral growth kinetics and infectivity (Akari et al., 2000; Chowers et al., 1994). But majority of the reports are based on studies using subtype B. These virological studies using subtype B viruses may not always be extrapolated /applied to other subtypes due to high sequence variability. Very limited information is available on role of Nef in subtype C virus from India and the effect of subtype associated sequence variability in Nef on its functionality.

1.8.2 Evolutionary Aspects

Epidemiological studies have shown the predominance of subtype C in geographically divergent regions of the world such as South Africa, China and India (Lal et al., 2005; Malim and Emerman, 2001). In, phylogenetic analysis for *gag*, *pol* and *env* sequences, Indian sequences tend to form a distinct subclade within clade C. We believe that these subtype determining sequence variations may not be only restricted to structural genes but can also be extrapolated to small accessory genes like *nef*. But information available on *nef* sequences from India is very limited for meaningful analysis. Hence, more Nef sequences from India need to be generated and compared with sequences found globally from other subtypes. This information will enable measuring
evolutionary/phylogenetic distances and monitoring the epidemic in India.

1.8.3 Immunological Aspects

The sequence variations influence may the immune recognition and may be responsible for the evolution of escape mutants. Efficient CTL immune response against the regulatory proteins have already been shown to correlate with the control of primary viremia in chronically SIV infection in monkeys and with a better prognosis for HIV-1 infected individuals (Daniel et al., 1992; Messmer et al., 2000). Nef being early viral protein may play important role in early immunological events. It has been earlier reported from our lab strong nef-specific CTL responses are seen in early seroconverters (Paranjape et al., 1998). Essex et al have also reported immunodominant CTLs epitopes in Nef by Elispot in African subtype C infected persons. This knowledge of CTL epitopes will prove crucial while designing multi-epitope vaccine candidates.

Nef has been one of the components of several vaccine formulations. However systematic studies to understand sequence variation in Nef, and its implications on immune system and viral
fitness are limited. Hence, this work was initiated to get insight for answering the following questions.

1. How genetically diverse is Indian subtype C nef from nef of closely related African subtype C, well studied subtype B and other major clades of HIV-1?

2. Whether any correlation exists between rate of disease progression and Nef of Indian subtype C virus?

3. Whether Nef from subtype C and subtype B differ in their ability to influence viral fitness?

4. Whether all the domains in Nef which can influence viral infectivity and replication kinetics are precisely mapped?

In brief, this work was aimed at studying a unique, unexplored niche in Nef characterization and our results will supplement other studies for better understanding phylogenetic, immunological and functional relevance of Nef in viral fitness.