Chapter 11

THE HIV-1 GROUP M EPIDEMIC IN THE ASIA-PACIFIC

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ABSTRACT

Human immunodeficiency virus type 1 (HIV-1) group M accounts for most HIV infections globally. The Asia-Pacific, with more than 60% of the world’s population, has the second highest regional disease burden. Many countries in the region are low- or middle-income economies where antiretroviral therapies (ARTs) are implemented as standardised regimens to facilitate ART rollout. Appropriate partnering of ART with virologic monitoring needs to be determined in resource-limited settings to give advance notice of treatment failure and HIV drug resistance (HIVDR) in the patient. Widespread transmitted HIVDR could threaten the efficacy of standardised regimens, consequently, regional capacity for monitoring resistant viral strains needs to be enhanced. Epidemic tracking of local HIV-1 genotypes may also help to more efficiently target prevention strategies in sub-populations of enhanced risk. This review summarises effects of HIV-1 on the host, processes underpinning viral quasi-species heterogeneity and serves as an introduction to the diverse regional epidemics in the Asia-Pacific.

FROM ORIGINS TO ISOLATION

Historically, the origins of the human immunodeficiency virus (HIV) have been controversial. Studies seeking to determine the mechanisms whereby selected simian retroviruses crossed over into human populations have suggested the involvement of both natural, epidemiological processes and human interventions [1-8]. Without sufficient data for conclusive scientific evaluations or the availability of substantiating physical evidence, questions relating to the origin of HIV have not been fully resolved. Proponents agree, however, that central western Africa is the likely geographic centre of the pandemic and the

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two earliest known HIV-positive specimens were sampled from the Democratic Republic of Congo (DRC) (formerly Zaire) in 1959 and 1960 [7, 9].

Sequencing technologies have been used to interpret the genetic code of HIV viral isolates since the late 1980s [10]. Collections of genetic sequences can be used to reconstruct viral relationships by phylogenetic methods. If appropriately modelled, the phylogenetic topology can provide insight into HIV ancestral states, genotypic variation and transmission dynamics in humans [11-13]. Evidence from phylogenetic studies suggest separate primate-to-human transmission events, for each of HIV type 1 (HIV-1) groups M (Main), N (Not-M/Not-O), O (Outlier) [reviewed in 14] and P [15]. The chimpanzee (Pan troglodyte troglodyte) has been determined as the natural host of groups M and N. Human isolates related to the simian immunodeficiency virus (SIV) of the western gorilla (Gorilla gorilla), SIVGOR, have been classified as group P [16]. It remains to be established whether the chimpanzee or western gorilla, is the natural host of Group O [17-20]. HIV type 2 (HIV-2) is distinct from HIV-1 and genetically more closely related to the sooty mangabey (Cercocebus torquatus atys) virus, SIVSM [21]. HIV-2 groups are also from separate transmission events [22] but are less pathogenic than HIV-1 and generally confined to West Africa[reviewed in 23].

HIV-1 group M viruses account for most infections globally [24]. Subtype B, the earliest characterised genotype common in developed countries, was first reported as a single infection cluster in 1981 [25]. Later that year came recognition of the acquired immunodeficiency syndrome (AIDS) and in 1983, HIV-1 was isolated and determined as the etiological agent of AIDS by Nobel Prize laureate (2008) Francois Barre-Sinoussi and colleagues [26-27]. However, despite 30 years of unprecedented technological, methodological and therapeutic advances, HIV-1 infection cannot be prevented by vaccination or eradicated from infected patients [28-33].

**THE HIV-1 GROUP M PANDEMIC**

Since the start of the pandemic, there has been an estimated 25 million AIDS-related deaths[34]. From 2000 to 2009, persons living with HIV-1 increased 20% to more than 33 million, reflecting new infections and longer survival times of infected persons in successful treatment[35-36]. Of 2.6 million estimated new infections during 2009, the resource-limited settings of Sub-Saharan Africa and South/South-East Asia featured disproportionately at 69% and 14%, respectively [36]. Although annual deaths from AIDS-related morbidity decreased to 1.8 million in 2009, compared to 2.1 million in 2004 at the pandemic peak [36], deaths due to HIV-1 are projected to be a continuing substantial cause of premature mortality internationally [37].

HIV-1 epidemic classifications are based on prevalence in high-risk sub-populations and the general population[38]. In countries with low-prevalence epidemics, infections are less than 5% in any sub-population and confined to the high-risk groups: injecting drug users (IDUs); men who have sex with men (MSM); sex workers (SW) and their clients; and sexual partners of these groups. In concentrated epidemics, infections are mainly confined to high-risk groups and prevalence has been consistently greater than 5% in at least one group but less than 1% in sentinel surveillance of pregnant women in urban areas. In generalised epidemics, sentinel surveillance estimates of HIV-1 prevalence among pregnant women are consistently
over 1% and high-risk groups may contribute disproportionately to new infections. Epidemics concentrated in high-risk groups can diversify into generalised epidemics which may then be maintained by heterosexual transmission in the general population [39].

**THE SITUATION IN THE ASIA-PACIFIC**

In 2009, it was estimated that approximately 4.2 billion people, more than 60% of the world’s population, were living in the Asia-Pacific [40-41]. The region has the second highest HIV-1 disease prevalence and incidence [35, 42]. Approximately 95% of people living with HIV-1 in the Asia-Pacific are from the ten low and middle-income countries of Cambodia, China, India, Indonesia, Malaysia, Myanmar, Nepal, Papua New Guinea (PNG), Thailand and Viet Nam [43]. Activities which disperse the human population, such as trade, travel, migration, trafficking (illicit drugs and/or people) and war also widen the distribution of the virus [44-45]. HIV/AIDS remains the most common cause of death and loss of work days among people aged 15-49 in the region [46] and of death among reproductive-age women [47].

**Table 1. HIV-1 epidemic characteristics in the Asia-Pacific**

<table>
<thead>
<tr>
<th>Country</th>
<th>HIV-1 Epidemic Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangladesh</td>
<td>- first case of HIV-1 infection reported in 1989&lt;br&gt;- low prevalence&lt;br&gt;- predominantly in IDUs; MSM and FSW less than 1% prevalence&lt;br&gt;- evidence of IDU epidemic spreading to other risk groups</td>
</tr>
<tr>
<td>Cambodia</td>
<td>- first case of HIV-1 infection reported in 1991¹&lt;br&gt;- concentrated in IDU, MSM, entertainment workers, SW, clients and partners&lt;br&gt;- predominantly sexual transmission among high risk groups&lt;br&gt;- decreasing prevalence in general population but increasing prevalence in women</td>
</tr>
<tr>
<td>China</td>
<td>- HIV/AIDS was first reported in China in 1985¹&lt;br&gt;- concentrated in high-risk groups but is spreading to the general population&lt;br&gt;- sexual transmission 59% (44.3% heterosexual, 14.7% MSM)&lt;br&gt;- MSM transmission is increasing</td>
</tr>
<tr>
<td>India</td>
<td>- first case of HIV-1 infection reported in 1986&lt;br&gt;- concentrated and generalised (state dependent)&lt;br&gt;- IDU and sexual transmission (FSW and clients, MSM)&lt;br&gt;- high risk males passing on to female partners</td>
</tr>
<tr>
<td>Indonesia</td>
<td>- first case of AIDS reported in 1987²&lt;br&gt;- concentrated and generalised (province dependent)&lt;br&gt;- mainly IDU and sexual transmission&lt;br&gt;- increasing in MSM and general population</td>
</tr>
<tr>
<td>Lao PDR</td>
<td>- first case of HIV-1 infection reported in 1990&lt;br&gt;- low prevalence&lt;br&gt;- heterosexual transmission&lt;br&gt;- two waves of epidemic: 1990s driven by HIV-positive male migrants returning from working in other countries in the region; early 2000s driven by FSW, clients and partners&lt;br&gt;- increasing MSM</td>
</tr>
<tr>
<td>Country</td>
<td>HIV-1 Epidemic Characteristics</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Malaysia     | - first case of HIV-1 infection reported in 1986  
- concentrated  
- driven by IDU and heterosexual transmission  
- 90.8% of cumulative cases are men  
- increasing prevalence in women                                                                                                                                  |
| Myanmar      | - first case of HIV-1 infection reported in 1988  
- concentrated  
- SW and their clients, MSM, IDU and the sexual partners  
- increasing prevalence in women                                                                                                                                       |
| Nepal        | - first AIDS case was reported in 1988  
- concentrated  
- epidemics differ according to regions/zones and districts  
- IDUs, MSM, FSWs and MSWs, clients and partners and seasonal male labour migrants (40% of HIV-infected) and wives of migrants                                                                 |
| Papua New Guinea | - first case of HIV-1 infection reported in 1987  
- the largest generalised epidemic in the Pacific  
- driven by heterosexual transmission  
- prevalence of HIV-1 among pregnant women 1% in 2003-04                                                                                                           |
| Philippines  | - first case of HIV-1 infection reported in 1984  
- low prevalence  
- in 2009, sexual transmission: 96% (of which homosexual contact: 41.8%; bisexual: 31.3%, heterosexual: 26.9%)  
- prevalence is increasing in high-risk populations                                                                                                               |
| Singapore    | - first case of HIV-1 infection reported in 1985  
- low prevalence  
- transmission: heterosexual 66%, homosexual/bisexual 28%  
- 88.5% of cumulative transmissions are in males                                                                                                                        |
| Sri Lanka    | - HIV-1 cases reported from 1987  
- low prevalence  
- heterosexual: 82.8%, MSM: 11.2%, MTCT: 5.4%, parenteral: 0.4%  
- increases in reported cases                                                                                                                                 |
| Thailand     | - first case of AIDS reported in 1984  
- generalised  
- sexual transmission SW, MSM, and IDU  
- increasing prevalence in pregnant women and remaining high in at-risk groups  
- only country in the region with general population prevalence in excess of 1%                                                                                      |
| Viet Nam     | - first HIV-1 case reported in 1990  
- concentrated  
- IDU, SW, MSM  
- epidemic appears to be stabilising  
- people aged 20-39 years account for more than 80% of cases                                                                                                          |

Source: UNGASS 2010 Country Reports, provided by respective countries [45].

1Other sources for first HIV/AIDS reports: Cambodia[52], China[53], Indonesia[54] and Viet Nam [54]. HIV-1 was first reported in a Pacific Islands country or territory in 1984 [55] and low-prevalence epidemics are reported from Timor Leste and the Pacific Island nations Fiji, Maldives, Republic of the Marshall Islands, Federated States of Micronesia, Palau, Samoa, Solomon Islands, Kingdom of Tonga, Tuvalu, Vanuatu. Nauru reports no people living with HIV-1 in 2009 [45]. Australia, one of the first countries to report an AIDS case [56], and New Zealand, first AIDS report 1983 [57], have low prevalence epidemics with infections historically concentrated in MSM.

Abbreviations: SW, sex worker; FSW, female sex worker; MSW, male sex worker; MSM, men who have sex with men; IDU, injecting drug user; MTCT, mother-to-child transmission.
Sexual transmission accounts for most infections globally but in the Asia-Pacific epidemics are diverse with concentrated, generalised and low-prevalence epidemics predominating in different countries [35]. The earliest reports of HIV-1 infection (Table 0-1) were from Thailand and the Philippines (1984). During the three years following, Singapore (1985), India, Malaysia (1986), Indonesia and Sri Lanka (1987) all reported cases of infection. In many countries, epidemics have been concentrated in high-risk groups such as IDUs (including street children) MSM, sex workers, clients and their partners [42, 45, 48]. In some countries the epidemic is transitioning or has transitioned (Thailand) from being concentrated in high-risk groups to disseminating into the general population[42, 48]. However, Thailand (and Cambodia) implemented early prevention strategies in their high-risk sex-worker and client populations effectively reducing levels of transmission in their local epidemics [42]. Areas where epidemics have been generalised include some provinces of Indonesia, PNG and certain states in India [35, 49-50]. The Philippines, Lao People’s Democratic Republic (PDR) and most Pacific Island nations are maintaining low-prevalence epidemics [45]. HIV-1 prevalence is decreasing in Cambodia and trajectory modelling (Figure 1) suggests HIV-1 incidence is decreasing or stabilising in India, Myanmar, Nepal and Thailand but increasing in Indonesia [45, 51].

For the Asia-Pacific in 2009, general population prevalence (Figure 0-2) was less than 1%, excepting Thailand (1.3%). PNG’s generalised epidemic had second highest regional prevalence (0.9%) [58]. However, country-level summary measures do not necessarily represent regional or risk-group-specific variation. In India, six high-prevalence states accounted for more than two-thirds of the HIV-1 burden. In China, five provinces accounted for more than half of people living with HIV-1 and in Indonesia’s Papua province, infections were 15 times the national average [35, 45]. Injecting drug use remains an important transmission route in Asia with more than 4.5 million people estimated to inject drugs[36]. Regionally, 16% of IDUs are estimated to be HIV-infected but country prevalence varies (Table 2).
Figure 2. HIV-1 prevalence in the Asia-Pacific, 2009. HIV-1 prevalence data for 2009 was sourced from AIDSInfo Database (58). Prevalence estimates are mapped for Australia, Bangladesh, Bhutan, Cambodia, China, Fiji, India, Indonesia, Japan, Lao People’s Democratic Republic (PDR), Malaysia, Maldives, Myanmar, Nepal, New Zealand, Pakistan, Papua New Guinea, Philippines, Republic of Korea, Singapore, Sri Lanka, Thailand and Viet Nam.

Table 2. HIV-1 prevalence estimates for high-risk groups, selected countries 2007-2009

<table>
<thead>
<tr>
<th>Area Name</th>
<th>Injecting Drug Users (%)</th>
<th>Men who have Sex with Men (%)</th>
<th>Sex Workers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cambodia</td>
<td>24</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>China</td>
<td>9</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>India</td>
<td>9</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Indonesia</td>
<td>52</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Japan</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Lao PDR</td>
<td>-</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Malaysia</td>
<td>22</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Myanmar</td>
<td>36</td>
<td>29</td>
<td>18</td>
</tr>
<tr>
<td>Papua New Guinea</td>
<td>-</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Philippines</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Singapore</td>
<td>-</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Thailand</td>
<td>39</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Viet Nam</td>
<td>18</td>
<td>17</td>
<td>3</td>
</tr>
</tbody>
</table>

Source: AIDSInfo[58], estimates provided by respective countries from sentinel surveillance.

High transmission rates are continuing to be reported among MSM and, in some countries, the odds of MSM being infected are 18.7 times that in the general population [59]. Onward sexual transmission to female partners of high-risk groups is helping to sustain generalised epidemics [44] and where females have lower socio-economic status, otherwise
low-risk women and young girls are at increased risk [60]. The proportion of HIV-1 infections among heterosexual females has more than doubled from 17% to 35% between 1990-2008 and approximately 90% of the 1.7 million women were infected from male partners while in long term relationships [34]. Stigmatisation of injecting drug use, same sex attraction and sex work is counter-productive and Asia-Pacific nations adopting the Declaration of Commitment on HIV/AIDS have shown determination in reducing HIV-1 infections in all sub-populations [45]. Drivers of country-level HIV-1 epidemics are diverse in the Asia-Pacific [54] and targeted, local prevention strategies are necessary for regional reductions [46].

**NATURAL HISTORY OF HIV-1**

HIV-1 enters the human host via sexual transmission (vaginal or anal), parenteral methods (injecting drug use, exposure to contaminated blood products, tattoo needles, piercing tools or other unsterile equipment) or by transmission from mother-to-child perinatally, during birth or breast feeding [reviewed in 61]. CD4+ T cells, key immune system functionaries in the elimination of pathogens, are preferentially infected [62] and, in the absence of antiretroviral therapy (ART), HIV-1 induces a slow, continuous deterioration of the immune system [63]. Following seroconversion, infection progresses in three phases, primary infection (acute), clinical latency (asymptomatic) and clinical disease progression (Figure 3) [reviewed in 64]. Rarely diagnosed, primary infection is characterised by transient high levels of viremia, sometimes accompanied by nonspecific symptoms, such as fever, fatigue, muscular pain and headaches [65-66] developing within days or weeks following infection. Cellular immunity develops and HIV-1 viral load (VL) decreases to set-point, reflecting balance between virion production and destruction. The infected person enters an asymptomatic phase persisting 2-15 years (50% within 10 years), shorter durations being associated with higher levels of circulating virus during primary infection and at set-point [67-68]. Continuing viral replication and host immunologic response result in decline of immune system integrity and clinical disease progression [69].

Due to persistently high levels of viremia, the presence of pathogens and immune system deterioration, individuals during disease progression are susceptible to opportunistic infections, malignancies and serious non-AIDS-related events, the risk increasing as CD4 counts decrease below 350-500 cells/mm³ [70-71]. If left untreated, patients may present with fever, weight loss, diarrhea, bacterial, protozoal, fungal or viral infections, malignancies and/or other conditions defined in the Centers for Disease Control and Prevention (CDC) classification of AIDS [66, 70]. Concomitant infections activate CD4+ T cells thereby providing additional targets for HIV-1 to infect. The advanced disease burden from persistent HIV-infection results in death of the patient from AIDS-related complications.

However, 5%-15% of HIV-infected individuals remain asymptomatic, based on duration of infection and CD4+ T cell counts - the long-term nonprogressors (LTNP) [72-74]. VL assays report levels of circulating virus in plasma and when these assays became available, most LTNPs had moderate or increasing VLs. True “controllers”, less than 1% of untreated patients, maintain VL <50 copies/mL for up to 25 years [75-76]. Suggestions were that controllers were not infected with replication-competent, pathogenic virus, however, studies have shown that this is not necessarily the case [77].
Risk of HIV-1 transmission is greater from individuals having high levels of circulating virus, as in primary infection or during disease progression[78-80]. However, transmissions during the asymptomatic period translate to a higher absolute number of infections [8, 79, 81]. When virus is selected from the infected person’s circulating population and transferred to the new host, the transmission bottleneck reduces genetic diversity in the viral founder population [82]. A single virus or number of strains may be transmitted, depending on the exposure. One study on IDU exposure found transmission of 1-16 viruses[83]. Studies on sexual transmission found 76%-90% of infections were from a single virus and remaining patients were infected with two to five viruses [84-85] although inflammatory, genital infections can reduce bottleneck effects [86]. Viral diversity is associated with the transmission surface. Greater variance in diversity during heterosexual exposures is reported in women, possibly reflecting the larger transmission surface of the vagina and cervix[reviewed in 61]. Higher levels of diversity during primary infection have been associated with accelerated disease progression [87].

Risk of infection differs by HIV-1 exposure. Receptive sex has a higher risk than insertive sex and highest infection risk estimates per coital act are for receptive anal intercourse (0.04%-3.0%) [reviewed in 88]. Compared to male-to-female (0.05%-0.50%) or female-to-male vaginal transmission (0.03%-0.14%), risk of transmission parenterally, through blood transfusion (95.0%), injecting drug use (0.67%) or nosocomial exposures, such as needlestick (0.50%), is significantly increased [reviewed in 89]. Mother-to-child transmission, without perinatal prophylaxis, results in HIV-1 transmission to approximately 26% of babies [90]. However, approved short course prophylactic regimens, are effective in reducing peripartum transmission [91].
COINFECTIONS AND COMORBIDITIES

HIV-1 coinfection, with diseases such as tuberculosis (TB) or hepatitis B (HBV) or C (HCV), is more frequently seen in individuals from resource-limited settings. One third of people living with HIV-1 are coinfected with TB [39] and TB is the leading cause of mortality among people living with HIV-1 [92]. Infection is usually by inhalation of airborne particles and the bacterium primarily affects the lungs but may manifest elsewhere. Being HIV-immunocompromised increases the risk of a new TB infection, progression of latent TB to active disease or relapses in previously treated patients [92-93]. In 2008, Africa represented 12% of the global population but was over-represented with 30% of new TB cases (Table 3). However, 55% of all new cases globally were from the Asia-Pacific with India, China and Indonesia accounting for 40% of infections [94].

Table 3. Tuberculosis incidence estimates by region, 2008

<table>
<thead>
<tr>
<th>Region</th>
<th>Population</th>
<th>Global Population (%)</th>
<th>Incidence</th>
<th>Global Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>804,865,016</td>
<td>11.9</td>
<td>2,828,485</td>
<td>30.2</td>
</tr>
<tr>
<td>The Americas</td>
<td>919,896,357</td>
<td>13.6</td>
<td>281,682</td>
<td>3.0</td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>584,354,906</td>
<td>8.7</td>
<td>674,585</td>
<td>7.2</td>
</tr>
<tr>
<td>Europe</td>
<td>889,169,869</td>
<td>13.2</td>
<td>425,038</td>
<td>4.5</td>
</tr>
<tr>
<td>South-East Asia</td>
<td>1,760,485,706</td>
<td>26.1</td>
<td>3,213,236</td>
<td>34.3</td>
</tr>
<tr>
<td>Western Pacific</td>
<td>1,788,176,627</td>
<td>26.5</td>
<td>1,946,012</td>
<td>20.8</td>
</tr>
<tr>
<td>High ranked asian countries*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>1,181,411,968</td>
<td>17.5</td>
<td>1,982,628</td>
<td>21.2</td>
</tr>
<tr>
<td>China</td>
<td>1,337,411,200</td>
<td>19.8</td>
<td>1,301,322</td>
<td>13.9</td>
</tr>
<tr>
<td>Indonesia</td>
<td>227,345,088</td>
<td>3.4</td>
<td>429,730</td>
<td>4.6</td>
</tr>
<tr>
<td>Global Aggregate</td>
<td>6,746,948,481</td>
<td>100</td>
<td>9,369,038</td>
<td>100</td>
</tr>
</tbody>
</table>

* In 2008, in terms of new TB cases, India and China were ranked globally as 1 and 2, respectively. Indonesia was ranked 5th. Adapted from [94].

HCV is caused by a viral infection from infected body fluids or parenteral exposure[95]. HIV-1 and HCV share common transmission pathways and coinfection is increasing in countries with concentrated IDU epidemics [39]. Of people currently living with HIV-1 internationally, 20%-30% are chronically coinfected with HCV and prevalence is increasing [96-97]. Tolerance of antiretroviral (ARV) agents is poorer in HIV/HCV coinfection [98] and coinfection has been associated with increased disease progression and death in observational cohorts [99-100].

HBV is a viral infection transmitted through contact with the blood, semen or vaginal fluid of an infected person. HIV/HBV coinfection prevalence estimates for the resource-limited compared to developed countries are 20% to 5%, respectively [96]. HIV/HBV coinfection rates are lower than HIV coinfection and HCV or TB but more studies are necessary to quantify the problem.
HIV-1 IMMUNOPATHOGENESIS

During immunopathogenesis, persons demonstrate both humoral (antibody) and cellular immune system responses. Humoral responses are mediated by antibodies produced in B lymphocytes (B cells) and although HIV-1 does not replicate in B cells, it causes severe dysfunction and depletion through viral protein toxicity and cytokine dysregulation [reviewed in 101]. The main targets for replicating virus, however, are activated CD4 T lymphocytes, essential for mediating cellular and coordinating antibody responses [102]. HIV-1 induces a selective decrease in these cells through mechanisms including the cytopathogenic effects of apoptosis (programmed cell death), formation of syncitia (fusions of infected cells) and cell death from cytotoxicity or the effects of viral replication[103, reviewed in 104]. Normal CD4 T cell count ranges are from 500 to 2,000 cells/μl and change in CD4 T cell count is used as surrogate measure to monitor immunologic function. Low CD4 T cell counts indicate increased risk of HIV-1 complications[105] and is one criteria on which a diagnosis of AIDS is made [70].

HIV-infected activated CD4 T cells are responsible for producing most virus in plasma [106]. These cells are short-lived, dying within one to two days but some differentiate into resting memory cells, carrying integrated HIV-1 provirus, manufacturing virus only when stimulated [103, 107-109]. Non-activated CD4 T cells can carry pre-integrated provirus but do not immediately commence virus production. Long lived cells in the lymph nodes, central nervous system, gut-associated lymphoid tissue (GALT) and other compartments are potential reservoirs for HIV-1 [110-113]. More research is needed to determine the role of viral reservoirs in HIV-1 persistence in treated persons [114].

VIROLOGY OF HIV-1

HIV-1 is from the family Retroviridae, known for inducing slow progressive disease states and immune system dysfunction. The clinical symptoms of HIV-1 infection and disease progression are the manifestation of the pathogenic features of the viral replication cycle on infected individuals[reviewed in 115]. HIV-1 is a single-stranded (ss)RNA lentivirus with a genome between 9-10 Kb in length [30, 116], each genome carrying two copies of ssRNA. HIV-1’s nucleotide sequence, translated in groups of three nucleotides called codons, determines the amino acid composition of viral proteins[117]. The virus uses host cell attributes and functionality during infection and replication [118-119]. The genome RNA sequence, reversed transcribed to DNA, integrates into the host genome. Using host cellular machinery, viral RNA is transcribed from the integrated DNA template and translated from nine open reading frames into 15 proteins, including three structural proteins (Gag, Pol and Env) and six regulatory and accessory proteins (Tat, Rev, Nef, Vif, Vpr, and Vpu) [116].

HIV-1’s viral envelope (Env) protein sits within a lipid bilayer, formed from infected host cell membrane during virion release and contains the structural features surface glycoprotein 120 (gp120) and transmembrane glycoprotein 41 (gp41) (Figure 4). HIV-1 infection depends on the interaction of gp120 with the target cell CD4 receptor (attachment) [62]. This interaction promotes binding to a coreceptor, usually host surface cell protein C-C chemokine receptor type 5 (CCR5) or C-X-C chemokine receptor type 4 (CXCR4), viral tropism being determined by the Env amino acid sequence and structure. Coreceptor binding
results in a conformational change in gp120 inducing structural rearrangement of gp41 facilitating fusion of the virus and target cell membranes. The virion core particle, or nucleocapsid (NC), enters the host cell and releases two copies of the RNA genome, the essential viral replication proteins, reverse transcriptase (RT), integrase (IN), protease (PR) [116] and other viral proteins into the cytoplasm[120-121].

RT mediates reverse transcription of the viral genome from RNA templates to DNA in the cell cytoplasm prior to its integration into the host, requiring transcription of ssRNA to an RNA/DNA double helix by the RT polymerase active site. The RNaseH active site breaks down the RNA component and RT polymerase completes the remaining DNA strand to form a double helix [reviewed in 116]. Since RT has no proof reading mechanism, genome transcription errors cause mutations, ie substitutions, insertions or deletions, having effects which range from conferring selective advantage to, if codons which terminate translation are encoded, lethality [117]. In an infected individual with high levels of circulating virus, based on experimental data, it is estimated that each possible point mutation can occur throughout the HIV-1 genome daily [103, 122-123]. Although some positions are conserved due to functional features, such as active sites, DNA or deoxyribonucleotide triphosphate (dNTP) binding regions [124].

Pause sites, for example breaks in RNA, can trigger template switching during reverse transcription[125-127]Template switching between the two co-packaged RNA genomes can repair damaged segments or purge lethal mutations in HIV-1. One virus has established infection in a cell, a recombinant mosaic genome can be generated [127-129]. When reverse transcription of viral DNA is finalised, it is transported from the cytoplasm to the nucleus and integrated into the host cellular DNA [118, reviewed in 130].

IN mediates processing of the viral DNA ends and strand transfer events which join the viral and host DNA to form functional integrated provirus. Activation of the host cell induces transcription of proviral DNA to messenger RNA (mRNA) which migrates into the cytoplasm where new viral components are synthesised, some of which require processing by PR. In addition to its role in viral assembly [131], PR cleaves precursor proteins necessary for maturation of viral particles [132] required for infectious virion release. The immature viral particles leave the cell acquiring an envelope of host and viral proteins, maturing and going on to infect other cells.

A host protein, APOBEC3G, hypermutates guanine (G) to adenine (A) during viral transcription, increasing incorporation of lethal mutations in HIV-1[133-134], however, HIV-1 Vif induces the degradation of APOBEC3G. HIV-1 Tat increases the transcription of HIV-1 genes, Rev allows the expression of the different HIV-1 genes by regulating splicing of the mRNA. Nef downregulates CD4 and MHC class I surface expression on the membrane of infected cells, potentially aiding viral escape from host immune surveillance. Vpr and Vpu proteins may be involved in intracellular transport of viral proteins and Vpr and Rev proteins have been found to enhance replication and infectivity [116].

HIV-1 populations are generated as a genetically heterogeneous, quasi-species [135] diverging 10% within an individual, due to replication errors during reverse transcription and, in cases of dual infection, recombination[reviewed in 136]. Variants differ in replicative fitness and during transmission majority and/or minority populations may be transferred into the new host environment, becoming the basis for viral diversification and evolution in the newly infected individual [137-138].
Figure 4. HIV-1 structure and replication cycle.
The HIV-1 Group M Epidemic in the Asia-Pacific

**HIV-1 Group M Diversity**

Based on genetic similarity HIV-1 group M viruses are classified into nine different subtypes (A–D, F–H, J, K) (Figure 5) diverging 25%-35% [reviewed in 14, reviewed in 136]. Subtypes A and F are further classified into sub-subtypes (A1, A2, F1, F2) and although subtypes B and D are as closely related as sub-subtypes, they maintain current classification for historical reasons [reviewed in 14].

Subtype B, referred to as B’, BB or Thai-B in some studies on Asia, is the most studied genotype, represented by 62% sequences in the HIV Sequence Database (http://www.hiv.lanl.gov) at January 2011. To describe mutations or polymorphisms, naturally occurring variations existing in more than 1% of the population, substitutions in the HIV-1 genome are made in reference to subtype B wild-type HXB2 strain (Figure 6) [27, 30]. Subtype B accounted for 10% of global infections in 2000-2004, after subtypes C and A with 50% and 12% of infections, respectively [139].

Circulating recombinant forms (CRFs) result from recombination between HIV-1 genotypes within a dually infected person and are important contributors to some regional epidemics [139]. A CRF classification requires a viral strain to infect three or more individuals, not epidemiologically linked. CRF names contain a “CRF” prefix, an integer indicating order of discovery, and letters identifying its contributing genotypes. If there are more than two ancestral genotypes, the suffix “cpx” (complex) replaces the contributing genotypes [14].

Earlier in the pandemic, the env region was used to determine subtype classifications [143]. When gag and pol regions were genotyped, subtype E env viruses included subtype A sections in the other regions of the viral genome resulting in subtype E’s reclassification as an A/E recombinant, CRF01_AE (Figure 7) [141]. However, no full genome for subtype E genome has been found leaving CRF01_AE’s recombinant status inconclusive [14]. By the end of 2010, 49 CRFs had been classified, consisting of combinations of all M group subtypes and three CRFs (CRF01_AE, CRF02_AG and CRF06_cpx) also contributing to new recombinants [142].

Viral genotypic heterogeneity may have implications for rates of transmission and disease progression. During transmission and in early infection, most genotypes use CCR5 (R5) coreceptors, CXCR4 (X4)-using syncytium inducing variants emerging later in infection [reviewed in 144, 145].

Subtype C studies generally report a lack of coreceptor switching from R5 to X4, possibly affecting transmission [144], and dual-tropic virus (X4/R5) found in other genotypes were not found in subtype D viruses [146]. Where subtypes A and D co-circulate, more rapid disease progression has been found for subtype D compared with subtype A [147] although evidence suggests that subtype A infections are outpacing subtype D [148].

In the Asia-Pacific 2000-2004, predominant genotypes were subtypes B and C, CRF01_AE and their recombinants, with country-specific epidemics dominated by different genotypes [139]. In India, 97% of infections were from subtype C while four Mekong River countries (Cambodia, Myanmar, Thailand and Viet Nam) were dominated by CRF01_AE (84%). In China’s Special Administrative Region of Hong Kong, subtype B and CRF01_AE co-circulated. Mainland China infections also comprised of 45% of B/C recombinants, primarily CRF07_BC or CRF08_BC.
Figure 5. HIV-1 Group M Reference Subtypes. The HIV-1 Subtype Reference Set sequence alignments (2008) for Pol region reverse transcriptase (RT) HXB2 positions 2550-3869 were sourced from the Los Almos HIV Sequence Database (http://www.hiv.lanl.gov). The most studied genotype is subtype B (in bold) and reference sequences have been sampled from France (FR), Thailand (TH) the US and the Netherlands (NL). Reference sequences are selected from HIV-1 M group subtypes to represent diversity within each subtype. The HIV Sequence Database FindModel tool determined that the general time reversible plus gamma (GTR+) was the most appropriate nucleotide substitution model for maximum likelihood (ML) estimation of the phylogenetic topology. The phylogenetic tree was generated using the HIV Sequence Database PhyML interface tool [140]. Branch length units are nucleotide substitutions per site. Countries of sampling for these reference specimens can be determined from the two letter ISO 3166 country codes following the Ref.Subtype. The subtype, country, and year of isolation are given if defined otherwise the GenBank accession number is provided.
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Source: http://www.hiv.lanl.gov/. Open reading frames are shown as rectangles. The gene start, indicated by the small number in the upper left corner of each rectangle, normally records the position of the “A” in the ATG start codon for that gene while the number in the lower right records the last position of the stop codon (30).

Figure 6. HIV-1 group M subtype B gene map based on strain HXB2.

Source: http://www.hiv.lanl.gov/. A breakpoint is the region on a recombinant genome delineating the change from one genotype to another. Figures 6-7 shows the CRF01_AE genome with A and E breakpoints (141).

Figure 7. Circulating recombinant form (CRF) 01_AE.

Subtype B infections predominated in Australia New Zealand (88%) and Japan (81%). No data for PNG were available during the period and representative studies are needed to better quantify the genotype distribution.

During 2007-2009, 728 patients from Thailand, Hong Kong and Malaysia were genotyped for PR and RT, the genomic regions of HIV-1 under selection pressure from regionally available ARVs [149]. Recombination, evidenced by CRFs and discordant genotypes (where PR/RT genotypes differ), excluding CRF01_AE and CRF02_AG, was reported in 4% of patients. The diversification and spread of HIV-1 strains is of international concern and the dynamic contributions of local epidemics may be better quantified if subtype and recombinant dispersion is tracked longitudinally. The availability of these data could allow improved precision in modelling estimates evaluating the effects of preventive strategies targeting HIV-1 transmission in high-risk networks.

**Antiretroviral Therapy (ART)**

The first medication shown to inhibit HIV-1 replication was zidovudine (AZT), approved by the US Food and Drug Administration (FDA) in 1987 [150]. ARTs are classified based on
where the HIV-1 life cycle is interrupted and are commonly designed on subtype B. FDA-approved drugs target attachment, fusion, reverse transcription, strand transfer events and protein cleavage[151]. Potent combinations of ARVs, known as highly active antiretroviral therapy (HAART), suppress viral load (VL), enhance patient immune function and reduce risk of opportunistic infections and death. The surrogate markers, CD4 cell count (immune function) and HIV-1 RNA in plasma (VL) are used to evaluate patient responses to treatment [152]. Studies have demonstrated the efficacy of combination regimens based on nucleoside/nucleotide reverse transcriptase inhibitor (NRTIs), non-nucleoside reverse transcriptase inhibitor (NNRTIs) and protease inhibitors (PIs) [153-154] available in resource-limited settings [149]. HIV-1 RT is the drug target for NRTIs and NNRTIs. NRTIs act as chain-terminators in DNA synthesis[reviewed in 155] and NNRTIs induce conformational changes in RT inhibiting catalytic activities [reviewed in 156]. PIs bind viral protease with high affinity and are generally supported by an additional PI, ritonavir, to boost levels in plasma [reviewed in 157]. However, HAART in developing Asia-Pacific economies does not generally incorporate the newer classes of entry and integrase inhibitors or newer generation ARVs, due to cost, limiting regimen choices for patients [149]. Additional challenges to ART rollout in these settings include limited health infrastructure and medical expertise. Therefore, treatments are delivered as standardised regimens, in contrast to the individualised patient management strategies, based on diagnostic monitoring, routinely available in developed economies [71]. Recommended first-line regimens incorporate a two NRTI backbone and a NNRTI. PIs are used for second-line therapy supported by novel/recycled NRTIs [158]. Despite differences among HIV-1 genotypes, studies show that virologic suppression is successful with HAART in pandemic regions [159-160].

In developed countries, patients have had access to regimens of NRTIs, NNRTIs and PIs for approximately 25, 20 and 15 years, respectively [155-157]. Low- and middle-income countries in the Asia-Pacific, have not enjoyed the same historical level of access (Figure 8) [48] although production of local generics has improved availability [42].


Figure 8. Antiretroviral therapy programme implementation in low- and middle-income countries.
Based on treatment initiation at CD4 cell count < 350 cells/mm$^3$, coverage in Asia increased from 25% in 2008 to 31% in 2009 [48] but country-specific estimates do differ (e.g. Thailand 61%, India 26%, Cambodia 94%) [48].

Even when patients have access to ARTs, circulating virus may not be durably suppressed to nondetectable levels. Regimen efficacy may be affected by suboptimal concentrations of drug due to lack of adherence, possibly due to low-level patient toxicities, interactions between co-administered ARVs, or co-administration of other medications such as rifampicin for TB which affects some NNRTI concentrations [161]. Furthermore, secondary effects of ARVs, such as metabolic disorders may require regimen discontinuation [162-163]. For patients in resource-limited settings, additional complications for maintaining virologic suppression are unplanned treatment interruption, due lack of funds to purchase medications, lack of transportation to collect prescribed ARVs and drug stock-outs at local pharmacies.

Patient management guidelines in developed countries recommend periodic collection of HIV-1 RNA and CD4 cell counts [71, 164]. In resource-limited settings where ART failure is generally determined by clinical evaluation, additional partnering of routine CD4 T cell count has a role in determining when to switch from failing first-line ART [165]. However, HIV-1 RNA informs knowledge of trends in viral replication and gives advance notice of non-adherence, treatment regimen failure and HIV drug resistance (HIVDR) [152]. VL monitoring is not widely available in asian countries and suboptimal access has negative implications for disease progression[100], possibly related to the increased risk of patients being maintained on failing regimens [166]. With treatment failure detected late, unabated viral replication coupled with drug selection pressure leads to acquisition and accumulation of resistance-associated mutations (RAMs) [167]. More studies are needed on how to determine appropriate partnering of ARTs and VL monitoring to maximise ART effectiveness for the patient and reduce the emergence and transmission of drug resistance into the general population.

**HIV Drug Resistance (HIVDR)**

Acquired (secondary) drug resistance emerges in ARV-experienced patients during suboptimal virologic suppression, causing primary resistance when transmitted to other individuals. Natural drug-resistant polymorphisms may pre-exist therapy with higher frequencies being found in non-B subtypes [168]. Drug-resistant variants may circulate as majority or minority populations proliferating under drug selection pressure [137]. Mutations generally confer a fitness cost and variants differing in fitness and drug susceptibility can replicate or persist, archived in viral reservoirs [107] At least 60 years of complete viral suppression on ART is required to eradicate archived HIV-1 in viral reservoirs [108], consequently, drug-resistant variants can re-emerge when failed therapies are reintroduced [169].

RAMs can confer resistance to a single ARV, a drug class or more than one drug class [170-171] reducing regimen efficacy and causing therapy failure [172]. HIVDR evaluations include assessment of virus drug susceptibility (phenotype) or viral sequence (genotype). Phenotypic assays measure in vitro replicative ability of viral isolates in the presence of ARV drug, in comparison to a drug-susceptible wild-type virus. The IC$_{50}$ (concentration reducing
response by 50%) of the drug being tested for patient-derived virus, divided by the wild-type reference virus IC50, is the fold change in susceptibility. Fold changes greater than one indicate that patient-derived virus is less drug susceptible than wild-type virus [173]. However, phenotypic assessments are time consuming and expensive. Genotypic resistance testing evaluates the genetic sequence of HIV-1 for the presence of RAMs [174] and is recommended, in guidelines for developed economies, for optimising patient regimens [71, 164]. Genotypic mutations are identified by three components, the wild-type consensus subtype B amino acid, the position of the amino acid (codon) and the substitution in the mutated virus genotype, where it differs from wild-type. Insertions and deletions in the viral genome may also confer HIVDR [171]. However, the quasispecies nature of HIV-1 complicates the detection of drug-resistant variants. Routine resistance testing, by bulk sequencing, does not reliably detect mutants circulating at less than 20% [reviewed in 138].

Depending upon the number of mutations required to confer resistance and their fitness cost to the virus, ARVs can have high or low genetic barriers to resistance [175]. Mutational pathways can involve intermediary or revertant substitutions and accumulating mutations may be compensatory or antagonistic and some examples follow. First generation NNRTIs, important ARVs in developing countries, have low genetic barriers, requiring one mutation to confer drug resistance, and high cross-class resistance [171]. High genetic barrier PIs accumulate RAMs with primary (major) mutations reducing drug susceptibility and secondary (minor) mutations, compensating for reductions in viral replicative capacity [reviewed in 157]. The signature mutation of NRTI lamivudine is M184V/I. Evaluations, based on monotherapy, found substitutions from wild-type methionine (M) (ATG) to intermediary isoleucine (I) (ATA) after two weeks of therapy. Due to replicative advantage, valine (V) (GTG) outgrew I within 9-20 weeks. However, both substitutions reduce processivity and replication, compared to wild-type [reviewed in 176]. NRTI mutations T215F and T215Y, require two nucleotide substitutions on the evolutionary pathway to resistance. Revertant single nucleotide substitutions (T215A/C/D/E/G/H/I/L/N/S/V) are indicative of ARV selection pressures, in treated patients, or signify primary resistance in untreated patients with increased risk of virologic failure [171, 177]. The K65R mutation, selected by NRTIs tenofovir, didanosine, abacavir, or stavudine, particularly in patients with nonsubtype-B clades [171], exhibits bidirectional antagonism with thymidine analogues mutations (TAMs), selected by zidovudine and stavudine. Therapies selecting for TAMs and K65R can prolong patient response to treatment via mutually antagonistic interactions [178].

Standard lists for monitoring and surveillance of HIVDR are used to characterise and compare the epidemiology of drug resistance[179]. Algorithms, assessed by expert panels, incorporate findings on correlations between viral genotype and one or more of the following: ARTs, phenotypic evaluations or virologic response[180]. In one study in Asia, 13.8% of ARV-naive patients from Hong Kong, Malaysia and Thailand, 2007-2009, had at least one RAM in any drug class, suggesting primary HIVDR is increasing [181]. Prevalence of mutations[182], or polymorphisms conveying resistance[183], has been shown to differ among genotypes [184-185]. Measuring the rate of RAMs conferring resistance in newly infected patients longitudinally in HIV-1 genotypes will help quantify the effects of HIVDR on the international pandemic.
CONCLUSION

In 2010, the United Nations General Assembly reported that 10 times more infected individuals have access to treatment than 5 years previously but new infections continue to outpace persons initiating treatment at a rate of 5:2 [47]. The search for a vaccine continues [33] although progress is complicated by increasing HIV-1 genetic diversity[186]. Research to eradicate HIV-1, focussing on latency and persistence in viral reservoirs [114], is ongoing, however, viable alternatives to ART will be unavailable in the near future. Consequently, currently available treatment options need to be preserved.

Lagging behind the international ART rollout in developing economies is access to disease staging diagnostics, primarily due to the prohibitive cost of commercial assay kits and reagents, equipment and infrastructure [reviewed in 100]. Studies suggest that appropriate monitoring of CD4 count [165] and VL [100] may decrease disease progression, possibly due to earlier implementation of second-line ART following treatment failure. Earlier switches to second-line ART has been shown with VL monitoring [166] and late detection of virological breakthrough, without VL monitoring, raises concerns that drug-resistant mutations may accumulate in the individual and be transmitted into the wider population. Widespread HIVDR would reduce already limited treatment options in the Asia-Pacific region [187]. Consequently, more work is needed to determine appropriate partnering of ARTs and VL monitoring in resource-limited settings.

Vaccines seek to target transmitted virus and further quantification of HIV-1 quasispecies diversity and genotypic proliferation are essential [188]. Where genotypes co-circulate, individuals coinfected with multiple HIV-1 variants may increase, providing potential for recombination and augmenting viral diversity. Strategies such as serosorting, where same HIV-status partners are sought for unprotected sex[reviewed in 189], have been reported in MSM. Serosorting is not supported as a risk reduction strategy [190] as it increases opportunities for recombination and superinfection. Some HIV-1 variants differ in responses to therapy [168, 185, 191] and dispersion of virus with reduced drug-susceptibility in regions where therapeutic options are already limited is of public health concern. In the Asia-Pacific, due to the heterogeneity of the sub-populations driving regional epidemics, country-specific prevention strategies are needed to prevent new infections. Monitoring HIV-1 genotype transmission dynamics and proliferation in at-risk population networks, will give advance notice of increases in circulating genotypes, quantify their roles in the epidemic and allow more effectively targeted prevention strategies in sub-populations and areas of concern.

In summary, to preserve treatment options for the individual, and at the population level, more work needs to be done to determine best practice guidelines for VL and HIVDR monitoring in resource-limited settings. HIV-1 epidemics are increasing in viral diversity, evidenced by ongoing recombination between HIV-1 group M genotypes, and virus founder population quasispecies, human mobility and transmission methods explain genotype geographic distributions. Monitoring VL and HIVDR in patients, coupled with sentinel surveillance of HIVDR, will help to maximise ART efficacy and effectiveness in the region for the longer term. Epidemic tracking of local genotypes will provide insight into the dynamics of HIV-1 in the Asia-Pacific region and, where genotype-specific prevalence is increasing, help to direct scarce resources towards prevention strategies for sub-populations at enhanced risk.
REFERENCES


