HIV infection in India: Epidemiology, molecular epidemiology and pathogenesis.

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The year 1986 saw first case of HIV infection as well as first report of AIDS case in India. Since then the epidemic has spread throughout the country. In the recent years there is evidence of epidemic being stabilized with decrease in new infections reported from some parts of the country. The absolute number of HIV infections in the country is expected to be close to 2.5 million and National AIDS Control Programme, phase III is geared to contain the epidemic. HIV viruses circulating in India predominantly belong to HIV-1 subtype C. However, there have been occasional reports of HIV-1 subtype A and B. Matter of concern is reports of A/C and B/C mosaic viruses that are being reported from different parts of the country. The data on HIV drug resistance from India is rather limited. Most of the studies have shown that the virus strains from drug naïve patients do not show significant level of drug resistance mutations. The few immunological studies in Indian patients show that the Indian HIV infected patients show both HIV-specific CTL responses as well as neutralizing antibody response. Mapping of CTL epitopes showed that while Indian patients identify same regions of Gag antigen as recognized by South African subtype C infected patients, some regions are uniquely recognized by Indian patients. There are very few studies on host genetic factors in India in context with HIV infection. However there are evidences reported of association of host genetic factors such as HLA types and haplotypes and HIV disease.

[Lakhashe S, Thakar M, Godbole S, Tripathy S and Paranjape R 2008 HIV infection in India: Epidemiology, molecular epidemiology and pathogenesis; *J. Biosci.* **33** 515–525]

1. Introduction

The first case of HIV infection as well as first case of AIDS was reported in India in 1986, within five years of first description of AIDS in North America. The Governmental and Non-governmental sectors have responded to the growing epidemic with increasing access to testing, prevention and anti-retroviral treatment. However, the research efforts have remained relatively low key in spite of India being country with second largest number of HIV infected persons. In this paper we review the research carried out in the field of epidemiology, molecular virology and immunopathogenesis in India.

The progress of HIV epidemic was relatively slow initially. Over the years the number of HIV infected persons increased and it is a major public health problem now. Based

Keywords. Epidemiology; HIV; immunology; India; subtypes

Abbreviations used: Ad5, Adenovirus serotype 5; AIDS, acquired immunodeficiency syndrome; ANC, antenatal care; ART, anti-retroviral treatment; ARV, anti-retroviral; BRTI, bacterial respiratory tract infections; BSS, behavioural surveillance surveys; CRF, circulating recombinant form; CTL, cytotoxic T-lymphocytes; DC-SIGN, dendritic cell-specific intercellular adhesion molecule 3 grabbing non-integrin; ELISPOT, enzyme-linked immunosorbent spot; FSW, female sex worker; HIV, human immunodeficiency virus; HIVDR, human immunodeficiency virus drug resistance; HLA, human leucocyte antigen system; IBBA, integrated behavioural and biological assessment; IDU, intra-venous drug user; IFNg, interferon gamma; LTR, long terminal repeats; MSM, men having sex with men; NACO, National AIDS Control Organization; NFHS, National Family Health Survey; NRTI, nucleotide reverse transcriptase inhibitor; NNRTI, non-nucleotide reverse transcriptase inhibitors; PBMC, peripheral blood mononuclear cells; PI, protease inhibitors; PLHIV, people living with HIV; RT, reverse transcriptase; STI, sexually transmitted infections; STD, sexually transmitted diseases; TI, targeted intervention; URF, unique recombinant forms

on the data from sentinel surveillance and National Family Health Survey III in year 2005–2006 it has been estimated that 2 to 3.1 million people were living with HIV/AIDS in India in year 2006

Since the number of HIV infected persons who come forward to undergo HIV testing is only fraction of those actually infected, it is important to estimate the disease burden for making future projections as well as planning control strategies. 2006-2007 was a watershed year for the process of estimating the number of HIV infected persons in the country. Since 1998 data from annual sentinel surveillance has been used for HIV disease burden estimation in India. In 2006, the surveillance network was expanded to 1,122 sentinel sites covering almost every district in the country, from 703 that existed the previous year. In the same year results from the third round of the National Family Health Survey (NFHS-III), which had included HIV testing of adult men and women (102,000) in the population based household survey became available and were also used in the generation of the HIV estimates. Additionally, data from the second round of national Behavioral Surveillance Survey (BSS-2) and the 1st round of Integrated Biological Behavioral Assessments (IBBA) survey, also conducted during the same year were available which were used for the estimation process.

A series of regional workshops in the country organized by National Institute of Medical Statistics and WHO, the 'workbook' estimation process and two consultative meetings of National and International experts held by National AIDS Control Organization (NACO) in June 2007, completed a complex process of data analysis and estimation, in order to produce revised estimates on India's AIDS epidemic.

The June 2006 estimates placed the national adult HIV prevalence in India at approximately 0.36% (0.29%-0.46%), which corresponds to an estimated 2 to 3.1 million people living with HIV in the country. Although this is almost half of earlier estimation, this reflects the application of more robust methodology used after the NFHS-III, 1st round IBBA and BSS-2 data were available. The new robust methods developed for the revised estimates were also used to "back calculate" the prevalence for years since 2002 based on the new set of assumptions and measures. These figures allow a fair comparison of year-on-year trends in HIV prevalence. They show an epidemic that is stable over time, with marginal decline in 2006.

The back calculations revealed that the adult HIV prevalence during last five years remained almost stable at 0.4% varying between 0.45% in 2002 and 0.36% in 2006. People living with HIV (PLHIV) were 2.47 million in 2006. Around 4% of them were children, 8% among the above-49 age group and the remaining 88% in 15–49 age groups. Although the epidemic has shown signs of stabilization high HIV prevalence in some hot spots and the increase in prevalence in the districts that were moderate or low prevalence districts is a cause of worry. Sentinel Surveillance 2006 identified 104 Districts with more than 1% HIV sero-positivity in women attending ante-natal clinics (ANC), and 14 Districts with more than 3% in ANC settings (Annual HIV Sentinel Surveillance Country Report 2006).

The order of magnitude of prevalence among different risk groups in descending order was 8.7%, 5.7%, 5.4%, 2.4% and 0.3% among IDUs, MSMs, FSWs, long-distance truckers and general population, respectively.

Other studies have also added to the early evidence of stabilization and showing even decline of the epidemic. A study published by Rajesh Kumar *et al* in 2006, analyzed trends in HIV Sentinel Surveillance data from 294050 women attending 216 antenatal clinics and 58790 men attending 132 STI clinics between 2000 and 2004. The results showed a significantly reducing trend in HIV prevalence among women aged 15–24 in Southern states (Kumar *et al* 2006b). Prevalence also fell significantly in men (20–29 years) attending STI clinics in the South, while this was not seen in the northern states. The study estimated that this reducing trend in the two groups was probably due to increasing condom use by men and female sex workers in south India thus reducing transmission in wives.

A population based study in the high prevalence Guntur district of Andhra Pradesh (Dandona *et al* 2006) identified reasons for a likely overestimation of HIV disease burden data (about 2.5 times) using sentinel surveillance data and the methods prevailing then.

While population based studies provided evidence of stabilization of epidemic, a cohort study among men and women attending STD clinics in Pune India provided first direct evidence of a decline in HIV incidence rates in FSW and male STD patients over the last 10 years (Mehendale *et al* 2007). However, there was no evidence of a change in the risk of HIV infection for wives of male STD patients. This stresses the need for additional targeted HIV prevention interventions**.**

Countrywide sentinel surveillance data indicates that the heterosexual contact is the main mode of HIV transmission in India. However, a localized epidemic driven by intra-venous drug use has been reported in the north eastern state of Manipur. Epidemic in Manipur began in the core group of intravenous drug users and spread explosively since the detection of the first case in 1989. The prevalence of HIV infection among injecting drug users in Manipur State increased from 2–3 per cent in 1989 to over 50 per cent in 1991 (Sarkar *et al* 1993) and reaching 80% in 1997. However, more recent sentinel surveillance data for the year 2006, revealed an HIV prevalence of 19.8% (95% CI: 17.33–22.27) among IDUs in Manipur (Annual HIV Sentinel Surveillance Country Report 2006)

Majority of the injecting drug users in Manipur; primarily young males, using drugs for a median of five years, in spite of high level of awareness about the risk of HIV transmission through sharing of needles routinely share injecting equipment (Godbole and Mehendale 2005). Transmission from IDU's to their partners or spouses and then their children also occurred rapidly helping in generalization of the epidemic and a study among couples showed that 45% of wives of IDUs were infected (Panda *et al* 2000). A study among women in Manipur (Panda *et al* 2001) in 1997 revealed a high HIV prevalence of 57% among injecting drug users compared to 20 per cent among non-injecting drug users. Another study among female sex workers noted that while the HIV prevalence was (12%) in sex workers who did not inject drugs, it was 9.4 times higher among those who were also IDU's (Agarwal *et al* 1999).

To control HIV epidemic in India, National AIDS Control Organization has currently launched the National AIDS Control Programme (NACP)-III. The overall goals of NACP-III are to halt and reverse the epidemic in India over the next five years by integrating programmes for prevention, care, support and treatment. This will be achieved through a four-pronged strategy:

- Preventing infections through saturation of coverage of high-risk groups with targeted interventions (TIs) and scaled up interventions in the general population.
- Providing greater care, support and treatment to larger number of PLHIV.
- Strengthening the infrastructure, systems and human resources in prevention, care, support and treatment programmes at district, state and national levels.
- Strengthening the nationwide Strategic Information Management System.

The specific objective of NACP-III is to reduce the rate of incidence by 60 per cent in the first year of the programme in high prevalence states to obtain the reversal of the epidemic, and by 40 percent in the vulnerable states to stabilise the epidemic.

2. Molecular epidemiology of HIV

HIV-1, demonstrates high genetic diversity due to lack of proof reading ability of its enzyme, reverse transcriptase. As a result of high mutation rates HIV-1 virus strains show extreme genetic divergence and have been classified into subtypes (A to K), circulating recombinant forms (CRFs) and unique recombinant forms (URFs). The distribution of subtypes is largely geographically restricted such as HIV-1 Subtype A in Africa, Subtype B in North and South Americas and Europe, Subtype C in India, China and South Africa. HIV molecular epidemiology studies carried out in different parts of India suggest that subtype C is the most prevalent among all HIV-1 genetic subtypes. Other subtypes that have been reported include subtype B, subtype A and few cases of subtype E (CRF01_AE).

Although there is no strong evidence of the influence of subtype on the disease progression, recent evidence from viral fitness studies indicate that subtype C viruses from India have fitness advantage over the subtype A viruses in India (unpublished data). This may explain why subtype A viruses although detected for over a decade remain minority among HIV viruses isolated in India. Molecular virology studies are important to keep vigil for emergence of recombinant strains. Such molecular virology data is also crucial for vaccine development strategies.

Table 1 lists various studies carried out in different parts of the country and highlights the fact that HIV-1 subtype C is the most prevalent subtype in India. Few cases of individuals infected with more than one subtype have also been reported (Mandal *et al* 2002).

Although HIV-1 epidemic in India has spread to practically all states over last twenty years the Indian HIV-1 subtype C strains have shown a remarkable homogeneity.

Analysis of HIV-1 subtype C genome sequences from India showed that these sequences were closely related to each other. In a phylogenetic analysis, these sequences clustered together and showed monophyletic lineage. These viruses are likely to be descendents of a single founder strain that was introduced in the beginning of the epidemic in India (Novitsky *et al* 2002; Novitsky *et al* 1999; Shankarappa *et al* 2001). Low genetic diversity has been reported in *gag* (Gupta *et al* 2005; Kurle *et al* 2004; Mullick *et al* 2006), *nef* (Jere *et al* 2004; Kumar *et al* 2006a), *tat* (Mullick *et al* 2006) as well as in the *env* (Agnihotri *et al* 2006; Kalpana *et al* 2004; Khan *et al* 2007; Mullick *et al* 2006) genes among subtype C viruses from India (table 3). In a phylogenetic analysis of 192 HIV-1 subtype C *env* sequences (including 42 sequences from India), majority of sequences from India clustered together away from sequences from other countries ($P<0.0001$) (Shankarappa *et al* 2001). During analysis of 73 near full-length genome sequences of HIV-1 subtype C from 9 different countries, 8 of 9 sequences from India formed monophyletic lineage at a bootstrap value of >95% (Novitsky *et al* 2002). The V3 loop of HIV-1 Env protein is known as one of the important neutralization determinant. Changes in amino acid sequence and glycosylation sites in HIV-1 Env V3 loop play an important role in protecting the virus from neutralizing antibodies. During analysis of V3 loop sequences of Indian HIV-1 subtype C viruses, it was found that the amino acid sequences as well as all potential glycosylation sites were well conserved (Kalpana *et al* 2004).

The epidemics in different countries where more than one subtypes coexist have shown the emergence of mosaic viruses that carried genomes from more than one subtype. In some of the African countries recombinant viruses have

Table 1. HIV-1 subtype analysis from different parts of India

State / Region	No of samples	Method	Findings	Reference
Maharashtra and New Delhi	2	Nucleotide sequencing of *pol*	Both sequences were of subtype C	(Soto-Ramirez *et al* 1996)
Maharashtra and New Delhi	9	Nucleotide sequencing of *env*	subtype C: 8 subtype B: 1	(Tripathy *et al* 1996)
Bihar, Haryana, West Bengal, Goa, New Delhi	6	Nucleotide sequencing of *gag*	subtype C: 5 subtype B: 1	(Voevodin *et al* 1996)
Maharashtra	46	Heteroduplex Mobility Assay (HMA) for V3-V5 region of *env*	Subtype C: 44 Subtype B: 1 Subtype A: 1	(Gadkari *et al* 1998)
Maharashtra	6	Nucleotide sequencing of full-length HIV-1 genome	subtype C: 5 A/C recombinant: 1	(Lole *et al* 1999)
Maharashtra and Delhi	28	Nucleotide sequencing of LTR	All samples were of subtype C	(Choudhury *et al* 2000)
West Bengal	54	HMA and nucleotide sequencing of C2-V5 region of *env*	95% samples were of subtype C	(Mandal *et al* 2000)
West Bengal	54	HMA and nucleotide sequencing of C2-V5 region of *env*	50 were subtype C and remaining were non-typable.	(Mandal *et al* 2002)
Manipur	25	HMA and nucleotide sequencing of C2-V5 region of *env*	subtype C: 17, subtype B: 5 Remaining were co-infected with subtype C and B	(Mandal *et al* 2002)
Referred samples from different parts of country	125	HMA of C2-V5 region of *env*	subtype C: 98 subtype B: 11 subtype A: 3 subtype E: 2 remaining were not typable	(Sahni *et al* 2002)
Maharashtra	128	Nucleotide sequencing of *pol* and C2-V3 of *env*	subtype C: 123 A/C recombinant: 3 subtype A: 1 CRF01_AE: 1	(Deshpande *et al* 2004)
Southern India and West Bengal	256	Subtype C specific-PCR	subtype C: 253 subtype A: 1 B/C recombinant: 2	(Siddappa *et al* 2004)
Southern India and West Bengal	115	Subtype C specific-PCR	subtype C: 112 subtype B: 1 subtype A: 1 B/C recombinant: 1	(Siddappa *et al* 2005)
Maharashtra	12	Nucleotide sequencing of *pol*	All sequences were of subtype C.	(Eshleman *et al* 2005)
Southern India	50	Nucleotide sequencing of *pol*	All sequences were of subtype C.	(Balakrishnan *et al* 2005)
Manipur	14	HMA for *gag* and *env*	subtype C: 9 subtype B: 1 B/C recombinant: 4	(Tripathy *et al* 2005)

increased to substantial proportions of all infections. The first genetically recombinant strain from India was reported in 1999 from Pune, Maharashtra. The recombinant strain showed mosaic between subtype A and C. The nucleotide sequencing of near full-length genome showed the backbone of subtype C and insertion of two segments of subtype A in the *env-nef* region (Lole *et al* 1999). Three more A/C recombinant viruses have been reported from Maharashtra (Deshpande *et al* 2004) based on *pol* and C2-V3 region of *env* gene sequence analysis. Three B/C recombinant viruses have been reported from Karnataka. These viruses showed subtype C in LTR and subtype B in *env* region (Siddappa *et al* 2005; Siddappa *et al* 2004). The B/C recombinant viruses have also been reported from Manipur (Bhanja *et al* 2005; Tripathy *et al* 2005) and Calcutta (Bhanja *et al* 2007). The features of recombinant viruses reported from India are summarized in table 3.

Full length sequences of four recombinant viruses isolated from Manipur have recently been reported (Lakhashe *et al* 2008a, b). The structures of these viruses are shown in

Table 2. Studies that show monophyletic lineage among HIV-1 viruses from India

HIV-1 gene	Number, source and genome region of virus studied	Reference
gag	p24-p7 region of 14 viruses from IDUs residing in Darjeeling	(Mullick *et al* 2006)
	Full-length *gag* gene of 24 viruses from north India isolated in year 1995-1999.	(Gupta *et al* 2005)
	Full-length *gag* gene of 6 viruses from recently infected patients from Pune isolated in year 2000-2001.	(Kurle *et al* 2004)
env	Full-length *env* gene of 28 viruses from north India isolated in year 1995-2004	(Khan *et al* 2007)
	gp120 region of 14 viruses from IDUs residing in Darjeeling	(Mullick *et al* 2006)
	gp120 gene of 6 viruses from recently infected patients from Pune isolated in year 2000-2001.	(Kalpana *et al* 2004)
	*gp41*region of 8 viruses from Pune	(Agnihotri *et al* 2006)
nef	43 viruses from different states of India isolated in year 1999-2001.	(Kumar *et al* 2006a)
	14 viruses from Pune isolated in year 1996-2001.	(Jere *et al* 2004)
tat	14 viruses from IDUs residing in Darjeeling	(Mullick *et al* 2006)

figure 1. Three recombinant viruses (NARI-FL-RC1, RC2 and RC3) showed a backbone of subtype C virus with a single insertion of the subtype B genome in the envelope region whereas one virus showed backbone of subtype B virus with three subtype C insertions (NARI-FL-RC4). The sequences indicated that these recombinant viruses were distinct from B/C recombinant CRFs CRF_07, CRF_08 circulating in China and CRF_04BR137 circulating in Brazil. Two of the viruses (NARI-FL-RC1 and RC3) showed mosaic identical to the Argentinean B/C recombinant ARE195FL. However, neighbor-joining analysis followed by phylogenetic clustering showed that *gp120* sequence of these viruses clustered with Thai B sequences, while *gag* sequences clustered with an Indian subtype C sequence, suggesting a unique ancestral origin of these recombinants. Also it needs to be seen whether these recombinant viruses represent an emerging circulating recombinant form. This is significant as it may have impact on the molecular virology of HIV.

3. Drug resistance in HIV infection in India

High genetic diversity in HIV strains also has implications on the success of antiretroviral treatment. High mutation rates and selection of drug resistant mutants in presence of anti-retroviral drugs can lead to the failure of ART. Multidrug treatment and strict adherence to ARV regimens can reduce the chance of emergence of drug resistance mutations. In 2004, free anti-retroviral treatment was made available by Government of India and as of today more than 150000 HIV infected persons are receiving the treatment. For ensuring the success of ART rollout plan it is necessary to ensure the monitoring of drug resistant mutants.

In one of the earlier reports from India, Eshleman *et al*. reported the absence of HIVDR among newly infected individuals, and limited evolution of HIVDR mutations during 2 years of follow-up (Eshleman *et al* 2005).

Deshpande *et al* genotyped the *pol* gene (PR: 1–99; RT: 1–243) from 128 drug-naive patients in early stages of HIV disease from Mumbai, India. The M184V resistance mutation was observed in two of the 128 samples studied (Deshpande *et al* 2004).

Sen *et al* estimated the prevalence of HIV drug resistance (HIVDR) mutations in the HIV protease (PR) and reverse transcriptase (RT) genes from peripheral blood mononuclear cells (PBMCs) in a study population of 25 antiretroviral (ARV) therapy-naive and 50 ARV-experienced chronically infected patients from Pune city, India. There were no observable HIVDR mutations in ARV-naive patients. The ARV-experienced patients had a history of exposure to nucleoside reverse transcriptase inhibitor and non-nucleoside reverse transcriptase inhibitor combinations. At least one HIVDR mutation in RT was observed in 29 (80.55%) of 36 ARV-experienced patients with evidence of failing therapy. M184V was the most common observed HIVDR mutation. No PR major mutations were observed among ARV-experienced patients. Higher prevalence of proviral HIVDR mutations in PBMCs was associated with irregular adherence to therapy ($p<0.05$) and HIV-1 RNA levels <1000 copies/ml ($p< 0.001$) (Sen *et al* 2007a).

Similar findings have been reported by Arora *et al* (2008) and Balakrishnan *et al* (2005) reported 6%, 14%, and 20% mutations at subtype B-defined NRTI, NNRTI and major protease drug resistance positions, respectively, in 50 treatment-naive patients from southern India, all being infected with subtype C. Though these mutations were present at ARV resistance positions, none has been associated with ARV drug resistance.

In anti-retroviral experienced patients, a large number of drug resistance mutations were observed. In a report

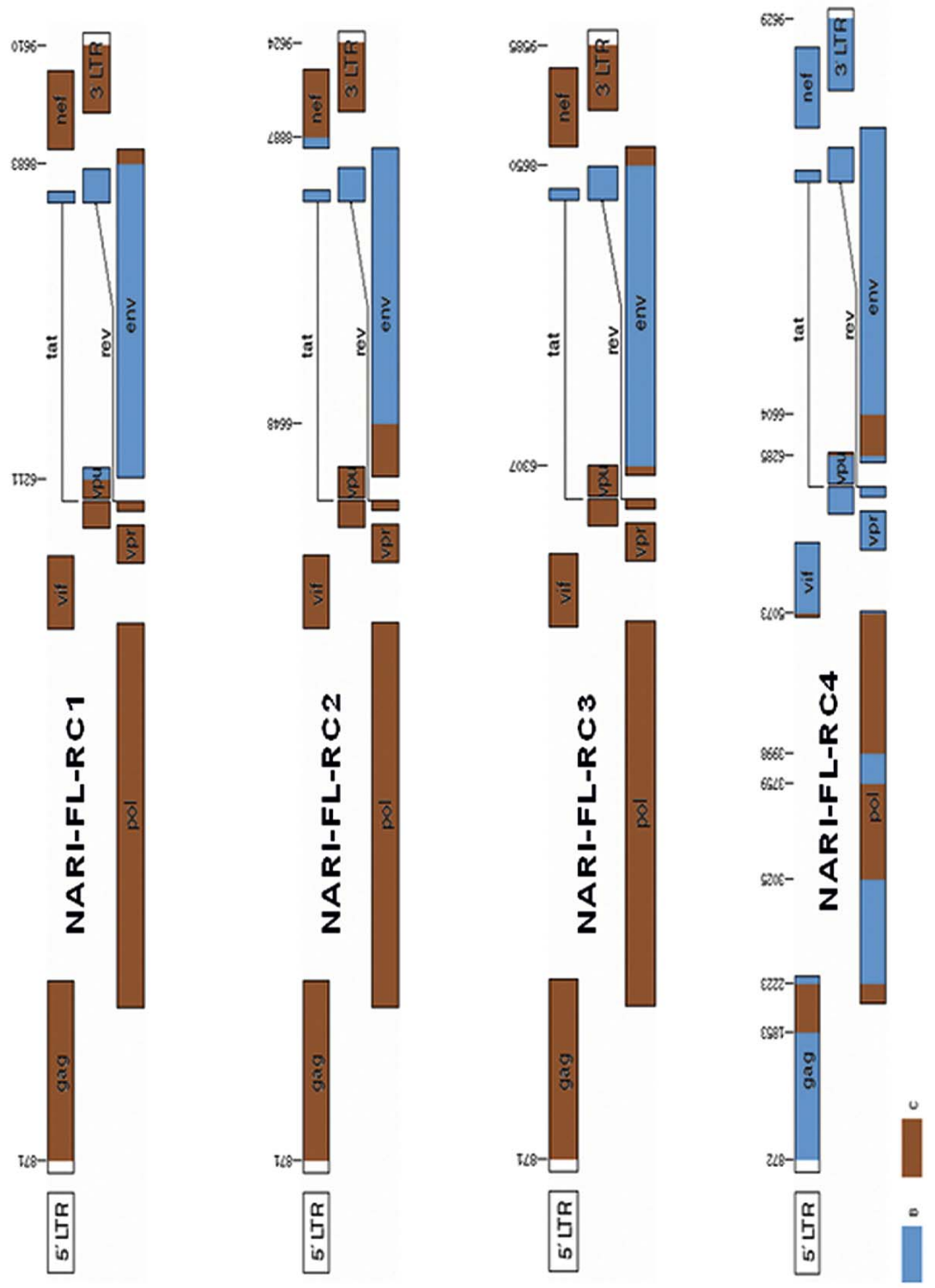

Figure 1. Genomic organization of B/C recombinant viruses identified in Manipur, India.

Table 3. Genetically recombinant HIV-1 strains from India

State /region	Recombination between	No of strains	Reported features	Reference
Maharashtra	A and C	1	Multiple breakpoints in *env, nef* and 3'LTR	(Lole *et al* 1999)
Maharashtra	A and C	3	Discordant subtype for *env* and *pol*	(Deshpande *et al* 2004)
Karnataka	B and C	3	LTR: subtype C *env:* subtype B	(Siddappa *et al* 2005; Siddappa *et al* 2004)
Manipur	B and C	3	*gag:* subtype C *env*: subtype B	(Lakhashe *et al* 2008a; Tripathy *et al* 2005)
Manipur	B a nd C	2 1	*gag:* subtype B *env*: subtype C *gag:* subtype C *env*: subtype B	(Bhanja *et al* 2005)
Manipur	B and C	1	subtype B backbone with three subtype C insertions in *gag* and *pol*	(Lakhashe *et al* 2008b)
Calcutta	B and C	16 1	*env:* subtype C *nef*: subtype B *env:* subtype B *nef*: subtype C	(Bhanja *et al* 2007)

from NARI (Sen *et al* 2007b), one or more HIV resistance mutations were observed in 81.81% of the 33 antiretroviral treatment-experienced study participants with evidence of virologic failure, with M184V being the most commonly observed resistance mutation (69.7%). Two out of four participants with protease inhibitors (PI) experience harbored multiple PI-associated resistance mutations. No resistance mutations were observed in 22 treatment-naive study plasma sequences.

In a study in infants whose mothers had received a single dose Nevirapine as a prophylaxis against mother to child transmission of HIV, high percentage of HIV resistant mutations were seen at 48 hours (10.5 %) and at two months (46.15 %) after birth (Kurle *et al* 2007).

Data regarding resistance to NRTIs and NNRTIs are scarce in children. Soundararajan *et al* evaluated the pattern of polymorphism and potential drug resistance mutations in HIV-1 isolates from 48 children naive to antiretroviral therapy attending the outpatient clinics of the Tuberculosis Research Center in Chennai (Soundararajan *et al* 2007). All the samples showed significant polymorphisms in both RT and protease genes, but none had major drug resistance mutations.

The findings overall show that HIV drug resistance mutations are not common in drug-naïve populations. However, drug experienced patients show HIVDR mutations at higher frequency.

4. HIV-specific Immune response in Indian patients

Although considerable data has been generated on the molecular virology of HIV in India the data on the immune response in Indian HIV-1 subtype C infection is very limited. Such data is crucial for the development and selection of HIV preventive vaccines suitable for use in India. India's first HIV vaccine trial was conducted using an Adeno-Associated Virus based vaccine that carried *gag*, RT and truncated Protease genes from HIV-1 subtype C strain from South Africa. This strain has 98% homology with consensus Indian subtype C sequence. The trial showed very low HIV specific immune response in the vaccine recipients (Mehendale *et al* 2008). A Phase IIb clinical trial of Merck's Adeno-virus(Ad5) based vaccine was stopped before completion as there was no protection offered. This Ad5 based vaccine was a trivalent vaccine that encodes the Gag, Pol and Nef proteins of HIV. The analysis of the unblinded data showed that there were more breakthrough infections in vaccine group (Robertson *et al* Abs No 89LB CROI 2008). This has prompted the scientific community to return to the drawing board and reemphasize the fact that there is need for greater research in immune responses against HIV infection.

A series of studies published by the National AIDS Research Institute have looked at the Cytotoxic T lymphocyte (CTL) responses in Indian patients. The CTL responses in recently infected individuals were found to be strong and polyclonal in nature. The Gag, Nef and Env-specific responses (frequency of the CTL precursors) were measured using vaccinia expressing subtype B antigens in ^{51}Cr release assay (Paranjape *et al* 1998). The study was extended further and the Gag-specific responses were estimated using IFN-γ secretary ELISPOT assay in recently infected Indian patients from Pune. The study showed marginally significant negative correlation between subtype C Gag-specific CTL response and plasma viral load (Thakar *et al* 2002). However the presence and the intensity of CTL responses in chronic HIV infection did not show any correlation with the indicators of disease progression, CD4 count or plasma viral load. Thakar *et al* (Thakar *et al* 2005) demonstrated that the patients infected with HIV-1 C from India recognize the antigenic determinants from the same segments of Gag and Nef proteins that are recognized by the HIV-1 subtype C infected patients from South Africa and Botswana. This observation has implications in vaccine

development strategies for HIV-1 infected population. There is possibility that a vaccine candidate that may generate immune response against CTL epitopes recognized across different strains of subtype C.

The CTL epitopes recognized by Indian patients were mapped using algorithm where peptides recognized by a person were identified in the IFN-γ secretary ELISPOT and the Computer aided algorithm was used to define the epitope within peptide in context with HLA type of the individual. This algorithm predicted 26 epitopes with ninety percent of them in the conserved region in HIV-1 C Gag and Nef proteins. Six of these epitopes were novel and newly identified in Indian patients. Similar observations were reported by a group from All India Institute of Medical Sciences. They used intracellular cytokine estimation to identify HIV-1 C Gag and Nef peptides recognized by Indian patients (Kaushik *et al* 2005). All these studies revealed that the conserved regions are recognized by Indian patients.

A study by Cecilia *et al* has demonstrated that Indian subtype C viruses do not show shift from R5 (virus strains using chemokine receptor R5) phenotype to X4 (virus strains using chemokine receptor X4) even after progression to AIDS (Cecilia *et al* 2000). Such shift is seen in HIV-1 subtype B infections. However, the significance of this observation has not yet been understood. This might be the result of lesser CXCR4 expression on the CD4 lymphocytes in comparison with CCR5 expression in Indian patients (Ramalingam *et al* 2002).

Along with HIV-specific CTL responses, neutralizing antibody response may play an important role in HIV immunopathogenesis. Although a considerable work has been carried out on the neutralizing antibody response worldwide some of the challenges in studying neutralizing antibody response include selection of optimal panel of viruses for neutralizing antibody assays, identification of neutralizing antibody epitopes shared by the primary isolates representing virus strains circulating in the population. Lakhashe *et al* (2007) studied neutralizing antibody response in 239 HIV-1 subtype C infected persons from India against a panel of 12 primary HIV-1 subtype C isolates from India. The study revealed that there is considerable cross reactivity in neutralizing antibody response against different strains and by different plasma. This is indicative of the extensive sharing of neutralizing antibody determinants. This provides a window of opportunity for immunological interventions such as vaccine.

5. Host genetic factors and HIV disease

The host genetic factors that have been found to influence acquisition of HIV infection or course of disease progression include HLA polymorphism, polymorphism in the chemokine receptor/ligand genes. Several studies on Indian population have reported association of host factors with HIV infection and disease. The table 4 summarizes the results of the studies. In a countrywide study on 1871 samples representing large fraction of people of India, it was reported that the frequency of CCR5Δ32,rs333 was extremely low in Indian Population with only clusters from north showing the mutation.(Indian Genome Variation Consortium 2008) The C-type lectin DC-SIGN which is expressed on dendritic cells is involved in capture and transmission of HIV-1 from mucosal surface to lymph nodes. A potential association of DC-SIGN neck domain repeats polymorphism and risk of HIV infection is currently under debate. Rothore *et al* (2008) showed that risk for HIV infection is not associated with repeat region polymorphism in the DC-SIGN neck domain among north Indians (Rathore *et al* 2008). A study among Western Indian population showed that HLA types B*3520, B*1801 and Cw*1507 alleles were independently associated with HIV-1 infection. Further, it was observed that A*110101-B*3520-Cw*1507 haplotype was significantly increased among the AIDS patients (Shankarkumar *et al* 2003). Several novel HLA types have been reported from India (Mehra *et al* 2002). The role of genetic factors in HIV infection is far from clearly defined. More and more genes are being identified to be influencing HIV susceptibility and disease

Table 4. Studies from India on host genetic factors that have been found to influence HIV disease

Gene	Mutation/ Polymorphism that influence HIV acquisition/ disease progression	Study population	Frequency of mutation	Reference
CCR5	32 base pair deletion	100 healthy individuals	1%	(Husain *et al* 1998)
		500 healthy individuals from north India	1.5%	(Verma *et al* 2007)
CCR2	Valine to Isoleucine mutation at 64th amino acid position	500 healthy individuals from north India	9.1%	(Verma *et al* 2007)
SDF 1	Our of two polymorphic forms i.e. SDF 1-3'A and SDF 1-3'G (found in the 3' untranslated region), SDF-3'A has been found to be associated with slow disease progression	100 healthy individuals from north India	SDF-3'A was found in 40% individuals	(Ramamoorti *et al* 2001)
		500 healthy individuals from north India	SDF-3'A was found in 20.4% individuals	(Verma *et al* 2007)

progression. Studies in Indian population that address this issue are necessary.

HIV-1 infection presents a complex scenario. Both viral and host factors are important in deciding the way the HIV infection will progress. The host factors include the innate immunity, host genetic factors and host immune response. The molecular virology studies indicate that Indian HIV-1 subtype C viruses segregate away from the subtype C viruses from other geographical areas. The limited immune response studies have indicated that there is considerable cross reactivity in CTL responses and neutralizing antibody responses. This offers an opportunity to develop effective tools for immunological intervention.

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*e*Publication: 15 October 2008