

# HIV/AIDS research in India: past, present and future

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Since the first report on the serodiagnosis of HIV-1 infection in 1987, several laboratories from India have contributed significantly to the knowledge of HIV/AIDS over the years. These reports span a wide range of disciplines including serological and molecular characterization of the viral strains circulating in India; elucidation and characterization of the recombinant strains; study of the economic impact of the viral infection; examination of the immune profile of the host; delineating the natural history of HIV-1/AIDS; evaluation of the awareness among general populations; molecular validation of the primary viral isolates and study of their pathogenic properties including neuro-pathogenesis; developing and evaluating alternative therapeutic strategies for HIV/AIDS; vaccine development and validation in human clinical trials, etc. The quantum of academic research in India is driven mainly by individual investigators affiliated to research institutions, universities and clinics spread across the country, with a few research institutes and non-government organizations making significant contributions. Given the space restriction and the volume of the research that has appeared in peer-reviewed journals, it would not be possible to encompass all this work in the present review. This review primarily focuses on a few basic and application-oriented research reports that appeared over the years from India. Additionally, attention will be drawn to several other elegantly written reviews that focused on areas that have not been covered here.

**Keywords:** HIV/AIDS, seroconverters, viral infection, viral molecular epidemiology.

## Viral molecular epidemiology

In India, since the first report of HIV infection in 1987 (ref. 1), the rate of viral incidence and prevalence remained low and stable over the years. According to the National AIDS Control Organization (NACO), the over-

all viral prevalence among general adult population of India remains at 0.36% as of 2006 which translates to approximately 2.5–3.0 million cases. Although the reasons for significantly low levels of viral incidence in India as compared to African countries, where subtype C is also the predominating strain, are not completely understood, socio-economic conditions, cultural factors, host genetics and family traditions may have played or are playing an important role. Of note, among the high-risk groups, including injecting drug users (IDU), men who have sex with men (MSM) and female sex workers (FSW), the infection rates are higher than 5% (NACO, India), suggesting that host resistance may have a limited significance. Although India harbours the second largest number of HIV infections in the world, the number of scientific studies and research publications appearing from India rather remains small and inadequate.

The topic of molecular epidemiology and distribution of diverse viral subtypes in India has been reviewed earlier<sup>2–8</sup>. Lakhashe *et al.*<sup>9</sup> have recently reviewed the sentinel surveillance system of India which is mainly responsible for the periodical evaluation of seroprevalence of HIV-1 infections in India. The molecular nature of the HIV-1 strains in circulation in India, the magnitude of genetic variations among the circulating viral strains, and the implications of such genetic variations for drug resistance and control measures have been reviewed<sup>9–11</sup>. The present review will attempt at highlighting a few unique characteristics of the HIV-1 infections in India. A few research publications that appeared during the past one or two years will also be discussed.

Several characteristics of the viral epidemics of India are unique and distinct from the epidemics of other countries. First, the viral epidemics of India by and large have been driven primarily by subtype C strains<sup>12–17</sup>. Although over the decades, several non-subtype C strains of group M have been reported by several groups, subtype C strains have been responsible for viral incidence up to as high as 85–99% (refs 12, 18–21). Depending on the phylogenetic relatedness of the viral strains of the two countries, the viral infections of India are believed to have been introduced from South Africa<sup>22</sup>. A limited number

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of publications suggest further that following the initial introduction, the viral strains of India have undergone additional genetic variation quite unique for the Indian context such that the sequences of the viral genes typically form a monophyletic cluster<sup>23,24</sup>. Heterosexual contact remains the primary route of entry for HIV in India<sup>25-29</sup>. Second, molecular nature of the viral strains circulating in the north-eastern states of India is quite distinct from that of the mainland India and forms the second focus of the viral epidemics. These strains are spread mainly through drug abuse and are phylogenetically related to the subtype B viral strains of Myanmar and China<sup>12,13,27,30</sup>. Third, several non-subtype C viral strains have been reported from different parts of India<sup>12,15,18-20,31-37</sup>. Subtype A appears to be most common among such strains closely followed by subtype B viruses and additionally, with subtypes D and A/E also documented. Despite the regular appearance of the non-subtype C strains and the reporting by several groups, it is not clear why these viral strains do not seem to initiate their own epidemics and infection foci in India. Fourth, a large number of publications reported the presence of recombinant viruses from different regions of India. These publications have been reviewed recently<sup>11</sup>. Most of these studies are not only limited in sample size but also a lack of follow up of the findings makes it unrealistic to estimate the real incidence of the recombinants in circulation. Given the regularity and generality with which the recombinant viruses have been identified by several groups and the extensive variation of the sporadic viral sequences reported, it is rather certain that a large number of diverse HIV-1 strains are in circulation in India. Lastly, in addition to HIV-1, infections of HIV-2 have been detected from different parts of the country. Since the first report in 1991 (ref. 38), a large number of publications reported the presence of HIV-2 in India. Given that the initial studies primarily used serological detection for HIV-2 diagnosis, the real incidence of the viral infections with HIV-2 alone and dual infections with both HIV-1 and HIV-2 reported during the early phase was perhaps unreliable<sup>39-45</sup>. Several later studies confirmed HIV-2 using different molecular approaches<sup>46-54</sup>. Two different groups from India generated molecular clones of HIV-2 from primary clinical isolates<sup>55,56</sup>. The incidence of HIV-2 infection and that of coinfections with HIV-1 appears to be rather small. A consensus emerged that the HIV-2 strains of India are closely related to subtype A phylogenetically. Although the presence of HIV-2 in India represents a unique situation, given the small numbers and the limited expansion of this viral group, HIV-2 may not be considered to be a significant problem for India.

Rao *et al.*<sup>57</sup> recently developed a mathematical model to predict the number of people living with HIV/AIDS over the next five years period taking into consideration targeted interventions among high risk groups, provision of anti-retroviral therapy, and behaviour change among

HIV-positive individuals. Their work identified the spread of the viral epidemics in India to be less severe compared to the trend observed in the late 1990s.

### Natural history of viral infection in India

Unlike in the industrialized countries, the Indian HIV epidemics are characterized by heavy incidence of several opportunistic infections, a situation similar to that of various African countries. The most common infections associated with AIDS development are pulmonary tuberculosis (up to 49%), *Pneumocystis carinii* pneumonia (6%), cryptococcal meningitis (5%), central nervous system toxoplasmosis (3%) and cytomegalovirus retinitis, among others<sup>58-62</sup>. Infections of the nervous system have been reviewed and tuberculosis followed by cryptococcosis and toxoplasmosis in various combinations are the major neuropathologies in the Indian clinic<sup>63,64</sup>. Viral infections and HIV-associated neoplasia seem to be infrequent, including primary CNS lymphomas. HIV encephalitis and HIV-associated dementia too are considered infrequent.

The excessive parasite and microbial burden is believed to constitute a constant source of chronic immune activation leading to enhanced immune activation markers in the normal population of Africa<sup>65</sup>. A positive correlation between immune activation and the rate of disease progression has been observed<sup>66</sup> and proposed to be an important parameter underlying faster disease progression in Africa<sup>67</sup>, although a difference in the rate of disease progression between industrialized and low-income countries remains highly controversial<sup>68</sup>. No studies from India examined the important association between chronic immune activation due to heavy parasite and microbial burden, and its influence on the dynamics of disease progression.

Considering the presence of subtype C in India, the existence of excessive parasite burden and the differences in the profile of host-restriction factors including the HLA variations, it is of concern whether the rate of disease progression in India differs significantly as compared to what has been reported from the industrialized nations. Unlike in Africa, studies considering important questions on natural history of the infection have been scarce from India and as a consequence, no clear understanding has emerged on any of these important issues.

In addition to the problem of inadequate information on the viral natural history, the applicability of the CDC classification system to the Indian context appears to be controversial. In 1993, CDC revised its definition of AIDS-related illness by including the CD4 cell count drop below 200 cells/ $\mu$ l in addition to including tuberculosis, recurring bacterial infections and invasive cervical carcinoma<sup>69</sup>. In the developed countries, CD4 cell dynamics including the absolute numbers, percentage of CD45 cells and the rate of decline with disease progression play an important role as a valuable prognostic marker, although

in combination with the viral load<sup>70-72</sup>. CD4 cell count, however, may vary significantly depending on several factors including geographic location, ethnic origin, age, gender and changes in total and differential leucocyte count. The reliance of the CDC classification system on the CD4 cell count to fall below 200 cells/ $\mu$ l to predict AIDS-related illness has led to serious confusion according to its validity in the underdeveloped countries including Africa<sup>73-75</sup>, China<sup>76,77</sup> and India<sup>78-81</sup>. Since the baseline CD4 cell count in the healthy individuals could have a direct impact on HIV disease progression and the dynamics of CD4 cell drop, several research groups attempted to determine the ranges of CD4 profile in normal Indian populations. In one of the largest studies reported from India, a national task force constituted by the Indian Council of Medical Research (ICMR), performed immunophenotyping by flow cytometry for CD3, CD4, CD8, CD19, CD16 and CD56 populations in peripheral blood samples from a total of 1027 healthy Indians hailing from different parts of India<sup>82</sup>. This study did not find significant differences in the ranges of several T- and B-cell sub-populations tested, with the exception of the CD4 cells, in healthy Indians as compared to American standards reported previously<sup>83</sup>. Importantly, this study identified significantly low CD4 numbers in the healthy populations of the southern Indian states as compared to those of the northern and western populations. The validity of this finding is questionable given that no clinical validation of the flow cytometry assays was established among the various laboratories that participated in this study and additionally different laboratories used different antibodies in the analysis. Furthermore, in a different study, Kannangai *et al.*<sup>84</sup> from Vellore in southern India identified significantly higher CD4 cell numbers ( $1048 \pm 210$  cells/ $\mu$ l) in their clinical cohort as compared to the ICMR study. Kannangai *et al.*, however, used a non-standard assay, the Capcellia CD4/CD8 whole blood assay, for the evaluation of the CD4 count in their clinical cohort. The reliability and significance of these observations remain to be ascertained in future studies. Several other reports from India too identified relatively low levels of CD4 numbers in healthy adult populations as compared to the reference standards of the developed countries<sup>78,80,81,85-87</sup> whereas one report did not<sup>88</sup>. Many of these studies are limited with respect to smaller sample size, restricted geographical distribution and less stringent technology employed to measure CD4 cell count.

A small number of reports attempted to study the profile of disease progression in India. Mehendele *et al.*<sup>89</sup>, in a prospective cohort study of 46 drug-naïve recent seroconverters, determined a median HIV viral load set point of 28,729 RNA copies/ml and a corresponding median CD4 count of 644 cells/mm<sup>3</sup>. Interestingly, the study found that the initial changes in viral load and CD4 values in Indian seroconverters were similar to those of untreated HIV-infected seroconverters from a US Multicentric

AIDS Cohort study of 33,759 RNA copies/ml and 599 cells/mm<sup>3</sup> respectively, at six months from seroconversion<sup>90</sup>. Despite these similarities, the study proposed a rapid disease progression in the Indian clinical cohort with a median decline of CD4 count of 120 CD4 cells/year and a concomitant median early increase in plasma viral load of 10.17 log<sub>10</sub> RNA copies/ml/year (8274 RNA copies/ml/year) during the study period of 720 days. In a different study by Ding *et al.*<sup>91</sup> that appeared recently from India, the authors failed to find such sharp depletion in CD4 cell count with disease progression. Hira *et al.*<sup>92</sup> detected faster rate of disease progression among the heterosexual cohort in Mumbai in a study involving a prevalent cohort of 1009 seropositive and drug naïve subjects comprising of 488 asymptomatic, 259 with AIDS-related complex (ARC), and 262 with AIDS as compared to what was reported for homosexual men and haemophiliacs of the developed countries<sup>93</sup>. The study determined the time taken from the asymptomatic stage to ARC, ARC to AIDS using time series analysis and AIDS to death using Weibull curves. The median incubation periods for progression were found to be 7.9 years for HIV to AIDS, 1.9 years for ARC to AIDS and 19.2 months for the median survival after developing AIDS. Kumarasamy *et al.*<sup>94</sup> in a retrospective analysis of 594 patients in Chennai, southern India, found that the mean duration of survival for the study participants was 92 months from the date of HIV serodiagnosis. This study was based only on CD4 cell count and concomitant viral load changes have not been studied in disease progression<sup>95</sup>. Improved survival among HIV seropositive subjects has been recorded in a clinical cohort by the same research group. Singh *et al.*<sup>10</sup> have recently reviewed the natural history of HIV infection among children in India.

### Immunology of viral infection

HIV-1 is a uniquely difficult target to develop immunological intervention against. In this regard, one of the main hindrances has been our lack of understanding as to what constitutes protective immunity to the virus. A small number of studies from India have focused on examining immune response to HIV. Anti-HIV-1-specific T cell responses in early HIV-1 infection have been found to be important in determining the course of disease progression. A number of studies have examined cytotoxic T cell (CTL) responses in HIV seropositive subjects. In one of the first reports from India, Paranjape *et al.*<sup>95</sup> identified strong CTL responses to Gag and Nef in recent seroconverters. A subsequent report from the same group extended this work and demonstrated that immune responses especially to Gag controlled viral replication in the early phase of the infection<sup>96</sup>. Studies from Delhi in northern India indicated that both CD4(+) and CD8(+)T cells display higher magnitude of immune response to p24/Gag with the induction of IFN- $\gamma$  secretion being significantly

higher than that of IL-2 (ref. 97). Studies from Pune, western India detected several immunodominant epitopes in Gag and Nef proteins of which a few epitopes were novel<sup>98</sup>. A similar study recently has identified additional novel epitopes in Gag and Nef proteins<sup>99</sup>. The identification of Gag- and Nef-specific responses across HIV-1 infected Indian populations and targeting epitopes from multiple immunodominant regions may provide useful insights into the designing of new immunotherapy and vaccines.

Study of the cytokine dysregulation has been a research priority for some of the Indian laboratories. Studies from CMC, Vellore in southern India identified a direct correlation between increased levels of plasma interleukin (IL)-10 and a rapid fall in CD4 cell count with an associated disease progression to AIDS<sup>100</sup>. In a comparative analysis from Vellore, plasma IL-10 levels were found to be significantly higher among HIV seropositive subjects as compared to normal healthy controls<sup>21</sup>. Furthermore, the same study also reported that in this southern Indian cohort, IFN- $\gamma$  levels among symptomatic and AIDS groups were significantly elevated as compared to asymptomatic subjects. This study also identified a shift in the cytokine profile from Th1 to Th2 with disease progression similar to what was previously observed in the West. In a study from AIIMS, Delhi, northern India, TNF- $\alpha$  levels were also found to be higher in HIV seropositive subjects as compared to normal healthy controls or patients with tuberculosis with wasting<sup>101</sup>. Wanchu *et al.*<sup>102</sup> found that  $\beta$ -2 microglobulin levels in their northern Indian clinical cohort were significantly elevated in the viral infection and could be used as a potential marker for disease progression. A study from Vellore in southern India reported that the mean MIP-1 $\alpha$  and RANTES levels among the HIV-1 infected individuals were significantly higher while those of MIP-1 $\beta$  were lower in their cohort as compared to healthy controls<sup>103</sup>.

### Host and viral factors

Susceptibility to the viral infection, viral transmission, disease progression and response to antiviral therapy all have been attributed to variability in multiple host genes as well as viral factors. One of the most exciting discoveries in the field of HIV-1 infection and progression has been the remarkable protection against HIV-1 among individuals who were homozygous for CCR5  $\Delta$ 32 mutation. This deletion common in the USA and the UK is extremely rare in India<sup>104,105</sup>. The promoter regions of CCR5 and other chemokine receptors are highly polymorphic and haplotype variants possibly accelerating disease progression of HIV-1 have been reported from the All India Institute of Medical Sciences (AIIMS), Delhi in northern India<sup>106,107</sup>. Clearly multiple factors regulate and modulate viral proliferation including several cellular genes, virus factors, micro-RNAs, HLA antigens, NK-cell receptors, etc. A computational prediction by Brahma-

chari's group at the Institute of Genomics and Integrative Biology, Delhi identified several targets for specific human miRNAs in the HIV-1 genome<sup>108</sup>. Subsequent biological evaluation confirmed that the cellular miRNA, hsa-miR29a, interferes with HIV-1 replication by down regulating Nef expression from the cell surface<sup>109</sup>. The copy number of the CCL3L1 gene, which exerts a powerful influence on disease progression to AIDS, has been shown to vary among different regions in the world<sup>110</sup>. In a north Indian clinical cohort, a detailed analysis of the variations in several chemokine and chemokine receptor genes failed to detect an association between the genetic variations studied and susceptibility<sup>111</sup>. Evidence exists that Indian populations preferentially display those HLA antigens that support rapid proliferation of the virus<sup>112</sup>.

Several immunomodulatory functions have been ascribed to the HIV-1 accessory protein, Nef, including down regulation of CD4, MHC-I and MHC-II. Shahid Jameel's group at the International Center for Genetic Engineering and Biotechnology, Delhi identified a novel immunomodulatory function of Nef which possibly contributes to viral pathogenesis. Their studies demonstrated a novel role for Nef in redistributing the costimulatory molecules CD80 and CD86 away from the cell surface in human monocytic cells thus interfering with T-cell activation<sup>113</sup>. Subsequent studies from the same laboratory demonstrated binding of Nef to the cytosolic tails of CD80 and CD86, triggering their endocytosis via Rac-based actin polymerization<sup>114</sup>. Furthermore, they also showed that Nef exploits the same pathways involved in normal MHC-II recycling to target MHC-II to the lysosomal destination<sup>115</sup>. Additional studies from this group also point to the immunomodulatory properties of the viral accessory protein Vpu when they identified MHC-II invariant chain as a Vpu-interacting protein. They demonstrated that Vpu can down modulate the surface expression of mature MHC-II molecules thus contributing to viral persistence by attenuating immune responses during the viral infection<sup>116</sup>.

The vast majority of the publications on biologic and pathogenic properties of HIV-1 have originated from subtype B strains prevalent in North America and Europe. In contrast, little is known on subtype C strains prevalent in Africa and Asia and it is not understood why these strains are responsible for the large majority of the global epidemics. A collaborative effort between two research groups from Bangalore, southern India at the Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR) and the National Institute of Mental Health and Neurosciences (NIMHANS) and one at the Albert Einstein College of Medicine (AECOM), USA proposed that natural variation at position 31 of Tat of subtype C is correlated to the reported low incidence of HIV-associated dementia in India<sup>117-119</sup> as this variation disrupted the monocyte chemokine nature of Tat<sup>120</sup>. This was perhaps the first demonstration of clade-specific molecular variations modulating viral biologic properties and possibly influ-

encing clinical manifestation<sup>121</sup>. Subsequent studies from Pankaj Seth's laboratory at the National Brain Research Institute, Manesar, Haryana using human fetal central nervous system progenitor cell-derived astrocytes and neurons demonstrated clade-specific functional differences in Tat-induced apoptosis in the neurons. The study identified significantly higher levels of neuron death when exposed to clade B Tat protein or DNA as compared to those of clade C<sup>122</sup>. Vinayaka Prasad's laboratory at AECOM and other groups in the USA in collaboration with the JNCASR laboratory using the severe combined immune deficiency (SCID) mouse HIV encephalitis model and intracranial injection of macrophages infected with representative viral strains demonstrated that clade C-induced fewer cognitive and memory errors compared to clade B<sup>123</sup>. A full-length infectious molecular clone of subtype C viral strain was isolated from a subject diagnosed with HIV-associated dementia at the JNCASR laboratory. Unlike the typical subtype C strains that use only CCR5, this molecular clone demonstrated expanded coreceptor requirement including the use of CXCR4 possibly underlying the cause of dementia in this subject<sup>124</sup>.

As suggested in many studies, HIV-1 Tat is considered to be a potential immunomodulator. Studies from National Centre for Cell Science, Pune demonstrated that coadministration of Tat as a genetic vaccine along with gp120 suppressed immune responses to the latter in mice and this immunosuppression was IL-10 mediated<sup>125</sup>. Additional work from this group identified a phase-specific role of IL-12 in regulating the CTL response to gp120 in DNA immunization specifically in the priming of CD4+ T cells that provide help to CD8+ T cells. These data underlie the importance of IL-12 for the priming of antigen-specific T cells and its essential role in the induction of IFN- $\gamma$  by T cells<sup>126</sup>. Previous work from Mitra's group demonstrated a direct binding of Tat to the nuclear factor kappa B (NF $\kappa$ B) sites in the HIV-1 promoter, both *in vitro* and *in vivo*, thus not only providing a novel molecular basis to explain TAR-independent transactivation of the viral promoter, but also pointing toward a potential mechanism of Tat-mediated modulation of cellular genes<sup>127</sup>. Work from the same laboratory pointed at the crucial role played by the heat shock protein 40 (Hsp40) in viral pathogenesis. Their demonstration that Nef not only interacted with Hsp40 but also induced the expression of Hsp40 in HIV-1 infected cells which alludes to the fact that Hsp40 is crucial for Nef-mediated enhancement of viral gene expression and replication<sup>128</sup>. Work from this group also identified that viral infection causes apoptotic cell death by interfering with host cell energy generating machinery thus providing a novel molecular explanation for HIV-induced T cell depletion<sup>129,130</sup>.

In a series of publications, Mahalingam's laboratory from southern India worked out the mechanism of nuclear export of SIV mediated by the accessory protein Vpx. They not only identified a novel import pathway for Vpx

but also proved the significance of Vpx for the optimal virus replication in non-dividing cells such as macrophages<sup>131</sup>. In an extension of this work, they demonstrated that the cellular MAPK signal transduction pathway regulated an early step in SIV infection and that the structural integrity of helical domains is critical for Vpx functions<sup>132</sup>. Subsequent analysis identified two distinct non-canonical nuclear localization signals in Vpx, one at the N-terminal domain interacting with importin alpha and the other at the C-terminal domain also interacting with importin beta<sup>133</sup>. Furthermore, they have shown that Vpx is a nucleocytoplasmic shuttling protein and that the cellular Fyn kinase phosphorylates Vpx and regulates its export from nucleus<sup>134</sup>.

### Intervention strategies and technology development

Despite the success of antiretroviral treatment, HIV/AIDS still poses a major public health problem globally. Development of viral resistance to drugs is common besides toxicity and pharmacokinetic differences between individuals. In the absence of an efficacious HIV-1 vaccine in the near future, other antiviral approaches are being actively considered along with the conventional antiretroviral therapy. These approaches at the global level have been reviewed extensively<sup>135</sup>.

Considering enormous potential of the virus to undergo rapid genetic variation, targeting host factors essential for virus proliferation is expected to offer a great advantage. Several efforts have focused on the coreceptor CCR5 given that the individuals with CCR5  $\Delta$ 32 mutation seem to lead a normal life. As demonstrated initially by Banerjee's laboratory at the National Institute of Immunology<sup>105</sup> and later confirmed by Mehra's group at AIIMS<sup>107</sup>, CCR5  $\Delta$ 32 mutation is quite rare in India. Banerjee's group designed an anti-CCR5 hammerhead ribozyme and a 10–23 motif containing DNA-enzymes<sup>136</sup>. An indeed large number of strategies have also targeted various viral proteins in isolation or combinations. Catalytic RNAs with hammerhead catalytic motif and catalytic DNAs with the 10–23 or 03 8–17 motif have been exploited to knock off either HIV-1 coreceptors or other viral genes. Given the high variation rate, multiple regions of the HIV-1 genes should be targeted, alternatively, a judicious combination of viral as well as host factors must be used<sup>136</sup>. RNA aptamers have attracted a great deal of attention because they are generated via ligand evolution and bind ligands with high affinity. Greater understanding of the biology of HIV-1 replication also allowed the use of TAR and RRE decoys. These small RNA molecules can potentially bind Tat and Rev proteins, and interfere with HIV-1 replication. Thus, multiple HIV-1 interfering RNAs (Rzs, aptamers and siRNAs) can be engineered in the lentiviral vector under different promoters to provide long-term expression. Most of the lentivirus-based gene expression

systems have used HIV-1 backbone. In contrast, Robin Mukhopadhyay's group in Mumbai has recently developed an HIV-2 based gene transfer system<sup>137</sup>.

Significant progress has been made in advancing the application of microbicides in women despite earlier failures. Talwar's group from Delhi developed a novel polyherbal microbicide (Basant) with potent anti-HIV activity which is presently under clinical evaluation<sup>138</sup>.

### Vaccine research

Although development of an AIDS vaccine continues to remain a research priority, few laboratories in India have been actively pursuing this goal. Practical problems, including the lack of a research primate model in the country, are a setback for launching serious vaccine development efforts in India. Vaccine research work in the Indian laboratories, therefore, is mainly restricted to immunization of small experimental animals without the necessary confirmation of the quality of the immune responses against virus challenge. Additionally, an even smaller number of efforts are currently in progress to evaluate a few clade C-based HIV vaccines in human clinical trials.

D. N. Rao's group at AIIMS, Delhi evaluated peptide immunogens derived from Env along with the application of diverse novel adjuvants for inducing immune responses in mice<sup>139-143</sup>. Pradeep Seth's laboratory from the same institute demonstrated antigen-specific immune responses in mice to HIV-1 gp160 delivered as a DNA vaccine<sup>144</sup> or subtype C envelope and core proteins delivered as recombinant modified Vaccinia Ankara (MVA) viruses<sup>145</sup>. Subsequently, using a DNA-MVA vaccine prime-boost formulations encoding *env*, *gag* and protease genes of Indian subtype C, they could elicit high levels of humoral and cell-mediated immune responses<sup>146</sup>. Varadarajan's group at the Indian Institute of Science, Bangalore is actively pursuing the design of novel immunogen entities of gp120 to elicit neutralizing antibodies<sup>147,148</sup>. Ranga's group at JNCASR, Bangalore has been developing Tat-based DNA vaccines for eliciting cell-mediated immune responses<sup>149,150</sup>. Recent work from this group identified a novel B-cell epitope in the cysteine-rich domain of Tat recognized only by the seropositive subjects but not by the natural antibodies present in the healthy controls<sup>151</sup>. Studies performed at NARI, Pune for neutralizing antibody response in HIV seropositive subjects showed extensive cross-neutralization, suggesting presence of shared neutralization determinants among circulating HIV-1 subtype C viruses in India<sup>152</sup>. A recent study from NARI has suggested that HIV-1 envelope regions other than the V3 domain may be involved in generating a neutralization response in HIV-1 subtype C infected individuals<sup>153</sup>.

In a randomized, multicentric, dose escalation phase I clinical trial, 30 HIV-uninfected healthy volunteers at

NARI, Pune (and 50 volunteers in Europe) were recruited between February 2005 and January 2006 to evaluate a recombinant adeno-associated virus serotype-2 protein capsid (tgAAC09) vaccine, developed by the USA-based Targeted Genetics Corporation, encoding HIV-1 subtype C *gag*, protease, and part of reverse transcriptase genes. In the clinical trial that ended in December 2007, the vaccine was found to be safe and well-tolerated in human volunteers. Modest antigen-specific T cell responses were detected to Gag in the vaccinees<sup>154</sup>. Sekhar Chakrabarti's laboratory at the National Institute of Cholera and Enteric Diseases, Kolkata constructed a recombinant MVA TBC-M4 consisting of *env*, *gag*, *tat*, *rev*, *nef* and reverse transcriptase genes derived from clade C of HIV-1. TBC-M4 was evaluated in a second human clinical trial that started in January 2006, at the Tuberculosis Research Centre (TRC) in Chennai, under the auspices of the ICMR in collaboration with the International AIDS Vaccine Initiative (IAVI). This two-year trial phase-I clinical trial involving 32 healthy men and women was found to be safe and elicited virus-specific immune responses in all the study participants although at moderate levels. A scientific communication on the trial results is awaited. The momentum generated by the Chennai trial is leading to a third phase-I clinical trial to be evaluated simultaneously at two different sites in India TRC, Chennai and NARI, Pune. The third trial monitored by the ICMR, IAVI and the NACO is likely to enroll a larger number of participants. Considering the low quality immune responses generated in the Chennai clinical trial by TBC-M4, the new clinical trial will employ DNA priming using ADVAX DNA vaccine encoding HIV clade C *env*, *gag*, *pol*, *nef* and *tat* genes developed at the Aaron Diamond AIDS Research Center in New York City in collaboration with Rockefeller University and IAVI. This will be followed by boosting with TBC-M4 to enhance the breadth and magnitude of the immune response. The TBC-M4/ADVAX combination is also being evaluated in 32 volunteers by St Stephen's AIDS Trust in London in a different Phase-I clinical trial initiated in December 2008 by the IAVI.

### Research priorities for the future

With approximately 2.7–3 million infections, India harbours the second largest viral incidence of HIV in the world. In contrast to the original fears and projections, and despite the presence of the virus in the country for more than two decades, the viral infection has not crossed a danger limit. In many parts of the country, the incidence of the infection has remained below 1% level among general population. With prevalent illiteracy and poverty, unorganized health care system, inadequate knowledge, rampant private blood bank industry, perennial drug trafficking and extensive commercial sex work, it is rather surprising that HIV/AIDS has not become a more serious

medical hazard for India over the years. Furthermore, the viral incidence is even projected to have plateaued in India in the recent years although such a proposition must be carefully evaluated in the coming years because such an assumption could lead to complacency and neglect which could be harmful and counterproductive in the long run.

On one hand, the unique properties of HIV infection of India offer several interesting research questions of academic and medical importance whereas on the other, several practical considerations make it a daunting task to address many of these questions in an appropriate manner. These difficulties, however, are not unique for the HIV research community alone but applicable broadly for other areas of research. Some of these practical issues have been summarized below. First, the small size of the research community makes it far inadequate to address several research questions of significance. This situation leads not only to the lack of a breadth of expertise but also to the absence of meaningful academic collaboration. Second, interaction between basic and clinical scientists is far from being significant. There is an urgent need to bridge this gap. Special grants must be offered by the government funding agencies to promote active research collaboration between basic research laboratories and the clinics including those in the private industry. Third, lack of skilled and trained personnel is seriously hampering research activity in the Indian laboratories. The driving force in most of the laboratories is the involvement of graduate students and project assistants. Absence of more experienced postdoctoral scientists and trained laboratory technicians is a serious setback and refractory to address research questions within a reasonable period of time. Fourth, lack of adequate funds and local technical resources is a serious handicap. An average grant awarded by the Indian funding agencies is significantly small in comparison with international standards compelling researchers to resort to multiple projects and thus spending significant amount of time on administrative and regulatory issues. Some of these problems could be sufficiently addressed by elevating the grant size to the international standards and by increasing the duration of the grants to a period of five years, instead of the present three years, with annual monitoring. Finally, not commensurate with the magnitude of the viral infection, India does not have adequate number of quality clinical cohorts. Thus most of the basic research is seriously compromised. Likewise, there are no high-containment experimental facilities in India for primate/virus challenge experiments seriously affecting vaccine development efforts.

As mentioned above, several characteristics of the HIV infection of India are unique and provide opportunities for meaningful research. The domination of subtype C is intriguing. The host restriction factors, including the MHC, cytokines, chemokines, miRNA, etc. that may favour the selective proliferation of specific viral subtypes must

be identified. Given the size of the country and that the viral infection has spread to general populations in all the states and that the viral evolution is dynamic, independent foci of different subtypes or recombinants may be shaping up viral epidemics in different regions of the country. The finding of an indeed large number of recombinant forms from different parts of the country is quite alarming<sup>11</sup>. A recent study from Banerjee's group reported the presence of more complex forms of recombinants in India<sup>17</sup>. A molecular epidemiological study on a larger number of samples from different parts of the country is urgently warranted to determine the real incidence of the recombinant viruses of HIV-1 and to verify if new viral strains are in the process of emerging. The emergence of new recombinant strains could complicate the development of effective intervention strategies and their implementation. There have been few studies evaluating natural history of HIV-1 in India. Several aspects of the natural history of the HIV infection must be subjected for extensive evaluation including the CD4 cell count differences between the healthy and seropositive populations, the prognostic value of the CD4 cell dynamics for the disease progression, the average incubation periods for seroconversion from the time of infection, for the development of AIDS from seroconversion and the median duration from AIDS to death. The validity of the CDC classification system for the Indian context needs a serious consideration. The influence of chronic infections including the parasite load and malnutrition on the rate of disease progression must be examined. Individuals who might be protected against disease progression or capable of controlling the virus naturally must be identified and the host determinants underlying natural resistance must be evaluated. A nation-wide and coordinated campaign must be initiated to screen for individuals with broad level neutralizing antibodies. Such studies have a direct relevance to not only India but also to other countries where subtype C is the predominant viral strain. Now that first-line anti-retroviral therapy is being given out to people through government-sponsored programmes and that second-line medicines are being rolled out for those who have developed resistance for the first-line drugs, the drug resistance in these populations must be examined. Prognostic markers that might predict drug failure as early as possible must be identified given that CD4 is not a good correlate for this purpose. This could be an important area of research collaboration between the research and clinical groups. The nature of coreceptor requirement by subtype C strains and the near absence of coreceptor switch and its relevance to disease progression must be examined. The magnitude of genetic diversity of the virus is a formidable barrier for vaccine development. Efforts must be waged to develop multi-component vaccines to elicit broader, higher and long-lasting immune responses that offer cross-clade protection. As it is obvious that an effective vaccine will take sometime to come for human

use, serious efforts should also be initiated to rapidly develop an effective microbicide preparation.

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