Host genes that affect progression of AIDS/HIV in India and novel gene therapeutic approaches against HIV

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A multitude of host and viral factors play critical role in susceptibility to HIV-1 infection and its subsequent progression to AIDS. Various host factors involved in HIV-1 infection include the chemokine receptors CCR5, CX3CR1, their ligands, RANTES, SDF-1 and cytokines like IL-10, IL-4, among others. The CCR5∆32 allele is the most important genetic factor known to confer resistance to HIV-1 infection. However, other mutations in CCR5, CX3CR1 and SDF-1 have also been identified in Indian population. Polymorphisms in DC-SIGN, MHC class-I and II molecules are also known to affect HIV-1 progression. These polymorphisms can be utilized as genetic markers for evaluating disease progression and developing effective therapeutics. The review also describes the development of anti-viral therapy, involving the use of catalytic nucleic acids like DNA-enzymes and ribozymes and the expression of ribozymes and si-RNA using lentiviral vectors for stem cell based anti-HIV therapy.

Keywords: HIV-1, CCR5, Polymorphisms, Chemokine receptors, DNA-enzyme, Ribozyme, siRNA, Lentiviral vectors

Introduction

The HIV-1 genetic subtype-C is mostly responsible for driving the epidemic of HIV-1 in India and other regions in the world. The infection is initiated by the successful interaction of HIV-1 gp160 on the viral membrane, CD4 receptor glycoprotein and one of HIV-1 co-receptors on the host cells1-4. This results in membrane fusion and entry of the virus in a susceptible cell. The two major host cells for HIV-1 are macrophages and T-lymphocytes. It is believed that HIV-1 infection is established by CCR5 chemokine receptor (also known as HIV-1 co-receptor) using HIV-1 (called R5 tropic or macrophage tropic). During the long course of disease, viruses that can use other HIV-1 co-receptors are generated. At the terminal stages, viruses that predominantly use another HIV-1 co-receptor called CXCR4 usually predominates (X4 tropics). This co-receptor is predominantly found on T4 helper lymphocytes. As a result of massive replication of HIV-1 in CD4+ve lymphocytes, large-scale lysis of these cells occurs. This severely impairs the ability of the host to mount an appropriate immune response. Thus, detection of X4 tropic virus is a pathogenic event that leads to rapid development of HIV/AIDS. During the long incubation period of this disease, one can also isolate viruses that use both CCR5 and CXCR4 receptors – and are called dual tropic HIV-1 or R5X4 tropic.

There are several chemokine receptors that HIV-1, HIV-2 or SIV can use to gain entry and all are 7-transmembrane G-coupled protein receptors. These receptors play an important role in the early interaction with viruses, participate in signal transduction and play a key role in several immunological reactions that also involves the movement of lymphocytes and macrophages over a chemotactic gradient3. Besides chemokine receptors, other HIV-1 co-receptors such as D6 have also been identified in humans. D6 unlike “classical” chemokine receptors lacks a DRYLAIVHA G-protein interacting domain and fails to mobilize intracellular calcium4.

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AIDS/HIV-1 modifying genes

The CCR5 co-receptor is involved in the initiation and establishment of HIV-1 infection. Therefore, individuals who lack the complete copy of CCR5 gene should be protected against HIV-1 infection. A remarkable 32 base pair deletion (∆32) in the CCR5 gene is quite common among people of European descent. The consequence of such a mutation is that these individuals express a severe truncated form of CCR5 that can neither participate in viral infection nor in signal transduction. These individuals (1% of normal European population) are apparently healthy and seem to lead a normal life. This is not surprising because chemokine system is quite redundant and there are overlapping ligands that other chemokine receptors can potentially use. Several other chemokine receptors or mutants have also been linked with disease modulation, but their impact in the spread of HIV-1 is not as profound as that of CCR5 ∆32 (for reviews see ref. 5).

Besides the ∆32 mutation, several conserved and non-conserved mutations have been reported in the open-reading-frame (ORF) of CCR5 gene also. Recently, a comprehensive analysis of some of the AIDS modifying genes among North-Indians has been reported. They found the frequencies of CCR5 ∆32, CCR2 64I and SDF1-3’A alleles in 500 healthy North Indians as 1.5%, 9.1% and 20.4%, respectively, suggesting greater prevalence of SDF1-3’A and CCR2-64I mutations in comparison to CCR5 ∆32, the lower frequency of which may be related to a higher genetic susceptibility to HIV-1 infection in North Indians. They all confirmed the earlier findings that ∆32 mutation is rare among Indian individuals infected with HIV-1, but other mutations like SDF-1 and CCR264I are quite common.

HIV-1 protective mutation (CCR5∆32) is extremely rare in India

We screened about 500 normal healthy individuals from North-India and found an individual heterozygous for ∆32 mutation. The inheritance pattern of this mutation is also established. Although a rare mutation among North-Indians, this mutation is not that rare (3 heterozygous out of 100 screened) among normal Parsee people (heterozygous form) in Pune (Maharashtra). Precise 32 base pair mutation on a sequencing gel identified in our laboratory (National Institute of Immunology, New Delhi) is shown in Fig. 1 A & B. It is important to mention that this mutation is quite common among people of European descent. Thus, Indian population is genetically more prone to HIV-1 infection.

CCR5 Promoter is highly polymorphic

The cis-acting region of CCR5 gene controlling its expression is highly polymorphic in humans and combinations of haplotypes can dictate fast or slow progression of HIV-1. This probably reflects the varying amounts of CCR5 protein on the host cell membrane. Study suggests that the levels of this protein on membrane surface can influence the ability of the virus to initiate infection. We have carried out limited studies on this aspect among normal healthy North Indians and the mutations observed are shown in Table 1 and Fig. 2 A-D. It seems certain

![Fig. 1 — (A): Sequence showing a precise 32 bp deletion in CCR5 gene; and (B): comparison of wild type and ∆32 mutant sequence of CCR5](image-url)
combinations of haplotypes are predominant in North-Indian population. A more extensive work on CCR5 promoter haplotypes in India has recently been reported which suggests an association of CCR5*59402A with likelihood of acquisition of HIV-1 infection\textsuperscript{12}. These studies also suggested that CCR5 promoter mutations that up-regulate the expression is more frequently found among normal and HIV-1 infected individuals from India.

**CCR5 Promoter mutation (59353) in monkeys**

We have identified two highly polymorphic regions in the three species of monkeys (Langur, Baboon and Rhesus) and found all of them possess remarkable deletion of 5 bases (AACAA – position 225 to 229)\textsuperscript{13}.

**Table 1 — CCR5 promoter polymorphisms/haplotypes among normal healthy North-Indians**

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<tr>
<th>Nature of polymorphism</th>
<th>Normals (n =13)</th>
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For nucleotide positions please refer Gene bank Accession # U95626

**Multiple genes are involved in progression of HIV/AIDS**

**G to A mutation in SDF-1 gene is quite common in India**

The high levels of stromal cell derived factor-1 (SDF-1), a ligand for CXCR4 chemokine receptor in an infected individual may occupy CXCR4 chemokine receptor and not allow the X4 tropic virus to initiate infection\textsuperscript{14}. The G to A transition in SDF-1 gene is very common in India\textsuperscript{15-16}. The functional implication of this observation is currently controversial, but it may exert its effect in combination with other disease modifying mutations in the host. Fig. 3 A-D show the genotyping results from normal humans and monkeys. The monkeys possess a double transition (GG to AA) at the same location, besides other minor changes. The location of the mutation is shown by arrows.

**IL-4 and IL-10 genes also affect the progression of HIV-1**

Interleukins IL-4\textsuperscript{17} and IL-10\textsuperscript{18} may work indirectly by influencing the levels of two most important chemokine receptors CCR5 and CXCR4 in the host cells. Cytokines are highly polymorphic and both IL-4 689T and IL-10 -593 mutations have been shown to affect HIV-1 progression in humans. We screened for these mutations in limited number of normal healthy individuals and have not found these mutations. However, novel mutations in the promoter regions of...
both the interleukins have been observed in simians that may help explain their varying course of HIV/SIV disease (ref. 19 and unpublished observation).

DC-SIGN Intronic mutations among normal healthy individuals
DC-SIGN is involved in the pathogenesis of HIV-1 and probably many other pathogens 20. It consists of 7.5 repeat region of 23 amino acids in the 4th exon. Recent studies 21-23 suggest that variation in the repeat region can affect HIV-1 progression. We screened about 15 normal individuals from North-India, but have found no change in the repeat region. However, four unique intronic single nucleotide polymorphisms (SNPs) along with an insertion of a C nucleotide have been observed immediately before and after 4th exon, respectively (Khan, Sood and Banerjea, Unpublished observation, Fig. 4) among all individuals. The implications of these mutations in splicing reactions are currently being studied.

CX3CR1 mutations
Several mutations in the ORF of chemokine receptor CX3CR1 have been earlier observed among Europeans that modulate the disease progression 24. However, a second SNP of CX3CR1 has shown no significant effect on disease progression 25. We screened six normal individuals and none of them have shown the earlier reported mutations. One normal individual, however, has shown methionine to isoleucine change at the 3rd extracellular loop region (Khan, Sood and Banerjea, unpublished observation).

RANTES promoter mutations in humans and monkeys
Regulated upon activation, normal T-cell expressed, and secreted (RANTES) is a powerful ligand for CCR5 and its high amounts are associated with long-term non-progression to AIDS/HIV-1. It is a powerful chemokine that inhibits R5 tropic infection. There are two remarkable mutations in the promoter region, namely -28G and -403A in humans.
that affect progression of HIV-1. While we have not come across these mutations in humans, four highly polymorphic regions in the promoter region (spanning +78 to -413) have been observed in monkeys. All the monkeys possess -28A and have shown a conserved single nucleotide change in the middle of proximal NFκb sequence (G to A that created a GATA binding site). Recently, we reported that the basal RANTES promoter activity differs significantly among different species of monkeys.

**MIP-1α promoter polymorphisms**

Macrophage inflammatory protein-1 alpha (MIP-1α) is a ligand for CCR5 receptor along with other minor HIV-1 co-receptors. We analyzed 134 bases from the promoter region that constitutes the minimal promoter for genetic variations in humans and monkeys and found unique polymorphism among normal humans and remarkable insertion of specific sequences in monkeys.

**HIV-1 co-receptor CXCR4 promoter mutations in simians**

We compared the 400 bp promoter region of CXCR4 promoter between humans and three most common species of monkeys and observed that mutations are largely caused by single nucleotide changes as compared to CCR5 promoter, which is characterized by large deletions and substitutions. Interestingly, the rabbit CCR5 promoter lacks the second highly variable region.

**Role of major histocompatibility complex (MHC)**

Certain human leukocyte antigen (HLA) class I and II haplotypes can significantly influence the course of HIV-1 progression. Mehra and his co-workers (ref. 32 and references therein) have identified disease-specific genetic changes and observed that Indians display extreme diversity in the MHC region with unique alleles. It has also been reported that genetic make-up may support faster spread of HIV-1 among normal Indians.

**Novel antiviral approaches against HIV-1 co-receptor and HIV-1 genes**

**Ribozymes**

We designed a hammerhead ribozyme against the CCR5 gene. This host gene could be targeted because individuals who lack this gene (due to Δ32 mutation) do not show any abnormality in their growth. This ribozyme cleaves the CCR5 RNA very efficiently both in vitro and in vivo. Thus, efforts are in progress to exploit this by expressing in T-lymphocytes and macrophages via lentiviral vectors.

**DNA-enzymes**

Short stretches of DNA possessing either 10-23 or 8-17 catalytic motifs have been synthesized against the HIV-1 coreceptor-CCR5 as well as several HIV-1 genes. These DNA-enzymes cleave the target RNA very efficiently and interfere with the HIV-1 gene expression. We also designed multi-target DNA-enzymes by placing two DNA-enzymes in tandem. Such a multi-target approach for RNA based genes (for eg. RNA viruses) is required, as they tend to mutate quite frequently. Earlier, we reported the synthesis of mono and multi-target ribozymes against the S1 genome segment of reovirus.

Recently, we found that both ribozymes and DNA-enzymes against Tat/Rev RNA of HIV-1 can prevent intracellular gene expression and T-cell mediated apoptosis. These DNA-enzymes can now be expressed in a mammalian cell by novel single-stranded DNA expression vectors. Recently, we have shown that Rzs and DNA-enzymes can also act synergistically to knock down HIV-1 gene expression. Furthermore, since DC-SIGN receptors on dendritic cells react with HIV-1 envelope and promote replication, we have designed for the first time, DNA-enzymes, targeted against the repeat region of the DC-SIGN receptor. As expected, they cleave the DC-SIGN RNA multiple times in a sequence-specific manner. Further work is in progress to know, if this DNA-enzyme is capable of interfering with HIV-1 replication in a tissue culture system.

**Small interfering RNAs (siRNAs)**

SiRNAs are new gene therapeutic tool that regulate gene expression post-transcriptionally and have been exploited to knock-off the expression of several HIV-1 genes. Sequence-specific degradation of target RNA is achieved by short 21-23nt long siRNAs. We designed novel siRNAs against the host genes CCR5 and CXCR4 and the Tat/Rev regions of the HIV genome and found that all these siRNAs show significant inhibition of HIV-1 replication.
Use of lentiviral vector and stem cells as gene therapy for HIV-1

Once these novel antiviral molecules (ribozymes, siRNA) are obtained, the next task is to place them in a lentiviral vector to achieve a prolonged expression in target cells. Hematopoietic stem cells that form T-cells and macrophages have been transduced to express the antiviral molecules (ribozymes and siRNAs) and potent inhibition against HIV-1 challenge has been observed.

In conclusion, we have been able to identify several unique mutations in the host genes in humans and monkeys that may potentially affect the progression of HIV-1 infection. There are several challenges, most important being the extraordinary power of HIV-1 to mutate and escape any antiviral approach. There is a need for a multi-target approach to either stop or delay significantly the resistant virus from appearing. Besides genetic factors, several other factors including malnutrition and existence of co-pathogens (viral, bacterial and others) in India and other Asian countries may be influencing the course of HIV-1 epidemic. We have also been able to design some promising gene therapeutic tools (ribozymes, DNA-enzymes, siRNA) that may be exploited for therapeutic use.

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References


38 Unwalla H, Goila R & Banerjea A C (1999) Mono and Di-DNA-Enzyme against the regulatory gene TAT of HIV-1 also interferes with the production of virus particles. *AIDS Res Hum Retrovirology* 20, A83


46 Shahi S, Shanmugasundaram G K & Banerjea A C (2001) Ribozymes that cleave reovirus genome segment S1 also protects cells from pathogenesis caused by reovirus infection. *Proc Natl Acad Sci USA* 98, 4101-4106


