GENETIC FACTORS FOR AIDS PROGRESSION: ANALYSES OF HIV-1 RESISTANT GENES IN INDIA AND THERAPEUTIC POTENTIALS OF NOVEL CATALYTIC RNA AND DNA MOLECULES.

Samitabh Chakraborti and Akhil. C. Banerjee*.

Laboratory of Virology, National Institute of Immunology, New Delhi-110067.

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Chemokine receptors or HIV-1 coreceptors are intimately involved in the very early interaction of the host cell with the virus, which leads to the fusion of viral and host cell membrane. Although many coreceptors have been identified, the two most important are CCR5 and CXCR-4. The former receptor is used by the macrophage- tropic isolates during establishment of infection and CXCR-4 using viruses, which are T-lymphocytotropic in nature, are observed during the late stage of infection. Individuals who have a homozygous 32 base pair deletion corresponding to the second extracellular loop of the 7-transmembrane G-coupled protein receptor in CCR5 gene, have been found to resist HIV-1 infection, strongly suggesting that CCR5 is the principal coreceptor involved in transmission. Since chemokines (α & β) can inhibit infection of HIV-1 under *in vitro* conditions, mutations in the open reading frame of chemokine genes or their promoter regions have become important to study. These chemokines (besides others Rantes, Macrophage inflammatory protein 1 α & β) or chemokine receptors are, therefore, promising therapeutic targets for interfering with HIV-1 infection. Novel ribozymes and DNA-enzymes were designed to interfere with HIV-1 replication.

Role of HIV-1 coreceptors or Chemokine receptors in infection /progression

HIV-1 is spreading at an alarming rate in Asian countries including India. It is of great significance to understand what host factors are responsible for this spread. It is well established now that HIV-1, HIV-2 and SIV need an additional factor (called

coreceptors), besides CD4, to gain entry into a susceptible cell. Indeed many such coreceptors have been identified that the virus can use (reviewed in 1). The co-receptors are actually 7-transmembrane G coupled protein. The two major coreceptors are CCR5 and CXCR-4. The chemokine receptor CCR5 is used by the primary isolates of HIV-1 that are macrophage tropic (2). It is now believed that virus establishes the infection using almost exclusively CCR5 coreceptor (known as R5 viruses) and then after prolonged course of the disease, the virus usually shows the ability to use other coreceptors, the most prominent among them is the CXCR-4 (3). These viruses are called X4 and when the virus can use both the receptors, they are known R5X4 type. The virus that one isolates just after infection is predominantly R5 type and they usually do not cause syncytia formation, called non-syncytia inducing (NSI). On the contrary, the X4 viruses, which appear late in the disease, cause syncytia very efficiently. Very often, the appearance of X4 using viruses in the host is associated with rapid decline of CD4+ cells and accelerated progression of the disease. Other chemokine receptors, for eg.CCR3, could be important in infecting cells of the central nervous system (4). It has been shown that most primary isolates of HIV-1, HIV-2 and SIV use CCR5 to gain entry. It was remarkable that some of the laboratory adapted strains of HIV-2 could gain entry into CD4 negative cell line, but needed only one of the chemokine receptors (for review see 5). This observation has led to the suggestion that the original receptor for retroviruses must have been chemokine receptors, HIV then adapted or evolved to use CD4 receptor . A satisfactory answer to the question as to why only R5 viruses replicate in macrophages or Langerhan cells (these are the cells the virus comes in contact when transmitted through heterosexual route) has not been found, but it could be that certain cell types express more of CCR5 than other

Author for Correspondence :

Dr. A. C. Banerjee

Chief, Laboratory of Virology, JNU Campus,

National Institute of Immunology,

Aruna Asaf Ali Marg, New Delhi-110067.

Fax: 91-11-6162125

E mail: akhil@nii.res.in & akhil_banerjee@yahoo.com

coreceptors. Obviously, the regulation of such key molecules is being actively studied by many investigators.

Role of CCR5 in HIV-1 transmission: Role of mutations in CCR5 gene

It is known that not all individuals infected with HIV, come down with the disease at the same time. An average time for the progression of the disease is about 8 - 10 years. The exceptions are the long-term non-progressors and short term progressors. An extremely small percentage of Caucasian people of European heritage were found resistant to HIV-1 infection although they were exposed repeatedly to the virus. When the coding region of the CCR5 gene was analyzed, a 32 base pair deletion corresponding to the second extracellular region of the transmembrane was observed and they were homozygous for this deletion (6). This was the first indication that CCR5 may be the principal coreceptor involved in transmission. Later on, statistical analyses using large number of HIV-1 infected patients suggested that $\Delta 32$ mutation conferred an extremely strong protective effect against HIV-1 infection. This protection, however, is not complete, as few individuals who were homozygous for this deletion ($\Delta 32$), were found to be infected and the virus that was isolated from these individuals were found to be X4 type (7,8). CCR5 receptor using viruses are the major players in mother-fetal transmission of HIV. This deletion results in a frame shift mutation, due to which a severely truncated protein is synthesized (9). This truncated protein is not expressed on the cell surface and is non-functional for signal transduction and is unable to participate as a coreceptor.

Global distribution of $\Delta 32$ mutation of CCR5 gene & other gene mutations

This mutation was found to be very common among the normal healthy population of Caucasian people of European heritage. Approximately 10% people are heterozygous and 1% homozygous for this mutation (6,7, 9). Even heterozygous individuals showed delay in the progression of the disease (10). Interestingly, a north to south gradient was also observed among European countries, for e.g. more in Sweden (15%) Vs only ~ 5% in Southern France. It was extremely rare in African and Asian countries. Other minor mutations have been observed, which also results in premature termination. These mutations are extremely rare.

CCR5 $\Delta 32$ mutation in India

We have screened about 350 normal healthy individuals for this gene from North India, and found one individual who was heterozygous for the $\Delta 32$ allele of CCR5 (11). We then studied the inheritance pattern of this deletion mutation in this family. This gene was inherited in the Mendelian fashion (12).

Role of CCR5 promoter mutations

The entire promoter of the CCR5 gene has been mapped. Some of the point mutations in this region showed strong modulating effects on the disease process (13). We are now in the process of analyzing these mutations in the Indian context.

Role of chemokine Stromal cell derived factor SDF-1

SDF-1 is a ligand for CXCR-4 receptor, which the T-tropic virus uses to replicate. Furthermore, the chemokine could very efficiently inhibit the replication of virus under *in vitro* conditions. A transition from G to A was observed in the untranslated region of SDF-1 RNA in HIV-1 infected patients who showed a strong protective effect against HIV-1 infection (14) only if they were homozygous for this point mutation. The mechanism of this inhibition is not known but the easiest would be up regulation of the expression of SDF-1, which is known to directly interact with the CXCR-4 receptor. It seems this mutation is common in all the geographical regions of the world. We have screened about 100 normal healthy Indians and this mutation seems to be quite common in India. Approximately 40% individuals have this mutation with about 8% in the homozygous form (15).

Role of CCR2 mutation

A conservative valine to isoleucine substitution in the transmembrane region has been associated with the protection against HIV-1 but the mechanism for this is not known. Interestingly, all the chemokine receptor genes have been found to cluster on a single chromosome (16). The global distribution of this mutation is being studied.

Chemokine receptors and entry cofactors

The envelope protein of HIV-1 interacts specifically with CD4 and one of the chemokine receptors. This trimolecular interaction leads to the conformational

change in the HIV-1 envelope molecule, which exposes the fusion domain of gp41. The fusion domain of the HIV-1 envelope gets inserted into the membrane of the mammalian cell, which leads to fusion of cell membranes. This is the most important step in the virus entry and attempts are being made to interfere with this interaction from the therapeutic application point of view. The very fact that this trimolecular interaction exposes new epitopes led some investigators to ask questions whether the new epitopes could generate neutralizing type of response.

Genetic variation of HIV-1

HIV-1 being a member of lentivirus, is known to generate mutants, primarily because the reverse transcriptase lacks the proof reading mechanisms. The virus has been divided into two main groups, M and O. The M group consists of many genetic subtypes (A to I or more !). The subtype B predominates in European countries and in United States of America. In Thailand, it is subtype E but in India, it is predominantly subtype C. Other minor subtypes, A, E have also been reported. Genetic recombinants have also been reported from the Indian isolates (17).

Molecular approaches against CCR5 and HIV-1 genes

Nature has presented us with a perfect human knock out experiment with respect to CCR5 gene. As stated earlier, approximately 1% of normal Caucasian people with European heritage are homozygous for this mutation, i.e., they possess precise 32 base pair deletion corresponding to the second extracellular loop of the 7-transmembrane - G-coupled protein receptor. These individuals lead a normal healthy life which may be surprising considering this is an important housekeeping gene responsible for many key immunological reactions. This defect is not lethal in the host as there is redundancy of chemokine receptors, which have overlapping ligands. We have engineered a hammerhead ribozyme and an RNA-cleaving-DNA-enzyme against this important coreceptor. Further more, we have shown that when these ribozymes, now under a strong eukaryotic promoter - CMV, are introduced into a mammalian cell, could interfere

with the coreceptor function (18). This was studied by a novel cell membrane fusion assay which depended upon all the three molecules involved in fusion, CD4, coreceptor, and compatible HIV-1 envelope protein. We recently used this assay to study the efficacy of DNA-enzyme that was targeted to cleave the HIV-1 envelope gene (19), and also showed functional impairment of coreceptor function. We (Chakraborti & Banerjee; unpublished observation) have recently identified two DNA-enzyme (possessing 10-23 catalytic motif) cleavage sites in the HIV-1 TAR region – a characteristic stem-loop structure that is present at the 5'-end of all the HIV-1 transcripts.

Evolution of Chemokine genes:

Chemokine SDF-1 that is known to interfere with the replication of HIV-1 was examined very carefully in several species of monkeys and a remarkable double transition GG to AA at the same region that was observed in humans, was reported by us (20). Also highly polymorphic regions in the CCR5 promoter regions were observed in monkeys by us that might explain as to why the course of infection by either HIV or SIV in monkeys differs to a great extent (21). Another potent anti-HIV-1 chemokine is RANTES and we recently showed novel mutations in the selected group of monkeys (22)

Major unresolved questions:

1. Why R5 virus is involved in transmission and progression of HIV?
2. How and why is the appearance of X4 virus related with rapid CD4 decline?
3. What is the *in vivo* role of various chemokines?
4. The mechanism of HIV-1 protective genes, for eg., CCR2 V64I, SDF-1-A, etc.

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