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RESISTANCE AGAINST HIV-1: ROLE OF CHEMOKINE RECEPTOR, CCR5 AND RESTRICTION FACTOR, APOBEC3G

ARSHIA BERRY[#], SAKSHI GUPTA[+] AND DR NAVKIRAN KAUR[+]*

[#] Faculty of medicine, Friedrich Schiller, University of Jena, 07743, Germany
[+]Amity Institute of Biotechnology, Amity University, Sector-125, Noida-201303, Uttar Pradesh, India.

ABSTRACT

It can be rightly said that no one is really or truly "immune" from HIV/AIDS, however, there are a few individuals, around 1% of the world's total population who exhibit strong natural resistance against the virus. Scientists have discovered two different types of adaptations against HIV in human population: one, which is able to repel the virus itself and the infection and the second that is able to keep HIV from giving way to AIDS. Chemokine binding co-receptor, CCR5 and endogenous anti-retroviral protein, APOBEC3G has been well studied individually in controlling the viral infection. However, the role of these proteins cumulatively remains controversial and opens new research opportunities to find out how these proteins are capable of inculcating minute genome differences in some people together in order to achieve natural resistance from HIV-1.

KEYWORDS: *HIV-1, natural resistance, CCR5, APOBEC3G, mutation, infection*

DR NAVKIRAN KAUR
Amity Institute of Biotechnology, Amity University, Sector-125, Noida-201303, Uttar Pradesh, India.

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INTRODUCTION

Discovered in the laboratories of Dr. Luc Montagnier in Paris and Dr. Robert Gallo in Bethesda around 1983 and 1984[1], HIV-1, belonging to the Family *Retroviridae*, genus *Lentivirus,* species *Human immunodeficiency virus 1*, is responsible for approximately 1.5 million deaths round the globe each year, despite the evident drop in the rate of mortality brought about by antiretroviral therapy (ART) and highly active antiretroviral therapy (HAART), as stated in the global health observatory data, 2011-2015. HAART therapy helps in the reduction of viral load, leading to the declination in morbidity and mortality of HIV-infected individuals, but cannot eradicate the virus.[2,3] The basic hallmarks of HIV-1 infection consists of dysfunction of immune system, rapid and persistent decrease in immune cells (mainly CD4+ T cells), often leading to inflammation and finally occurrence of various opportunistic infections. The rapid decrease in the number of CD4+ T cells (<200cells/µl of blood) leads to the manifestation of Acquired Immunodeficiency Syndrome (AIDS) resulting in death of the patient or a natural progressor inflicted with the disease.[3] There is, however, a limited set of population that is able to control this infection naturally in the absence of ART/HAART. Of this HIV-resistant cohort population, there are a few individuals who have been exposed to HIV-1 repeatedly through sexual or other means over a long period of time, but they remain free of any detectable sign of infection. These individuals with natural resistance from HIV are referred to as "exposed seronegative/ uninfected". Such individuals who can keep a stable count of CD4+ cells for a prolonged period, after being infected with the HIV are referred to as Long Term Non-Progressors (LTNPs) or Elite Controllers (Ecs) depending upon their ability to maintain the undetectable levels of CD4+T cells and consistent low levels of *HIV*-1 RNA for many years after the infection.[4,5] The LTNPs comprise roughly about only 5% of the total population chronically infected with HIV. They are mainly characterised by being infected for over 6 to 7 years, yet exhibiting a stable CD4+ count (>500 cells/µl of blood) after sero-conversion along with very low, although detectable, viral plasma load. A further confined or restricted population, approximately 3 in 1000 individuals infected with HIV is represented by the ECs [6], who are able to maintain elevated CD4+ levels, despite being exposed to an almost 100-fold more virus dose than required to cause infection. The viral load of <50 copies/mL of blood for over 12 months post exposure are virtually undetectable. The viral plasma load in cerebrospinal fluid in case of ECs too, is virtually undetectable (<2.5 copies/mL). Recently, a few individuals were identified who exhibited the features of both ECs as well as LTNPs, and were termed as "elite LTNPs".[5,7] In order to infect human cells, HIV-1 needs two receptors including the CD4 receptors present on some cells of the immune system and the chemokine binding co-receptors. Two types chemokine co-receptors are used by the circulating strains of HIV-1, namely CCR5 and CXCR4. HIV strains that use CCR5 are known as macrophage tropic (M-tropic) or R5 HIV subtypes which account for more than 95% of incident HIV-1 infections. The other HIV subtypes, called T-tropic or X4 viruses, require CXCR4 co-receptor in addition to CD4. X4 viruses usually appear late in the course of HIV disease. As soon as, HIV has bound to both the receptors: CD4 and a chemokine, virus body is exposed through which the HIV-1 RNA can fuse and enter the human cell.[5] CCR5 is certainly the main HIV coreceptor, involved in the entry of virus and cell-to-cell spread. The discovery of CCR5 associated genetic polymorphism, plays a major role in counteracting HIV at its entry level in the host body, through the research and development carried out to identify the targeted inhibitor- drugs and antibodies. Some of these products are presently undergoing clinical trials evaluation or have even been licensed for therapy. Such potential therapies are thereby developing natural immunity against in special populations exposed to HIV, and are referred as HIV-exposed seronegative people and LTNP seropositive individuals. Surprisingly, such antibodies ─ found in serum, as well as in mucosal secretions ─ provides protection or controls the disease. These long-lasting, easy-to-use and cheap mucosal protections could significantly limit HIV spread, especially in countries like Africa, Eastern Asia, and others, where sexually transmitted diseases are major health and social issues.[8] These observations confirm that CCR5 is a promising target in the prevention or treatment of HIV and gives an innovative link towards developing approaches, such as anti-CCR5 vaccination, that can provide useful scientific insight to fight HIV. Another type of host genome polymorphism has been observed in case of an endogenous antiretroviral protein-APOBEC3G/ CEM15/ A3G. This is one of the enzymes belonging to the family of human cytidine deaminases, capable of inhibiting HIV replication through more than one mechanism.[9,10] In the replication cycle of HIV-1, reverse transcription is a critical step which is catalyzed by HIV-1 reverse transcriptase. During this step, when the HIV-1 viral infectivity factor (Vif) is absent, A3G interacts with the viral RNA and gets incorporated into the progeny viruses. Upon initiating a new infection cycle, during reverse transcription A3G will deaminate the cytidine (dC) to uridine (dU) in the viral minus-DNA strand, thereby resulting in hypermutation in the provirus. As a result, the proviral DNA of HIV-1will no longer be functional or degrade quickly. Moreover, the proviral DNA may now produce damaged or truncated viral polypeptides that signifies an important source of the restricted epitopes of major histocompatibility complex class I (MHC-I) to activateCD8 cytotoxic T lymphocytes which are specific to HIV-1.[10] In an attempt to understand the level of connectivity and productivity between CCR5 and APOBEC3G, in terms of acquiring natural resistance from HIV infections, following studies were taken into consideration. According to a study conducted by Pido-Lopez *et al.,*[11] the stimulation of cell surface CCR5 and CD40, with CCL3 and CD40L respectively, or both, with a microbial heat shock protein (HSP-70) upregulated A3G levels in human CD4+ T cells and monocyte-derived dendritic cells. This was demonstrated by real-time PCR and Western blots, respectively. A dose-dependent increase of A3G was associated with inhibition in HIV-1 infectivity. The ligation carried out at the cell surface activated the

ERK1/2 and p38 MAPK signalling pathways that induced the expression and production A3G protein. D.J.M. Lewis and colleagues[12] in 2014 reported that vaginal immunization with an HIVgp140 vaccine linked to the 70-kDa heat shock protein down-regulated the HIV coreceptor CCR5 and increased the expression of the HIV resistance factor APOBEC3G, in women. A 70-kDa heat shock protein (HSP70) has already been known to significantly inhibit HIV-1 infectivity of human $CD4^+$ T cells and elicit chemokine and cytokine functions by engaging CCR5 and CD40 molecules and stimulating A3G molecules.[13] Thus, vaccine-induced innate responses not only promote adaptive immunity– they may also help in preventing HIV transmission. To further understand the relation between CCR5 and A3G, one first needs to fully elucidate their roles against HIV at their respective individual levels. This review, therefore, discusses the roles of both of these factors, including the dual-action of CCR5 and A3G on HIV-1 post infection.

CCR5 AND HIV RESTRICTION

Chemokine receptors are cell surface molecules that induce the migration of cells bearing receptors towards injured tissues. This interaction in turn, secrete small peptide ligands called chemokines in the bloodstream- a mechanism commonly used to recruit leukocytes to the site of inflammation.[14] The C-C chemokine receptor type 5, more commonly known as CCR5 or CD195, is a receptor protein for chemokines on the cell surface of white blood cells, where they help in signaling and coordination of immune responses. In *Homo sapiens,* the CCR5 gene, encoding for the CCR5 protein receptor is situated on the short arm (p) of chromosome 3 at the 21st position.[15] The structure of the gene is as illustrated in figure I. CCR5 and other chemokine receptors belong to a larger family of seven transmembrane proteins coupled to G proteins.CCR5 is expressed on immature, memory and primed Thelper1 cells, monocytes and macrophages; on epithelium, endothelium, smooth muscle, and fibroblasts; on neurons, microglia, immature Dendritic cells and astrocytes.[16]

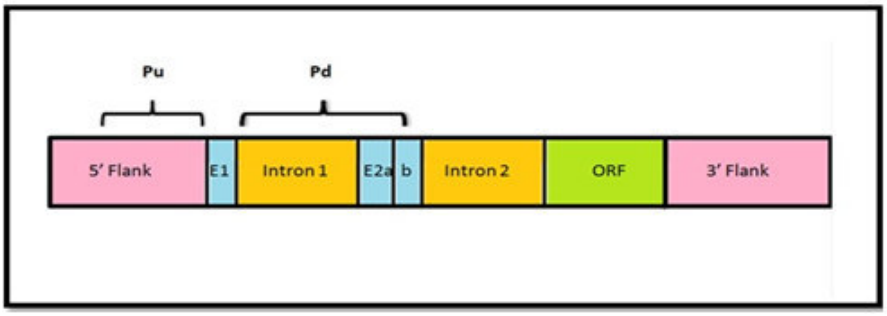

Figure I
Structure of CCR5 gene

The three natural ligands that bind to CCR5 are the pro-inflammatory cytokines CCL3 (MIP-1 α), CCL4 (MIP-1 β) and CCL5 (RANTES), which are required for the initiation of effector responses.[17] Since chemokine binding may interfere with the docking of HIV to the host cell, naturally available CCR5 ligands were considered as HIV competitors, with varying results. In vitro studies cited by Menten *et al.,*[18] proved that sufficient quantities of these natural ligands were able to suppress HIV-1 infection by increasing the competitive binding of CCR5, thereby inhibiting the viral entry. Thus, elevated levels of MIP-1α, a characteristic of elite controllers, have been associated with HIV resistance as well as delayed disease progression.[19]

HIV TROPISM AND CHEMOKINE RECEPTORS

All the primary HIV-1 isolates use CCR5 co-receptor for entry, regardless of their viral genetic type. The macrophage or the M-tropic HIV-1 strains make use of the β-chemokine co-receptor, called CCR5, to mark their entry inside the host macrophages and CD4+ T cells and replicate during the initial stages of HIV-1 infection. HIV strains using CCR5 cell co-receptor are called R5. Similarly, the T-tropic or T-cells strains of HIV-1 make use of the α- chemokine receptor called CXCR4, for their entry and replication in both, primary CD4+ T cells as well as macrophages at a much later stage of HIV-1 infection which shows progression towards AIDS. These strains are referred to as the X4 viral strains. In addition to these, there are certain dual-tropic strains of HIV-1 which use both CCR5 and CXCR4 co-receptors for viral entry. These strains are referred to as X4R5.[20]

CCR5 PROMOTER POLYMORPHISM

Speaking about HIV-1 R5 infection and progression of the disease, mutations observed in the promoter region of CCR5 gene are well documented, which in turn leads to the production of an aberrant CCR5 mRNA, affecting either the regulatory sites and/or the DNA transcription factor binding.[21] Analysis of single strand confirmation polymorphism using high pressure liquid chromatography (HPLC) in HIV seropositive individuals by Martin *et al.,*[22] helped in identification of 10 single nucleotide polymorphisms (SNPs), occurring in various combinations and giving way to four common haplotypes: CCR5 P1-4 and six infrequent haplotypes: CCR5 P5-10, each affecting the disease progression of HIV at variable rates. At position 59029 (GenBank Accession: U95626) in the CCR5 promoter region, an A/G polymorphism was shown to affect the progression of HIV. Individuals having a G/G genotype polymorphism show slower progression to AID ,almost by 3.8 years, in addition with 45% lower promoter activity of CCR5 gene whereas, individuals with an A/A

genotype are known to show rapid progression towards AIDS[23] and in case of homozygous conditions, the A/A genotype has also been associated with greater CCR5 expressing CD4+ cell levels. Despite its absence in the transcription factor binding regions, this A/A allele (found in CCR 5 P1 haplotype) has possibly a greater proficient promoter activity.[24] It is also believed that almost 10-17% of patients progressing towards AIDS within 3 to 3.5 years of acquiring the infection do so due to the presence of CCR5P1/P1 haplotype.[22] Further analysis of epidemiological studies with a substantially greater number of HIV cohorts is required to establish the role of CCR5 promoter region polymorphisms in AIDS progression. Nevertheless, CCR5 antagonists like, Maraviroc (Pfizer), Aplaviroc (GlaxoSmithKine) and Vicriviroc (Schering-Plough) are already being exploited as the new, emerging class of ARV (anti-retroviral) drugs that bind to the hydrophobic CCR5 residues in the transmembrane domains (TMDs) to prevent the receptor-HIV interaction.[25]

CCR5 DELETIONS AND CONSEQUENCES

CCR5 expression levels may usually vary in all individuals without affecting their immune function.[26] Depending on the availability of the exposed receptors, CCR5 expression in all individuals has been defined.[27] Changes in the CCR5 expression levels reflect genetic factors and environmental stimuli between individuals, as observed in a comparative report on describing higher levels of CCR5 expression and immune activation in European and African individuals residing in Africa, than in a cohort of the same ethnic groups residing in Europe.[28] Genetic factors have been described to prevent CCR5 expression in order to display natural resistance in HIV-exposed uninfected people.[29] Moreover, the enhanced expression of chemokines has been reported in providing natural resistance to HIV-1 infectants.[8] Soon after the discovery of CCR5 and its role as the co-receptor along with CD4 to mediate HIV-1 entry into host cells, its mutant allele was discovered in 1996.[30] This CCR5 variant was characterized by a 32 base-pair deletion in the single coding exon of the CCR5 gene, as shown in figure II. This mutation, identified in 4-16% Caucasians, is called *CCR5-Δ32.*[31] It has been estimated to have occurred about 700-2000 years ago and is said to have been a result of positive selection by nature, since its frequency has been increasing rapidly, almost by 13% per annum in the North European populations, especially in the Scandinavia. However, this mutation is absent in most of the East Asian populations as well as among the native Africans and American Indians.[32]

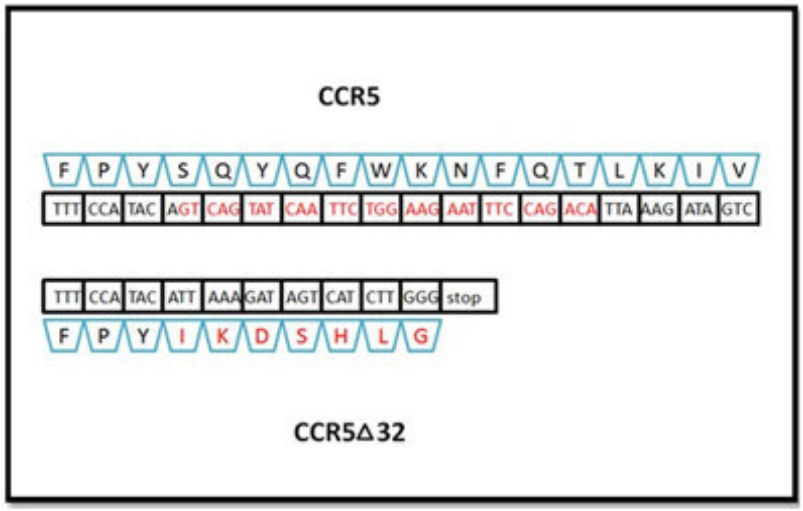

Figure II
Illustration of differences between the wild-type CCR5 gene translation and the mutant Δ32.

The mutation CCR5-Δ32 leads to the production of a non-functional protein, since the polymorphism introduces a premature stop codon in the CCR5 genic region. As a result, a truncated protein is produced, that remains trapped in the endoplasmic reticulum because of which, the R5 isolates of HIV-1 are unable to bind to the target cell surfaces, which demonstrates its near-complete protection against the virus.[33] Individuals who are homozygous for this mutation are completely resistant to the R5-tropic strains of HIV-1 infection.[30] In addition to this, the population homozygous for CCR5-Δ32 display no prominent clinical symptoms and appear to be almost perfectly healthy as other homologous chemokine receptors.[14] Individuals with one copy of CCR5-Δ32 and CCR5-wildtype (CCR5-Δ32 heterozygous or wt.) each may have altered chemokine receptor activity. The heterozygous nature of CCR5-Δ32 protects against sexual transmission of HIV infection both from male-to-male as well as from male-to-female.[34] Numerous studies have also found that LTNPs, as compared to the disease progressors, are expression of CCR5 on CD4+ T cells, which in turn leads to slower progression to AIDS.[35] Transmission of X4 viruses is not affected by the CCR5-Δ32 mutation.[5] A recent clinical study showed that an HIV-positive patient suffering from acute myeloid lymphoma (AML) who had CCR5+ genotype and had undergone CCR5-/- stem cells transplantation, observed a long-term control of infection without any antiretroviral therapy.[36] On performing a biopsy on the patient, CCR5+

macrophages were identified from intestinal mucosa several weeks after transplantation, reflecting that only the circulating CCR5+ cells had been revived by the transplant. However, despite the presence of HIV-permissive cells, viral RNA was undetectable and the patient did not experience an increase in the viremia in the absence of antiretroviral therapy.[8]

CCR5 AND HIV INFECTION

The mechanism of CCR5 signalling initiates by the binding of HIV to CCR5, followed by receptor dimerization and phosphorylation.HIV entry in the host cell engages the viral envelop (env) glycoprotein complex, the CD4 antigen and a chemokine receptor, CCR5, and sometimes CXCR4. The virus envelope consists of two proteins, gp120 and gp41, which mediates attachment of the virus on the host cell, followed by its binding and fusion with the target cell membrane.[37] This binding thereafter, generates a conformational change in the env complex and exposes the CCR5 binding site, comprising the bridging sheet and the variable V3 loop. On the interaction between the Env domains and CCR5, there occurs a change in the conformation of the coreceptor, which activates the coreceptor signalling. This further triggers some conformational changes, which leads to the extension of the gp41 fusogenic domain and its refolding, thereby bringing lipid bilayers in close contact, leading to the required fusion.[38] This intracellular signal transduction occurs through GDP release, thereby hydrolyzing a new GTP molecule. With the dissociation of the activated G protein CCR5 and the secondary-messenger cascade gets activated, sustained by the kinases.[39] The mechanism of CCR5 signalling and regulation is a complex phenomenon with many aspects yet to be understood.[8] Use of some monoclonal antibodies has also been found to promote receptor signalling. However, even though HIV may bind with wild-type and C-truncated CCR5 receptors (which are unable to be internalized or to induce signalling), this event is not required for efficient host cell entry.[40] In order to design and produce drugs like Maraviroc which target CCR5, crystallographic studies as well as indirect biochemical approaches or immunological studies may be useful.[8.41]

APOBEC3G AND HIV INFECTION

Among the identified proteins for inhibiting HIV-1 replication is apolipoprotein B mRNA-editing enzyme-catalytic, polypeptide-like 3G, also known as APOBEC3G or A3G, CEM15 and hA3G.[42] The APOBEC family proteins are identified with the presence of the zinc-dependent cytidine also referred as deoxycytidinedeaminase domain (ZDD). Zinc is coordinated between the H or C residues in the super secondary structure of the enzyme, consisting of a beta sheet formed by 5-antiparallel beta strands and two alpha helices. This deaminas eactivity of APOBEC involves conversion of cytidine (C) or deoxycytidine (dC) to form uridine (U) or deoxyuridine (dU).[43] APOBEC family consists of eleven members in humans, the most prominent ones being APOBEC1, APOBEC2, APOBEC3A-H, APOBEC4 and activation-induced cytidine deaminase (AID). All these enzymes affect a variety of physiological functions by converting dC (deoxycytidine) to dU (deoxyuridine) in single-stranded DNA (ssDNA) or RNA (ssRNA) of viral as well as human genomes[44,45], thus triggering DNA degradation via activation of DNA repair mechanisms. The main function of APOBEC3 (A3) proteins is to safeguard host cell innate immunity from mobile genetic elements such as endogenous retroelements and exogenous viruses). Different forms of A3 genes are found in mammals: 1 gene in pigs, rodents, cats, and sheep; 2genes in cows; 3genes in horses and dogs; and 7genes in primates.[43,46] The most studied A3 family member is A3G. A3G is predominantly expressed in macrophages, dendritic cells and $CD4^+$ T lymphocytes, all being the primary targets of HIV.[47] In 2002, the function of A3G as an antiviral host factor was discovered based on the experiments on cDNA transfer, which was designed to recognize a host cell suppressor of the HIV-1 accessory protein called as the viral infectivity factor (Vif). Vif binds to A3G and induces its destruction through proteasome degradation pathway and ubiquitination. Viruses lacking Vif had poor infectivity when produced in cell lines known as 'non-permissive' (express A3G), otherwise they exhibited wild type infectivity when produced in 'permissive' cell lines. To convert non-permissive phenotype for Vif-deficient HIV-1 infectivity, transfection of permissive cells with A3G was required.[43,47] Upon infecting a susceptible cell in the first step of reverse transcription, A3G remains connected to the reverse transcription complex, in order to edit dC residues to dU on the proviral minus strand. Thus, hA3G is similar to AID in that it edits single-stranded DNA, not RNA. Editing of the HIV-1 DNA minus strand by hA3G can be remarkably efficient, with up to 20% of all dC residues of proviral minus strand being deaminated to dU. Two possible effects on the HIV-1 proviral strand can be elucidated from such editing. Firstly, that the reverse transcription may proceed normally except that on the introduced dU residues, template addition of A residues in the proviral-plus strand, thus resulting in G-to-A hypermutation of the HIV-1 provirus.[48,49] While the resultant provirus can then be integrated normally into the target cell genome, rendering the provirus defective. Secondly, editing by A3G may destabilize and degrade HIV-1 reverse transcripts.[50] This phenomenon remains poorly understood, although it might occur from the action of cellular DNA repair enzymes.[50,51] This mechanism of action has been illustrated in figure III.

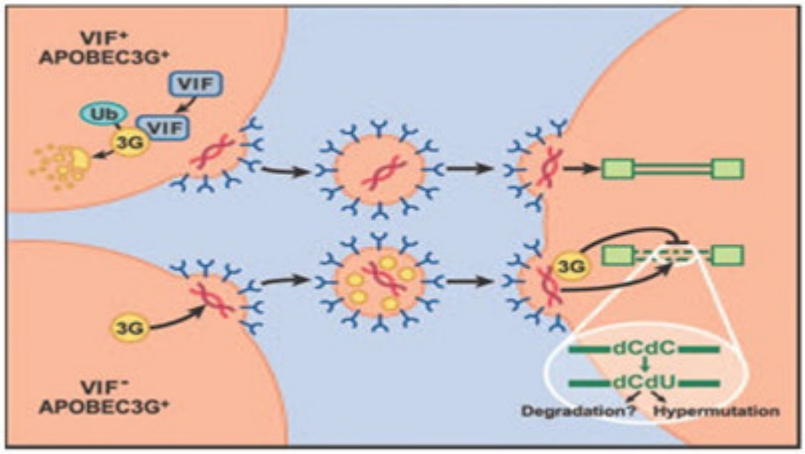

Figure III
Schematic representation of the mechanisms of action of APOBEC3G and the HIV-1 Vif protein.

APOBEC3G EFFICACY LIMITING VIRAL FACTORS- VIF AND HMM FORMATION

To counteract viral propagation by the inhibiting activities of A3G, several strategies, as illustrated in figure IV, have been acquired by lentiviruses, fundamentally in the form of various auxiliary proteins. One such lentivirus protein is Vif, which exclusively binds and targets A3G and thereby, degrading it via ubiquitin-dependent proteosomal pathway.[51] It also impairs its translation in the target cell and directly interferes with the encapsidation of A3G[52], thus decreasing its levels. For Vif-mediated A3G degradation, the viral auxiliary protein also utilises several co-factors present in the target cell itself, for example, Core binding factor beta (CBF-β), which aids in A3G ubiquitination and in turn, its degradation.[53] Therefore, in the presence of Vif, the inhibitory action of A3G as well as its efficacy is greatly reduced, making Vif an integral component to support HIV replication in A3G expressing host cells. However, in some cases, as reported by Gonclaves and Santa-Marta in 2004[54], the presence of Vif is not able to completely abolish the activities of A3G and this co-relation between A3G inhibition by Vif and viral infectivity level is also not absolute and A3G may still be able to inflict sub-lethal mutation levels, even in the presence of Vif.[55] Vif, therefore can act as a variable negative regulator of A3G, but not a complete inhibitor. Another such factor that limits the efficacy and action of A3G is its entrapment in the high-molecular-mass ribonuclear complex or HMM, that is mediated non-specifically by the binding of either cellular or/and viral proteins and RNA.[56] This shuttling of A3G into newly synthesized virions is important for its anti-viral activity, but sometimes also leads to the formation of HMM, thus, limiting its functions. In many cases, Vif is also known to either induce or directly promote the formation of HMM complexes.[57] However, conversion of anti-viral activity limiting HMM can be reversed to LMM (low-molecular-mass) A3G via RNase H/A.[58] Ironically, the very viral genome that binds to A3G leading to the HMM formation, also contains RNase H, as a part of the activity of reverse transcriptase, that releases the bound A3G, allowing it to act on the viral genome.[59] Therefore, this RNase activity of reverse transcriptase is now regarded necessary as well as detrimental to the propagation of virus, ascribing to its role in release of active A3G.[60] These active A3G-induced mutations in the viral genome may only partially succeed in inhibiting the viral replication, that too, at sub-optimal levels and consequently might assist in viral evolution by leading to generation of sequence variation.[48]

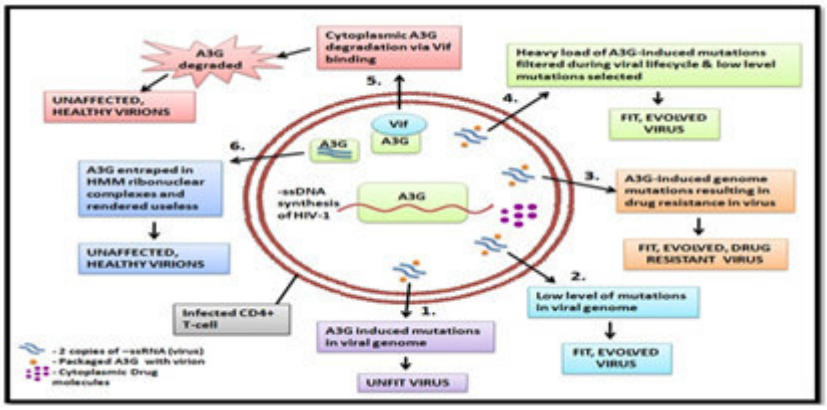

Figure IV
Pro- and anti- HIV actions of the APOBEC3G (A3G).

FIGURE IV illustrates various outcomes of A3G interactions with the viral cellular components and the viral genome inside the cytoplasm of an infected CD4+ T-cell. Step1- A3G acting as the classic innate host immunity defense agent, generating mutation the genome of the virus, resulting in the production of unfit viruses, incapable of infecting new cells. Step2- Depict the generation of fit and evolved viruses, when A3G induces low-levels of mutations that are used by the virus to gain resistance and fitness. Step3- In presence of the pool of various cytoplasmic, anti-viral drugs, A3G may induce certain mutations in the viral genome that results in gain of resistance against that particular drug, resulting in viral evolution into fit and drug-resistant strains. Step4- Depicts the process of purifying selection where the heavy or lethal mutations induced by A3G are filtered out from the genome throughout the viral life-cycle and a pool consisting of low-level mutation is specifically selected, leading to the production of fit virus particles. Step5- Illustrates the possible process of binding between A3G and the viral infectivity factor (Vif), where Vif marks the degradation of A3G, resulting in the production of healthy and unaffected viruses. Step6- Entrapment of the cytoplasmic A3G in HMM (high molecular mass) ribonuclear complexes that consequently renders A3G useless, again leading to the production of healthy and unaffected viruses.

APOBEC3G AND CYTIDINE DEAMINATION INDEPENDENT EFFECTS ON HIV-1 INFECTION

A3G is no doubt a potent mutator of HIV-1 DNA, recent studies has revealed that A3Gcan also exert cytidine deamination-independent effects during HIV infection. According to an experimental study, replacement of the conserved residues such as cysteine, glutamic acid or histidine of the C-terminal CDA domain demonstrated that editing is completely mediated by this domain[61], and the mutant proteins thus produced can still inhibit virus infection to a prominent extent.[62] However, this remains a controversial topic, since different investigations till now vary by the experimental work that is being performed in different laboratories. Anyhow, these effects of the wildtype A3G inhibiting the accumulation of the HIV-1 reverse transcripts have also been described for early and late reverse transcription intermediates.[63,64,65] In another study, on permeabilizing the purified virions with melittin followed by incubation with dNTPs and measuring the levels of cDNA synthesis in endogenous reverse transcription reactions, it was demonstrated that A3G inhibits reverse transcription in a dose-dependent fashion.[63] It was examined in the cell-based experiments that the extent of A3G inhibition progresses fastly among the later reverse transcription intermediates. Also, the addition of the first dNTP to the 30-OH of the tRNALys primer is not inhibited noticeably, implying that placement of tRNALys at the PBS of the viral RNA is unaffected by A3G. These observations cumulatively studied by Iwatani *et al.,* 2007 [64] indicated that A3G impedes the progression of reverse transcriptase enzyme along template RNA and does not directly inhibits its biosynthetic capabilities. A recent analyses of the unedited A3G proteins have indicated that the anti-HIV-1 properties exhibited at high concentrations titrate away at lower expression levels.[66,67] These findings indicate that the editing independent anti-viral property is possessed by A3G. However, such conclusion simply that it is not necessary to segregate the editing dependent and -independent activities of A3G. Interestingly, this dual nature of A3G may vary under different conditions. This behaviour could be reflected by a recent research on the two different cell lines: the human T-cell line, H9 and vif-deficient HIV-1 infection in primary blood lymphocytes (PBLs), neither of which is capable of productive viral replication.[68] It was observed that the viral cDNAs from the H9 cell lines produced enhanced levels of G-to-A mutation, as compared to PBLs. This suggests that high levels of unedited effects of A3G may contribute to its anti-HIV-1 phenotype in PBLs as compared to H9 cells. Hence, in different cell types A3G reflects its differential regulation.[69] Moreover, the observed alleviation of A3G expression in resting PBLs via RNA interference renders them susceptible to infection. This indicates that A3G expression is necessary for the barrier to HIV-1 infection in these cells. Also, G-to-A hypermutation modifications are only carried out in approximately 10% of the incomplete reverse transcripts. This implies that A3G's expression is not linked to cytidine deamination when it comes to inefficient reverse transcription in these cells.[70] Indeed, a combination of experimental protocols and systems are required to determine accurately how A3G inhibits HIV-1 replication.[71]

CONCLUSION

The preventive mechanism of action of both CCR5 and APOBEC3G individually, has been scrutinized thoroughly in the past; however their cumulative role in controlling this viral disease progression has not yet been studied properly. Reports so far contribute to the action of HSP-70 along with the use of HIV vaccines, which could bring about natural resistance against HIV infection by the dual role of CCR5 and APOBEC3G (Figure V). It might be suggested that HSP70 can be used as an effective HIV adjuvant in developing the vaccinations, since HSP70 may inhibit both pre-entry and post-entry infection of HIV by generating CC chemokines and up-regulating A3G. Thus, one needs to fully comprehend this inhibitory nature of A3G and the consequences of null CCR5 homozygotic phenotype, in the eye of the use of an HIV-adjuvant in order to exploit these potential targets towards discovering a potential cure or therapy against HIV. This study may be helpful in enhancing the innate antiviral HIV action by providing a complementary strategy in finding a protective and therapeutic immunization against HIV.

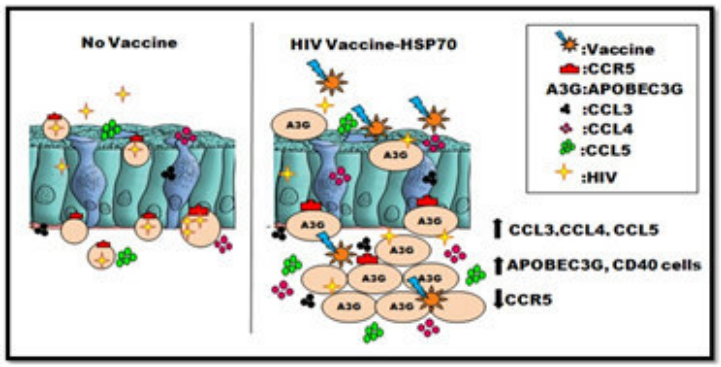

Figure V
Representing suggestive scheme of how the HIV vaccine linked to heat shock protein-70, may protect from HIV infection.

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ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the author

CONFLICT OF INTEREST

No Conflict of interest to declare

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Reviewers of this article

Dr. Nidhi Srivastava, Ph.D.

Assistant Professor, Amity institute of Biotechnology, Amity University, Noida, U.P., India

Prof. Srawan Kumar G.Y

Associate Professor,Nalanda Institute of Pharmaceutical Sciences, Sattenapalli, Guntur, Andrapradesh, India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

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