The role of HIV-1 on genetic diversity, drug resistance, response to anti-retroviral, disease progression, and on In vivo HIV control as potential target for therapeutic vaccines Development (Part Seventeen)

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Abstract

Current treatment of HIV/AIDS consists of a combination of three to five agents targeting different viral proteins, i.e. the reverse transcriptase, protease, integrase and envelope, and aims to suppress viral replication below detectable levels.

Recent data suggest that cellular factors also represent useful targets for therapy. Here, we summarize findings from several genome-wide screens that identified a large number of cellular factors exploited by HIV-1 at each step of its life cycle.

Studies on host genomics have revealed the existence of identifiable HIV-1 specific protective factors among infected individuals who remain naturally resistant viraemia controllers with little or no evidence of virus replication.

Several studies have highlighted the individual and population level gross differences both in the viral clade sequences as well as host determined genetic associations.

Accordingly, a number of viral factors and host genetic characteristics have been shown to play a crucial role in the control of HIV disease by delaying progression to AIDS or even preventing infection. There is also an improved understanding of humoral and cellular immune responses in terms of specificity, functional repertoire, longevity and tissue distribution and their ability to contain HIV replication.

There is considerable interest in small RNA molecules predominantly due to their in vivo stability and significant functional involvement in the regulation of a variety of cellular activities, encompassing cell proliferation, apoptosis, morphogenesis and cell differentiation, signifying their importance in biological processes and human diseases.

In this article, I discuss HIV-1 Genetic Diversity, Comparative genomics, Impact of host genetics on in vivo HIV contro, Association of HLA polymorphisms with HIV disease outcome, Clinical and Biological Relevance of HIV-1 Genetic Diversity, Structure of the virion and Structure of the viral genome

Key Words: HIV-1, HIV-1 Genetic Diversity, HIV Structure of the virion,HIV-1 viral genome Antiretroviral Therapy and Therapeutic vaccines

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1. Introduction

The human immunodeficiency virus (HIV-1) infection induces a wide range of immune responses in humans and depending on the level of immune resistance elicited, the host may or may not develop acquired immunodeficiency syndrome (AIDS). The arena of host genetics has progressed immensely owing to the recent advancements in analytical approaches, development of high throughput next generation sequencing platforms, genome and proteome wide microarrays, expression profiling screens, highly sensitive and specific immunological assays and other tools for gene function readouts. Genomic architecture of HIV-1 infection relates to a complex network of genes and their cumulative influence on predilection (or resistance on the contrary) to HIV infection and its progression to AIDS. Multiple host genetic factors regulate individual variations in acquisition of HIV-1 infection and disease progression (1). An improved understanding of the immunopathogenesis of HIV infection and the role of

host genetic markers and viral diversity in this control is urgently needed. Rather, specific markers that are directly mediating viral control need to be identified so that vaccine design is not misled by focusing on epiphenomena and functionally unlinked markers. The determinants of viral attenuation, specific HLA class I and II alleles, certain polymorphisms in co-receptor genes and ligands, the specificity and functionality of virus-specific CD4+ and CD8+ T-cell responses, as well as new insights into factors of the innate immune response in HIV control are being discussed. More recently, antiretroviral drugs that inhibit the viral integrase (IN) (raltegravir) or the six-helix bundle core formation of the gp41 trans membrane protein required for virus–cell fusion (enfuvirtide) have been approved for the clinic (2). While this growing repertoire of antiretroviral agents is impressive, none of these drugs is useful for the treatment of HIV/acquired immunodeficiency syndrome (AIDS) on its own because HIV-1 is highly variable and capable of developing resistance against all of them. This high genetic variability provided the rationale for the development of "highly active antiretroviral therapy" (HAART) consisting of combinations of three or more antiretroviral agents. Several studies there is clear evidence showing that HIV is able to subvert and manipulate host mRNA and miRNA machinery, therefore a clear understanding of complex aspects of the human genome and its regulation by miRNA harbors immense potential for not only developing new generation of biomarkers and therapeutic targets to control HIV, but also to delineate mechanisms regulating non-progressive HIV disease in Elite controllers in the absence of antiretroviral therapy.] MicroRNAs (miRNAs) are small (21-22nt), non-coding RNA fragments found in many organisms, from plants to humans, which function to negatively regulate gene expression (3),(4). Because of the drawbacks of current combination antiretroviral therapy, it remains a major interest to develop new antiretroviral drugs or innovative therapies to reduce undesired side effects, to prevent the emergence of drug resistance or even to attack the viral reservoirs (5),(6). Indeed, a major barrier to curing HIV infection remains the ability of HIV to integrate in the host genome and remain latent. currently only a single drug targeting a cellular protein has been approved for the clinic: Maraviroc binds to the HIV-1 entry cofactor CCR5 and blocks its interaction with the viral envelope gp120 to prevent the membrane fusion events necessary for viral entry (7),(8),(9).

2. HIV-1 Genetic Diversity

HIV-1 is characterized by extensive genetic diversity. Mutational escape results in a remarkable degree of viral diversity within HIV-1 and in its adaptation to both immune activity and antiretroviral therapy. However, not all escape mutations are advantageous to the virus since some of them can severely affect viral fitness (10),(11). The extensive genetic diversity of HIV-1 is due to its high replication rate, the error-prone reverse transcriptase, and recombination events that may occur during virus replication (12),(13).

2.1. Error-Prone Reverse Transcriptase Enzyme and High Replication Rate

The molecular basis of HIV-1 variabilityis a highly error-prone reverse transcriptase enzyme (14). The activity of this enzyme, essential for viral replication, is specifically required for the conversion of single-stranded genomic RNA into double-stranded viralDNA, which is later

integrated into the host genomic DNA (15). For this reason, HIV-1 reverse transcriptase inhibitors are powerful inhibitors of HIV-1 replication and represent an important class of antiretroviral agents (16). HIV-1 reverse transcriptase is a multifunctional enzyme that possesses RNA-dependent and DNA-dependent DNA polymerase activities as well as an RNase H activity that specifically degrades the RNA strand of RNA/DNA hybrids (15). As an intrinsic property, and in contrast to other DNA polymerases, HIV-1 reverse transcriptase lacks a proofreading function. This error-prone nature of reverse transcriptase, together with the high rate of virus production sustained by HIV-1 infection *in vivo*, strongly contributes to the continuous generation of newviral variants (17),(18). The rate of nucleotide substitutions introduced by reverse transcriptase is approximately 10−4 per nucleotide per cycle of replication, which is equal to one nucleotide substitution per genome during a single replication cycle (19). Insertions, deletions, and duplications also contribute to the genetic heterogeneity of HIV-1 (20). HIV-1 has a rapid turnover, and it is estimated that approximately 109 virions perday are generated in an infected individual. The composite lifespan of plasma virus and virus producing cells is very short with a half-life of approximately two days, and an almost complete replacement of wild-type strains by drug resistant virus occurs in plasma within 2– 4 weeks (18). During antiretroviral treatment, rapid viral turnover in combination with a high mutation rate is a primary factor behind the emergence of HIV variants with antiretroviral drug resistance.

2.2. Genetic Recombination

Each retroviral particle contains two copies of single-stranded RNA, and template switches occur frequently during reverse transcription, thus generating mutations and recombination by intramolecular and intermolecular jumps. Recombination may link drug resistant mutations in HIV-1, leading to increased resistance to a particular drug (21), or the generation of multidrug resistant variants (22). In addition, recombination may lead to the acquisition of mutations that compensate for a loss in viral fitness or replicative capacity due to previous acquisition of resistance mutations. Since recombination can create a multiple drug resistant virus out of two single drug resistant strains, it is generally believed that the capacity of the virus to recombine facilitates the evolution of drug resistance (21-24),. Recombination is a strategy for viral rejuvenation, and it is likely that recombination between HIV strains may lead to the evolution of fitter forms and viral strains acquiring drug resistance to all major classes of HIV-1 inhibitors. A different scenario could be that a fitter virus can be generated by recombining parts of two parental genomes with lesser fitness, or alternatively a less fit virus can be generated by breaking up favourable combinations of mutations in the parental genomes. The potential for genetic differences among subtypes to yield different patterns of resistance-conferring mutations is supported by natural variation among HIV subtypes in genetic content (40% variation in the *env* gene, and 8–10% variation in the *pol/gag* genes). This issue acquires special relevance in view of the fact that the HIV *pol* gene is the major target for all major classes of anti-HIV drugs and most HIV strains show hotspots for recombination in *gag-pol* and *env* regions.

3. Genome-wide Association Studies

Genome-wide association studies (GWAS) investigate genome for genetic variation or SNPs associated with a disease or condition of interest by utilizing high throughput technologies. If an SNP is overrepresented in the affected population vs. in the control population, then the SNP

is associated with the condition of interest. SNPs vary in a nonrandom manner within a chromosomal region and although SNPs may be distantly located on a chromosome, they can be inherited together as a block or haplotype and are said to be in linkage disequilibrium (25). It is therefore possible for researchers to scan the genome with only a limited number of "tag" SNPs (e.g. 500,000-2,000,000) and still detect polymorphisms associated with a phenotype. However, this strategy rarely identifies the causal variant associated with an observed effect. The causal variant SNP and the tag SNP are usually in varying degrees of linkage disequilibrium. The "tag" SNP generally marks a chromosomal region of interest but not a specific gene. The location and distribution of common SNPs and haplotypes is made possible by the HapMap project (26),(27). Compared to the traditional gene candidate approach, this strategy can identify functionally important polymorphisms in genes that have an unexpected role in disease pathogenesis. Results of GWAS are dependent on appropriate selection of control and affected populations, large sample cohorts, and adequate statistical analysis for use with large data sets. In general, GWAS are successful when the association is strong and/or when the variant is relatively common, but these conditions are frequently not met (28),(29).

4. Genes that code entry gatekeepers

Molecular events involved in the process of viral entry into host cells are highly intricate. Briefly, HIV-1 engages its envelope proteins (gp120, gp41) sequentially and exploits host cell surface co-receptors CCR5 or CXCR4 along with CD4 to gain entry into the cell. Cell surface density of vacant chemokine co-receptors CCR5/ CXCR4 may act as gatekeepers to the virus. On the contrary, their saturation with the corresponding ligands (MIP1a/b, Regulated upon activation normal T cell expressed and secreted (RANTES) for CCR5 and SDF-1 for CXCR4, respectively) could obstruct viral entry and retard its subsequent transmission (29),(30).

4.1. Chemokine co-receptors

CCR5: Genetic variability in the CCR5 co-receptor has been of considerable interest and it is so far the only genetic locus illustrated with translational value against HIV-1. A natural knockout deletion of 32 nucleotide bases (Δ32) renders this receptor non functional and blocks the virus from gaining entry.

The CCR5 promoter region embraces multiple SNPs that regulate its cell surface expression and hence influence viral entry. Several studies have shown that the CCR5 haplogroup HHE favours HIV-1 infection and development of AIDS in multiple populations including Caucasians, Thais and the North Indians (31).. Similarly, the haplogroup HHD is associated with fast progression among the African populations. Because of the population specific variations, the genetic influence on virus transmission and disease progression also vary in a race specific manner.

CCR2: The *CCR2* gene is located in the vicinity of *CCR5* on chromosome 3 and both the loci show strong linkage disequilibrium. A particular SNP (G190A or V64I) has been reported to be associated with slower progression to AIDS (32). These studies and those by others have suggested that although CCR2 is an important genetic marker, the influence of CCR2 V64I polymorphism on susceptibility to HIV may not be direct. It is now clear that it might affect the pace of progression in part or entirely through its linkage with other variants, particularly in the CCR5 promoter (31).

4.2. Chemokine ligands

MCP1: The monocyte chemoattractant protein-1 (MCP-1/CCL2) is a potent chemokine that mediates macrophage activation and recruitment. It is a ligand for CCR2 and has been reported to be associated with encephalitis and dementia among HIV infected individuals. The MCP-1 -2518 G allele in the promoter region has been reported to be associated with higher MCP-1 expression and with reduced risk of HIV-1 acquisition (33).

It has been shown that the 'G' allele occurs with a frequency of 23-25 per cent among Caucasians (25.8% in Germans, 25% in Italians, 23.9% in Hungarians and 23.8% in Czechs) (34). On the other hand, it occurs with a considerably higher frequency of 50-65 per cent among Asian populations (65% in Koreans, 63.8% in Japanese and 51% in Chinese population) (33). The allelic and genotypic frequencies of -2518MCP-1 A/G were found to be comparable between HIV +ve subjects and healthy controls (32). Further, a cumulative analysis of MCP-1 and its ligand CCR2 genetic variants together did not reveal association of this receptor-ligand genetic axis with susceptibility towards HIV infection in north Indians.

SDF1 (CXCL12): The stromal cell–derived factor 1 is the only chemokine ligand known for the HIV-1 co-receptor CXCR4. Transition from G to A at position +801 in the 3′ untranslated region of the CXCL12β gene transcript has been associated with delayed progression to AIDS (33). and with HIV-1 resistance in seronegative high-risk individuals 41.

The observed SDF1-3'A frequency (27.5%) and its lack of association with HIV susceptibility, viral acquisition and transmission in our results are in conformity with a previous report from north India ? (25) CCL5/RANTES: This chemokine is a ligand for CCR5 and therefore may inhibit viral entry by competitive binding and CCR5 down-modulation. The promoter genotype -403GA-28CC has been shown to be associated not only with HIV-1 susceptibility but also delayed onset of AIDS in European Americans (EA) (26).

MIP-1- α: The macrophage inflammatory protein-1-alpha is produced by stimulated T lymphocytes, macrophages, neutrophils and monocytes. This chemokine contributes to acute cellular immune responses via recruitment and activation of macrophages and T cells inducing the production of inflammatory cytokines. A biallelic dinucleotide (TA) repeat exists within the promoter region at -906 of the *MIP-1A* gene (27).

We also evaluated the role of genetic polymorphism of *MIP-1a* +459C/T in HIV infection. The homozygous TT genotype was found with a significantly lower frequency in HIV +ve subjects (5.45%) as compared to healthy controls (11.6%), suggesting its possible role in protection or linkage with some other genetic marker.

5. Comparative genomics

It is known that the chimpanzees can be infected with HIV-1/ SIVcpz virus but do not progress to AIDS like disease. It is believed that they experienced a selective sweep resulting in marked reduction in their MHC class I allelic and haplotypic repertoire in the past caused by an HIV-1/SIV like retrovirus pandemic(34). Similarly, there is evidence for unique patterns of natural selection among non MHC genes in chimpanzees that include relative conservation in CCR5 promoter, CXCR4 and CX3CR1 genes, high CNVs in CCL3L1 and long term persistence of advantageous alleles, *e.g.*, in T cell transmembrane immunoglobulin and mucin 1 (TIM1). A strong positive selection has been suggested among genes for post entry restriction factors like APOBEC family, TRIM5a and others1,(1). Lessons learnt from primate studies could help

identify analogous genes that can interfere with cross species transmissions and allow nonpathogenic outcomes.

6. Genomic portrait of HIV-1 studies in the Indian population

There was a progressive improvement in his circulating and gut mucosal CD4 T cell counts over the ensuing five years. Interestingly, the patient exhibited a high frequency of activated memory CD4+ cells. These were shown in *ex vivo* experiments to be the favoured targets for infection by any CXCR4-tropic HIV-1 strains. This report demonstrates that although the recovered T cell population is resistant to CCR5-mediated HIV cell entry, these are not resistant to CXCR4-mediated cell entry by X4 tropic HIV. While this case study indicates the scope of gene therapy as a possible cure for HIV, it also raises issues of enhancing sensitivity of currently employed viral assays, risks from long lived non haematopoietic cell reservoirs, and restraints of X4 viruses (35),(36),(37). These reports highlight the importance of identifying potential HIV patients at very early in their infection stage so that they could benefit from directed HAART interventions.

7. Impact of host genetics on *in vivo* HIV control

viral diversity is likely be shaped by differences in the frequency of different host genetic markers and, based on viral evolution, can lead to opposite effects of a specific genetic marker on HIV disease control (38). Thus, whole human genome approaches are severely complicated by viral diversity in different host ethnicities making comparisons across different clades of HIV and various geographically distinct human populations difficult. This consideration also points to the possibility that different clades of HIV may possess inherently different replication fitness and may drive disease development at variable levels, as recently considered as a possibly contributing factor in a case of severe acute HIV infection (39).

8. Association of HLA polymorphisms with HIV disease outcome

Many host genetic polymorphisms associated with levels of disease control involve genes encoding for receptors for viral entry and molecules expressed on the surface of cells of the innate or acquired immune system, such as HLA, CCR5 and KIR receptors. Moreover, it seems that in some cases their potential protective influence might have a cumulative effect as seen for the synergic effect of some KIR receptors and HLA-B complexes (38). The HLA class genes form highly polymorphic loci in the Major Histocompatibility Complex (MHC) located in the short arm of chromosome 6 and encode for cellular surface molecules that present foreign antigenic epitopes to T lymphocytes. There are two groups of HLA molecules including HLA class I and HLA class II antigens. The HLA class I molecules are divided into HLA-A, HLA-B, HLA-C all of which bind peptides derived from intracellularly processed proteins and present them to CD8+ cytotoxic T-cell lymphocytes (CTL). Among these, the HLA-B alleles, while most diverse (more than 1,000 HLA-B alleles have been identified to date) have also been shown to carry the bulk of the anti-viral T cell immune response in HIV infection (40). Accordingly, the number of well-defined HLA-B restricted epitopes exceeds the number of defined epitopes restricted by HLA-A and, particularly,HLA-C alleles. However, especially the HLA-C alleles are currently under more intensive investigation as larger HIV infected cohorts with more complete and high-resolution HLA-C typing have become available. HLA alleles are grouped into 9 supertypes based on their structure, peptide-binding motif, epitope representation and sequence similarity (41),(42). Particularly alleles included in the HLA-B7 (B*5101, B81), HLA-B27 (HLA-B27, B*1503)

and HLA-B58 supertypes (HLA-B57, B*5801, B*1516, B*1517) have been associated with improved or impaired levels of HIV control. Of note, almost all the alleles in the HLA-B58 supertype appear to mediate superior control of HIV infection (43), with the exception being the HLA-B*5802 allele, which is highly prevalent in South Africa and which is associated with elevated median viral loads (44). The reasons how subtle changes in the HLA sequence (HLA-B*5802 only differs in three amino acids from the "good" HLA-B*5801 allele) can so profoundly affect HIV disease outcome are still unclear and are not in all cases simply attributable to different CTL epitope repertoires presented on these alleles (44),(45). In addition toHLAallele frequency,the homocygous expression of individualHLA alleles has been associated with reduced viral control (46). Furthermore, the effects of particular HLA supertypes or of individual alleles have also been reported to provide the basis for immunologically mediated resistance to infection (47),(48). It will be interesting to confirm the potential protective effects of such alleles in additional cohorts with variable allele frequencies and to assess other mechanisms and markers present in genetic linkage to these alleles that may possibly be involved to at least some levels in protection from HIV infection (49). Associations between HIV control and specific polymorphisms in the HLA class II loci have been less welldefined, maybe reflecting a possibly only indirect antiviral effect of HLA class II restricted CD4+ T cells (50). The DRB1*13/DRQ1*06 haplotype has also been found at increased frequency in individuals who were treated early in HIV infection and who maintained virus suppression after treatment interruption (51). Furthermore, a protective role of DQB1*06 alleles, irrespective of their DR haplotype co-expression, has been identified(51). While the HLA class II associations have not produced as strong markers as HLA class I analyses, the representative studies given above highlight the importance to further explore the contribution of the specific CD4+ T cell responses and their genetic basis in the control of HIV.

9. Clinical and Biological Relevance of HIV-1 Genetic Diversity

9.1. Impact of HIV-1 Subtypes on the Drug Resistance

There is a solid body of evidence indicating that the type and degree of HIV-1 resistance to NRTIs, NNRTIs, and PIs vary between different subtypes (52),(53),(54),(55). The development of nelfinavir resistance in subtypes B and G represents a classic example of this phenomenon. A different level of resistance has been observed among different subtypes. Indeed, the recombinant form CRF02 AG is more susceptible to nelfinavir and ritonavir than subtypes C and F; subtype G is more sensitive to tipranavir and lopinavir than other subtypes (56), and the subtype C has accelerated risk in developing resistance to tenofovir (57),(58),(59). An explanation for the extreme variability of HIV-1 subtypes in the response to antiretrovirals can be given by the presence of some polymorphisms that can influence both the emergence of drug-resistance mutations and the response to drugs. For example, polymorphisms at residues 20 and 36 of HIV-1 protease decrease the genetic barrier to tipranavir resistance in subtypes A, C, F, and G (60). while nucleotide heterogeneity at 64 and 65 positions in the reverse transcriptase accelerates development of K65R in subtype C (61), (57). In some cases, drug exposure may lead to amplification of such polymorphisms as A98G/S in reverse transcriptase and M36I, K20I, and L89Min protease, leading to a potential for resistance (62). Continuous research on the role of polymorphisms in the development of drug resistance is therefore necessary. Studies are needed to assess genotypes both before and after therapy in the context of possible associations between polymorphisms and drug resistance. The use of

nontoxic, effective antiretroviral drugs should yield excellent clinical responsiveness, regardless of the viral subtype. Subtype differences, suboptimal therapies, and deficiencies in health care delivery systems can create conditions for accelerated development of resistance. Urgent recruitment for low-cost viral-load monitoring is needed to prevent and detect drug resistance, as well as to avoid unnecessary treatment switches (63),(64).

9.2. Impact of HIV-1 Subtypes on Response to Antiretroviral

Therapy
polymorphisms at resistance-associated positions in subtype B could not necessarily be interpreted as conferring resistance in non-B subtypes. Frater et al. retrospectively analysed the virological response in 362 patients: 265 Europeans infected with subtype B and 97 Africans infected with non-B subtypes (65). Subtypewas presumed fromethnic and epidemiological data, with confirmation further extrapolated from genotyping the samples from 60% of the Africans and 30% of the European patients. There was a significant imbalance between the two groups in several important parameters, including gender, transmission-risk groups, CD4 cell counts, and antiretroviral regimens used.

9.3. Impact of HIV-1 Subtypes on Disease Progression and Viral

Transmission; Several studies on disease progression showed that, among non-B subtypes, subtypes C and D were found to bemore aggressive, followed by G, AE, AG, and A, the least aggressive of all HIV-1 subtypes (66),(67),(68),(69). Viral factors that could explain some of the HIV-1 pathogenicity are higher *ex vivo* replicative capacity, higher genetic diversity, and CXCR4 coreceptor usage (70),(71),(72).

9.4. Immune Response according to Different HIV-1 Subtypes

Despite the importance of viral characteristics in determining the rate of HIV-1 disease progression, recent findings from genomic studies show that host genetic factors also play a crucial role. The genetic determinants that influence susceptibility to HIV-1 and limit AIDS vary in different populations and among individuals. Meta-analyses of large cohort studies have identified several genetic variants that regulate HIV cell entry (particularly chemokine coreceptors and their ligands with copy number variations), acquired and innate immunity (major histocompatibility complex (MHC), Killer immunoglobulin-like receptors (KIRs), and cytokines), and others (TRIM5-α and APOBEC3G) that influence the outcome of HIV infection (73),(74),(1). Of the various genes that contribute toward host genetic propensity, MHC turns out to be the major contributor because it is responsible both for restriction of cytotoxic T lymphocyte (CTL) epitopes and for the emergence of CTL escapemutants. Leukocyte antigen (HLA) alleles have been shown to be associated with the rate of disease progression in Africans and Caucasians (75),(76),(77). The interplay of viral, immune, and host genetic factors in the control of HIV-1 replication has been recently evaluated in HIV controllers (78),(79).

10. Structure of the virion

HIV-1 virions contain two copies of a single stranded RNA genome within a conical capsid surrounded by a plasma membrane of host-cell origin containing viral envelope proteins. The

RNA genome is 9750 nucleotides long (80),(81). and the virions measure approximately 120 nm in diameter. A detailed three-dimensional structure of HIV-1 envelope-glycoprotein spikes, which are required for the infection of host cells, has recently been elucidated by cryoelectron microscopy tomography (82). The HIV-1 RNA is tightly bound to the nucleocapsid proteins, p6 and p7, which protect it from digestion by nucleases. This viral core further contains reverse transcriptase, integrase, and protease. The entire complex is surrounded by an icosahedral capsid (p24). A myristoylated matrix protein (p17) surrounds the capsid. Also enclosed within the virion particle are the proteins Vif, Vpr, and Nef. The envelope is formed when the capsid buds from the host cell, taking some of the host-cell membrane with it. Embedded within the lipid bilayer are the viral envelope glycoproteins that form the HIV-1 spikes: the external surface glycoprotein (gp120), and the transmembrane glycoprotein (gp41) (83),(84),(85).

11. Structure of the viral genome

The HIV-1 genome, flanked by a long terminal repeat, contains the following genes
• *gag* (group-specific antigen): encodes p24 (viral capsid); p6 and p7 (nucleocapsid proteins); and p17 (matrix protein).
• b) *pol*: encodes the viral enzymes, which are reverse transcriptase (transcribes the viral RNA into double-stranded DNA), integrase (allows integration of the DNA produced by reverse transcriptase into the host genome), and protease (cleaves the proteins derived from gag and pol into functional proteins).
• c) *env* (envelope): encodes gp160, which is the precursor of the gp120 and gp41 proteins present in the viral envelope of mature virions. This protein forms spikes that allow the virus to attach to and fuse with target cells.
• d) *tat, rev, nef, vif, vpr, vpu*: each of these genes encodes for a single protein with the same name. Their function is described in Section 4. The structural biology of HIV-1 has been reviewed (83),(84),(85).

12. Target cells

HIV-1 enters cells through interaction with the CD4 receptor and a chemokine co-receptor (CXCR4 or CCR5). The virus infects CD4-positive T cells and macrophages expressing these receptors (86). HIV-1 can alsoinfect dendritic cells (87), which are thought to mediate transmission (88). HIV-1 can be assigned to one of three classes based on its ability to use the two co-receptors. Class R5 comprises the viruses that use CCR5 but not CXCR4; they were previously called nonsyncytia- inducing (NSI) or M-tropic viruses. The viruses that use CXCR4 are in class X4; they were previously called syncytia-inducing (SI) or T-tropic viruses. Viruses that can use either CCR5 or CXCR4 are referred to as R5X4 or dual viruses (89). Primary lymphocytes and macrophages express both co-receptors, so co-receptor use does not strictly define cell tropism (90).Thus, while X4 virus infects T-cell lines, and R5 virus infects macrophage cell lines, in primary cells, these definitions are not as clear. CD4-positive T cells in lymphoid tissues can express both CCR5 and CXCR4, and are the main target for replication *in vivo*. CCR5 is expressed predominantly on the CD45R0+ memory subset of CD4-positive T lymphocytes, while CXCR4 is expressed on CD4-positive CD45R0- and on CD4-positive CD45RAlow naive cells (91). Phenotypic assays and genotyping can be used to determine tropism, as the primary determinants of co-receptor tropism are located in the V3 region of the gp120 envelope protein. Most individuals have the R5 virus at the time of diagnosis, whereas

the presence of the X4 and dual virus is associated with progression to AIDS (90). HIV-1 can be present in a variety of tissues, which is to be expected given the distribution of T cells, macrophages, and dendritic cells throughout the body. HIV-1 has been shown to be associated with germinal centre follicular dendritic cells in lymph nodes, tonsils and adenoids, and mucosa-associated lymphoid tissue (MALT) as well as in T and B cells (92),(93),(94),(95). HIV-1 frequently infects the brain, and the microglial cells are the main location for viral replication in the central nervous system(96). In reproductive organs of infected men, HIV-1 is present in cells of lymphocytic/monocytic morphology in the seminiferous tubules and interstitium of the testis, in the epididymal epithelium, and in connective tissue of the epididymis and prostate (96).

13. **Conclusions and future directions**

The identification of numerous cellular factors that are exploited by HIV-1 at essentially every step of its replication cycle provides a large number of potential targets for antiretroviral therapy (97),(98). It is conceivable that HIV-1 has evolved to efficiently antagonize those host defenses that are most relevant for its control (99),(100). PolyfunctionalCD8+ T-cell immunity against particular viral proteins along with virusreactive CD4+ T-cell help have been most consistently implicated in modulating HIV infection *in vivo*. Viral factors such as specific mutations often emerging as a consequence of immune selection pressure and entire gene segment deletions have also been associated with reduced viral burden and slower progression of HIV disease. While such host genetic markers may provide great help in understanding the (immune)-pathologyof HIV, they will likely not be directly informative for HIV vaccine development. miRNAs are small molecules with bigger functional impact, as single miRNA can target hundreds of mRNAs at any given time in regulating gene function. Thus, miRNA-mediated regulation of host gene expression underlies complex relationship that controls host-virus interactions. Several viruses are also known to encode miRNAs, which play a vital role not only in the viral life cycle, but also in determining disease course in the host through host-virus interactions and subversion and manipulation of the host genome. Altogether, the recent scientific advances in our understanding of viral pathogenesis and on the cellular factors promoting or restricting HIV replication hold great promise for the development of improved treatment and prevention strategies. With immune parameters of controlled infection, the identification of host genetic markers may in the future facilitate the design of gene therapy approaches that would try to either block expression of unfavorable genes or introduce beneficial components. Although not based on gene-therapy, the case of the CCR5-Δ32 stem-cell transplanted individual referred to above, points towards the potential feasibility of such approaches. Moreover, the determination of such targets and their interaction during the disease course may provide a clear view of this host-virus interaction and may lead to the development of new therapeutic strategies. Similarly, the identification of dysregulated miRNAs during HIV infection may yield attractive targets for anti-HIV therapy. Given that HIV cure strategies are gaining momentum, analysing the role of miRNA in modulating the host gene expression in guiding HIV disease staging (progression or non-progression) is timely.

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