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Characteristics of human immunodeficiency virus-1 influencing the development and efficacy of anti-HIV-1 vaccines

Karakteristike virusa humane imunodeficijencije-1 koje utiču na razvoj i efikasnost anti-HIV-1 vakcina

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Introduction

The human immunodeficiency virus (HIV) belongs to the family *Retroviridae*, subfamily *Orthoretrovirinae*, genus *Lentivirus*, characterized by a long incubation period of several months to several years and the outbreak of diseases with a chronic course and fatal outcome. HIV-1 and HIV-2 viruses, derived from primate lentiviruses, have a tropism for the cells of the immune system, leading to a depletion of $CD4^+$ T lymphocytes and a lack of cellular immune response, and the final stage of HIV infection is characterized by the development of acquired immunodeficiency syndrome (AIDS). Since the beginning of the AIDS pandemic, 88.4 million people have been infected with HIV, and 42.3 million have died from AIDS-related diseases. According to the latest data, in 2023, 39.9 million people worldwide were living with HIV infection (38.6 million adults and 1.4 million children), 1.3 million were newly infected, and around 630,000 people died from AIDS-related diseases. Of those infected, 53% were young women and girls [1]. HIV is currently one of the most serious public health problems, and understanding the mechanisms of replication and spread of HIV infection is crucial for the development of new drugs and vaccines to combat this disease. The antiretroviral drugs (AD) that have come to market over the last forty years have significantly improved the quality of life and life expectancy of HIV-positive individuals. Due to the great genetic and antigenic variability of the virus, no effective vaccine has yet been developed that could significantly reduce the incidence of HIV infections in risk groups.

HIV-1 is a ribonucleic acid (RNA) virus with a protein capsid of atypical symmetry and a lipid bilayer envelope. The capsid consists of the small basic nucleoproteins p7, p9, and the protein p24. Inside the core are viral enzymes reverse transcriptase (RT), integrase, and protease, which are involved in the synthesis of proviral deoxyribonucleic acid (DNA) and its integration into the host genome, as well as in the maturation of viral particles. The matrix protein p17 is located between the capsid and the lipid envelope, while the transmembrane glycoprotein gp41 and the surface glycoprotein gp120 are integrated into the envelope and contribute to the adsorption and penetration of HIV-1 into the target cell. The virus has a diploid RNA genome consisting of two identical molecules of linear single-stranded $^{(+)}$RNA. The genome of HIV-1 contains three genes found in all retroviruses – *GAG*, *POL*, and *ENV*, which code for the structural and functional proteins of HIV-1, and six regulatory genes – *TAT*, *REV*, *VIF*, *NEF*, *VPR*, and *VPU*, whose products regulate viral replication and are responsible for evading the host immune response [2].

Variability of HIV-1 and its impact on vaccine development

Groups and subtypes of HIV

HIV-1 is responsible for the global AIDS epidemic and infects 95% of HIV-infected individuals, while HIV-2 is less prevalent and less virulent [3–5] but causes similar clinical

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symptoms to HIV-1 [6]. HIV-2 is most widespread in West Africa, while only a few people are infected in Europe, India, and the United States of America. Compared to HIV-1, it is characterized by a longer asymptomatic phase and a slower course of the disease. There are four different groups within the HIV-1 type: M (main), O (outlier), N (non-M, non-O), and P (pending) [7–11]. These groups are genetically related but have a different geographical distribution, and all four lead to similar clinical symptoms of HIV infection [12].

Group M is the most widespread and accounts for approximately 90% of all HIV-1 infections. It emerged in the 1920s in Kinshasa, Democratic Republic of Congo, and its zoonotic origin is the simian immunodeficiency virus (SIV) from wild chimpanzees (SIV_{cpz}), which infects chimpanzees. There are nine subtypes within this group, designated by the letters A to J (A1, A2, A3, A4, A6, B, C, D, F1, F2, G, H, J, and K) [13–18]. The geographic distribution of group M circulating subtypes is presented in Figure 1.

Group N was originally identified in Central Africa but is relatively rare and causes only a few infections worldwide [17]. Like group M, it is derived from the SIV_{cpz} [16].

Group O causes infections in West and Central Africa and is rarely found outside this region [17]. In contrast to groups M and N, group O is derived from the SIVs infecting the western lowland gorillas (SIV_{gor}), which infects gorillas. For this reason, detection of this group had been difficult with the original HIV-1 diagnostic kits until newer generation tests were developed [19].

Group P was first isolated in Cameroon in 2009 and is the least widespread of all groups. It has a high degree of genetic similarity with SIV_{gor}.

Circulating recombinant forms and unique recombinant forms

Infection of an individual with two or more HIV-1 subtypes can lead to their recombination and the formation of a unique recombinant form (URF) of the virus. This URF can be detected in an infected person by sequencing the viral genome and has no major epidemiologic significance. When the URF is detected in three or more geographically distant individuals who are epidemiologically unrelated, this recombinant genome is referred to as the circulating recombinant form (CRF). It has epidemiologic significance for the spread of the M-group HIV-1 epidemic [18]. The CRFs are designated by numbers and are numbered in the order of their discovery. Approximately 150 different CRFs and several URFs have been identified [20]. The HIV-1 subtypes E and I no longer exist today as independent entities, as recombination of these subtypes with other subtypes has taken place over the years, and CRF01_AE (recombination of subtypes A and E) and CRF04_cpx (complex recombination

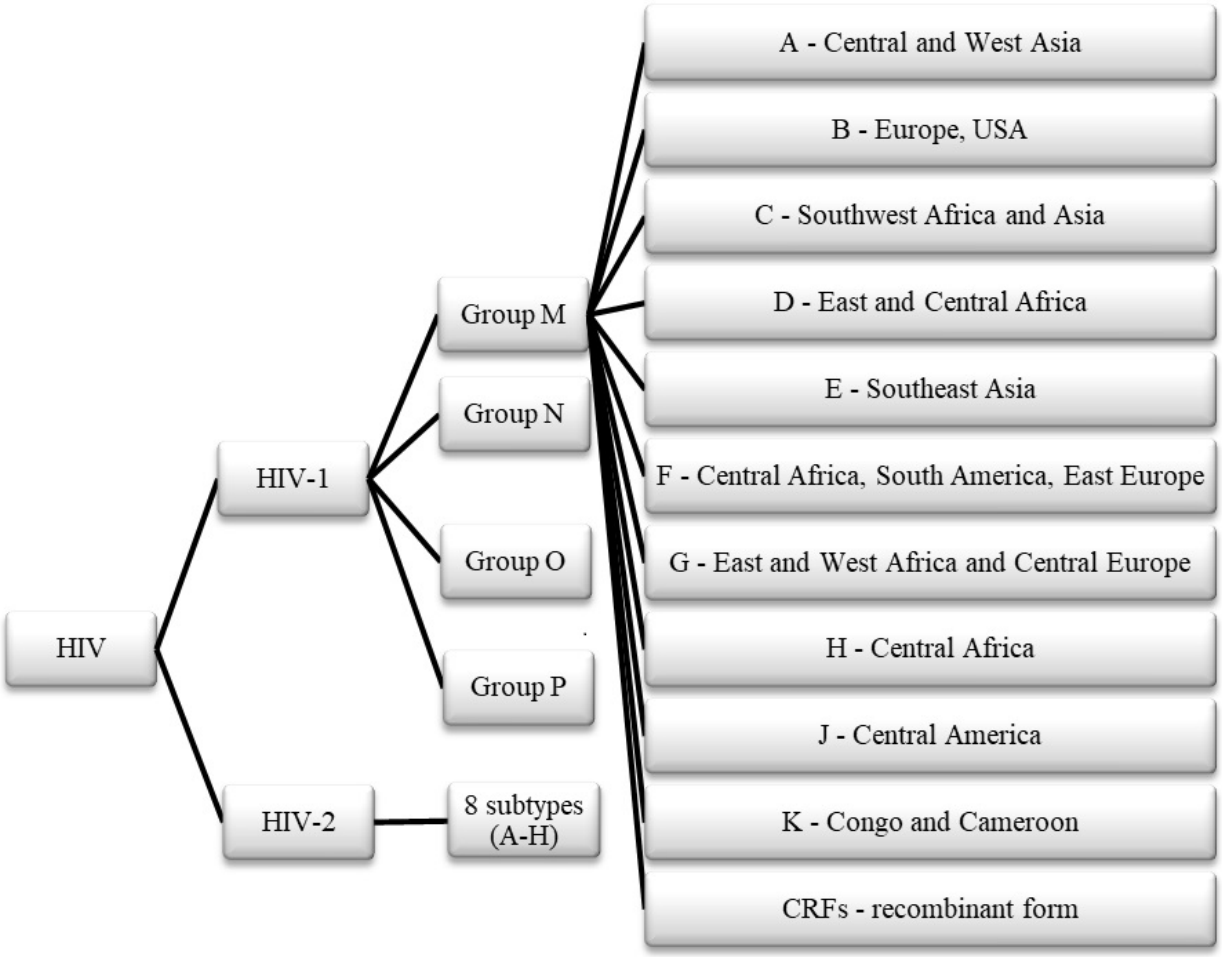

Fig. 1 – Geographic distribution of human immunodeficiency virus (HIV)-1 circulating subtypes.

of several subtypes) have taken subtypes E and I out of circulation. Most of the HIV-1 infections are caused by subtype C (46.6%), followed by subtype B (12.1%), subtype A (10.3%), CRF02_AG (7.7%), and CRF01_AE (5.3%). CRF01_AE is most widespread in Asia, and CRF02_AG in West Africa [11].

The geographical distribution of groups and subgroups of HIV-1, as well as CRFs, can change over time, and population migration and travel are the most important factors for the spread of different types of HIV-1 to geographically distant locations. In addition, most HIV-1 infections worldwide belong to group M and its subgroups, and different subgroups may predominate in various regions of the world. The Balkan Peninsula is characterized by the occurrence of different subtypes of HIV-1 of group M. Subtype B is predominant in Serbia, Slovenia, and Hungary, while subtype A is predominant in Albania, and subtype F in Romania [21, 22]. Determining viral subtypes is important for predicting therapeutic success, as certain HIV-1 subtypes may be resistant to some antiretroviral drugs. It is also of particular importance for the development of vaccines against HIV-1. An effective vaccine must protect against different types/subtypes of the HIV-1 virus and their recombinant forms. In addition, the diversity of HIV-1 subtypes also affects the accuracy of HIV diagnostic tests and viral load tests. The tests currently in use are able to identify and monitor all subtypes and recombinant forms that have been identified so far. However, it is expected that current recombination will lead to the emergence of new URFs and CRFs for which the tests have not yet been designed [19].

Genetic and antigenic variability of HIV-1

HIV-1 is characterized by a high rate of viral mutations that cause extreme genetic diversity of the virus. As a consequence, the virus adapts to the effector mechanisms of the immune response and evades it efficiently. It also develops resistance to antiretroviral therapy. However, some mutations are not beneficial for the virus, as they impede its survival and ability to cause the infection of the host (e.g., "fitness" of the virus) [23, 24]. The genetic diversity of HIV-1 is a consequence of a high rate of viral replication, the non-repairing function of the enzyme RT, and the recombinations that happen during the replication of the virus [25, 26].

High replication rate of HIV-1 and the reverse transcriptase enzyme

HIV-1 RT is a multifunctional enzyme that possesses both RNA-dependent DNA polymerase and DNA-dependent DNA polymerase activity as well as ribonuclease H activity, which specifically degrades the RNA strand of the resulting RNA/DNA hybrid. In contrast to other DNA polymerases, the HIV-1 RT does not have the function of correcting errors that occur during replication. The rate of nucleotide substitution introduced by the RT is approximately 10^{-4} *per* nucleotide *per* replication cycle, resulting in one nucleotide substitution *per* genome during a replication cycle [27]. The HIV-1 replicates daily at a high rate; it is estimated that an infected person produces about 10^9 virions *per* day. In contrast, the life span of the virus in plasma and virus-infected cells is quite short, with a half-life of about two days. Therefore, the wild-type strains that primarily infected humans are completely replaced by genetically similar variants of the same viruses (e.g., quasispecies) within two to four weeks [28]. This property of RT, together with the high replication rate of the virus *in vivo*, contributes to the continuous emergence of new viral variants [13, 29-31]. During viral replication, insertions, deletions, and duplications also occur, which also contribute to the genetic heterogeneity of HIV-1 [28].

Genetic recombination of HIV-1

Genetic recombination of HIV-1 occurs when two or more genetically distant viruses recombine during dual or multiple infections of a person, resulting in a new form of the virus with the original sequence (CRF or URF) [32]. Recombination increases the overall genetic complexity and diversity of the viral population, leading to faster viral adaptation, the emergence of resistance to AD, including multidrug resistance, evasion of the immune response, and disease progression [33-38].

Emergence of HIV-1 quasispecies

Quasispecies are a group of mutant viruses that develop during viral replication in an HIV-positive person. The HIV-1 can mutate into multiple quasispecies during infection, which reduces the ability of the immune response that has developed against the primary wild strain of the virus to control the infection and also leads to the emergence of HIV viral variants that are resistant to AD [39]. One of the main goals of early initiation of antiretroviral therapy during HIV infection is to reduce the rate of viral replication, thereby reducing the possibility of the emergence of quasispecies. For this reason, in several clinical trials investigating HIV vaccines, pre-exposure prophylaxis has been administered along with the vaccine to increase vaccine efficacy [40].

Antigenic epitopes of HIV-1 important for vaccine development

Antigenic epitopes that are potential candidates for HIV-1 vaccine development must be evolutionarily conserved and present in the majority of HIV-1 subtypes and their variants for the vaccine to be effective in a wider geographic area.

Antigenic epitopes of HIV-1 for B lymphocytes

Epitopes for B lymphocytes can be either linear or conformational [41]. Linear epitopes consist of a continuous (consecutive) sequence of amino acids on the antigen, whereas a conformational epitope is formed after the protein has folded and connects two or more non-consecutive linear sequences (e.g., discontinuous epitope). The epitopes of HIV for B-cell receptor (BCR) can be lipid, glycan and protein

antigens, or their combinations [42-45]. Numerous studies have shown that broadly neutralizing HIV-1 antibodies (bNAbs) bind more efficiently to conformational than linear epitopes [42-44, 46], which complicates their use in vaccine development, where single epitopes are used. HIV epitopes for bNAbs are thought to have an evolutionarily conserved sequence due to their broad affinity for multiple HIV-1 subtypes, making them cross-reactive [44, 47], but they may also contain highly variable segments [42]. Although these epitopes do not have the same amino acid sequence, the binding of bNAbs may result from the epitopes having a common conformation in the secondary or tertiary structure of the protein, which poses a challenge to the development of anti-HIV vaccines [48].

The presence of bNAbs was first detected in 20–50% of HIV-positive individuals who had had chronic HIV infection for more than 2–5 years [44, 49, 50]. Based on the study of bNAbs isolated from infected individuals, it was found that these antibodies mainly belong to different isotypes of the IgG class. They can bind to five different epitope regions of the HIV ENV protein: the CD4 binding site; the V2 proteoglycan motif at the top of the ENV trimer; the V3 proteoglycan motif with high mannose content; the membrane-proximal external region (MPER) of the ENV transmembrane domain; the gp120-gp41 protein junction with or without fusion protein [42-44]. Ideal vaccine antigens would be HIV-1 epitopes that induce bNAbs that bind to one or more of the above sites on the Env protein and induce the production of IgG. However, these have not been identified or developed to date [43, 44, 51]. An overview of BCR epitopes for bNAbs and their characteristics is given in Table 1.

Several studies have shown that bNAbs isolated from HIV-positive individuals with chronic infection share common features of paratopes [antigenic epitope binding sites in the complementarity-determining region (CDR)1, CDR2, and CDR3 of the immunoglobulin light and heavy chains], such as a high degree of somatic hypermutation in the genes of the V(D)J region, the presence of a long heavy chain in the CDR3, and polyreactivity or autoreactivity with self-antigens (proteins, glycans, and lipids), which can lead to autoimmune diseases [42-44, 52, 53].

In addition to the antigenic epitopes that lead to the formation of bNAbs, the HIV-1 also possesses epitopes that do not lead to the formation of neutralizing antibodies (i.e., eliciting non-neutralizing antibodies – nNAbs) but can lead to antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular viral inhibition (ADCVI) [54-57]. ADCC and ADCVI are mediated by the Fcγ receptor on the surface of effector cells (NK cells, macrophages, dendritic cells, or neutrophils) that produce the effector molecules perforin and granzyme, resulting in cytolysis (ADCC) or β-chemokines to inhibit the virus-infected cell (ADCVI). In addition to the beneficial functions of nNAbs, they can interfere with the functions of bNAbs by competing for the same antigenic epitopes, thereby reducing the function of bNAbs. They can also lead to increased infection of cells with HIV, as they bind to Fcγ receptors on the surface of macrophages and dendritic cells, which can promote the entry of HIV into these cells [58].

Antigenic epitopes of HIV-1 for T lymphocytes

Epitopes for T lymphocytes are short linear peptides generated by the processing of protein antigens and displayed by the major histocompatibility complex (MHC) molecules. Evolutionarily conserved epitopes of HIV-1 for T lymphocytes have an identical amino acid sequence in HIV-1 isolates of the same subtype (i.e., type-specific epitopes) or for several different HIV-1 subtypes [59-61]. They are also evolutionarily conserved in other human lentiviruses, primate [primate immunodeficiency lentiviruse (PIV)] and cat lentiviruses [HIV, SIV, feline immunodeficiency virus

Table 1

Characteristics of HIV epitopes for the B-cell receptor that induce broadly neutralizing antibodies (bNAbs)

Structure of the epitope	Epitope regions on the HIV ENV protein	bNAbs	Antibody isotype
Conformational	CD4 binding site	VRCO1, CH103, b12	IgG1
		3BNC117	IgG1κ
		PGV04, 8ANC13, CH235	IgG
	V2 proteoglycan	PG9, CHO1	IgG1
		PGT145, VRC2609	IgG
	V3 proteoglycan	PGT121, PGT128	IgG1
		PGT135	IgG
	gp120-gp41	PGT151, VRC34.01, 35022, 8ANC195	IgG
Linear	MPER	10E8, 2F5	IgG3
		4E10	IgG3κ

HIV – human immunodeficiency virus; MPER – membrane-proximal external region; the antibodies VRCO1, 3BNC117, b12, PG9, PGT145, PGT121, 35022, 10E8, 4E10, and 2F5 bind to the Fcγ receptor on the surface of effector cells and induce antibody-dependent cellular cytotoxicity.

Table 2

Example of HIV-1 epitopes for T lymphocytes

Epitope	Protein	HXB2 location	Subprotein	HXB2 DNA	Subtype	Species	HLA
WEKIRLRP	GAG	15-23	p17(15-23)	832..858	B	human	A*02:05
EDEGKISKI	POL	197-205	RT(42-50)	2673..2699	B	human	B*51:01
VWKDAETTL	ENV	44-52	gp120(44-52)	6354..6380	B	human	B*38:01

HIV – human immunodeficiency virus; RT – reverse transcriptase; HLA – human leukocyte antigens.

(FIV)] [62, 63]. Mutations in epitopes for T lymphocytes occur regularly, both in conserved and non-conserved epitopes with variable sequences, leading to differential retention of variants that occur after mutations. It is hypothesized that highly conserved epitopes occur in protein regions essential for viral survival and that any significant mutation would compromise viral viability [64-66]. Therefore, variants that occur after these mutations are eliminated by the mechanisms of natural selection.

To date, a large number of HIV-1 epitopes have been discovered for T lymphocytes that are candidates for vaccine development. HIV epitopes can vary in length, but the variant with the shortest epitope length is thought to elicit the strongest immune response, so this epitope variant is considered "optimal". HIV-1 epitopes shorter than 21 amino acids are included in a list of optimal epitopes, the so-called A-list of HIV epitopes [20]. Each HIV-1 epitope has a unique identification number, the position of the defined epitope site in relation to the HXB2 protein sequence (viral reference genome HXB2, GenBank code K03455), the protein on which it is located and its subunit, the virus subtype, the epitope sequence, and the host and the human leukocyte antigens (HLA) restriction epitope element [20]. An example of an epitope for T lymphocytes can be found in Table 2.

Evolutionarily conserved epitopes of HIV-1 for T lymphocytes must be able to induce activation of $CD8^+$ cytotoxic T lymphocytes (CTLs) [44, 67], $CD8^+$ and $CD4^+$ T lymphocytes [68, 69], and possibly activation of a subset of cytotoxic $CD4^+$ CTL [70]. All of the above activities of T lymphocytes are important for the development of prophylactic vaccines.

HIV-1 epitopes for T lymphocytes are subdivided into epitopes for CTL/$CD8^+$ and epitopes for T helper lymphocytes (T helper/$CD4^+$). To date, 2,067 epitopes for CTL and 725 epitopes for $CD4^+$ T lymphocytes have been identified, which are mainly located on the GAG, POL, and ENV proteins. The largest number of identified epitopes belongs to HIV-1 virus subtype B. A list of HIV-1 epitopes for CTL/$CD8^+$ and $CD4^+$ T lymphocytes is available in the Los Alamos HIV database [20]. According to the information in this database, 569 epitope sequences belong to subtypes B (537), C (9), and G (7), and 16 additional subtypes (01_AE: 4; 01B: 2; 02_AG: 4; A: 2; A1: 3; F1: 1) were identified in Serbia (date of access July 1, 2024).

HIV-1 antigen epitopes for T lymphocytes bind to MHC class I and MHC class II molecules coded by HLA genes and their alleles. Various HLA allotypes can be associated with susceptibility or resistance to HIV infection [71-74]. Certain alleles for HIV resistance/susceptibility also differ in correlation with ethnicity or the endemic prevalence of HIV subtypes that circulate in the country. For instance, the resistance of European and North American Caucasians to HIV-1 subtype B and African populations from Kenya, Tanzania, and sub-Saharan Africa to subtypes A, C, and D are associated with HLA-A2 alleles such as HLA-A2, HLA-A*0205, and HLA-A*6802 [71-73, 75, 76]. In contrast, susceptibility to HIV-1 is associated with HLA-B7 alleles: susceptibility to subtype B in Europe and North America with HLA-B*3501, HLA-B*3502, and HLA-B*5303 alleles [75], and to subtypes A, C, and D in Kenya with HLA-B*0702 and HLA-B*4201 alleles [76].

Conclusion

The existence of a large number of groups and subgroups of HIV-1 with a high degree of mutation and recombination leading to the emergence of circulating and unique recombinant forms and quasispecies of HIV-1 makes it difficult to develop a single vaccine against HIV-1 that would be effective against all strains of the virus in all geographic areas.

Given the effector functions of HIV-1 antibodies (bNAbs and nNAbs) and the risk of interference of these antibodies with other functions in the body, antigenic epitopes that lead to the production of bNAbs and/or nNAbs must be carefully selected when choosing a potential vaccine antigen candidate. In the development of vaccines containing antigenic epitopes of HIV-1 for T lymphocytes, the distribution of HLA allotypes in a given endemic area should be considered in addition to the selection of the appropriate epitope to induce a protective immune response.

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