

Antibodies Targeting the Envelope of HIV-1

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ABSTRACT Antibodies (Abs) are a critical component of the human immune response against viral infections. In HIV-infected patients, a robust Ab response against the virus develops within months of infection; however, due to numerous strategies, the virus usually escapes the biological effects of the various Abs. Here we provide an overview of the different viral evasion mechanisms, including glycosylation, high mutation rate, and conformational masking by the envelope glycoproteins of the virus. In response to virus infection and to its evolution within a host, “conventional Abs” are generated, and these can also be induced by immunization; generally, these Abs are limited in their neutralization breadth and potency. In contrast, “exceptional Abs” require extended exposure to virus to generate the required hypermutation in the immunoglobulin variable regions, and they occur only in rare HIV-infected individuals, but they display impressive breadth and potency. In this review, we describe the major regions of the HIV envelope spike that are targeted by conventional and exceptional Abs. These include the first, second, and third variable loops (V1, V2, and V3) located at the apex of the envelope trimer, the CD4 binding site, and the membrane-proximal external region of the gp41 ectodomain. Lastly, we discuss the challenging task of HIV immunogen design and approaches for choosing which immunogens might be used to elicit protective Abs.

INTRODUCTION

HIV continues to be a major global public health issue, with an estimated 35 million people living with the virus and more than 2 million new infections occurring yearly (1). As part of the natural immune response, antibodies (Abs) exert immune pressure on HIV and play a key role both in controlling the virus and in driving escape mutations in the viral envelope glycoproteins. Therefore, and because the elicitation of Abs is believed to be

crucial for an effective vaccine against HIV, Abs targeting HIV have been the focus of intense research in the past years.

ANTIBODY RESPONSE TO HIV

Upon infection with HIV, a strong Ab response occurs in essentially all infected individuals. These Abs are directed against several viral proteins, including the gp120 and gp41 envelope proteins that are found on the surface of the virus particles. While in many viral diseases, such as influenza and polio, the Abs against surface antigens can establish protective immunity, many HIV envelope-specific Abs have little neutralizing capacity due to the many complex escape mechanisms employed by the surface viral glycoproteins that occur as sparse trimeric spikes in the virus envelope (2). These virus escape mechanisms include the following.

Glycan Shield

One of the reasons why HIV is a difficult target for Abs that prevent infection is the dense glycosylation of the envelope proteins gp120 and gp41. With approximately

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25 N-linked glycosylation sites, gp120 is one of the most heavily glycosylated viral proteins described. These glycans are large, complex carbohydrate structures that shield vulnerable epitopes on the surface of HIV, leading to viral escape from Abs (3). The function of glycans can be demonstrated by the removal of certain glycosylation sites, leading to a significant increase in neutralization sensitivity of the virus (4, 5).

High Mutation Rate

The high mutation rate of HIV-1 is another obstacle for the development of immune protection by neutralizing Abs. With an *in vitro* mutation rate of approximately 2.2×10^{-5} to 5.4×10^{-5} per nucleotide base per replication cycle, the virus continuously and rapidly evolves to escape neutralization (6). In an attempt to recognize the mutated virus, neutralizing Abs (nAbs) evolve in tandem with the virus, resulting in successive waves of nAb maturation followed by viral escape. This leads to the phenomenon of nAbs often being able to neutralize virus from months earlier, but not concurrent circulating variants (7–9).

The high mutation rate and the fact that billions of new viral particles are produced daily within each patient leads to an enormous diversity of HIV-1 variants. Based on their sequence, the variants have been divided into nine distinct subtypes, or clades, designated by the letters A, B, C, D, F, G, H, J, and K. Overall, the amino acid sequence of the different subtypes can vary by as much as 30% from one clade to another, but in certain genomic regions the genetic variability can be as high as 42%. Further complicating the HIV landscape are recombinant forms of the virus in which heterologous virus strains combine segments of their genome during reverse transcription to produce genetically divergent new virus forms. Countless recombinants of virtually every virus strain combination have been identified worldwide, and their prevalence differs by geographic regions (10). A protective vaccine will need to induce Abs that target this tremendous viral diversity.

Conformational Masking

The envelope spike is a heterotrimer consisting of three gp120 molecules noncovalently anchored to the viral membrane via three gp41 molecules. In an attempt to shield neutralization-sensitive domains, the elements of the envelope spike adopt a quaternary conformation where domains of neighboring gp120 subunits can interact. For example, it was suggested that in the apex of the three-dimensional envelope spike, intersubunit contact between the V1, V2, and V3 loops occurs, protecting adjacent regions from recognition by Abs (11).

To enter its target cells, HIV must bind sequentially to cellular receptors, including CD4 and one of two chemokine receptors (CXCR4 or CCR5). The regions of gp120 that are involved with binding to these receptors must, therefore, be conserved to maintain the infectious potential of the virus. The CD4 binding site (CD4bs) and chemokine receptor binding sites on gp120 would thus appear to be good targets for Abs; however, while able to bind to some exceptional Abs (9, 12, 13), the CD4bs is partially obscured by glycans and variable regions and undergoes conformational reorganization, allowing it to evade neutralization by conventional CD4bs-specific Abs. Thus, the virus places an energetic barrier to Ab binding (14).

It is believed that dynamic conformational changes also play a role in masking conserved epitopes on chemokine receptor binding sites (15); regions of gp120 that are involved in binding to the chemokine receptor—the V3 loop and the $\beta 20/\beta 21$ strands of the bridging sheet—form an exposed surface only after binding to CD4 and are thus exposed to Abs only transiently (16).

TYPES OF ANTIBODIES

Conventional Abs

Abs that commonly occur during HIV infection and that are present in the majority of infected individuals are here defined as “conventional Abs.” These Abs do not exhibit unusual structural or genetic characteristics, and their immunoglobulin genes undergo relatively little somatic hypermutation from germline (17, 18). Conventional Abs have long been known to protect against infection in animal systems. This was established with passive immunization of chimpanzees using IgG preparations from the blood of HIV-infected individuals (19). Thus, the proof of principle was established more than two decades ago that Abs alone from unselected HIV-infected individuals could provide sterilizing immunity.

Conventional Abs are elicited as early as two weeks after seroconversion in acutely infected individuals; however, they display very limited neutralization breadth (20): using a standardized reference pseudovirus panel that represents genetically diverse subsets of viruses, conventional Abs targeting gp120 were shown to neutralize up to 50% of tier 1 pseudoviruses, but $\leq 9\%$ of tier 2 pseudoviruses, suggesting that these Abs target epitopes exposed on a minority of viruses and tend to be specific for the virus infecting the host. Conventional neutralizing Abs (nAbs) also have a limited neutralizing potency *in vitro*, and while most tier 1 pseudoviruses are

neutralized with low levels of nAbs (<1 to $10\text{ }\mu\text{g/ml}$), often $>10\text{ }\mu\text{g/ml}$ are required to neutralize tier 2 pseudoviruses (21, 22).

The Thai clinical HIV-1 vaccine trial RV144 provided additional support for the role of conventional Abs in protection: high levels of V1V2 and V3 IgG Ab levels, especially those of the IgG3 subclass, were found to be significantly associated with the reduced infection rate (estimated 31% vaccine efficacy) in vaccine recipients (23–28). Monoclonal Abs isolated from the RV144 vaccinees showed very low mutation rates from germline genes encoding the variable region of the heavy chain (VH)—mutation rate range of 1.5 to 4.5% (29, 30), which is even lower than that noted after influenza immunization (mean VH mutation rate of 8.1% [31]). These data further uphold the concept that conventional Abs can be associated with reduced infection rates.

Thus, even though conventional nAbs are limited in their breadth and potency, they are commonly made by HIV-positive individuals (32), by immunized humans (24), and by immunized animals (33, 34), and the previously mentioned studies suggest that they can reduce infection rates. Therefore, such Abs, induced by vaccines, have substantial potential for influencing the course of the HIV epidemic.

Exceptional, Broadly Neutralizing Abs

Unlike conventional HIV Abs, broadly neutralizing Abs (bNAbs) are found in relatively rare HIV-infected subjects (35), with the most broad and potent serum Abs being identified in only $\sim 1\%$ of “elite neutralizers” (36). Most of the broadly neutralizing monoclonal Abs (bNmAbs) have been developed in the last few years (37–41). In addition to wide breadth (neutralization of 100% of tier 1 viruses and 72 to 100% of tier 2 viruses), bNmAbs are very potent, and *in vitro* assays have shown that very small amounts are sufficient for neutralization of tier 1 and tier 2 viruses ($<1\text{ }\mu\text{g/ml}$ and 0.02 to $27.0\text{ }\mu\text{g/ml}$, respectively) (17).

For bNAbs to acquire their impressive breadth and potency, extensive somatic hypermutation is required—particularly in the variable heavy chain (VH) genes and in some framework regions—a process that usually requires more than a year of exposure to the virus after HIV infection (42, 43). Several additional unique characteristics of bNmAbs have been described, such as frequent auto- and/or poly-reactivity for host antigens and long heavy chain complementarity-determining region 3 (HCDR3) sequences composed of 20 to 34 residues, which stand in stark contrast to the length of HCDR3s produced by human B cells, which averages 16 residues (44–47). These features contribute to the rarity of bNAbs

and pose a major challenge for the development of an HIV-1 vaccine designed to induce such Abs.

While bNmAbs are exceptionally potent and provide protection in animal models (48–51), all attempts to elicit bNAb responses by vaccination have so far been unsuccessful, and it is believed that a panel of special immunogens which will both stimulate suitable naïve B cells and guide them through lineage maturation will be required to achieve this goal (52); this process of Ab maturation appears to be long and complex.

To design these immunogens, bNmAbs from infected donors are being isolated and sequenced to reconstruct the lineage of the Abs, including the sequence of the probable common unmutated ancestor of a given bNmAb. The envelope glycoproteins recognized by the Abs in the bNmAb lineage are being expressed, and these will serve as immunogens to be used sequentially to engage the naïve B cell receptor and to stimulate and guide the evolution of the nAb response (44).

While the ultimate success of this path toward the design of a prophylactic vaccine is unknowable at this point, there is, nevertheless, great potential for the use of bNmAbs for passive immunization to protect against and treat HIV infection. Thus, treatment of macaques with bNmAbs has been shown to completely protect against infection with simian/HIV when these Abs are present at serum concentrations as low as $30\text{ }\mu\text{g/ml}$ (53–55). Broadly neutralizing mAbs are also being developed for treatment of established HIV infection, a process in which bNmAbs will be infused into HIV-infected individuals to decrease and/or eliminate virus. Success has already been achieved in this area as demonstrated by treatment of humanized mice and macaques infected with HIV and simian/HIV, respectively (56–58).

There is also great interest in whether passively transferred bNmAbs can contribute to the prevention of mother-to-child-transmission of HIV-1, the continuing cause of a significant percentage of new infections in the developing world. During pregnancy, HIV-specific Abs can pass from an HIV-infected mother to the fetus through the placenta. These Abs, however, are not effective against later HIV infection, for example, during the breast feeding phase. Moreover, the transmitted virus variants have fewer potential N-linked glycosylation sites, a fact that could impact positively on the interaction of glycan-dependent bNAb with transmitter/founder viruses recognition (59). Indeed, transmitted HIV variants were shown to be susceptible to various bNmAbs, but a combination of potent bNmAbs targeting diverse epitopes might be needed to successfully protect against HIV infection in the mother-to-child-transmission context (60).

VACCINE DEVELOPMENT AND IMMUNOGEN DESIGN

As a result of the extensive data summarized above, the elicitation of Abs is clearly indicated as a requirement for a successful HIV vaccine. As noted, this is a challenging task. The effort to develop vaccine candidates capable of inducing protective Abs has accelerated dramatically since the Thai vaccine trial RV144 revealed that Abs were associated with a reduced rate of infection (24–26, 28).

Several envelope-based immunogens have been tested for their ability to induce nAbs. Monomeric gp120 is relatively easy to produce and has been widely used in animal studies. Initial human vaccine trials elicited only weak nAbs, and no protection against HIV infection was achieved (61, 62). The RV144 vaccine trial, however, demonstrated a beneficial effect of monomeric gp120 in combination with a canarypox prime (63). It was suggested that the epitopes exposed on monomeric gp120 are poor neutralization targets because they are occluded on the native envelope trimer (64), and therefore immunogens were designed that better mimic the native envelope spike. Since the trimeric envelope is highly unstable and difficult to produce, a variety of different immunogens using gp140, the ectodomain of trimeric gp160, have been made (65–67). To date, immunizations using trimeric envelope immunogens have not been successful in the induction of potent nAbs (68, 69). Recent studies, monitoring the coevolution of virus and bNAbs in patients during natural infection, demonstrated that a tight interplay between Ab maturation and subsequent viral escape drives the development of bNAb responses (9, 70, 71). These studies provide important new insights into the elicitation of bNAbs and will advance the development of better immunogens.

Rather than using whole Env monomers or trimers, another approach to vaccine design is the use of recombinant immunogens that target the Ab response to particular epitopes. Computationally designed vaccines that mimic viral and bacterial epitopes have been shown to induce potent conventional protective nAbs against various viruses and bacterial pathogens (72, 73). This same approach is being applied to the design of recombinant vaccines that target specific HIV epitopes. At the present time, the type of epitopes to be targeted by conventional Abs include the glycan-independent V1V2 and V3 regions of the HIV envelope, whereas the type of epitopes to be targeted by bNAbs include “sites of vulnerability” (74) defined as the glycan-dependent V2 epitope (see “V2q epitope” in the list below), the glycan-dependent epitope at the base of V3, the CD4 binding site in gp120, and the membrane proximal external region (MPER) in gp41.

Abs Targeting Variable Loops 1 and 2 (V1V2)

Electron tomography, cryo-electron microscopy, and biochemical studies have shown that the V1V2 domain is localized at the apex of the trimeric HIV-1 Env structures, and therefore at least some of the V1V2 epitopes are accessible to Abs (75, 76). V2 loop sequences differ in length, but the majority of amino acids are highly conserved, suggesting conserved structural elements (77). The V1V2 region forms four antiparallel β -strands (A, B, C, and D) which are linked via disulfide bonds (78). Via a conserved tri-peptide, the $^{179}\text{LDV}/^{181}$ binding motif, V2 can bind to $\alpha 4\beta 7$, an integrin expressed on activated CD4⁺ T cells that is required for the homing of CD4⁺ T cells to the gut mucosa (79).

Abs targeting the V1V2 region were associated with a lower risk of infection in the RV144 clinical vaccine trial, thus making this area a promising target for vaccine development and the focus of intense research (24–26). To date, three different epitope types have been defined in the V1V2 region:

V2i epitope. A group of seven human mAbs recognizes a conformation-dependent epitope, designated V2i since these Abs target the disordered region in V2 that connects the C and D strands and includes the $\alpha 4\beta 7$ -integrin binding site (hence the term “V2i”) (80, 81). The structure of this region has so far not been solved, suggesting that it is highly flexible and dynamic. Abs targeting V2i are highly cross-reactive in binding to monomeric gp120 but do not neutralize HIV well (18), suggesting that the epitope is mostly occluded from Ab recognition in the trimeric envelope.

V2p epitope. This epitope is defined by two mAbs (CH58 and CH59) that were isolated from an RV144 vaccinee. The epitope is glycan-independent, and these mAbs bind V2 peptides (thus, “V2p”) and selected monomeric gp120, recognizing an epitope composed of helical or helical-coil structures in the C strand of V1V2 (30, 82).

V2q epitope. This is a quaternary epitope, which is preferentially expressed on the trimeric structure of the gp120 spike. Crystallographic studies with a V1V2-fusion protein show that broad and potentially neutralizing V2q mAbs bind to relatively conserved residues within V2 as well as to N-linked glycans—most importantly the N160 glycan. Earlier studies also showed that the binding of V2q-specific mAbs was influenced by residues in V3 (37, 83, 84). V2q-specific bNAbs, including PG9, PG16, and CH01, are extremely potent and broad

in their reactivity and have long CDRH3 loops that interact with N-linked glycans and reach around them to contact amino acids of V2. These V2q mAbs are highly mutated from germline (44).

Abs Targeting Variable Loop 3 (V3)

The V3 loop is located in close proximity to the V1V2 domain at the apex of the envelope trimer (85) and is involved in CCR5 or CXCR4 coreceptor tropism and binding. It thus plays an important role in virus entry into the host cell, and it is required for infectivity since V3-deleted mutants are noninfectious (86).

While, by definition, there is considerable amino acid sequence variation in V3, about 60% of the amino acids are conserved, and the variation occurs at restricted positions (87, 88). The region is characterized by a conserved length of 34 to 35 amino acids, the presence of N-linked glycosylation sites at its N- and C-terminal ends, and several conserved structural features. The V3 loop can be divided into three structural regions: (i) a base region that is located in the gp120 core and includes a disulfide bond, (ii) a flexible stem region, and (iii) a distal crown that contains the highly conserved GPGR/Q motif at its tip. The recognition of conserved V3 elements contributes to the broad cross-reactivity of V3-specific Abs (89, 90).

V3 Abs are present in essentially all infected individuals (91), and V3 Abs have been elicited by several types of vaccines (4, 92, 93). Moreover, the first demonstration of the successful use of “reverse vaccinology,” i.e., the design of vaccines based on epitopes recognized by biologically active mAbs, was achieved using V3-scaffold immunogens which targeted the immune response to this single epitope of the HIV envelope. For this, the V3 loop was spliced into a conformationally correct site on the highly immunogenic protein, cholera toxin subunit B, a protein which forms a pentameric structure and therefore presents five copies of V3 (94), serving as a particularly strong antigen for induction of Abs (95). High anti-V3 Ab titers were elicited in rabbits with one or a combination of V3-cholera toxin subunit B immunogens, and these immune sera were able to neutralize numerous diverse HIV strains (33, 94).

Just as Abs to V2 target three regions (V2i, V2p, and V2q), three types of V3 Abs have been described.

Glycan-independent “ladle-like” V3 Abs

The V3 crown is an immunodominant region, and Abs targeting the epitopes in the crown are made by essentially all HIV-infected individuals (91). Abs to this region are

glycan-independent. The ladle-like anti-V3 mAbs bind to the tip of the V3 crown which sits in the “bowl” of the ladle while the N-terminal V3 beta-strand adheres to the “handle” of the ladle. Representative mAbs of this type include 447-52D, where the long CDRH3 forms the handle of the ladle that interacts with the main chain of the N-terminal beta-chain of the V3 crown.

Glycan-independent “cradle-like” V3 Abs

The second type of V3 Ab uses an antigen-binding mode typified by mAb 2557. In such cradle-like Abs the antigen binding site consists of a groove in the Fab fragment, and the epitope lies in this groove, resembling a baby in a cradle; in this case, the major binding site is the hydrophobic core of the V3 crown, usually composed of hydrophobic, conserved residues 307, 309, and 317 (89, 96, 97).

Both types of glycan-independent Abs specific for the V3 crown can neutralize most laboratory-adapted HIV-1 strains and tier 1 viruses but neutralize relatively few tier 2 viruses using standard neutralization assays (21, 98, 99). This is largely due to masking of the V3 loop by glycans (100) and by the V1V2 domain that is situated atop the trimer. Deletion of V1V2 leads to a better exposure of V3 epitopes and thus better neutralization by V3 mAbs (101, 102). Importantly, it was recently shown that anti-V3 Abs are effective against tier 2 viruses if the Ab and virus are coincubated for several hours rather than for 1 to 2 hours, which is the norm in standard neutralization assays (103). These results suggest that the V3 loop is meta-stable on the virus surface, flickering between a cryptic and exposed conformation, the latter being both required for interaction with the chemokine receptor and available for Ab binding leading to neutralization. Additionally, CD4 binding induces a conformational change in gp120, releasing the V3 crown from the surface of the envelope trimer and thus augmenting V3 epitope exposure and sensitivity to V3 Ab neutralization (90).

Glycan-dependent V3 Abs

The base of V3 is poorly immunogenic, eliciting Abs in a relatively small proportion of infected individuals. Nonetheless, mAbs that target this region, such as the PGT121-like and the PGT128-like Ab families, are extremely potent and broadly reactive. These mAbs are highly somatically mutated and require specific glycan interactions, particularly at position N332 (38, 104). The crystal structure of PGT128 in complex with an engineered outer domain of gp120 recently showed that this Ab also interacts with the N301 oligomannose glycan, a position that is not recognized by PGT121-like

Abs (104). Even though the two Ab families are suggested to approach gp120 from different angles, both block HIV-1 infection by interfering with CD4 binding through allosteric mechanisms (105).

Abs Targeting the CD4 Binding Site (CD4bs)

The CD4bs is functionally highly conserved and thus seems to be an ideal target for Abs. However, it is well hidden by surrounding glycans and variable regions (74), and Abs are obstructed from binding due to steric and conformational hindrance (see section on conformational masking, above). Studies have shown that many CD4bs mAbs can bind with high affinity to recombinant gp120 but cannot access the CD4bs on the envelope trimer. The mAb b12 was the first neutralizing mAb discovered to successfully target the CD4bs, but its breadth and potency are restricted due to amino acid variation both within and outside of the CD4bs (44, 106, 107).

More recently, several bNmAbs have been isolated that mimic binding of CD4 to gp120 and as a result neutralize HIV-1 potently and with broad reactivity. These CD4bs-specific bNmAbs, isolated from various HIV-infected individuals, share several genetic and structural characteristics. First, their heavy chains all derive from the VH1-2 or the closely related VH1-46 germline genes. These Abs are also highly somatically mutated, with ~20 to 30% of nucleotide changes in their heavy chains compared to germline. However, attempts to induce these CD4bs bNAbs have been problematic, even using “engineered gp120” and recombinant “designer immunogens” modeled on the structure of the epitopes contacted by the very effective bNmAbs (108–110). Cross-clade nAbs were induced in rabbits via the reverse vaccinology approach. Immunogens were designed based on the epitope recognized by the mAb IgG1b12. Thus, fragments of gp120 containing 70% of the b12 epitope were used for priming of rabbits. The animals received a boost with full-length gp120 after 16 and 51 weeks. Cross-clade neutralizing HIV-specific Abs were elicited in the rabbits, which neutralized tier 1, 2, and 3 viruses (111), providing a maximum geometric mean of IC₅₀ titers against five tier 2 and 3 viruses of 1:134.

Abs Targeting the Membrane-Proximal External Region (MPER)

The MPER consists of the last 24 C-terminal amino acids of the gp41 ectodomain. Its sequence is highly conserved, contains many hydrophobic residues, and is usually rich in tryptophans. It is believed that the MPER undergoes significant conformational changes during viral entry (112–114).

Different epitopes have been described in the MPER: the very potent human mAb 10E8 recognizes an α -helix in this region, while other human mAbs, including 2F5 and 4E10, target an overlapping region that additionally includes residues of the transmembrane spanning domain. As opposed to mAbs 2F5 and 4E10, bNmAb 10E8 can neutralize ~95% of viruses tested and lacks detectable reactivity with self-antigens, a feature of the less potent 2F5 and 4E10 (40, 44).

Like the bNAbs targeting gp120, bNAbs specific for the MPER of gp41 have been extremely difficult to induce with vaccines (115). They are present in only a minority of HIV-infected individuals (35, 116), suggesting that this is a poorly immunogenic region. This is supported by the finding that MPER mAbs such as 2F5 and 4E10 appear to mimic self antigens, and therefore the responses of B cells with MPER specificity are downmodulated (46).

CONCLUSIONS

Three decades of study have established the important role of Abs in protecting against HIV infection. However, it has become quite clear that HIV uses many mechanisms to protect itself from the biologic effects of Abs that would block infectivity. Design of an effective vaccine must take into account the presence of glycans and masking phenomena to induce Abs that can penetrate or circumvent these protective shields employed by the virion. Current immunogen design is affected profoundly by whether the aim is to induce conventional Abs or exceptional broadly neutralizing Abs. Induction of exceptional Abs with a vaccine may require the use of a series of immunogens that “guide” the immune response through the mutations required for the specificities displayed by broad and potent neutralizing Abs. However desirable this goal is, whether it is achievable has yet to be established. Alternatively, conventional Abs, while not as broad or as potent as exceptional Abs have already been elicited by vaccine trials and are correlated with a reduced rate of infection in the RV144 phase III clinical vaccine trial. Induction of conventional protective Abs is therefore possible. The vaccine regimens and reagents to be used in vaccine development are many, ranging from DNA and viral vector priming immunogens to proteins representing the trimeric envelope proteins or portions thereof spliced onto immunogenic scaffolds. Many more clinical trials for safety, immunogenicity, and protection are required to establish which of these many regimens and reagents will result in a prophylactic vaccine.

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