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HIV-1 Entry Inhibitors

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Abstract

The discovery of chemokine receptors as essential cofactors for HIV-1 entry into target cells, as well as the publication of crystal structures of viral molecules involved in the entry process, has stimulated the development of a broad spectrum of novel antiviral substances targeting this initial step in the virus replication cycle. The aim of this article is to review the antiviral compounds targeting different steps during HIV-1 entry: 1) attachment inhibitors, which block the initial binding of the virus to the cell, 2) compounds interfering with subsequent coreceptor binding, and 3) fusion inhibitors, which prevent the fusion process between viral and cellular membranes. Some of these compounds have already entered clinical phase I/II trials and are promising drugs due to their mode of action, i.e. inhibition of *de novo* infection of cells and their potent antiviral activity. Thus, new therapeutic options will be available to be used in combination with highly active antiretroviral therapy (HAART) to treat drug-naive, but also drug-experienced, HIV-positive persons. Furthermore, insights into the process of HIV-1 entry also stimulate new approaches for vaccine development.

Key words

HIV-1. Entry inhibitors. Attachment. CD4. Chemokine receptors. Fusion.

Introduction

Drugs currently approved for the treatment of infections with the human immunodeficiency virus type I (HIV-1) target two key viral enzymes, reverse transcriptase (RT) and protease[1,2] (Fig. 1). Today, 16 such antiviral drugs are available, and generally 2-3 RT inhibitors are combined with 1-2 protease inhibitors to achieve highly active antiretroviral treatment (HAART). Despite the success of HAART in terms of successful reduction of the viral load in patients and a remarkable decline in morbidity and mortality, HAART is not able to completely suppress viral replication in the patients[3,4]. Besides insufficient drug potency and the presence of sanctuaries in the body that are not accessible for certain drugs, severe adverse effects frequently result in non-adherence of the patients to the strict drug regimens. This scenario favours the emergence of drug-resistant virus variants, which is an increasing problem in today's HIV-1 therapy (reviewed in[5]).

In order to augment the potency of currently available antiretroviral drug combinations and to achieve the inhibition of drug-resistant virus variants, more effective drugs, which target additional steps in the viral replication cycle, are urgently needed. Very promising candidates are emerging in the class of HIV-1 entry inhibitors (Fig. 1). New insights into the molecular details of HIV-1 entry into target cells, as well as the knowledge of the crystal structures of viral molecules involved in this process[6-12], have led to the development of antiviral molecules targeting different steps during HIV-1 entry. As these drugs interfere with the *de novo* infec-

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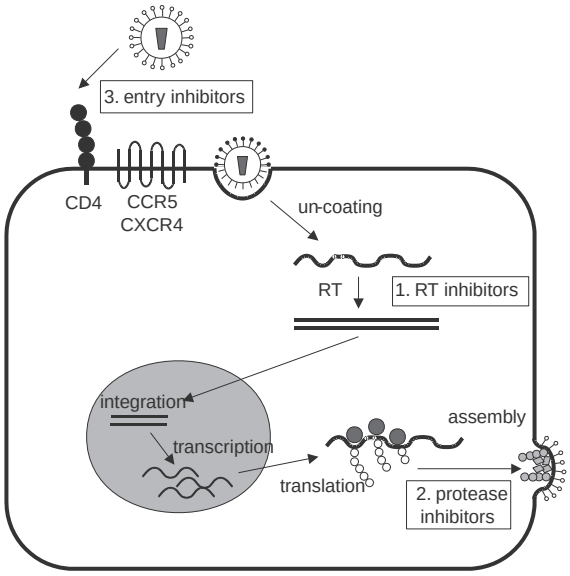

Fig. 1. *Therapeutic approaches against HIV-1: current treatment options are based on the combination of drugs targeting reverse transcriptase (RT)(1) and the viral protease(2) to achieve highly active antiretroviral therapy (HAART). A new class of therapeutic drugs is emerging, which target viral entry(3) by interfering with attachment, receptor binding or fusion.*

tion of cells, they should limit the spread of the virus in the body very efficiently and be active against drug-naive viruses as well as against variants resistant for RT and protease inhibitors.

HIV-1 entry into target cells

Entry of HIV-1 into target cells is a multi-step process involving the timely and locally ordered exposure of previously occluded entry domains within the viral glycoproteins gp120 and gp41 (reviewed in[13,14], Fig. 2). HIV-1 has probably evolved this complex mechanism of entry to protect the functionally important and necessarily conserved entry domains from the attack of the immune system. The gradual exposure of entry domains results from conformational changes within the viral glycoproteins, which are triggered by multiple receptor interactions. By this mechanism, the crucial entry epitopes are only exposed when the virus is already close enough to the cell membrane to initiate entry, thus minimising exposure of these domains to antibodies.

Infection of cells by HIV-1 begins with the interaction of the viral surface glycoprotein gp120 with specific receptors on the target cells, thereby limiting the host range of the virus to the cells bearing these receptors. The main receptor for HIV-1,

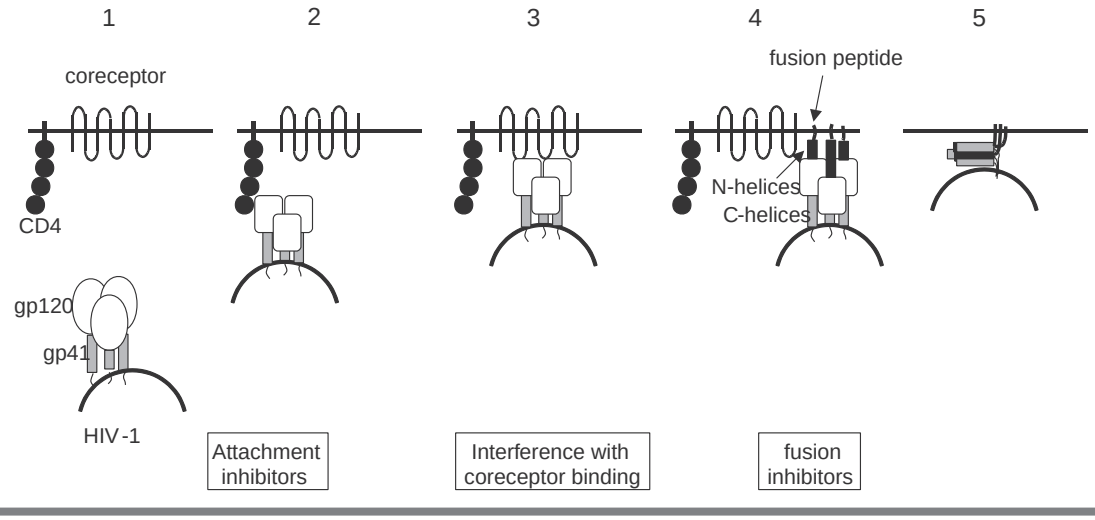

Fig. 2. *Summary of the different structural intermediates involved in HIV-1 entry that are targets for therapeutic intervention. 1) Viral and cellular molecules involved in the entry process; 2) upon binding of trimeric gp120 to CD4, conformational changes are induced in gp120, which result in the exposition of coreceptor-binding domains; 3) these conserved CD4-induced epitopes in gp120 bind to the coreceptor, mediating closer approximation between virus and cell; 4) conformational changes in gp41 are induced, resulting in the formation of a pre-hairpin structure and in the insertion of the fusion peptide into the target cell membrane; 5) formation of the hairpin structure is induced by folding-back of the C-helices into a groove in the N-helices, which finally allows viral and cellular membranes to come into contact and to form the fusion pore complex.*

and the first recognized as such, is the CD4 receptor present on T4-lymphocytes and monocytes/macrophages[15,16]. The interaction between gp120 and CD4 is necessary to trigger conformational changes within gp120, resulting in the exposure of previously occluded, conserved epitopes, which then bind to a second receptor belonging to the large family of chemokine receptors, a subclass of G-protein coupled receptors (reviewed in[17,18]). Although many chemokine receptors are known, and a substantial amount of those are known to bind HIV or the simian immunodeficiency virus SIV *in vitro,* mainly two chemokine receptors, CCR5 and CXCR4, are relevant for infection *in vivo*[19-21]. CCR5 is the main receptor for non-syncytium inducing (NSI) HIV-1 variants (today referred to as R5) in the early phase of the infection and, therefore, is the essential receptor during primary HIV-1 infection. Syncytium inducing (SI) virus variants appearing later during the course of the disease use CXCR4 (R4)[22]. After coreceptor binding, additional conformational changes within gp120 lead to exposure of the fusion peptide of the viral transmembrane protein gp41 and to the activation of gp41 from a pre-fusogenic into a fusogenic conformation, which ultimately mediates fusion between the viral and cellular membranes.

The sequential exposure of highly conserved *env* domains critical for the HIV-1 entry process offers multiple opportunities for therapeutic intervention, as all structural intermediates represent potential targets for antiviral drugs aiming at interfering with HIV-1 entry (Fig. 2, table 1).

1. Attachment inhibitors

Polyanionic substances

The interaction of the viral glycoprotein gp120 with the CD4 receptor can be inhibited by polyanionic substances such as polysulphates, polysulphonates or polycarboxylates[23-25]. On the other hand, polyanionic cell surface heparans are known to be involved in the attachment process between virus and cell before the specific interaction with the receptors[26,27]. Polyanionic molecules probably exert their antiviral activity by neutralizing positively charged amino acids within gp120, which are necessary for receptor binding. Consequently, resistance development to one such compound, dextran sulphate, is associated with single amino acid substitutions within gp120[28]. The inhibitory effect of polyanionic molecules increases with molecular weight and the degree of sulphation. Sulphated polysac-

Table 1. *Antiviral substances targeting HIV-1 entry*

Compounds	Characteristics	Clinical status	References
1. Attachment inhibitors			
polysulfates polysulfonates polycarboxylates	interaction with viral envelope; mainly against R4 HIV-1	under consideration as vaginal microbicides	23, 24, 25, 29 34, 35
PRO 2000 Cyanovirin-N	naphtalene sulfonate polymergel formulation in 11 kDa protein from Cyanobacterium, interacts with sugars in Env	phase I pre-clinical	36, 37, 38 51-54
PRO 542	soluble CD4-IgG	phase I	47-50
2. Coreceptor interference			
Met-RANTES L-RANTES AOP-RANTES NNY-RANTES 3-68 RANTES 9-68 RANTES C1.C5-RANTES	RANTES derivatives blocking interaction of gp120 with CCR5	mostly pre-clinical	61 60 62 63 64 65 63
TAK-779	0.5 Da small molecule inhibitor, targets pocket between in TM of CCR5	pre-clinical	66, 67
Schering-C	small molecule CCR5 antagonist	pre-clinical	68
AMD3100 T22, T134, T140	bicyclam, CXCR4 antagonist peptide antagonists of CXCR4	phase 2 pre-clinical	78, 79 81-83
ALX40-4C CGP64222	selected as Tat/TAR inhibitors, inhibit CXCR4	pre-clinical	86 85, 87
3. Fusion inhibitors			
T20 = DP178 C34 N36 Cyclic D-AA-peptides	C-peptide binding to N-helices of gp41 C-peptide extending into the pocket of N-helic N-peptide target pocket in N-helices	phase 2 pre-clinical pre-clinical pre-clinical	96-98 90-94 90-94 99

charides are able to block HIV-1 replication *in vitro* at concentrations as low as 0.1 to 0.01 µg/mL[29]. However, the inhibitory effect is mainly restricted to CXCR4 using HIV-1 isolates due to their increased basic charge in the V3 loop. In contrast, infection of macrophages by CCR5 using HIV-1 isolates can even be enhanced by high molecular weight dextran sulphate[30].

Clinical trials of polyanionic compounds did not result in remarkable antiviral effects, probably due to low bioavailability and the fact that *in vivo* CCR5 using HIV-1 isolates predominate, which can hardly be inhibited[31-33]. However, some substances are under consideration as vaginal microbicides, especially since their antiviral effect is not limited to HIV-1, but also includes other viruses like herpes viruses CMV or HSV[34,35].

PRO 2000, a naphthalene sulphonate polymer, is a promising candidate for antiviral vaginal microbicides[36]. This substance was able to completely block proviral formation *in vitro*[36] and in ectocervical explants exposed to HIV-1BAL and three other primary HIV-1 strains at a concentration of 100 µg/mL[37]. A gel formulation of PRO 2000 was tested in a clinical phase I trial in healthy women, showing good tolerance[38].

Soluble CD4 and derivatives

Another possibility to interfere with the binding of gp120 to cell-bound CD4 is the use of soluble CD4 receptor preparations (sCD4). The obligatory interaction of primary HIV-1 isolates with CD4 has made this molecule an attractive antiviral target since the late eighties. Recombinant sCD4 was shown to inhibit infection of T-cell lines with laboratory HIV-1 strains at concentrations of 2-10 µg/mL[39,40]. However, primary HIV-1 strains require a much higher dose of sCD4 than laboratory HIV-1 strains in order to be neutralized, the 90% inhibitory dose (ID_{90}) being 200- to 2700-fold higher for the patients' isolates[41]. Clinical trials showed sCD4 to be pharmacologically safe; however, the plasma half-life of sCD4 was only 45 minutes and peak plasma levels of only 300 ng/mL were achieved at the highest dose (30 mg per day)[42-44]. This was too low by far to neutralize patients HIV-1 isolates.

Thus, the pharmacokinetic properties of sCD4 had to be modified in order to achieve a more stable and prolonged production of the molecule. Genetic engineering of cells *in vitro* for the continuous production of sCD4 after retroviral transfection resulted in a maximum of 15 ng/mL sCD4[45,46]. Although the number of infected cells could be reduced, complete protection was never achieved and the number of transfected cells was low, especially for primary cells.

Another option to increase the stability of sCD4 is through multimerisation. PRO 542 is a recombinant, tetravalent, antibody-like fusion protein, where the Fv portions of the immunoglobulin (Ig) heavy and light chains have been replaced by the two N-terminal Ig-like domains of CD4[47]. Compared to monomeric sCD4, PRO 542 has demonstrated 100-fold greater activity against primary HIV-1 isolates[48,49]. The concentration required to achieve 90% reduction in viral infectivity *in vitro* (IC_{90}) was 20 µg/ml. *in vivo*, serum concentrations of > 500 µg/mL were obtained after administration of a single dose (10 mg/kg) of PRO 542 and concentrations remained above the *in vitro* IC_{90} for longer than one week[50]. One subject showed >2-log reduction in plasma viral load 1 to 2 weeks after a single-dose intravenous administration of PRO 542. Further antiviral activities of this promising compound have to be tested in phase 2 clinical trials.

Other substances

Cyanovirin-N is an 11-kDa protein isolated from the cyanobacterium *Nostoc ellipsosporum* with broad neutralizing activity against HIV-1 and HIV-2, and also other enveloped viruses like the feline immunodeficiency virus FIV, human herpesvirus HHV6 and measles virus[51]. The inhibiting mechanism of Cyanovirin-N is not very clear. The molecule is known to bind to a conserved region of gp120, inhibiting binding to CD4[52]. However, Cyanovirin-N also seems to act at later stages during coreceptor binding and membrane fusion[53]. The broad antiviral effect may be due to interactions of Cyanovirin-N with high-mannose oligosaccharides present on Env proteins[54].

2. Compounds interfering with coreceptor binding of HIV-1

The family of chemokine receptors, which are G-protein coupled 7-transmembrane receptors, and their ligands (chemokines), are involved in the trafficking of leukocytes in immune surveillance and inflammation (for review see[17,18]). As such, chemokine receptors play an important role in the pathophysiology of inflammatory and allergic diseases, but also in hematopoiesis, angiogenesis, differentiation and development and become an attractive therapeutic target in a variety of diseases like asthma, autoimmune diseases, etc.[55]. Some chemokine receptors are used by intracellular pathogens like *Plasmodium vivax*[56] or HIV-1[18] for entry and transmission and, thus, represent novel anti-parasitic and antiviral targets.

Chemokine receptors CCR5 and CXCR4 are the essential coreceptors for HIV-1 *in vivo*, although nine additional coreceptors are able to mediate infection with HIV-1, HIV-2 or SIV *in vitro*[17]. The discovery of CCR5 as coreceptor for HIV-1 was based on the knowledge that β-chemokines RANTES, MIP-1α and MIP-1β, the natural ligands of CCR5, have antiviral activity against primary HIV-1[57], the antiviral activity being mediated either by steric hindrance or by chemokine-induced receptor internalisation. Therefore, the development of receptor antagonists, which are still able to bind the receptors but do not activate them, was an obvious aim in the development of antivirals interfering with HIV-1 entry at the level of coreceptor binding. Furthermore, the fact

that individuals with a 32-basepair deletion in the CCR5 gene, which results in premature termination of the protein and the lack of expression at the cell surface, are highly resistant to infection with HIV-1 (however these people are infectable by rare virus variants able to use CXCR4 during primary infection), proves an essential role of CCR5 for primary HIV-1 infection[58,59].

As the absence of CCR5 from the cell surface in these individuals does not impair their health, blocking of CCR5 by receptor antagonists should also signify the absence of drastic side effects upon therapeutic treatment. For this reason, CCR5 is particularly interesting as an antiviral target.

Agents targeting CCR5

Chemokine derivatives

The easiest way to produce CCR5 antagonists is by modification of the natural chemokine ligands for CCR5. For RANTES, the chemokine with the highest affinity for CCR5, it was shown that antiviral activities could be uncoupled from signalling functions. This opens new perspectives for the development of chemokine-based therapeutic approaches against HIV-1 in the absence of inflammatory side effects[60]. A number of amino-terminal RANTES modifications, which show antiviral activity against HIV-1, have been developed. Met-RANTES[61] and L-RANTES[60] differ from natural RANTES by the addition of an extra methionine or leucine at the N-terminus of the protein. AOP-RANTES was produced by chemical coupling of an alkyl chain (aminooxypentan) to the amino terminal serine of RANTES[62]. NNY-RANTES (N-nonanoyl) is a nonanoic acid derivative of RANTES[63]. Other variants resulted from deletions of 2 (3-68 RANTES[64]) and 8 amino acids (9-68 RANTES[65]) at the N-terminus of RANTES. In C1.C5-RANTES, serine 1 and 5 of RANTES were substituted with cysteines. Although AOP-RANTES showed the best antiviral activity *in vitro*, with an IC_{50} about 10-fold lower than natural RANTES, this molecule behaved as a CCR5 agonist; i.e. it also activated the receptor in terms of intracellular calcium mobilization[60]. C1.C5-RANTES was the best receptor antagonist, mediating good antiviral activity (IC_{50} 5-fold lower than RANTES) without activating CCR5 and thus may represent a good lead-compound for HIV-specific intervention.

However, one has to be aware that blocking of CCR5 alone may result in the selection of virus variants able to use CXCR4 or even other coreceptors. In fact, this could be nicely shown in the hu-PBL-SCID mouse model[63], where treatment with NNY-RANTES was shown to partially protect mice from infection with HIV-1. However, infected mice contained amino acid substitutions in the V3-region known to confer a coreceptor switch to CXCR4.

Non peptidic small molecule inhibitors

TAK-779 is a small molecule (531Da) known to specifically target CCR5[66].

The compound inhibits infection of target cells with HIV-1 isolates using CCR5 as coreceptor and also inhibits ligand-induced signalling. The IC_{50} values in peripheral blood mononuclear cells range from 10 to 100 nM, depending on the HIV-1 isolates used. Interestingly, TAK-779 blocks the interaction between viral gp120 and CCR5 by binding to a pocket located between transmembrane helices 1, 2, 3 and 7 of CCR5[67]. Probably, the molecule interacts first with the extracellular domains of CCR5 by its hydrophilic part dictating the coreceptor specificity, and then inserts its hydrophobic moiety into the transmembrane pocket. Nothing is known yet about the pharmacological properties of TAK-779.

Schering-C is another small molecule antagonist for CCR5 having sub-nM activity in HIV-1 entry assays[68]. This compound and others have been isolated in high-throughput screenings for CCR5 antagonists.

In addition, a number of monoclonal antibodies against CCR5, which inhibit HIV-1 infection of target cells by CCR5-using HIV-1 isolates[69,70], are known. Although these antibodies are not directly useful as therapeutic agents, they are extremely valuable to identify the domains in CCR5 involved in HIV-1 binding[71] and, consequently, for the development of small molecule inhibitors targeting these sites.

Agents targeting CXCR4

Chemokine derivatives

Antiviral agents targeting CXCR4 will be useful to inhibit infection of cells with the more pathogenic CXCR4 using HIV-1 strains often found at later stages of disease progression, but also to be given in combination with CCR5 blocking agents in order to avoid a coreceptor shift towards CXCR4. The natural ligand for CXCR4 is stromal cell derived factor SDF-1α, which inhibits infection of X4 HIV-1 strains both by receptor blocking and internalization[72,73]. As CXCR4, besides acting as coreceptor for HIV-1, plays an important role in the development of B-cell and myeloid lineages and in T-cell homing[74], it is particularly important to dissociate the signalling and the antiviral properties of SDF-1α for specific therapeutic intervention against HIV-1. By synthesizing overlapping 13 amino acids, long peptides corresponding to SDF-1α, an N-terminal peptide, could be identified that showed antiviral activity without interfering with signalling[75]. Also, single amino acid exchanges within the N-terminus of natural SDF-1α led to potent antagonistic molecules[76]. Furthermore, a common polymorphism in the 3´ untranslated region of SDF-1α (SDF1-3´A) correlates with delayed disease progression in HIV-1 infected individuals[77].

Small molecule inhibitors

AMD3100 (830 Da) is a potent CXCR4 antagonist, which inhibits X4 HIV-1 strains at nanomolar concentrations by binding to anionic residues within the extracellular loop of CXCR4[78]. It belongs to the

bicyclams, which were known to inhibit HIV-1 long before the identification of CXCR4 as coreceptor for HIV-1. AMD3100 has been shown to reduce HIV-1 viral load in the SCID-hu mouse model and in phase I clinical trials[79]. The substance is currently in phase II clinical trials involving intravenous administration. Bioavailability is poor and the substance also inhibits binding of SDF-1α to CXCR4.

In addition, several peptidic antagonists of CXCR4, which inhibit X4 HIV-1 with IC_{50}s of 2-50 nM[80], are in pre-clinical development. The most active peptides are T22 (an 18 amino acids analogue of polyhemusin II)[81], T134[82] and T140[83].

Unexpectedly, two additional cationic peptides have been shown to be CXCR4 antagonists, although they were originally selected as peptides binding to TAR, the Tat-responsive region in the viral m-RNA[84,85]. ALX40-4C is a 9 amino acid peptide, which mimics the basic domain of the viral transactivator protein Tat and inhibits binding of gp120 and SDF-1α to CXCR4[86]. The same dual mechanism of action could be demonstrated for the peptoid CGP64222, which also inhibits Tat/TAR and gp120/CXCR4 interaction[87,85].

3. Fusion inhibitors

The viral glycoprotein gp41 is responsible for the fusion of viral and cellular membranes to finally allow the virus entry into the target cell. In free virus particles in the plasma, the highly conserved gp41 fusion domains are buried in the interior of the *env* trimers on the viral surface and are thus protected from the immune system. It is only after the sequential conformational changes in gp120 induced by the previously described receptor and coreceptor interactions, that these functionally important domains are exposed, once the virus is close enough to the cell membrane (Fig. 2). After the conformational changes in gp120 leading to gp41 exposure, gp41 itself undergoes conformational changes, switching from a prefusogenic into an active fusogenic state. During this conformational change, the C-terminal region of gp41 (C34) contacts a hydrophobic groove in the N-terminal trimeric domain of gp41 (N36) to create a six-helix bundle (hairpin-structure). The formation of the six-helix bundle structure facilitates approximation between viral and cellular membranes and finally leads to the insertion of the fusogenic peptide at the very N-terminus of gp41 into the target cell membrane and the formation of the fusion pore in a highly cooperative manner. The interaction of the fusogenic peptide with the target cell membrane may be facilitated by specific interaction with heparan sulphate on the cell surface[88]. It is during this time, from the exposure of the gp41 pre-hairpin structure to the formation of the fusogenic state, that the virus is vulnerable to antiviral molecules targeting the structural intermediates.

Peptides inhibiting HIV-1 fusion

A number of peptides are available today that are derived from the α-helical regions at the N-terminus (N-peptides) and C-terminus (C-peptides) of gp41, mediating potent antiviral activity in the nM range against HIV-1 (for review see[89], Fig. 3). C-peptides, which are more potent inhibitors than N-peptides, interact with the groove in the N-terminal heptad of gp41 and viceversa[90-92]. C34 tightly packs into the grooves of the N36 coiled coil, thereby inhibiting binding of the natural gp41 C34 region from doing

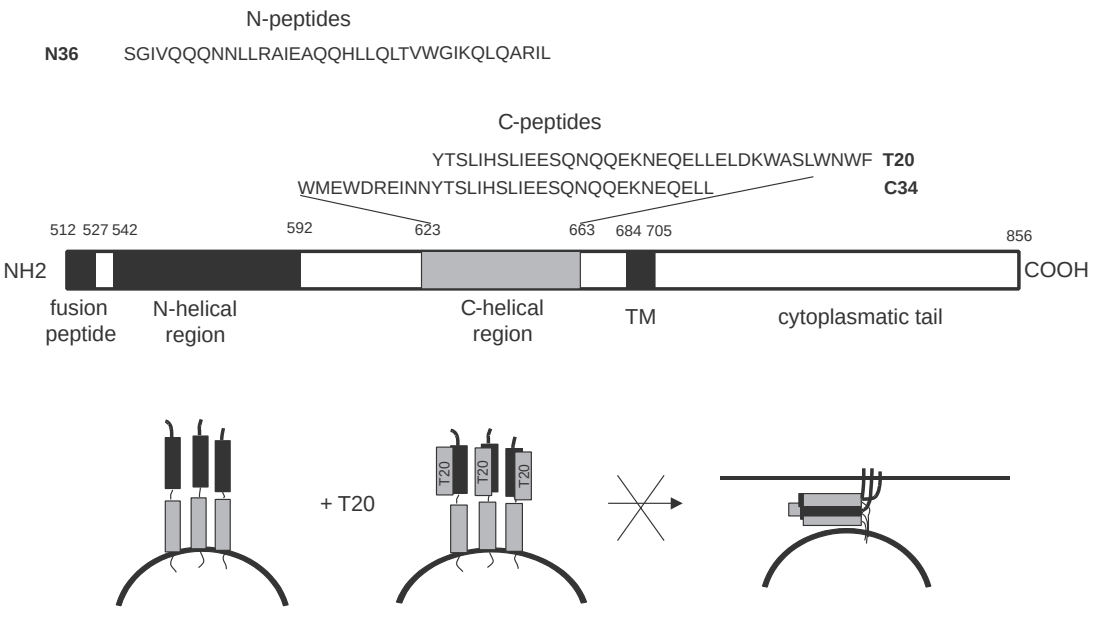

Fig. 3. *Functional domains in the viral gp41: the N-helices and C-helices are indicated as shown in fig. 2. Folding back between C-helices and N-helices can be inhibited by peptides derived from these regions, which target the pre-hairpin intermediate, as shown for T20.*

so. The X-ray crystal structure of the N36-C34 complexes shows a large hydrophobic groove in the N36 trimer, which is contacted by the C34 helices[91,93,94]. A pocket at the end of the groove accommodates three hydrophobic amino acids of C34 (I635, W631 and W628). C-peptides that extend into the pocket-like C34 have more potent antiviral activity and cause less resistance *in vitro* than peptides exclusively targeting the groove, like T20[95]. T20 (also called DP178)[96] has been shown in phase I/II clinical trials to reduce viral load in HIV-1 positive individuals by about 2 log after intravenous application[97]. However, large amounts of peptide are required to achieve the antiviral effect and peptides have a short half-life. Therefore, gene-therapeutic approaches are underway in order to express the inhibitory peptide in the target cells[98].

Currently, new smaller fusion inhibitors are being developed, which target the pocket in the N-terminal heptad. Cyclic D-amino acid peptides fitting into the pocket were identified in a phage display approach starting with a soluble N36 target; however, antiviral activity was less efficient than for C34[99]. Screening of combinatorial chemical libraries is expected to result in non-peptidic molecules with better fitting into the pocket and consequently with better antiviral activities. Besides the pocket, amino acids in the middle and at the N-terminus of the N-terminal heptad seem to be important for binding of the C-terminal heptad, as the NEQE and the WNWF amino acid motifs in the C-peptides (middle and at the C-terminus of the C-heptad) are important for antiviral activity[96,100].

Outlook

The structural intermediates in the multi-step process of HIV-1 entry into target cells offer various opportunities for therapeutic interventions. The fact that different steps during entry can be targeted also allows the combination of classes of drugs interfering at different levels, like, for example, coreceptor binding and fusion. This may result in synergistic effects. The combination of the CXCR4 blocker AMD3100 and the fusion inhibitor T20 has already been shown to have synergistic effects *in vitro*[101]. Clinical trials have to show if this is reflected in clinical benefits *in vivo*.

Furthermore, the different classes of entry inhibitors offer new opportunities for combination with HAART. Due to their completely different mode of action, these drugs are active against viruses resistant for RT or protease inhibitors and, thus, offer new opportunities for the treatment of drug-experienced HIV-1 positive persons. Furthermore, as entry inhibitors prevent the *de novo* infection of target cells, viral dissemination in the body should be limited.

However, the development of resistance will also be a problem for entry inhibitors, and the appearance of resistant viruses *in vitro* has already been described for some of these drugs. On the other hand, entry epitopes are functionally conserved and the virus may not have too many options to escape from drug pressure, especially if different entry steps are targeted in combination.

There may also be an additional entry target, for which no drugs are yet available. This is the domain in the viral gp120, which interacts with coreceptors after activation by CD4. Structural information on this conserved domain is available from the crystal structure of gp120[9] and from studies that identified contact amino acids for neutralizing antibodies known to bind to CD4-induced epitopes[102]. These CD4-induced epitopes are also interesting for vaccine development, as immunization with these structures is expected to induce broadly neutralizing antibodies. In fact, immunization of mice with whole cell fusion-competent vaccines representing transient Env- CD4-coreceptor fusion intermediates elicited antibodies neutralizing 23 of 24 primary HIV-1 isolates[103]. This proved the principle of such immunizations and has to be proven with pure immunogens corresponding to these structures, once they are ultimately identified.

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