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Enfuvirtide, the First Fusion Inhibitor to Treat HIV Infection

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Abstract

Entry inhibitors are a new class of drugs for the treatment of HIV infection. Enfuvirtide is the first compound of this family to be approved for clinical use. It blocks HIV fusion to host cells. It is a synthetic peptide that mimics an HR2 fragment of gp41, blocking the formation of a six-helix bundle structure which is critical in the fusion process. Enfuvirtide is a good therapeutic option as rescue therapy in combination with other active antiretrovirals and works against different HIV-1 variants, including all group M subtypes and group O. However, it is not active against HIV-2. The main mechanism of resistance to enfuvirtide depends of the selection of changes in a 10-amino acid domain between residues 36 to 45 in the HR1 region of gp41. Single and double mutations in this region have been shown to result in high-level resistance to enfuvirtide. A negative impact of enfuvirtide-resistance mutations on viral fitness has been postulated, since resistance mutations tend to disappear soon after drug discontinuation and because immunologic benefits have been noticed despite virologic failure in patients undergoing enfuvirtide treatment. **(AIDS Reviews 2005;7:139-47)**

Key words

HIV. Enfuvirtide. Fusion inhibitors. Resistance. Viral fitness.

Introduction

Highly active antiretroviral therapy (HAART) has dramatically changed the prognosis of HIV-infected individuals in the developed world[1]. However, given that HIV cannot be eradicated, most patients select drug resistance over time and need a change in their treatment combination. Ultimately, a growing proportion of subjects accumulate multiple resistance mutations, which is a major obstacle for the indefinite control of viral replication[2]. This fact validates the continuous need for new drugs, particularly compounds belonging to new classes which target different steps of the HIV replicative cycle, and lack cross-resistance with current antiretrovirals.

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Viral entry is currently one of the most important targets in the search for new drugs to treat HIV infection. Advances achieved in the knowledge of the molecular mechanisms involved in the different stages of the entry process have enabled the production of molecules which block each step: i) attachment of the viral gp120 to the CD4 cell receptor; ii) binding of gp120 to CCR5 or CXCR4 coreceptors; and iii) fusion of the viral and cellular membranes. Entry inhibitors are the latest family of antiretroviral compounds, the first of which to be approved has been enfuvirtide (Fuzeon®), a fusion inhibitor[3,4]. Many other entry inhibitors are currently in clinical development, and hopefully will soon be part of the therapeutic armamentarium against HIV. This new family of antiretrovirals is eagerly awaited by the growing number of patients carrying drug-resistant viruses to reverse transcriptase and protease inhibitors. This article will review the history of the development of enfuvirtide, from basic investigations to its current clinical use.

Viral entry

The HIV envelope glycoprotein is the mediator of the entry process into the host cell. It is an integral mem-

brane protein that is generated as a polyprotein precursor (160 kDa) named gp160. In the Golgi compartment, after cleavage by a cellular protease, the mature envelope protein is generated. A non-covalent association links its two components: gp120 and gp41 glycoproteins[5]. The mature envelope is found in the viral membrane forming trimers.

The first step in the viral entry process is the attachment of the viral gp120 to the CD4 receptor present in the cell surface. It is mainly driven by electrostatic forces between the positive charge of the CD4 molecule and the negative charge of the gp120 cavity. Van der Walls' forces and hydrogen bonds help to stabilize the initial CD4/gp120 interaction. Only amino acid Phe 43 in the CD4 receptor accounts for 23% of the binding with HIV-1 gp120. After CD4/gp120 binding, gp120 experiences conformational changes allowing a subsequent interaction with chemokine coreceptors CCR5 or CXCR4, present on the cell surface. This is the second step in the viral entry process. The HIV-1 gp120 V3 loop is the main domain involved in this interaction and V3 amino acid sequences largely determine the use of CCR5 or CXCR4 by HIV in the entry process into the cells. Accordingly, HIV isolates may be classified as R5, X4, or R5/X4 strains, depending on their coreceptor use.

Finally, another conformational change in the envelope follows the interaction of the CD4/gp120 complex with the coreceptor. The result is a shift from a non-fusional to a fusional state, in such a way that gp41, which is constituted by repeat regions 1 (HR1) and 2 (HR2), drive the subsequent fusion process. The N-terminus domain of gp41 is exposed and inserted through the fusion peptide (FP) into the cellular membrane, allowing viral and cellular membrane fusion. Thereafter, the viral capsid enters into the cytoplasm[6,7].

The fusion mechanism

The gp41 is mainly responsible for the HIV fusion process. The lineal structure of gp41 shows that the N-terminus extreme harbors the FP domain. The hydrophobic characteristic of the FP permits its insertion into the cellular membrane. Adjacent to the FP are two repeat regions, HR1 and HR2, with a characteristic repeating pattern of seven residues (abcdefg) in which, the "a" and "d" positions correspond to hydrophobic amino acids. Through them, the binding of gp41 monomers occurs, forming trimeric structures at the viral surface confronting the cell membrane (Fig. 1).

The HR1 region is rich in leucines and during the fusion process adopts a coiled-coil structure through the formation of a leucine zipper. The HR2 region is rich in tryptophans, as is the transmembrane domain (TM) which is close to the C-terminus extreme of gp41. Between the HR1 and HR2 regions there is a five-amino acid hydrophilic loop, defined by two cysteine residues (CC)[8-10] (Fig. 2).

During the fusion process, gp41 experiences a structural reorganization that provokes the interaction between HR1 and HR2, forming a thermostable, six-helix bundle structure, which is critical for the viral and cellular membrane fusion[11]. An inner trimer of the coiled-coil HR1 structure and an outer trimer of HR2 form the six-helix bundle structure. The HR2 regions fold in an anti-parallel manner towards the HR1 regions through the hydrophobic grooves[7,12]. The hydrophobic interactions between HR1 and HR2 offer a high stability to the six-helix structure. The change in free energy associated with the formation of the six-helix bundle provides the force needed for the formation of the fusion pore, throughout which the viral capsid enters within the target cell[13] (Fig. 3).

This model of fusion is not unique for HIV and has also been described for the influenza virus, and more recently for other agents such as the coronavirus responsible for the severe acute respiratory syndrome (SARS-CoV)[14,15].

Mechanism of action of fusion inhibitors

Since the early 1990s it has been known that peptides synthesized on the basis of the amino acid sequence of HR1 and HR2 of gp41 may show antiviral properties against HIV[16-18]. The first HIV peptide inhibitor described was DP106, which mimicked a fragment of the HR1 amino acid sequence[16]. In 1993, the *in vitro* potency of another peptide, DP-178, which was synthesized on the basis of the amino acid sequence of HR2, was demonstrated. This molecule was renamed enfuvirtide or T-20[19], and moved to clinical development soon thereafter. Enfuvirtide is a synthetic peptide of 36 amino acids, which mimics the HR2 region of gp41[6,7] (Fig. 2) and is the first fusion inhibitor approved for clinical use.

The second generation of fusion inhibitors was represented by T-1249, a 39-amino acid peptide, which like enfuvirtide, was synthesized on the basis of the HR2 sequence, but overlapping a different region of HR1[10] (Fig. 2). T-1249 is active against HIV-1 enfuvirtide-resistant strains as well as against HIV-2 and simian immunodeficiency virus (SIV)[20,21]. However, the clinical development of this drug was put on hold in January 2004.

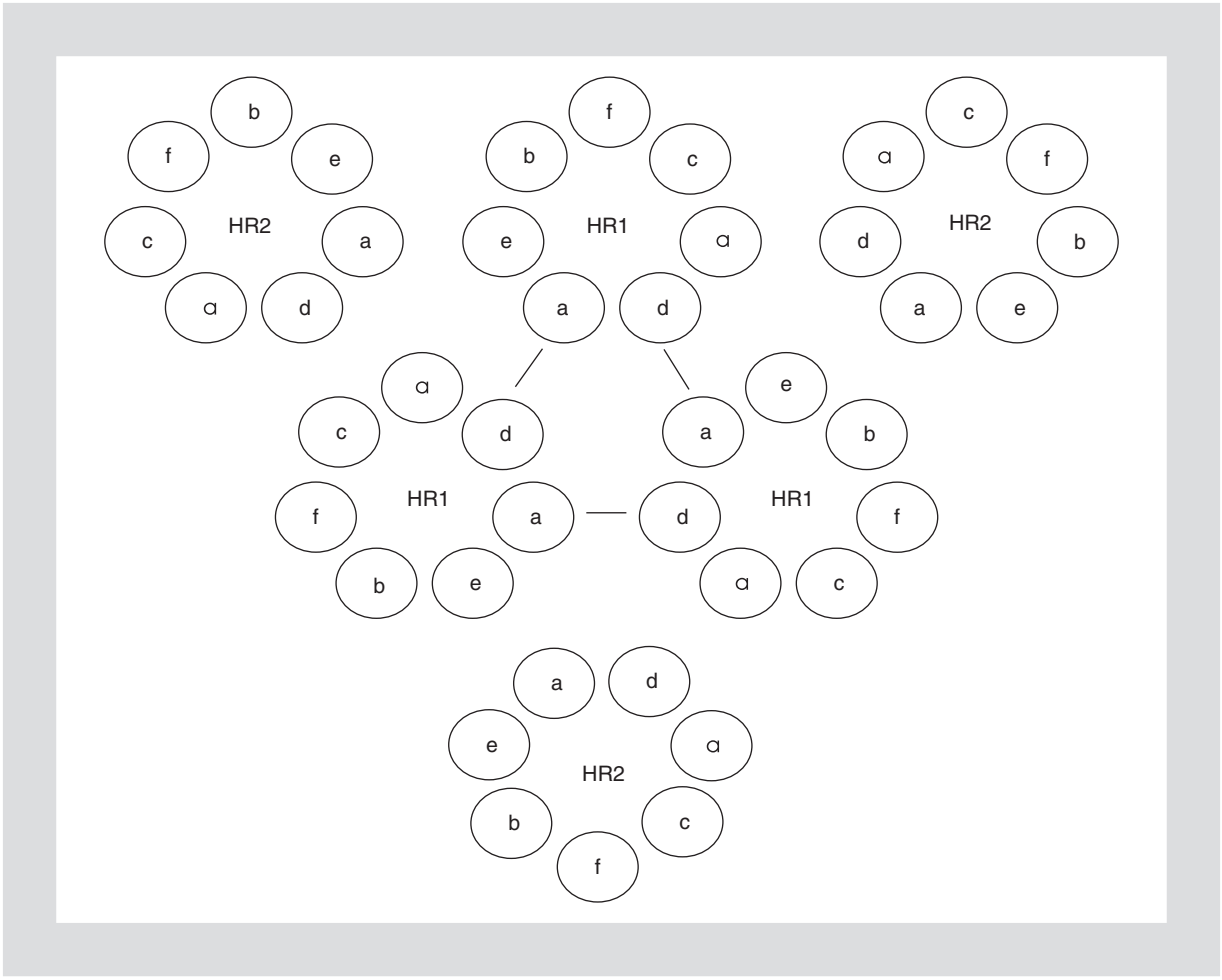

Figure 1. *Cross-sectional view of the six-helix bundle structure of HIV, formed by an inner trimer of HR1 and an outer trimer of HR2.*

The most accepted hypothesis for understanding the molecular basis for the inhibition of HIV entry using these synthetic peptides is the "dominant-negative inhibition model"[22,23]. According to this, the mode of action of enfuvirtide, which mimics HR2, is by inhibitory competition with HR2. Both peptides have an affinity for binding to the HR1 region of gp41. Using enfuvirtide, the formation of the six-helix bundle structure, which is critical for the formation of fusion pore, does not occur.

Clinical use of enfuvirtide

The clinical efficacy and safety of enfuvirtide was demonstrated in the T-20 *vs.* Optimized Regimen Only (TORO) 1 and 2 studies, two phase III clinical trials with enfuvirtide that enrolled almost 1000 patients in the USA, Brazil, Europe, and Australia. These studies proved the virologic and immunologic benefit of adding enfuvirtide along with an optimized antiretroviral regimen in multidrug-experienced patients[3,4]. The FDA approved enfuvirtide for the treatment of infection in March 2003[24], and soon thereafter it was also approved by the EMEA.

Enfuvirtide has been licensed for the treatment of patients suffering failure to prior therapies. The peptidic nature of enfuvirtide does not permit its oral administration. Therefore the drug is administrated by subcutaneous injection with an approved adult dose of 90 mg twice daily. The mode of administration is its main disadvantage and local injection site reactions are the most common adverse events, which appear in more than 90% of patients[25].

The TORO 1 and 2 trials revealed that the greatest virologic success was obtained in patients in whom enfuvirtide was administered along with two or more active drugs in the optimized regimen[26]. In fact, the benefit of the drug is only transient in patients in whom the drug is used as the single active antiretroviral agent, indirectly suggesting that it has a relatively low genetic barrier for resistance[27]. Thus, ideally, enfu-

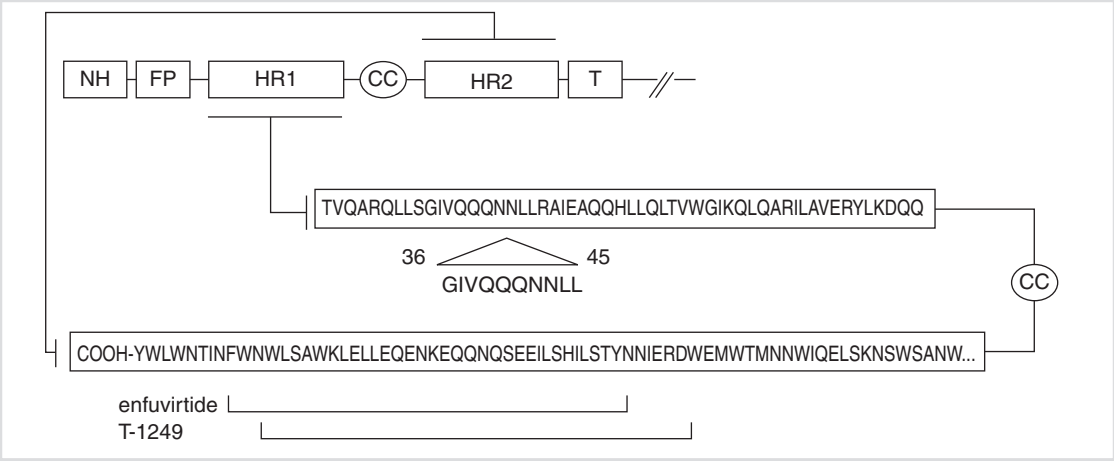

Figure 2. *Scheme of the gp41 lineal structure and enfuvirtide and T-1249 sequences that mimic HR2. FP: fusion peptide; CC: cysteine-cysteine; TM: transmembrane domain.*

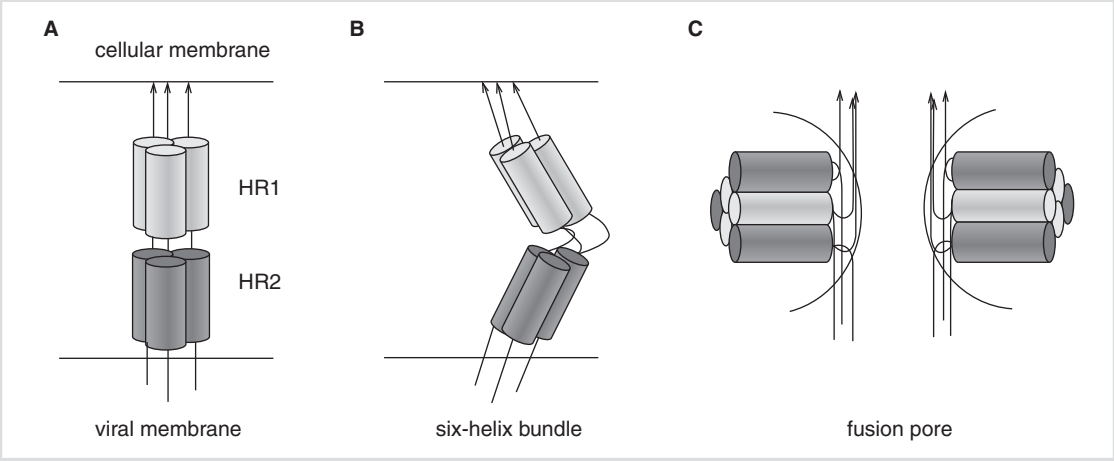

Figure 3. *Scheme of the fusion process driven by gp41.* ***A:*** *gp41 trimer formed by the hepta-repeat regions HR1 and HR2;* ***B:*** *six-helix bundle structure formed by an inner HR1 trimer and an outer HR2 trimer; and* ***C:*** *viral and cellular membrane fusion through the fusion pore formation.*

virtide therapy should be considered after the second or subsequent treatment failure and in combination with one or preferably two other active drugs. In this situation, the prospects for achieving complete suppression of viral replication are high and the benefits of the drug will be maximized[28,29].

Resistance to enfuvirtide

In early *in vitro* studies, enfuvirtide resistance was associated with the selection of mutations in a three-amino acid domain (positions 36-38) within the HR1 region of gp41[30]. However, results obtained subsequently in clinical studies have shown that virologic resistance in patients receiving enfuvirtide may be also due to changes expanding from codon 36-45 in HR1 (GIVQQQNNLL)[31-33] (Fig. 2).

A variety of different mutations have been described in this amino acid region (positions 36-45), each one reducing to a different extent the susceptibility to the drug. Although two changes within the 36-45 domain have been observed in some individuals failing enfu-

virtide, in most cases a single mutation is selected and brings resistance to enfuvirtide. Genotypic and phenotypic correlates of resistance to enfuvirtide are available and summarized in table 1[27,32,34,35].

Mutations selected at the time of enfuvirtide failure may persist throughout the whole period of enfuvirtide therapy. However, a genetic evolution within the 36-45 amino acid domain is observed during enfuvirtide therapy in most patients, which may reflect the acquisition of further changes leading to higher levels of enfuvirtide resistance[27]. Alternatively, these changes may be compensatory and restore the lower replicative capacity of viruses carrying the initial resistance mutation[36].

There is a wide range of susceptibility to enfuvirtide in viral isolates derived from enfuvirtide-naive patients, as well as from individuals undergoing enfuvirtide therapy, even for viruses apparently carrying the same resistance mutations in HR1[27,32,37] (Table 1). The determinants of this variability are unknown, but polymorphisms in the HR2 region of gp41 and changes in HR2 selected during enfuvirtide therapy might explain this variability[38]. In patients on long-term enfuvirtide therapy, changes in HR2 have been noticed. However, they do not follow a recognizable pattern and therefore it is difficult to establish whether they may influence enfuvirtide susceptibility[27,39]. Alternatively, changes in HR2 could represent compensatory mutations selected in an attempt to restore the impaired fitness of viruses with some mutations in HR1[36].

Other regions of the viral envelope involved in the fusion process might also influence the susceptibility to enfuvirtide. Early *in vitro* studies suggested that the tropism of viral strains for the chemokine receptors CCR5 and CXCR4 might influence enfuvirtide susceptibility. Viruses using CCR5 to enter the cells could be more resistant to enfuvirtide than those using CXCR4[40,41]. However, *in vivo* studies have not confirmed these differences in enfuvirtide susceptibility when comparing patients harboring R5 or X4 strains[42,43].

Besides viral factors, host determinants may also influence the susceptibility to enfuvirtide. A relationship between the level of coreceptor expression on target cells and fusion kinetics has been found, in such a way that the presence of high levels of CCR5 on the cellular surface results in more rapid membrane fusion, reducing the time in which gp41 could be targeted by enfuvirtide. Thus, individuals carrying Δ32-CCR5, who express low levels of CCR5, might consequently respond more favorably to enfuvirtide[41].

Impact of enfuvirtide-resistance mutations on viral fitness

It is well known that the accumulation of specific resistant mutations in the HIV protease (D30N) and reverse transcriptase (M184V, K65R) have been particularly associated with a reduced viral replicative fitness[44-47]. Consequently, those viruses bearing these resistance mutations seem to be less pathogenic, and several clinical studies have demonstrated a virologic and immunologic benefit of the antiretroviral treatment in patients harboring multidrug-resistant viruses[48].

Several *in vitro* studies have examined the impact of enfuvirtide-resistance mutations on viral fitness, with discordant results. While some authors have recognized that viruses harboring mutations within the 36-45 region have a lower replicative capacity than wild-type isolates[49,50], others have not confirmed these findings. The preexistence of some genetic polymorphisms, or selection of compensatory mutations at other regions of the *env* gene, could explain these discordances[51]. Moreover, differences in methodologies between these studies could also contribute to explain their disparity.

In spite of the conflicting *in vitro* studies, clinical observations seem to be coincident. Patients who discontinue enfuvirtide therapy after virologic failure uniformly show a disappearance of gp41 resistance mutations and reversion to wild-type within 12-24 weeks. This observation supports that these mutations negatively impact on the virus replicative capacity[27,52].

Immunologic benefit using enfuvirtide despite virologic failure

Despite sustained high levels of viral replication, some individuals harboring multidrug-resistant viruses have shown to keep stable or even raise CD4 counts. This discordant viro-immunological outcome has been explained in some cases by the presence of viruses with a reduced replicative capacity. This possibility has already been proven for isolates harboring specific resistance mutations against reverse transcriptase and protease inhibitors such as proD30N, rtK65R and rtM184V[44-47]. Generally it is believed that viruses with impaired reverse transcriptase and/or protease activities could be less pathogenic and result in less CD4+ T-cell loss. Similarly, an immunologic benefit despite virologic failure has been recognized in some individuals under long-term enfuvirtide therapy, harboring viruses with enfuvirtide-resistance mutations[33,53]. Although a negative impact of these mutations on the

Table 1. Relationship between genotypic and phenotypic resistance to enfuvirtide

Authors	Study	Range of baseline susceptibility (μg/ml)	Mutations gp41 aa 36-45	Phenotype		Methodology
				IC_{50} (μg/ml)	Susceptibility folds decrease	
Greenberg, et al. 2004[34]	Enfuvirtide phase II clinical trials	–				Site-directed mutagenesis
			G36D	0.091	8	
			G36S	0.088	7	
			V38A	0.188	16	
			Q40H	0.256	21	
			N42T	0.045	4	
			N43D	0.210	18	
			L44M	0.021	2	
			L45M	0.017	1	
			G36S+L44M	0.181	15	
			N42T+N43K	0.388	32	
			V38A+N42T	1.782	149	
Sista, et al. 2004[32]	Enfuvirtide phase II clinical trials	0.001-0.480				Virus isolates from patients under enfuvirtide
			G36D	0.242	17	
			G36S	0.499	12	
			Q40H	0.536	19	
			L44M/L	2.034	36	
			N43D	0.996	249	
			G36S+L44M	3.791	632	
			N42T+N43K	1.762	252	
Menzo, et al. 2004[35]	TORO 2 study	0.001-0.033				Gp41-recombinant viruses
			V38A	4.6	255	
			V38M	3.2	80	
			N43D	1.1	1100	
			G36D+N42T	12.8	1829	
			N42T+L45M	5.6	207	
			V38A+L44M	3.4	283	
Poveda, et al. 2005[27]	Enfuvirtide-treated patients	0.02-0.40				Phenoscript (Viralliance)
			G36D	5.22	44	
			G36V	3.87	194	
			G36G/V	4.77	239	
			N43N/D	4.39	24	
			N43D	> 10	> 83	
			G36D+L44V	8.9	445	

virus replicative capacity has been hypothesized, it is intriguing that viral loads were very high in some of these patients. A residual activity of enfuvirtide in these cases, as well as a shift in the main source of cells producing HIV from lymphocytes to monocyte-macrophages, could contribute to explain these observations (Table 2).

In support of the latest hypothesis is the observation from Schaeffer, et al.[54], which suggested that an inhibition of HIV entry into the cells using the fusion pathway is uniformly associated with a compensatory increase in the endocytosis pathway. While virion endocytosis in macrophages results in a productive infection, this is not the case when endocytosis occurs in CD4+ T-lymphocytes, which then are unable to produce viral particles. Enfuvirtide blocks the HIV entry by fusion and could promote the endocytosis pathway. Although resistance could attenuate this effect, any residual activity of the drug, along with an impaired replicative capacity of resistant viruses, could explain a relative CD4 preservation in the case of high levels of virus replication, driven by the release of viral particles from infected macrophages. These findings could support a clinical benefit of enfuvirtide therapy, beyond its direct antiviral activity.

Table 2. Possible mechanisms involved in the immunologic benefit in patients experiencing virologic failure under enfuvirtide

– Impaired replicative capacity of viruses with enfuvirtide-resistance mutations.
– Shift in the main source of cells producing HIV from lymphocytes to macrophages.
– Reduced immune activation with less destruction of T-lymphocytes.

Another explanation for the discordant viro-immunological response in patients on enfuvirtide relies on a reduced immune activation despite high levels of viremia. An ameliorated T-cell activation, with low levels of T-cell turnover and cytotoxic T-lymphocytes (CTL) have been recognized in patients under enfuvirtide therapy with sustained high levels of viremia and preserved CD4 counts, compared with untreated patients with similar viral-load levels[33]. A low immune activation could reduce the confinement of CD4+ T-cells into the lymphoid tissue and might permit their redistribution, thereby increasing their absolute number in the bloodstream[55]. Furthermore, low CTL responses seen in enfuvirtide-treated patients might account for a lower T-cell destruction in the periphery. The high plasma viremia and the low T-cell activation observed in these patients might reflect an increased production of viral particles from sources other than CD4+ T-lymphocytes, as previously discussed.

In experimental models, Rhesus monkeys infected with highly pathogenic SIV developed a complete depletion of CD4+ T-cells while plasma viremia was sustained, mainly by tissue macrophage[56]. In nature, infection of sooty mangabeys with SIV provides a similar model, in which high levels of viral replication do not result in CD4+ T-cell depletion[57,58], apparently due to a lack of the exaggerated immune activation which is characteristic of HIV-1 infection in humans[59]. Clearly, further studies are needed to demonstrate the impact of these observations in patients treated with enfuvirtide.

Enfuvirtide against different HIV variants

Enfuvirtide was originally designed based on the HR2 region from HIV-1_{LAI}, a subtype B virus isolate. Its potent antiviral activity was demonstrated in early studies conducted with this laboratory-adapted strain[17].

Although HIV-1 subtype B is the most commonly circulating variant in developed countries, non-B variants are on the rise, spreading rapidly in the USA and Western Europe[60-64]. Moreover, in other regions (i.e. South-east Asia and sub-Saharan Africa), non-B viruses have since the beginning been mainly responsible for the AIDS epidemics. Therefore, it is crucial to know the activity of any new antiretroviral drug coming onto the market against the distinct HIV variants.

Genetic analyses of gp41 sequences from different HIV-1 group M non-B subtypes have not found amino acids which could be related with resistance to enfuvirtide[65,66]. Phenotypic studies, although scarce, have confirmed the susceptibility of most non-B subtypes to enfuvirtide[67,68].

HIV-1 group O shows a highly genetically diverse gp41 compared to HIV-1 group M, with one change within the 36-45 aa domain (N42D) which might compromise the antiviral effect of enfuvirtide[69]. Nevertheless, the antiviral efficacy of enfuvirtide against HIV-1 group O seems to be preserved both *in vitro* as well as *in vivo*[70]. In contrast, enfuvirtide does not work against HIV-2. Preliminary *in vitro* studies demonstrated a diminished activity of the drug against HIV-2 isolates in comparison with HIV-1[19], and this has been recently confirmed[71]. Genetic analyses have shown a high variability in the transmembrane protein (gp36) of HIV-2 compared to the corresponding gp41 of HIV-1, with changes (N42Q and N43Q) inside the critical domain involved in enfuvirtide resistance[69].

Conclusions

The introduction of enfuvirtide, the first fusion inhibitor, as part of the HIV armamentarium represents the beginning of a new period in the story of HIV chemotherapy. Enfuvirtide is active against different HIV-1 variants (group M and O) and is a good option for treatment-experienced patients, as long as it is combined with other active compounds. The selection of changes in a 10-amino acid domain within the HR1 region of gp41 results in high-level resistance to the drug. Interestingly, virologic failure is not always followed by immunologic deterioration in patients receiving enfuvirtide. Hopefully, other HIV entry inhibitors currently in clinical development will follow in the steps of enfuvirtide and soon will be available for the growing number of HIV-infected persons who have failed the currently available therapeutic options.

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