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Discovery of natural mouse serum derived HIV-1 entry inhibitor(s)

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Summary. – Among rationally designed human immunodeficiency virus 1 (HIV-1) inhibitors, diverse natural factors have showed as potent anti-HIV activity in human blood. We have discovered that the boiled supernatant of healthy mouse serum could suppress HIV-1 entry, and exhibited reduced inhibitory activity after trypsin digestion. Further analysis demonstrated that only the fraction containing 10–25 K proteins could inhibit HIV-1 mediated cell-cell fusion. These results suggest that the 10–25 K protein(s) is novel natural HIV-1 entry inhibitor(s). Our findings provide important information about novel natural HIV entry inhibitors in mouse serum.

Keywords: HIV-1; HIV-1 entry; natural inhibitor; mouse serum

Introduction

The human immunodeficiency virus (HIV), which attacks the function of immune cells, continues to be a threat to global public health (http://www.who.int/mediacentre/factsheets/fs360/en/). Up to now, effective and safe HIV vaccine is still absent. In the attempts to restrain HIV infection, an increasing body of studies has focused on the development of antiretroviral drugs targeting multistep process of HIV entry (Henrich and Kuritzkes, 2013). HIV entry is initiated by interaction of CD4 on the T-cell surface and gp120, a subunit of an envelope glycoprotein spike. This interaction subsequently triggers gp120 conformational changes that permit binding to a chemokine receptor CXCR4 or CCR5, leading to gp41 fusion complex formation and cell fusion (Courter *et al*., 2014; Henrich and Kuritzkes, 2013). Efforts to develop the rationally designed HIV entry inhibitors lead to the appearance of two antiretroviral drugs approved for clinical use, i.e. Enfuvirtide (T20), a HR2-based fusion inhibitor (Champagne *et al*., 2009; Kilby *et al*., 1998; Matthews *et al*., 2004) and Maraviroc, a CCR5 antagonist (Dorr *et al*., 2005).

Among designed antiretroviral drugs, a wide range of natural anti-HIV factors in human blood have been identified. It is proposed that a variant of hemofiltrate CC chemokine (HCC)-1, a common component of healthy human plasma, is a potent CCR5 agonist which is able to block HIV-1 entry (Detheux *et al*., 2000). The group of Frank Kirchhoff identified a natural efficient inhibitor of the HIV-1 gp41 fusion peptide named VIRIP through a systematic screening of peptide library generated from human hemofiltrate (Munch *et al*., 2007). Through screening of hemofiltrate-derived peptide library, this group also discovered another endogenous effective and specific CXCR4 antagonist, a 16 amino acid long fragment of serum albumin, the most abundant protein in human plasma, as an inhibitor of CXCR4-tropic HIV-1 (Zirafi *et al*., 2015). Moreover, it has been reported that Palmitic acid (PA), which was isolated from *Sargassum fusiforme*, inhibited HIV entry through interference in gp120-CD4 interaction (Lee *et al*., 2009; Paskaleva *et al*., 2010). Besides *Sargassum fusiforme,* PA is also present in human plasma as one of the most common saturated fatty

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Abbreviations: gp = glycoprotein; HIV = human immunodeficiency virus; molecular weight MW; MLV = murine leukemia virus

acids (Denke and Grundy, 1992). All these studies indicate that antiviral factors circulating in human blood can be used as novel therapeutic and prevention agents to block HIV-1 replication.

Serum is component of blood without white blood cells, red blood cells and clotting factors. It contains soluble proteins and various small molecules, i.e. serum albumins, globulins, transferrin, haptoglobin, lipoproteins, electrolytes, antibodies, antigens, hormones, and any exogenous substances (Adkins *et al*., 2002). Blood serum is the most abundant library of biomarkers, whether for diagnostics or therapeutics.

In this study, we focused on the healthy mouse serum in attempt to identify novel natural HIV-1 inhibitor(s). Interestingly, only the supernatant of mouse serum heated at 99°C had the ability to inhibit HIV-1 entry. Furthermore, after digestion by trypsin the inhibitory activity of the HIV-1 inhibitor(s) decreased, so we have proved that the inhibitor is protein. The supernatant of mouse serum heated at 99°C was separated by size-based fast performance liquid chromatography (FPLC). We have finally identified that proteins with molecular weight range of 10–25 K residing in supernatant were the most potent fraction to inhibit HIV-1 entry. Thus we found novel natural healthy mouse serum derived HIV-1 inhibitor(s).

Materials and Methods

Heat treatment of serum. Blood of 12–14-week-old C57 mice was collected from retro-orbital plexus. To obtain serum, blood was left to sediment at 37°C for 30 min, and then 6 hours prior to centrifugation at 1,500 × g for 5 min three times, kept at 4°C to obtain serum. Serum was heated at 37°C, 56°C, and 99°C for 1 hr at each temperature and centrifuged at 12,000 × g for 10 min to separate the supernatant and sediment.

Inhibition of HIV-1 mediated cell-cell fusion. A dye transfer assay as previously described was used to detect HIV-1 mediated cell-cell fusion (Jiang *et al*., 1993). HIV-1_{IIIB}-infected H9 (H9/IIIB) and MT-2 cells were obtained from NIH AIDS Research and Reference Reagent Program. Calcein-AM-labeled H9/IIIB cells (2.0×10^5/ml) cultured in 96-well plate were incubated with or without 50 µl of serum at a serial 2-fold dilutions using 1×PBS at 37°C for 45 min before adding 100 µl of MT-2 cells (10^6/ml). After additional co-incubation for 2 hr, the fused and unfused calcein-labeled HIV-1-infected cells were counted under an inverted fluorescence microscope (Zeiss). The inhibition percentage of cell-cell fusion and the IC_{50} values were calculated using previously developed methods (Jiang *et al*., 2004).

Serum digestion by trypsin. Samples were treated with same volume of 0.25% trypsin at 37°C for 30 min.

Separation of serum fractions. Different fractions of serum heated at 99°C were separated for size-based fast protein liquid chromatography (FPLC) using a superose 12 10/300 GL high performance column (GE Healthcare). As a mobile phase we have used 1× PBS, with flow rate 0.8 ml/min, and the analytes were detected at the wavelength of 280 nm.

Results

Serum heated at 99°C effectively inhibited HIV-1 mediated cell-cell fusion

Mouse serum was heated at 37°C, 56°C, and 99°C (Fig. 1a, lanes 1, 2, 3). The relative amount of proteins with MW

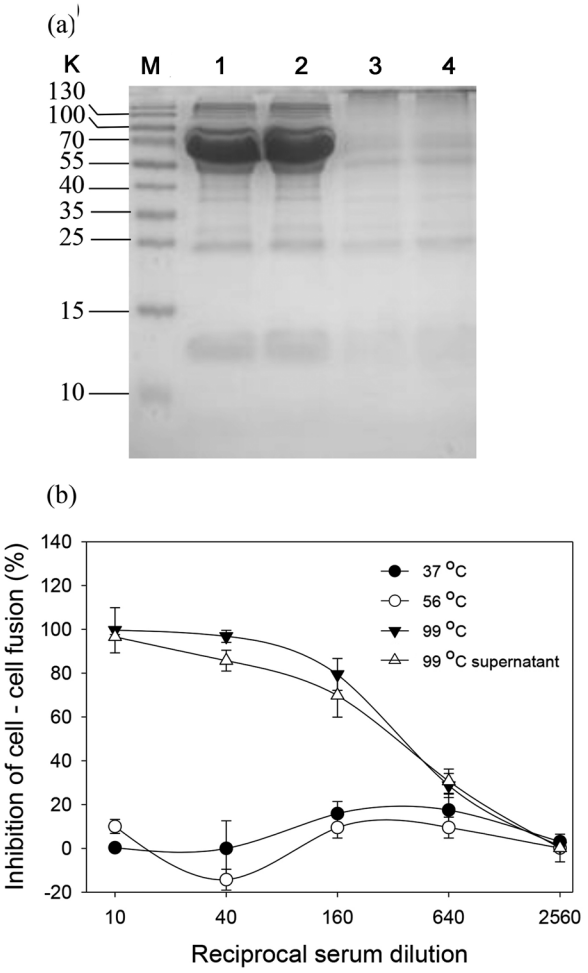

Fig. 1

Inhibitory activity of serum heated at different temperatures to HIV-1 mediated cell-cell fusion

(a) SDS-PAGE of mouse serum heated at different temperatures. Protein size marker (lane M). Serum was heated at 37°C (lane 1), 56°C (lane 2), and 99°C (lane 3). The supernatant of serum heated at 99°C (lane 4). (b) The anti-HIV-1 entry activities of mouse serum with different temperature treatment were determined using the HIV-1-mediated cell-cell fusion assay. Each sample was tested in triplicates and was presented in mean ± SD.

higher than 55 K was significantly reduced after heating at 99°C (Fig. 1a, lanes 3, 4). The inhibitory activity to HIV-1 entry was detected by using HIV-1 mediated cell-cell fusion assay. Notably, the serum heated at 99°C and its supernatant effectively inhibited HIV-1 mediated cell-cell fusion compared with serum heated at 37°C or 56°C. Moreover, the inhibition percentage of cell-cell fusion was up to 80% even though the serum heated at 99°C and its supernatant were diluted 160-fold. (Fig. 1b).

Proteins in serum heated at 99°C were the primary HIV-1 mediated cell-cell fusion inhibitors

Except proteins, serum contains various small molecules including salts, lipids, and sugars. To investigate whether the active substances residing in supernatant of serum heated at 99°C were proteins, we digested serum by trypsin. Most of high molecular weight (MW) proteins (size >55 K) were digested to low MW proteins (size <25 K) (Fig. 2a), accompanied by the loss of inhibitory activity to HIV-1 mediated cell-cell fusion (Fig. 2b). These results suggested that the active substances in supernatant of serum heated at 99°C were proteins.

Inhibitory activity of low molecular weight peptides in serum heated at 99°C to HIV-1 mediated cell-cell fusion

Different fractions of supernatant from serum heated at 99°C were separated with FPLC to further analyze the inhibi-

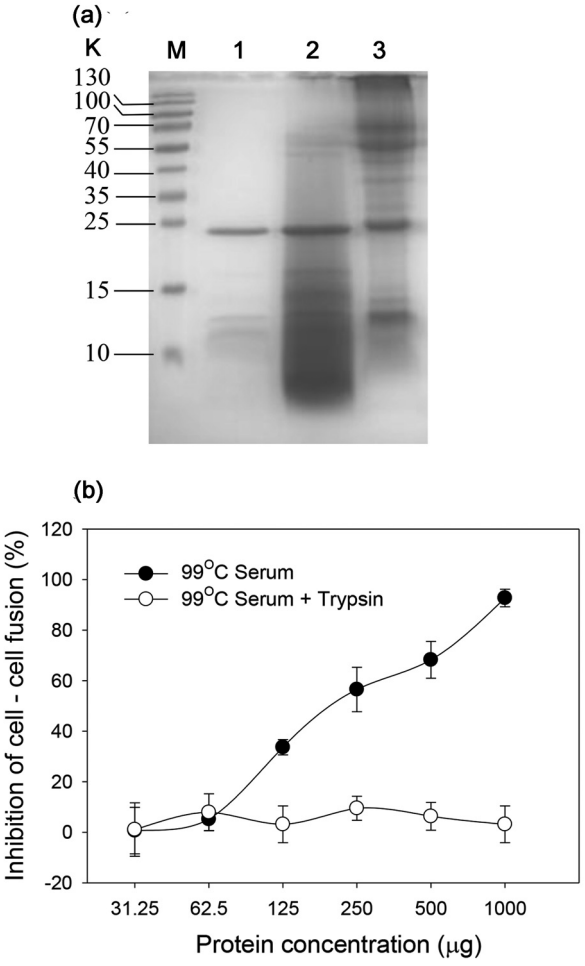

Fig. 2

The inhibitory activity of proteins of serum heated at 99°C to HIV-1 mediated cell-cell fusion

(a) SDS-PAGE of trypsin (lane 1); serum heated at 99°C and digested by trypsin (lane 2); serum heated at 99°C (lane 3). Protein size marker (lane M). (b) The anti-HIV-1-mediated cell-cell fusion activities of serum heated at 99°C and serum heated at 99°C and digested by trypsin.

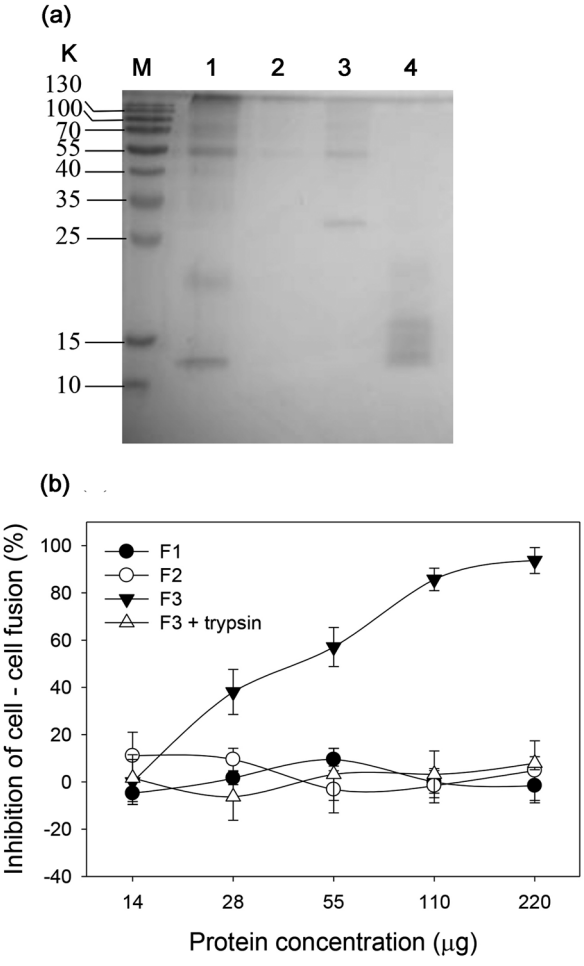

Fig. 3

Inhibitory activity of different molecular weight proteins in serum heated at 99°C to HIV-1 mediated cell-cell fusion

(a) Different fractions of serum heated at 99°C (lane 1) were separated by size-based FPLC. Protein size marker (lane M). Fraction 1 (F1 lane 2); F2 (lane 3); F3 (lane 4). (b) The anti-HIV-1-mediated cell-cell fusion activities of F1, F2, F3, and F3 digested by trypsin.

tory activity of the HIV-1 entry inhibitor(s). Three fractions of different MW were collected as shown in Fig. 3a (lane 1, 2, 3). MW higher than 55 K fraction (F1), MW range of 25–55 K fraction (F2), and MW less than 25 K fraction (F3) were separated. Particularly, the F3 proteins, with size range of 10–15 K, exhibited inhibitory activity against HIV-1 mediated cell-cell fusion with IC_{50} of 40 μg in a dose-dependent manner (Fig. 3b). However, the inhibitory activity decreased after trypsin treatment. These data indicated that F3 was the most potent fraction to inhibit HIV-1 entry.

Discussion

Mice can be infected by retrovirus such as the LP-BM5 retrovirus which causes murine acquired immunodeficiency (MAIDS) (Buller *et al*., 1987; O'Connor *et al*., 2016) and murine leukemia virus (MLV) which has the ability to cause cancer in murine hosts. The mechanism of LP-BM5 pathogenesis has not been fully understood to date. The maturation of MLV envelop precursor protein (80 K trimeric transmembrane protein) is promoted by two cleavage events. The cellular furin cleaves the precursor into surface (SU) subunit and transmembrane (TM) subunit, and then the viral protease removes the R-peptide from the TM (Ng *et al*., 1982). The second cleavage is necessary to prime MLV entry because R-peptide binds the TM legs together and hinders conformational changes in the fusion progression (Loving *et al*., 2012).

There are several differences between MLV and HIV-1 entry process. MLV envelope mediated cell fusion is pH-independent (Ragheb and Anderson, 1994), while vesicle fusion mediated by HIV gp41 ectodomain is pH-dependent (Ratnayake *et al*., 2015; Sackett *et al*., 2011). It has been reported that the entry of amphotropic MLV takes place through macropinocytosis which is independent of coat proteins (Rasmussen and Vilhardt, 2015). Nevertheless, HIV-1 utilizes clathrin-coated-pit endocytosis and plasma membrane fusion pathways to enter host cells (Janas *et al*., 2008).

HIV-1 entry inhibitor blocks virus entry into the target cells and thereby intervenes in the HIV-1 life cycle at an early stage and subsequently limits viral replication and spread. Nevertheless, the clinical application of T20 peptide, the first U.S. FDA-approved HIV entry inhibitor, is limited by the high drug dosage (90 mg) and T20 resistance-related mutations (Oliveira *et al*., 2009). Therefore, it is essential to develop novel HIV-1 entry inhibitors.

Although most of studies focus on the antiviral factors involved in the immune system of HIV-infected individuals (Alcena *et al*., 2013; Andrabi *et al*., 2014; Gach *et al*., 2014), the antiviral factors like broadly neutralizing antibodies are found only in a limited extent of persons who are infected for many years, and majority of the neutralizing antibodies are strain-specific and can suppress autologous virus but not heterologous virus. It is necessary to develop more ubiquitous and effective HIV-1 inhibitor. Up to now, several natural small molecular peptides in human blood and tissues have been identified as HIV-1 entry inhibitors (Munch *et al*., 2007; Zirafi *et al*., 2015). However, it is tough to purify and characterize the endogenous antiviral factors circulating in the human body. For example, the specific antiviral factor(s) released from $CD8^+$ T cells has not been identified although the factor(s) has been described for years (Chang *et al*., 2002; Levy, 2003). Nevertheless, this antiviral factor(s) has distinct biochemical and physical properties including size range of 10–15 K, resistance to heat (86°C, 10 min) and to low pH (2.0) (Levy, 2003).

In this study, we proved that there is a potential HIV-1 entry inhibitor(s) in healthy mouse serum heated at 99°C. The entry inhibitor(s) was resistant to heat similarly to the antiviral factor(s) released from $CD8^+$ T cells. Further analysis demonstrated that the low molecular weight protein(s) was the most potent suppressor(s). In addition to mouse serum, the proteins (10–15 K) in calf, rabbit, and human serum after 99°C heating could also prevent HIV-1 mediated cell-cell fusion (data not shown), revealing that there are some antiviral factors in serum of other mammals. These results enlighten us that there may probably be some common and conservative antiretroviral proteins/peptides circulating in mammal serum. Based on the mass spectrometry of proteins from fraction 3 (unpublished data), we confirm that this natural HIV inhibitor(s) is still not discovered by other groups. The specific HIV inhibitory factor(s) will be addressed in ongoing studies.

In conclusion, the evidence presented in this work suggests that low molecular weight protein(s) in mouse serum heated at 99°C is potential HIV-1 entry inhibitor(s). Our study provided significant information on discovery of novel natural HIV-1 entry inhibitor.

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References

Adkins JN, Varnum SM, Auberry KJ, Moore RJ, Angell NH, Smith RD, Springer DL, Pounds JG (2002): Toward a human blood serum proteome: analysis by multidimensional

separation coupled with mass spectrometry. Mol. Cell. Proteomics **1**, 947–955. https://doi.org/10.1074/mcp.M200066-MCP200

Alcena DC, Kobie JJ, Kaminski DA, Rosenberg AF, Mattiacio JL, Brewer M, Dewhurst S, Dykes C, Jin X, Keefer MC, Sanz I (2013): 9G4+ antibodies isolated from HIV-infected patients neutralize HIV-1 and have distinct autoreactivity profiles. PLoS One 8, e85098. https:/doi.org/10.1371/journal.pone.0085098

Andrabi R, Makhdoomi MA, Kumar R, Bala M, Parray H, Gupta A, Kotnala A, Thirumurthy V, Luthra K (2014): Highly efficient neutralization by plasma antibodies from human immunodeficiency virus type-1 infected individuals on antiretroviral drug therapy. J. Clin. Immunol. 34, 504–513. https:/doi.org/10.1007/s10875-014-0010-y

Buller RM, Yetter RA, Fredrickson TN, Morse HC, 3rd (1987): Abrogation of resistance to severe mousepox in C57BL/6 mice infected with LP-BM5 murine leukemia viruses. J. Virol. 61, 383–387.

Champagne K, Shishido A, Root MJ (2009): Interactions of HIV-1 inhibitory peptide T20 with the gp41 N-HR coiled coil. J. Biol. Chem. 284, 3619–3627. https:/doi.org/10.1074/jbc.M809269200

Chang TL, Mosoian A, Pine R, Klotman ME, Moore JP (2002): A soluble factor(s) secreted from CD8(+) T lymphocytes inhibits human immunodeficiency virus type 1 replication through STAT1 activation. J. Virol. 76, 569–581. https:/doi.org/10.1128/JVI.76.2.569-581.2002

Courter JR, Madani N, Sodroski J, Schon A, Freire E, Kwong PD, Hendrickson WA, Chaiken IM, LaLonde JM, Smith AB, 3rd (2014): Structure-based design, synthesis and validation of CD4-mimetic small molecule inhibitors of HIV-1 entry: conversion of a viral entry agonist to an antagonist. Acc. Chem. Res. 47, 1228–1237. https:/doi.org/10.1021/ar4002735

Denke MA, Grundy SM (1992): Comparison of effects of lauric acid and palmitic acid on plasma lipids and lipoproteins. Am. J. Clin. Nutr. 56, 895–898.

Detheux M, Standker L, Vakili J, Munch J, Forssmann U, Adermann K, Pohlmann S, Vassart G, Kirchhoff F, Parmentier M, Forssmann WG (2000): Natural proteolytic processing of hemofiltrate CC chemokine 1 generates a potent CC chemokine receptor (CCR)1 and CCR5 agonist with anti-HIV properties. J. Exp. Med. 192, 1501–1508. https:/doi.org/10.1084/jem.192.10.1501

Dorr P, Westby M, Dobbs S, Griffin P, Irvine B, Macartney M, Mori J, Rickett G, Smith-Burchnell C, Napier C, Webster R, Armour D, Price D, Stammen B, Wood A, Perros M (2005): Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. Antimicrob. Agents Chemother. 49, 4721–4732. https:/doi.org/10.1128/AAC.49.11.4721-4732.2005

Gach JS, Achenbach CJ, Chromikova V, Berzins B, Lambert N, Landucci G, Forthal DN, Katlama C, Jung BH, Murphy RL (2014): HIV-1 specific antibody titers and neutralization among chronically infected patients on long-term suppressive antiretroviral therapy (ART): a cross-sectional study. PLoS One 9, e85371. https:/doi.org/10.1371/journal.pone.0085371

Henrich TJ, Kuritzkes DR (2013): HIV-1 entry inhibitors: recent development and clinical use. Curr. Opin. Virol. 3, 51–57. https:/doi.org/10.1016/j.coviro.2012.12.002

Janas AM, Dong C, Wang JH, Wu L (2008): Productive infection of human immunodeficiency virus type 1 in dendritic cells requires fusion-mediated viral entry. Virology 375, 442–451. https:/doi.org/10.1016/j.virol.2008.01.044

Jiang S, Lin K, Strick N, Neurath AR (1993): HIV-1 inhibition by a peptide. Nature 365, 113. https:/doi.org/10.1038/365113a0

Jiang S, Lu H, Liu S, Zhao Q, He Y, Debnath AK (2004): N-substituted pyrrole derivatives as novel human immunodeficiency virus type 1 entry inhibitors that interfere with the gp41 six-helix bundle formation and block virus fusion. Antimicrob. Agents Chemother. 48, 4349–4359. https:/doi.org/10.1128/AAC.48.11.4349-4359.2004

Kilby JM, Hopkins S, Venetta TM, DiMassimo B, Cloud GA, Lee JY, Alldredge L, Hunter E, Lambert D, Bolognesi D, Matthews T, Johnson MR, Nowak MA, Shaw GM, Saag MS (1998): Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. Nat. Med. 4, 1302–1307. https:/doi.org/10.1038/3293

Lee DY, Lin X, Paskaleva EE, Liu Y, Puttamadappa SS, Thornber C, Drake JR, Habulin M, Shekhtman A, Canki M (2009): Palmitic Acid Is a Novel CD4 Fusion Inhibitor That Blocks HIV Entry and Infection. AIDS Res. Hum. Retroviruses 25, 1231–1241. https:/doi.org/10.1089/aid.2009.0019

Levy JA (2003): The search for the CD8+ cell anti-HIV factor (CAF). Trends Immunol. 24, 628–632. https:/doi.org/10.1016/j.it.2003.10.005

Loving R, Wu SR, Sjoberg M, Lindqvist B, Garoff H (2012): Maturation cleavage of the murine leukemia virus Env precursor separates the transmembrane subunits to prime it for receptor triggering. Proc. Natl. Acad. Sci. USA 109, 7735–7740. https:/doi.org/10.1073/pnas.1118125109

Matthews T, Salgo M, Greenberg M, Chung J, DeMasi R, Bolognesi D (2004): Enfuvirtide: the first therapy to inhibit the entry of HIV-1 into host CD4 lymphocytes. Nat. Rev. Drug Discov. 3, 215–225. https:/doi.org/10.1038/nrd1331

Munch J, Standker L, Adermann K, Schulz A, Schindler M, Chinnadurai R, Pohlmann S, Chaipan C, Biet T, Peters T, Meyer B, Wilhelm D, Lu H, Jing W, Jiang S, Forssmann WG, Kirchhoff F (2007): Discovery and optimization of a natural HIV-1 entry inhibitor targeting the gp41 fusion peptide. Cell 129, 263–275. https:/doi.org/10.1016/j.cell.2007.02.042

Ng VL, Wood TG, Arlinghaus RB (1982): Processing of the env gene products of Moloney murine leukaemia virus. J. Gen. Virol. 59, 329–343. https:/doi.org/10.1099/0022-1317-59-2-329

O'Connor MA, Vella JL, Green WR (2016): Reciprocal relationship of T regulatory cells and monocytic myeloid-derived suppressor cells in LP-BM5 murine retrovirus-induced immunodeficiency. J. Gen. Virol. 97, 509–522. https:/doi.org/10.1099/jgv.0.000260

Oliveira AC, Martins AN, Pires AF, Arruda MB, Tanuri A, Pereira HS, Brindeiro RM (2009): Enfuvirtide (T-20) resistance-related mutations in HIV type 1 subtypes B, C, and F isolates from Brazilian patients failing HAART. AIDS Res. Hum. Retroviruses 25, 193–198. https:/doi.org/10.1089/aid.2008.0160

Paskaleva EE, Xue J, Lee DY, Shekhtman A, Canki M (2010): Palmitic acid analogs exhibit nanomolar binding affinity for the HIV-1 CD4 receptor and nanomolar inhibition of gp120-to-CD4 fusion. PLoS One 5, e12168. https:/doi.org/10.1371/journal.pone.0012168

Ragheb JA, Anderson WF (1994): pH-independent murine leukemia virus ecotropic envelope-mediated cell fusion: implications for the role of the R peptide and p12E TM in viral entry. J. Virol. 68, 3220–3231.

Rasmussen I, Vilhardt F (2015): Macropinocytosis is the entry mechanism of amphotropic murine leukemia virus. J. Virol. 89, 1851–1866. https:/doi.org/10.1128/JVI.02343-14

Ratnayake PU, Sackett K, Nethercott MJ, Weliky DP (2015): pH-dependent vesicle fusion induced by the ectodomain of the human immunodeficiency virus membrane fusion protein gp41: Two kinetically distinct processes and fully-membrane-associated gp41 with predominant beta sheet fusion peptide conformation. Biochim. Biophys. Acta 1848, 289–298. https:/doi.org/10.1016/j.bbamem.2014.07.022

Sackett K, TerBush A, Weliky DP (2011): HIV gp41 six-helix bundle constructs induce rapid vesicle fusion at pH 3.5 and little fusion at pH 7.0: understanding pH dependence of protein aggregation, membrane binding, and electrostatics, and implications for HIV-host cell fusion. Eur. Biophys. J. 40, 489–502. https:/doi.org/10.1007/s00249-010-0662-3

Zirafi O, Kim KA, Standker L, Mohr KB, Sauter D, Heigele A, Kluge SF, Wiercinska E, Chudziak D, Richter R, Moepps B, Gierschik P, Vas V, Geiger H, Lamla M, Weil T, Burster T, Zgraja A, Daubeuf F, Frossard N, Hachet-Haas M, Heunisch F, Reichetzeder C, Galzi JL, Perez-Castells J, Canales-Mayordomo A, Jimenez-Barbero J, Gimenez-Gallego G, Schneider M, Shorter J, Telenti A, Hocher B, Forssmann WG, Bonig H, Kirchhoff F, Munch J (2015): Discovery and characterization of an endogenous CXCR4 antagonist. Cell Rep. 11, 737–747. https:/doi.org/10.1016/j.celrep.2015.03.061