RME-BASED PHARMACOLOGY: THE INHIBITION OF VIRAL ENTRY AS THERAPEUTIC PERSPECTIVE IN VIRAL DISEASES INCLUDING AIDS. HYPOTHESIS UPDATED AND ENLARGED

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In 1990, one of us (GNC) for the first time reported a hypothesis of receptor-mediated endocytosis (RME)-based pharmacology relevant to the possible antiviral therapy including in acquired immunodeficiency syndrome (AIDS). Then, RME using clathrin-coated pits/vesicles was the best-characterized endocytic pathway. Since then now, intensive research on the mechanisms of both RME and receptor-mediated virus-cell fusion (receptor-mediated fusion - RMF) helped to expand the list of chemical compounds with potential clinical application as antiviral agents, the so-called entry inhibitors, e.g. (i) inhibitors of clathrin-, dynamin-2-, caveolin- and/or lipid rafts-dependent RME, and (ii) inhibitors of RMF. Accordingly, in the present Dance Round we update and enlarge our hypothesis of RME-based antiviral pharmacology. Biomed Rev 2018; 29: 109-118

Keywords: receptor-mediated endocytosis, receptor-mediated fusion, clathrin, caveolin, dynamin-2, lipid rafts, viruses, HIV-1, AIDS, therapy

INTRODUCTION

Viruses have evolved a complex multistep process to infect target cells (entry, endosomal processing, replication, and dissemination to other cells). Each of these entry and post-entry intermediates offers the opportunity for therapeutic intervention. Although some viruses release their genomes into the cell by direct fusion with the plasma membrane, most viruses enter cells via receptor-mediated endocytosis (RME) or receptor-mediated virus-cell fusion, the latter being named herein receptor-mediated fusion (RMF). It is the process of internalization (entry) of extracellular material, including proteins, toxins, bacteria and viruses into cells through clathrin-coated vesicles, caveolin-coated vesicles and/or lipid rafts (1-9; for clathrin-independent endocytosis see 10) (Fig. 1, 2).
Figure 1. Adsorptive (receptor-mediated) endocytosis and fluid phase endocytosis.

Figure 2. Electron micrographs illustrating caveolae (A – by Palade’s transmission electron microscopy method, B – by Heuser’s quick-freeze deep-etch method; at least 20 caveolin-coated caveolae can be seen; one clathrin-coated vesicle is located at upper left corner of the micrograph; the typical pentagonal/hexagonal image of the clathrin coat is evident). Bar represents 200 nm – caveolae diameter is about 100 nm, whereas clathrin-coated vesicles are 2-3 time bigger in diameter. From: Rothberg KG, Heuser JE, Donzell WC, Ying Y-S, Glenney JR, Anderson RGW. Caveolin, a protein component of caveolae membrane coats. Cell 1992; 68: 673-682.
Short history of the knowledge of RME

In their Nobel lecture entitled “A receptor-mediated pathway for cholesterol homeostasis” delivered on 9 December 1985, Michael S. Brown and Joseph L. Goldstein stated: “We began our work in 1972 in an attempt to understand a human genetic disease, familial hypercholesterolemia (FH). In these patients the concentration of cholesterol in blood is elevated many fold above normal and heart attacks occur early in life. We postulated that this dominantly inherited disease results from a failure of end-product repression of cholesterol synthesis. The possibility fascinated us because genetic defects in feedback regulation had not been observed previously in humans or animals, and we hoped that study of this disease might throw light on fundamental regulatory mechanisms. Our approach was to apply the techniques of cell culture to unravel the postulated regulatory defect in FH. These studies led to the discovery of a cell surface receptor for a plasma cholesterol transport protein called low density lipoprotein (LDL) and to the elucidation of the mechanism by which this receptor mediates feedback control of cholesterol synthesis. Familial hypercholesterolemia was shown to be caused by inherited defects in the gene encoding the LDL receptor, which disrupt the normal control of cholesterol metabolism. Study of the LDL receptor in turn led to the understanding of receptor-mediated endocytosis, a general process by which cells communicate with each other through internalization of regulatory and nutritional molecules” (1, 11).

In 1975, a paper entitled “Ultrastructure of the arterial smooth muscle cell, with special reference to coated vesicles and microtubules” was published (12). “It is well known that coated vesicles are cellular transport devices for selective uptake of proteins. Therefore, the smooth muscle coated vesicles may be involved in the uptake and transport of specific macromolecular substances essential for smooth muscle cell functions. One may speculate that both basement membrane and sarcolemma may be genetically endowed with properties for special uptake and transport of some macromolecules as implicit in Bennett’s theory of pinocytosis and Singer and Nicolson’s fluid mosaic model for membrane structure.”

In 1990, a hypothesis of “Inhibition of receptor-mediated cellular entry of viruses including HIV: a perspective on further researches on chemotherapy in viral diseases including AIDS” was published (13). In essence, one of us (GNC) wrote therein: Although the kinetics and the pharmacological control of RME are different depending on the nature of ligands, the route of entry of many enveloped viruses it shares the following common features: (i) a glycoprotein component(s) of the viral envelope has the capacity to bind to a specific plasmalemmal receptor, (ii) concentrating into clathrin-coated pits-vesicles-endosomes, where (iii) the low endosomal pH induces fusion of the viral envelope with the endosomal membrane, and (iv) ejecting the viral nucleoid into the cytoplasm (3-6, 14-16). Our 1990 hypothesis, addressing mostly RME of viruses including human immunodeficiency virus type-1 (HIV-1), describes a perspective of further basic studies seen through the current knowledge about pharmacological control over various steps of the RME of different ligands including viruses (3-5, 17-23). The hypothesis proposes an experimental study of different chemicals in order to elucidate their eventual potential as chemotherapeutic drugs in some viral diseases including acquired immunodeficiency syndrome (AIDS). The following steps of cellular entry of viruses are suggested as possible targets for actions of these chemicals: (i) inhibition of viral internalization, and (ii) inhibition of viral release into the cytoplasm.

Inhibition of viral internalization: role of transglutaminase, calmodulin, protein kinase C, and cytoplasmic pH

The intracellular enzyme transglutaminase (TGase) catalyzes the cross-linking of proteins by forming an isopeptide bond between a lysine residue of one protein and a glutamine residue of another (4, 24). It was shown that clustering of hormone-receptor complexes and/or their internalization was inhibited by various primary amines (3, 4, 20) and by bacitracin (4). These authors suggested that these steps of the RME may require the TG-ase to be active, because TG-ase was a major cellular enzyme to be inhibited by such amines. The activity of both TG-ase and ornithine decarboxylase, a key cell enzyme producing primary amines, and the intracellular concentration of these amines in virus-infected (also HIV-infected) cells could reasonably be studied.

However, neither chloroquine nor dimethyllysylcadaverine are inhibitors of TG-ase (24), but they inhibited the RME of fibronectin in macrophages (23), whereas methylamine, a good inhibitor of both TG-ase and RME of different ligands (3, 4, 18, 20), did not cause any inhibition of RME of fibronectin (23). There is a possibility that the primary action of some amines and possibly chloroquine is not related to the activity of TG-ase: their antagonistic effect on calmodulin was suggested.
Indeed, calmodulin was shown to be involved in controlling coated pit/vesicle formation (25) and RME of transferrin (19). Because dansylcadaverine has a structure similar to that of W7, a well known calmodulin antagonist (26), W7 as well as other calmodulin antagonists may also be considered in the context of the present hypothesis. Whatever the primary mode of action, it seems reasonable that various primary amines and calmodulin antagonists should be experimentally studied for their potential inhibitory effect relative to viral internalization.

Another possibility might be substances that inhibit the activity of protein kinase C (PKC), because it was recently shown that phorbol-12-myristate-13-acetate, via its activation of PKC, stimulated coated pits to internalize, using transferrin and insulin as ligands (27). Hence, staurosporine, an alkaloid isolated from Streptomyces SP, which is a potent and specific inhibitor of PKC (28), as well as other PKC inhibitors (H7, polymycin B), may be included in the list of chemicals proposed. Clearly, we need further data about the turnover of inositol phospholipids, diacylglycerol, Ca²⁺ and PKC in virus-infected cells including HIV-infected ones. Although the PKC is independent of calmodulin it is inhibited by a large number of phospholipid-interacting drugs (chlorpromazine; trifluoperazine; dibucaine); which are known calmodulin antagonists (see (29).

Several methods have been developed to inhibit the formation of coated pits/vesicles: potassium depletion, hypertonic medium and cytoplasmic acidification (30). The two former results in inhibition of formation of these organelles, whereas the latter in “paralyzing” coated pits so they can no longer pinch-off from the plasma membrane to form coated vesicles (30). Because cytoplasmic acidification is a critical condition that paralyses coated pits, pharmacological control of intracellular pH may either stimulate or block this phenomenon. Activities of the ATP-dependent proton pumps, the Na⁺/H⁺ antiporters and the anion antiporters in virus-infected cells may provide some clues to this process. For instance, amiloride, a well-established inhibitor of Na⁺/H⁺ ATP-ase, and colchicine (an antitubulin), a recently recognized activator of this enzyme (31), may be involved in the regulation of intracellular pH, via the membrane-bound tubulin (31).

Colchicine as well as other antitubulins may also be involved in coated pit/vesicle formation, because tubulin is a molecular component of coated vesicles (32). Further, the calmodulin antagonist, trifluoperazine, and the actin filament disintegrator, cytochalasin B, may inhibit formation of coated vesicles (25).

**Inhibition of viral release into the cytoplasm, including virus receptor recycling: role of intraendosomal pH**

The low endosomal pH is an essential condition for ligand-receptor uncoupling (2-4) and for the release of viruses into the cytoplasm (5). The ionophore monensin as well as weak organic bases accumulate in the acidic endosomal compartment and lysosomes, increase pH and thereby block viral release. Monensin, via raising the pH in acidic vesicles like medial-Golgi, inhibits transport of Semliki Forest virus membrane proteins (33) and of very low-density lipoproteins (34; for chloroquine and verapamil see 35).

Organic amines (dansylcadaverine, dimethyl-dansylcadaverine, chloroquine), monensin, and trifluoperazine inhibit receptor recycling (19, 21-23, 36). How these substances may work in virus-infected cells, needs further experimental attention.

**NEW VIIRAL ENTRY INHIBITORS AVAILABLE FOR THERAPY IN AIDS AND AIDS-RELATED DISEASES**

Human immunodeficiency virus-1, an envelope lentivirus of the Retroviridae family, the causal agent of AIDS, delivers its RNA into cells by fusing the viral envelope with the plasmalemma (cell surface membrane) of target cells. This fusion process is mediated by viral envelope surface glycoprotein, a trimer of heterodimers consisting of glycoprotein 120 (gp120) and gp41 subunits. The process of fusion is initiated by gp120 interactions with CD4 and one of the two coreceptors CCR5 and CXCR4 at the target cell surface (37, 38). As mentioned above, we dubbed this process receptor-mediated fusion (RMF) (also see (39).

Every single step of HIV cellular infection offers a potential therapeutic strategy to inhibit its progression. Controlling the initial event, namely the viral entry – via RME or RMF – is one of the most effective ways to control viral dissemination (40, 41).

The gp120 interacts with T-cells CD4 receptor which complex further interact with a co-receptor (C-C chemokine receptor type 5 - CCR5) or CXC chemokine receptor type 4 (CXCR4) and virus-cell membrane fusion was mediated by viral trans-membrane (TM) gp41 subunit transformation into a six-helix bundle structure (42). In their chemical nature, the entry inhibitors are different synthetic peptides homologous to crucial viral glycoproteins. Despite the numerous compounds tested in various clinical trials, currently there are only two preparations approved as adjuvant drugs for AIDS chemo-
therapy with such mechanism of action.

The compounds acting on different stages of viral entry process can be grouped as follows: (i) blocking interaction of gp120 with CD4, co-receptor (CCR5), and (ii) “freezing” gp41 subunit transformation thus hindering the fusion process (43).

a. CD4 attachment inhibitors
Multiple studies were directed to inhibit viral attachment to CD4 receptor as well as the CD4 binding site of gp120. The first approach targets the viral proteins mainly through locking gp120 into nonfunctional conformation as PRO 542 recombinant fusion protein (44) or inhibiting gp120 interaction with CD4 receptor by small molecules (BMS-378806) blocking access to F43 important functional gp120 cavity (45). Second approach to block viral attachment is to target CD4 through antibody binding. The humanized monoclonal antibody ibalizumab in practice block the conformational changes of gp120 after binding to CD4 receptor thus blocking further viral entry process (46).

b. CCR5 or CXCR4 blockers
Small molecules antagonists blocked gp120-CCR5 interaction after binding to the co-receptor. Main representative from this group is Maraviroc which passed all phases of clinical trials (47) and is already available in clinical practice under trade name Celsentri. Maraviroc stabilizes CCR5 in an inactive conformation (48). It has been developed for treatment-experienced HIV-infected patients who have only CCR5-tropic HIV-1 detectable. These compounds are with very high adverse effects as they target essential host molecules rather than specific viral target.

c. Peptides blocking gp41 subunit transformation - fusion inhibitors
The gp41 subunit represent several distinct domains: transmembrane domain, long cytoplasmic tail of approximately 150 amino acids, two heptad repeat domains (HR1 and HR2) separated by disulfide bond and hydrophobic region known as a fusion protein (FP). Three HR1 and HR2 consecutively form the six-helix bundle which juxtaposes the viral and cellular membranes for the fusion event. Depending of the different amino acids sequences synthetic peptides targeting HR1 can be classified as (49):

1. First generation fusion inhibitors
   The first fusion inhibitors were peptides with shifted 36 amino acids which mimics a portion of HR2 domain.

   They inhibited virus entry by binding HR1 core. The most active and most developed is T20 (enfuvirtide, trade name Fuzeon). T20 is active in nM range but must be injected subcutaneously. The effective treatment protocol includes T20 as adjuvant drug with other anti-HIV agents.

2. Second generation fusion inhibitors
   The new peptide variants include amino acids sequences form HIV-1, HIV-2 and simian immunodeficiency virus (SIV) strains. The prototype T1249 is longer than T20 (39 amino acids) and shows increased potency in vitro (50).

3. Third generation fusion inhibitors
   Introducing of negatively and positively charged residues in the peptide helix of T20 and T1249 increased serum stability and loss of conformation entropy. The main representative T2635 with some additives of hydrophobic residues showed 100-times improvements of the half-life in serum of cynomolgus macaques and greatly improved potency against T20 and T1249 resistant variants (51). Similar increased stability after changes in the C34 peptide helix was obtained in Sifuvirtide with half-life of 20 hours compared to 4 hours half-life of T20 (52). The improved efficiency of sifuvirtide relative to enfuvirtide might be related to its ability to adsorb on rigid lipidic areas, such as the viral envelope and lipid rafts, which results in an increased sifuvirtide concentration at the fusion site (53). Phase Ia clinical studies of sifuvirtide in 60 healthy individuals demonstrated good safety, tolerability, and pharmacokinetic profiles (54).

d. Peptide fusion inhibitors against other pathogenic human viruses
The last step of protein transformation for virus cell entry is characteristic for many other viruses, e.g. the influenza virus HA2 protein, the Ebola GP2 protein and the SARS virus S2 protein. Respiratory viruses are attractive targets for local peptide drugs delivery by inhalation into the lung. Significant in vitro inhibition was achieved by some peptides analogues of coronaviruses (55) and paramyxoviruses (56).

Taken together the present updated hypothesis suggests further studies on chemicals for their eventual effects relative to inhibition of viral entry including HIV-1 (Table 1).
Table 1. Chemicals proposed to be tested for their potentials relative to inhibition of viral entry including HIV-1 (14, 15, 17-36, 42-45, 57-61).

| Possible mode of actions | Chemicals proposed to be tested for their potentials relative to inhibition of viral entry including HIV-1 |
|--------------------------|----------------------------------------------------------------------------------------------------------|
| Internalization          | Release into the cytoplasm including receptor recycling                                                  |
| Transglutaminase inhibition | primary amines, methylamine, bacitracin, rimactadine, lysine and glutamine analogues                        |
| Calmodulin antagonism    | trifluoperazine, chlorpromazine, dibucaine, W7, Dansylcadaverine (?)                                      |
| Cytoplasmic acidification ('paralysis' of coated pits) | Amiloride                                                                                           |
| Coated pit/vesicle formation inhibition | trifluoperazine, cytochalasin B, colchicine (?), K+ depletion hypertonic medium                        |
| Dynamin-2 inhibition*    | Dynasore                                                                                              |
| Endosomal alkalization including trans-Golgi | monensin, chloroquine, verapamil, week organic bases                                                |
| Blocking viral glycoprotein 120/CD4 interaction | Ibalizumab                                                                                           |
| Blocking viral glycoprotein 120/co-receptor (CCR5) interaction | Maraviroc                                                                                           |
| Blocking gp41 subunit transformation | Enfuvirtide, sifuvirtide                                                                              |
| Purinergic antagonists    | NF279, a selective P2X1 receptor antagonists                                                           |

* Dynamin-2 (DNM2) is known to contribute to clathrin-mediated endocytosis by pinching the clathrin-coated pit upon forming an octameric ring like structure that is important for its GTPase activity (Fig. 3). The finding that DNM2 can contribute to HIV-1 entry suggests that HIV-1 may indeed enter cells via RME, and DNM2 might also be directly involved in the process of virus-cell fusion (see (59).

Figure 3. Schematic illustration of the formation of clathrin-coated pit and vesicle (their coat is not depicted). Endophilin and cholesterol are required for the invagination of plasma-lemma. After that, dynamin takes its function: wrapping up around the neck of coated vesicles, and by using the energy of its own GTP-ase activity, pinching off the vesicle.

From: Alberts B, et al. Molecular Biology of the Cell. 5th edition. 2008.
CONCLUSION

The current clinical practice for the effective antiviral therapy requires combination therapy by attacking various stages in the life of viruses, including HIV-1: entry inhibitors, nucleoside/nucleotide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and protease inhibitors. A proper study of the chemicals proposed in Table 1 may prove (or disprove) to be (i) inhibitors of clathrin-, dynamin-2-, caveolin- and/or lipid rafts-dependent RME, or (ii) inhibitors of RMF. Tentatively, some of them or a combination with antiretroviral therapy (ART) may possess a therapeutic value in AIDS as well as other viral diseases. This might help finding the keys to close the door for HIV-1 entry (62), thus being an additional state-of-the-ART in the therapy of AIDS and AIDS-related diseases, e.g. HIV-associated neurocognitive and cardiometabolic diseases (63-66).

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CONFLICT OF INTEREST STATEMENT

The authors certify that they have no affiliations with or involvement in any organization with any financial interest in the subject matter discussed in the present Dance round.

REFERENCES

1. Goldstein JL, Anderson RGW, Brown MS. Coated pits, coated vesicles, and receptor-mediated endocytosis. Nature 1979; 279(679). [DOI: 10.1038/279679a0]
2. Wileman T, Harding C, Stahl P. Receptor-mediated endocytosis. Biochemical Journal 1985; 232(1): 1-14. [DOI: 10.1042/bj2320001]
3. Pastan I, Willingham MC. Receptor-mediated endocytosis: coated pits, receptosomes and the Golgi. Trends in Biochemical Sciences 1983; 8(7): 250-254. [DOI: 10.1016/0968-0004(83)90351-1]
4. Pastan I, Willingham MC. Receptor-Mediated Endocytosis of Hormones in Cultured Cells. Annual Review of Physiology 1981; 43(1): 239-250. [DOI: 10.1146/annurev.ph.43.030181.001323]
5. Willingmann P, Barnert H, Zeichhardt H, Habermehl K-O. Recovery of structurally intact and infectious poliovirus type 1 from HeLa cells during receptor-mediated endocytosis. Virology 1989; 168(2): 417-420. [DOI: 10.1016/0042-6822(89)90286-9]
6. Tooze J. Blocked coated pits in AtT20 cells result from endocytosis of budding retrovirions. J Cell Biol 1985; 101(5): 1713-1723. [DOI: 10.1083/jcb.101.5.1713]
7. Lin S, Wang XM, Nadeau PE, Mergia A. HIV Infection Upregulates Caveolin 1 Expression To Restrict Virus Production. Journal of Virology 2010; 84(18): 9487-9496. [DOI: 10.1128/jvi.00763-10]
8. Kalia M, Jameel S. Virus entry paradigms. Amino Acids 2011; 41(5): 1147-1157. [DOI: 10.1007/s00726-009-0363-3]
9. Wilen CB, Tilton JC, Doms RW. HIV: cell binding and entry. Cold Spring Harbor perspectives in medicine 2012; 2(8): a006866. [DOI: 10.1101/cshperspect.a006866]
10. Howes MT, Mayor S, Parton RG. Molecules, mechanisms, and cellular roles of clathrin-independent endocytosis. Current Opinion in Cell Biology 2010; 22(4): 519-527. [DOI: https://doi.org/10.1016/jceb.2010.04.001]
11. Brown M, Goldstein J. A receptor-mediated pathway for cholesterol homeostasis. Science 1986; 232(4746): 34-47. [DOI: 10.1126/science.3513311]
12. Chaldakov GNN, S-D. Ultrastructure of the arterial smooth muscle cell. Adv Exp Med Biol 1975; 57(14-20.
13. Chaldakov GN. Inhibition of receptor-mediated cellular entry of viruses including HIV: a perspective on further researches on chemotherapy in viral diseases including AIDS. Med Hypotheses 1990; 33(4): 265-268.
14. Andersen KB, Nexø BA. Entry of murine retrovirus into mouse fibroblasts. Virology 1983; 125(1): 85-98. [DOI: 10.1016/0042-6822(83)90065-X]
15. Mitsuya H, Broder S. Strategies for antiviral therapy in AIDS. Nature 1987; 325(773. [DOI: 10.1038/325773a0]
16. Dimitrov DS. HIV-1 infection of cells and AIDS progression. Biomed Rev 1993; 2(1-8.
17. Pastan I, Willingham M. Journey to the center of the cell: role of the receptosome. Science 1981; 214(4520): 504-509. [DOI: 10.1126/science.6170111]
18. Davies PJ, Davies DR, Levitzki A, Maxfield FR, Milhaud P, Willingham MC. Transglutaminase is essential in receptor-mediated endocytosis of alpha 2-macroglobulin and polypeptide hormones. Nature 1980; 283(5743): 162-167. [DOI: 10.1038/283162a0]
19. Hunt RC, Marshall-Carlson L. Internalization and recycling of transferrin and its receptor. Effect of trifluoperazine on recycling in human erythroleukemic cells. J Biol Chem 1986; 261(8): 3681-3686.

20. Maxfield FR, Willingham MC, Davies PJ, Pastan I. Amines inhibit the clustering of alpha2-macroglobulin and EGF on the fibroblast cell surface. Nature 1979; 277(5698): 661-663. [DOI: 10.1038/277661a0]

21. Tietze C, Schlesinger P, Stahl P. Chloroquine and ammonium ion inhibit receptor-mediated endocytosis of mannose-glycoconjugates by macrophages: Apparent inhibition of receptor recycling. Biochemical and Biophysical Research Communications 1980; 93(1): 1-8. [DOI: 10.1016/S0006-291X(80)80237-3]

22. Van Leuven F, Cassiman JJ, Van Den Berghe H. Primary blasts. Cell 1980; 20(1): 37-43.

23. Molnar J, Hoekstra S, Ku CS, Van Alten P. Evidence for alpha2-macroglobulin in virally infected cells treated with monensin. J Cell Biol 1983; 96(3): 851-856. [DOI: 10.1083/jcb.96.3.851]

24. Lorand L, Conrad SM. Transglutaminases. Mol Cell Biochem 1984; 58(1-2): 9-35. [DOI: 10.1007/BF00240602]

25. Salisbury JL, Condeelis JS, Satir P. Role of coated vesicles, microfilaments, and calmodulin in receptor-mediated endocytosis by cultured B lymphoblastoid cells. J Cell Biol 1980; 87(1): 132-141. [DOI: 10.1083/jcb.87.1.132]

26. Cornewell MM, Juliano RL, Davies PJ. Inhibition of the adhesion of Chinese hamster ovary cells by the naphthylsulfonamides dansylcadaverine and N-(6-aminohexyl)-5-chloro-1-naphthylenesulfonamide (W7). Biochim Biophys Acta 1983; 762(3): 414-419. [DOI: 10.1016/0006-291X(83)90006-X]

27. Iacopetta B, Carpentier JL, Pozzan T, Lew DP, Gorden P, Orci L. Role of intracellular calcium and protein kinase C in the endocytosis of transferrin and insulin by HL60 cells. J Cell Biol 1986; 103(3): 851-856. [DOI: 10.1083/jcb.103.3.851]

28. Yamada S, Hirot a K, Chida K, Kuroki T. Inhibition of phorbol ester-caused induction of ornithine decarboxylase and tumor promotion in mouse skin by staurosporine, a potent inhibitor of protein kinase C. Biochemical and Biophysical Research Communications 1988; 157(1): 9-15. [DOI: 10.1016/S0006-291X(88)80003-2]

29. Nishizuka Y. The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature 1984; 308(693). [DOI: 10.1038/308693a0]

30. Heuser J. Effects of cytoplasmic acidification on clathrin lattice morphology. The Journal of Cell Biology 1989; 108(2): 401-411. [DOI: 10.1083/jcb.108.2.401]

31. Berlin RDC, Huntley R, Melmed R, Oliver J. New roles for tubulin in membrane function. In: Sakai HM, Borisy G, ed. Biological Functions of Microtubules and Related Structures. Tokyo, New York, London: Academic Press, 1982; 405-424.

32. Kelly WG, Passaniti A, Woods JW, Daiss JL, Roth TF. Tubulin as a molecular component of coated vesicles. J Cell Biol 1983; 97(4): 1191-1199. [DOI: 10.1083/jcb.97.4.1191]

33. Griffiths G, Quinn P, Warren G. Dissection of the Golgi complex. I. Monensin inhibits the transport of viral membrane proteins from medial to trans Golgi cisternae in baby hamster kidney cells infected with Semliki Forest virus. J Cell Biol 1983; 96(3): 835-850. [DOI: 10.1083/jcb.96.3.835]

34. Quinn P, Griffiths G, Warren G. Dissection of the Golgi complex. II. Density separation of specific Golgi functions in virally infected cells treated with monensin. J Cell Biol 1983; 96(3): 851-856. [DOI: 10.1083/jcb.96.3.851]

35. Rustan AC, Nossen JØ, Tefre T, Drevon CA. Inhibition of very-low-density lipoprotein secretion by chloroquine, verapamil and monensin takes place in the Golgi complex. Biochem Biophys Acta (BBA) - Mol Cell Res 1987; 930(3): 311-319. [DOI: 10.1016/0167-4889(87)90004-8]

36. Basu SK, Goldstein JL, Anderson RGW, Brown MS. Monensin interrupts the recycling of low density lipoprotein receptors in human fibroblasts. Cell 1981; 24(2): 493-502. [DOI: 10.1016/0092-8674(81)90340-8]

37. Zaitseva E, Zaitsev E, Melikov K, Arakelyan A, Marin M, Villasnil R, et al. Fusion Stage of HIV-1 Entry Depends on Virus-Induced Cell Surface Exposure of Phosphatidylserine. Cell Host Microbe 2017; 22(1): 99-110 e117. [DOI: 10.1016/j.chom.2017.06.012]

38. Yang S-T, Kiessling V, Simmons JA, White JM, Tamm LK. HIV gp41-mediated membrane fusion occurs at edges of cholesterol-rich lipid domains. Nature Chemical Biology 2015; 11(424. [DOI: 10.1038/nchembio.1800 supplimentary-information]

39. Chou T. Stochastic entry of enveloped viruses: fusion versus endocytosis. Biophysical Journal 2007; 93(4): 1116-1123. [DOI: 10.1529/biophysj.107.06708]
40. Haqqani AA, Tilton JC. Entry inhibitors and their use in the treatment of HIV-1 infection. *Antiviral Research* 2013; 98(2): 158-170. [DOI: https://doi.org/10.1016/j.antiviral.2013.03.017]

41. Malik T, Chauhan G, Rath G, Murthy RSR, Goyal AK. “Fusion and binding inhibition” key target for HIV-1 treatment and pre-exposure prophylaxis: targets, drug delivery and nanotechnology approaches. *Drug Delivery* 2017; 24(1): 608-621. [DOI: 10.1080/10717544.2016.1228717]

42. Baldwin CE, Sanders RW, Berkhout B. Inhibiting HIV-1 entry with fusion inhibitors. *Curr Med Chem* 2003; 10(17): 1633-1642.

43. Venner CM, Ratcliff AN, Coutu M, Finzi A, Arts EJ: HIV-1 Entry and Fusion Inhibitors: Mechanisms and Resistance. In: Mayers DL, Sobel JD, Ouellette M, Kaye KS, Marchaim D, eds. Antimicrobial Drug Resistance, Volume 1. Cham: Springer International Publishing, 2017; 545-557.

44. Mukhtar M, Parveen Z, Pomerantz RJ. Technology evaluation: PRO-542, Progenics Pharmaceuticals inc. *Curr Opin Mol Ther* 2000; 2(6): 697-702.

45. Lalezari J, Latiff GH, Brinson C, Echevarria J, Treviño-Pérez S, Bogner JR, Stock D, *et al.* HIV-1 attachment inhibitor prodrug BMS-663068 in antiretroviral-experienced subjects: week 24 analysis. *Journal of the International AIDS Society* 2014; 17(4Suppl 3): 19530. [DOI: 10.7448/IAS.17.4.19530]

46. Moore JP, Sattentau QJ, Klasse PJ, Burkly LC. A monoclonal antibody to CD4 domain 2 blocks soluble CD4-induced conformational changes in the envelope glycoproteins of human immunodeficiency virus type 1 (HIV-1) and HIV-1 infection of CD4+ cells. *Journal of Virology* 1992; 66(8): 4784-4793.

47. van Lelyveld SFL, Dreylewicz J, Krikke M, Veel EM, Otto SA, Richter C, *et al.* Maraviroc Intensification of cART in Patients with Suboptimal Immunological Recovery: A 48-Week, Placebo-Controlled Randomized Trial. *PLOS ONE* 2015; 10(7): e0132430. [DOI: 10.1371/journal.pone.0132430]

48. Tan Q, Zhu Y, Li J, Chen Z, Han GW, Kufareva I, *et al.* Structure of the CCR5 Chemokine Receptor–HIV Entry Inhibitor Maraviroc Complex. *Science* 2013; 341(6152): 1387-1390. [DOI: 10.1126/science.1241475]

49. Eggink D, Berkhout B, Sanders RW. Inhibition of HIV-1 by fusion inhibitors. *Curr Pharm Des* 2010; 16(33): 3716-3728.

50. Eron JJ, Gulick RM, Bartlett JA, Merigan T, Arduino R, Kilby JM, *et al.* Short-Term Safety and Antiretroviral Activity of T-1249, a Second-Generation Fusion Inhibitor of HIV. *The Journal of Infectious Diseases* 2004; 189(6): 1075-1083. [DOI: 10.1086/381707]

51. Dwyer JJ, Wilson KL, Davison DK, Freel SA, Seedorff JE, Wring SA, *et al.* Design of helicoidal, oligomeric HIV-1 fusion inhibitor peptides with potent activity against enfuvirtide-resistant virus. *Proceedings of the National Academy of Sciences of the United States of America* 2007; 104(31): 12772-12777. [DOI: 10.1073/pnas.0701478104]

52. Dai S-j, Dou G-f, Qian X-h, Song H-f, Tang Z-m, Liu D-s, *et al.* Pharmacokinetics of sifuvirtide, a novel anti-HIV-1 peptide, in monkeys and its inhibitory concentration in vitro. *Acta Pharmacologica Sinica* 2005; 26(12): 1576-1579. [DOI: 10.1111/j.1745-7254.2005.00163.x]

53. Cao P, Dou G, Cheng Y, Che J. The improved efficacy of Sifuvirtide compared with enfuvirtide might be related to its selectivity for the rigid biomembrane, as determined through surface plasmon resonance. *PLOS ONE* 2017; 12(2): e0171567. [DOI: 10.1371/journal.pone.0171567]

54. He Y, Xiao Y, Song H, Liang Q, Ju D, Chen X, *et al.* Design and Evaluation of Sifuvirtide, a Novel HIV-1 Fusion Inhibitor. *Journal of Biological Chemistry* 2008; 283(17): 11126-11134. [DOI: 10.1074/jbc.M800200200]

55. Liu S, Xiao G, Chen Y, He Y, Niu J, Escalante CR, *et al.* Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: implications for virus fusogenic mechanism and identification of fusion inhibitors. *The Lancet* 2004; 363(9413): 938-947. [DOI: 10.1016/S0140-6736(04)6736(04)15788-7]

56. Porotto M, Carta P, Deng Y, Kellogg GE, Whitt M, Lu M, *et al.* Molecular determinants of antiviral potency of paramyxovirus entry inhibitors. *J Virol* 2007; 81(19): 10567-10574. [DOI: 10.1128/JVI.01181-07]

57. Chen W, Feng Y, Prabakaran P, Ying T, Wang Y, Sun J, *et al.* Exceptionally Potent and Broadly Cross-Reactive, Bispecific Multivalent HIV-1 Inhibitors Based on Single Human CD4 and Antibody Domains. *Journal of Virology* 2014; 88(2): 1125-1139. [DOI: 10.1128/jvi.02566-13]

58. Daecke J, Fackler OT, Dittmar MT, Kräusslich H-G. Involvement of Clathrin-Mediated Endocytosis in Human Immunodeficiency Virus Type 1 Entry. *Journal of Virology* 2005; 79(3): 1581-1594. [DOI: 10.1128/jvi.79.3.1581-1594.2005]
59. Jakobsdottir GM, Iliopoulou M, Nolan R, Alvarez L, Compton AA, Padilla-Parra S. On the Whereabouts of HIV-1 Cellular Entry and Its Fusion Ports. *Trends in Molecular Medicine* 2017; 23(10): 932-944. [DOI: https://doi.org/10.1016/j.molmed.2017.08.005]

60. Giroud C, Marin M, Hammonds J, Spearman P, Melikyan GB. P2X1 Receptor Antagonists Inhibit HIV-1 Fusion by Blocking Virus-Coreceptor Interactions. *Journal of Virology* 2015; 89(18): 9368-9382. [DOI: 10.1128/jvi.01178-15]

61. Swartz TH, Esposito AM, Durham ND, Hartmann BM, Chen BK. P2X-selective purinergic antagonists are strong inhibitors of HIV-1 fusion during both cell-to-cell and cell-free infection. *Journal of Virology* 2014; 88(19): 11504-11515. [DOI: 10.1128/JVI.01158-14]

62. Hertje M, Zhou M, Dietrich U. Inhibition of HIV-1 Entry: Multiple Keys to Close the Door. *ChemMedChem* 2010; 5(11): 1825-1835. [DOI: doi:10.1002/cmdc.201000292]

63. Garaci E, Caroleo MC, Aloe L, Aquaro S, Piacentini M, Costa N, Amendola A, *et al.* Nerve growth factor is an autocrine factor essential for the survival of macrophages infected with HIV. *Proceedings of the National Academy of Sciences of the United States of America* 1999; 96(24): 14013-14018.

64. Williams KS, Killebrew DA, Clary GP, Meeker RB. Opposing Effects of NGF and proNGF on HIV Induced Macrophage Activation. *Journal of Neuroimmune Pharmacology* 2016; 11(1): 98-120. [DOI: 10.1007/s11481-015-9631-z]

65. Souza TML, Temerozo JR, Giestal-de-Araujo E, Bou-Habib DC. The Effects of Neurotrophins and the Neuropeptides VIP and PACAP on HIV-1 Infection: Histories with Opposite Ends. *Neuroimmunomodulation* 2014; 21(5): 268-282. [DOI: 10.1159/000357434]

66. Bourgi K, Wanjalla C, Koethe JR. Inflammation and Metabolic Complications in HIV. *Current HIV/AIDS Reports* 2018; 15(5): 371-381. [DOI: 10.1007/s11904-018-0411-2]