ISSN 0975-6299

Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

REVIEW ARTICLE

BIOTECHNOLOGY

"RANTES- A NEW TARGET FOR FIGHTING AGAINST HIV INFECTION"

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ABSTRACT

Although treatments for AIDS and HIV can slow the course of the disease, there is currently no vaccine or cure. Antiretroviral treatment reduces both the mortality and the morbidity of HIV infection, but these drugs are expensive and routine access to antiretroviral medication is not available in all countries. Due to the difficulty in treating HIV infection, preventing infection is a key aim in controlling the AIDS pandemic, with health organizations promoting safe sex and needle-exchange programmes in attempts to slow the spread of the virus. RANTES was shown to be the most potent member of a trio of CC chemokines released by CD8+ T cells that were able to suppress the replication of non-syncitium-inducing (NSI) HIV-1 strains *in vitro* the others were macrophage inflammatory protein 1 α (MIP-1α) and MIP-1β. Some studies *in vivo* have supported a role for RANTES in HIV suppression, with the demonstration of unusually high levels of RANTES production in both HIV exposed humans and vaccinated monkeys who appear to be resistant to infection with HIV or simian immunodeficiency virus (SIV). These encouraging observations have led to further studies to test the potential therapeutic use of RANTES, both in the treatment and prevention of HIV infection.

KEY WORDS

HIV, RANTES, Chemokines.

INTRODUCTION

Acquired Immune Deficiency syndrome *or* ***acquired immunodeficiency syndrome*** *(**AIDS**)* is a disease of the human immune system caused by the human immunodeficiency virus (HIV) [1-3]. This condition progressively reduces the effectiveness of the immune system and leaves individuals susceptible to opportunistic infections and tumors. HIV is transmitted through direct contact of a mucous membrane or the bloodstream with a bodily fluid containing HIV, such as blood, semen, vaginal fluid, pre seminal fluid, and breast milk [4][5]. This transmission can involve anal, vaginal or oral sex, blood transfusion, contaminated hypodermic needles, exchange between mother and baby during pregnancy, childbirth, breastfeeding or other exposure to one of the above bodily fluids.

AIDS is now a pandemic. In 2007, it was estimated that 33.2 million people lived with the disease worldwide, and that AIDS killed an estimated 2.1 million people, including 330,000 children. Over three-quarters of these deaths occurred in sub-Saharan Africa. Genetic research indicates that HIV originated in west-central Africa during the late nineteenth or early twentieth century. AIDS was first recognized by the U.S. Centers for Disease Control and Prevention in 1981 and its cause, HIV, identified in the early 1980s. Although treatments for AIDS and HIV can slow the course of the disease, there is currently no vaccine or cure. Antiretroviral treatment reduces both the mortality and the morbidity of HIV infection, but these drugs are expensive and routine access to antiretroviral medication is not available in all countries. Due to the difficulty in treating HIV infection, preventing infection is a key aim in controlling the AIDS pandemic, with health organizations promoting safe sex and needle-exchange programmes in attempts to slow the spread of the virus [5,6].

Human Immunodeficiency Virus: An Overview:

HIV is a retrovirus from the lentivirus subfamily. Two major subtypes of HIV, HIV-1 and HIV-2, have been characterized. HIV-1 is the predominant HIV type throughout the world while HIV-2 is mostly found in West Africa [7]. Of HIV-1, two subtypes can be distinguished based on nucleotide sequence relationships, i.e. group M ('majority') and group O ('outlier'). In group M, at least ten genetically different subtypes have been identified (from A to J). Group O contains virus strains that are very divergent compared to those of group M [8]. The following part presents more information on the structure and replication cycle of HIV and highlights the different classes of antiretroviral therapeutics investigated.

HIV structure:

The mature HIV virion is an icosahedral sphere with a diameter of approximately 100 nm. The outer shell, the viral envelope is a lipid bilayer, from host membrane origin, that embeds host cell proteins and spikes. These spikes consist of two viral envelope proteins (env): an outer protruding cap glycoprotein (gp) 120 and a stem gp41, which are non-covalently attached to each other. These glycoproteins are formed by cleavage of a larger precursor gp160, by a cellular protease. Located within the viral envelope is the matrix, made of an HIV protein called p17, and herein the viral conical core or capsid, which is made of the viral protein p24 (core antigen). The viral core contains two single strands of HIV RNA and multiple reverse transcriptase, the nucleocapsid proteins p6 and p7, the protease p11 and the integrase p32. The HIV genome exists of nine different genes where of three (gag, pol and env) are common in all retroviruses. Gag is the gene coding for the viral core proteins, pol codes for the viral

enzymes reverse trancriptase, integrase and protease and env codes for the envelope glycoproteins. The other genes (tat, rev, vpr, nef, vif and vpu) are responsible for the organisation of the virus life cycle. To ensure the effective production of newvirions by its host cells the viral genome is flanked at each site by long terminal repeat (LTR) sequences that can bind cellular proteins to activate transcription under control of viral signals [9].

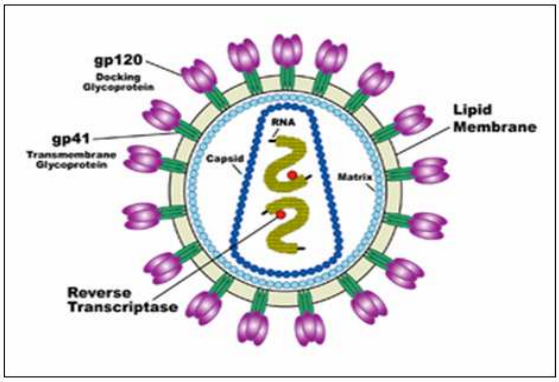

Fig 1
The structure of HIV.

HIV Entry to the cell:

HIV enters macrophages and $CD4^+$ T cells by the adsorption of glycoproteins on its surface to receptors on the target cell followed by fusion of the viral envelope with the cell membrane and the release of the HIV capsid into the cell. Entry to the cell begins through interaction of the trimeric envelope complex (gp160 spike) and both CD4 and a chemokine receptor (generally either CCR5 or CXCR4, but others are known to interact) on the cell surface. Gp120 binds to integrin $\alpha_4\beta_7$ activating LFA-1 the central integrin involved in the establishment of virological synapses, which facilitate efficient cell-to-cell spreading of HIV-1. The gp160 spike contains binding domains for both CD4 and chemokine receptors. The first step in fusion involves the high-affinity attachment of the CD4 binding domains of gp120 to CD4. Once gp120 is bound with the CD4 protein, the envelope complex undergoes a structural change, exposing the chemokine binding domains of gp120 and allowing them to interact with the target chemokine receptor. This allows for a more stable two-pronged attachment, which allows the N-terminal fusion peptide gp41 to penetrate the cell membrane. Repeat sequences in gp41, HR1 and HR2 then interact, causing the collapse of the extracellular portion of gp41 into a hairpin. This loop structure brings the virus and cell membranes close together, allowing fusion of the membranes and subsequent entry of the viral capsid. After HIV has bound to the target cell, the HIV RNA and various enzymes, including reverse transcriptase, integrase, ribonucleases, and protease, are injected into the cell. During the microtubule based transport to the nucleus, the viral single strand RNA genome is transcribed into double strand DNA, which is then integrated into a host chromosome. HIV can infect dendritic cells (DCs) by this CD4-CCR5 route, but another route using mannose-specific C-type lectin receptors such as DC-SIGN can also be used. DCs are one of the first cells encountered by the virus during sexual transmission. They are currently thought to play an important role by transmitting HIV to T-cells when the virus is captured in the mucosa by DCs. The presence of FEZ-1,

which occurs naturally in neurons, is believed to prevent the infection of cells by HIV [10,11].

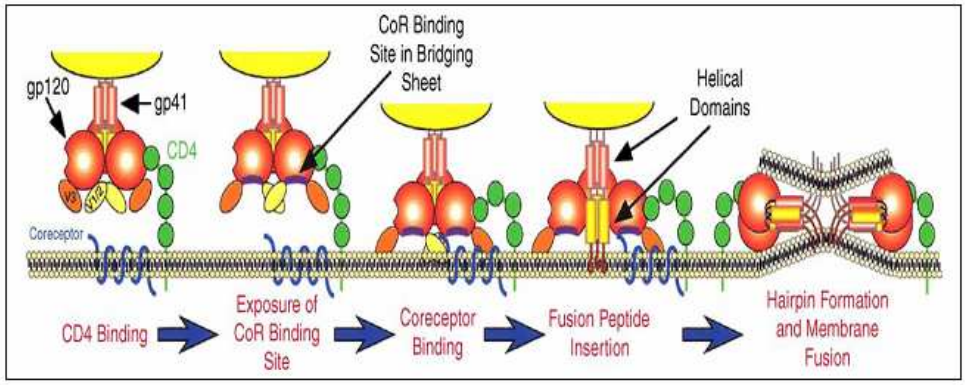

Fig 2

Schematic presentation of the HIV-1 entry process. The entry process is initiated by the attachment of the viral envelope protein gp120 to the CD4 receptor, which induces conformational changes in the gp120 subunit setting free the co-receptor binding site on gp120. After binding of the glycoprotein with the chemokine/ coreceptor, HIV-1 gp41 'unfolds' by a hinge mechanism followed by insertion of the fusion peptide into the cell membrane, anchoring the virus to the cellular membrane. Then, gp41 folds into a 'hairpin structure' and brings the cell membrane and the viral membrane into close proximity where after fusion can take place. Following membrane fusion, the viral contents are expelled into the cell.

RANTES – a new target for inhibiting the entry of HIV-1:

The discovery that the CC chemokines RANTES, MIP-1_ and MIP-1_ act as potent natural inhibitors of HIV-1, the causative agent of AIDS, and the subsequent identification of CCR5 as a major virus co-receptor have triggered a wealth of basic and applied research approaches aimed at developing safe and effective viral entry inhibitors. Some of these efforts have focused on RANTES engineering with the goal of enhancing the antiviral activity of the native molecule while reducing or abrogating its inflammatory properties. The wave front generated a decade ago is still on its course, with a flow of promising leads constantly emerging and being evaluated in preclinical studies. Here, we present an overview of this rapidly evolving field, highlighting the most important features of RANTES molecular architecture and structure—function relationships. CC chemokines come into action during the HIV-1 entry process as CCR5 ligands with natural antiviral activity. Among these chemokines, RANTES is the most powerful HIV- 1 blocker. Given the central role of RANTES and CCR5 in HIV-1 pathology, much of the research in the field of HIV-entry inhibitors has been generated focusing on these two molecules. However, the three-dimensional structure of CCR5 is still unsolved, essentially due to its seven transmembrane domain structure, and therefore the fine structural details of the RANTES—CCR5 interaction remain unknown. On the contrary, structural data on CC chemokines are abundant, including nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography studies on wild-type molecules, mutants and chemically modified variants, as well as the characterization of their interaction with glycosaminoglycans(GAGs). These studies have built fundamental knowledge to drive the rational engineering of chemokines with improved antiviral activity and pharmacological properties. In this review, we summarize the accomplishments achieved in this rapidly evolving field with particular focus on the RANTES—CCR5 interaction, as well as RANTES-engineering strategies for the development of novel HIV-1 entry inhibitors.

Although the complex role of the chemokine system in the regulation of immune functions is of fundamental importance, a discussion of the immunologic role of RANTES is beyond the scope of the present review and has been extensively reviewed elsewhere [12].

RANTES binding to GAG's and CCR5 on cell surface:

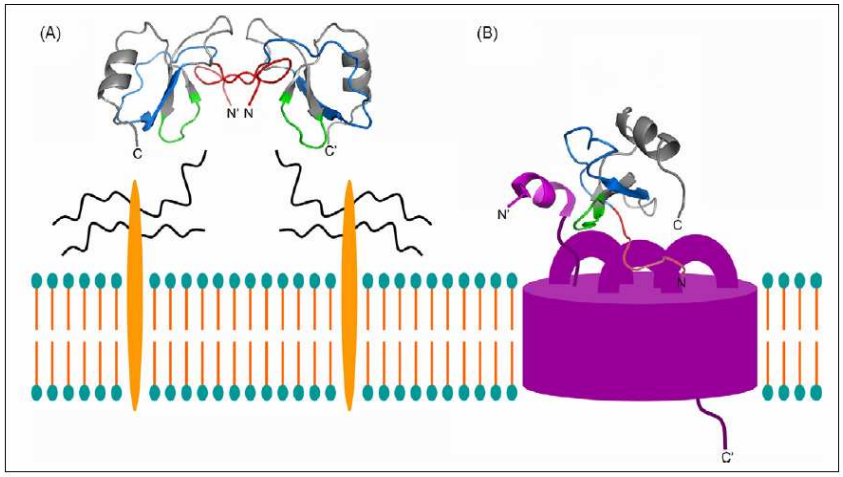

Fig 3

Schematic representation of RANTES binding to GAGs and CCR5 on the cell surface. (A) Ribbon side view representation of the RANTES dimer (PDB entry 1HRJ) and its interaction with cell surface glycosaminoglycans (the protein core is represented in orange; the carbohydrate chains in black). The N-terminus (aa 1—10) is colored in red; the N-loop/_1-strand (aa 11—29) in blue; the GAG-binding 40's loop (aa 43—48) in green. N and C denote the N- and C-terminus, respectively (N_ and C_, the termini of the second RANTES monomer within the dimer). (B) Side view of the RANTES monomer (color code as in (A)) in a proposed complex with CCR5 (pink). The RANTES monomer is shown with a 45 anti-clockwise rotation compared to (A), and is slightly tilted towards the observer. The _-helix conformation adopted by the CCR5 N-terminal peptide, spanning residues 7—15, corresponds to that illustrated in PDB entry 2RLL. As previously suggested, the RANTES N-terminus is likely to be embedded within transmembrane CCR5 helices, while the extracellular CCR5 loops, particularly ECL2, should surround RANTES. N and C denote RANTES N- and C-terminus, respectively (N_ and C_, denote the CCR5 termini). In both (A) and (B), RANTES orientation towards GAGs, CCR5 and the cell membrane have been chosen arbitrarily.

Molecular Architecture of RANTES:

Similar to other chemokines, RANTES is a small globular protein with a very stable fold, which represents an invaluable advantage for successful protein engineering. Its three-dimensional structure, solved by NMR, showed that the protein is present in solution predominantly as a dimer. In the dimer context, each monomer presents a partially disordered N-terminal region, followed by a short-strand leading to the signature two-cysteine (CC) motif, an extended region (N-loop) ending with a 310 turn, three anti-parallel-strands (1-3) connected by loops, and a C-terminal-helix (Fig. 3). RANTES dimerization and oligomerization, as well as the role of its different domains, are described below, particularly for their differential contribution to the anti-HIV activity [12].

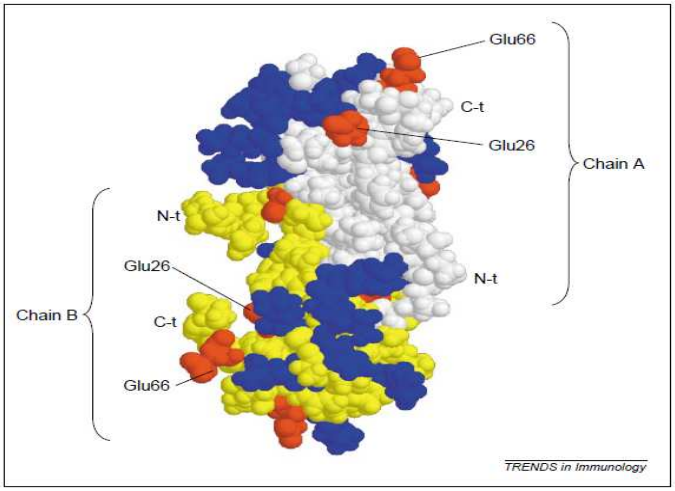

Fig 4

Structure of a dimer of human RANTES. Positively charged residues are colored in blue and negatively charged residues are in red. The dimerization of RANTES has a structural basis (white colored residues belong to chain A, and yellow-colored residues belong to chain B) but, in addition, RANTES self-aggregates to form multimers. The dimerization occurs at the N-terminal part of the molecule, which is also essential for receptor binding and signaling. The residues involved in the multimerization, Glu66 and Glu26, are found at the surface of the protein, suggesting that ionic binding with positively charged surface residues from other RANTES molecules occurs and leads to self-aggregation. Changing these residues to serine abrogates aggregation.

Diverse effects of RANTES on HIV replication:

In 1995, RANTES was shown to be the most potent member of a trio of CC chemokines released by CD8+ T cells that were able to suppress the replication of non-syncitium-inducing (NSI) HIV-1 strains *in vitro* the others were macrophage inflammatory protein 1 α (MIP-1α) and MIP-1β [13]. These observations were fundamental to the identification of the receptor for these chemokines, CCR5, as the major co-receptor for cell entry of primary NSI HIV-1 strains [14]. The precise mechanism of HIV suppression by RANTES is not yet fully understood but does not require full signal transduction via RANTES-specific receptors. It might be mediated by simply preventing access of the viral envelope protein (gp120) to CCR5, but it might also involve CCR5 dimerization and internalization following RANTES binding12. The HIV-suppressive effect of RANTES appears to be critically dependent on the presence of glycosaminoglycans (GAGs) on the target cell surface [15], although their precise role is not entirely clear. Controversially, several isolated but consistent studies have also reported increased HIV replication following signal transduction by RANTES via its specific receptors [16][17]. There has been no consistent demonstration of an inverse relationship between levels of RANTES and HIV disease progression, largely because of the inherent difficulties in measuring circulating RANTES concentrations. However, some studies *in vivo* have supported a role for RANTES in HIV suppression, with the demonstration of unusually high levels of RANTES production in both HIV exposed humans and vaccinated monkeys who appear to be resistant to infection with HIV or simian immunodeficiency virus (SIV) [18-20]. These encouraging observations have led to further studies to test the potential therapeutic use of RANTES, both in the treatment and prevention of HIV infection. A range of N-terminus-modified RANTES derivatives, such as N-terminal-truncated RANTES, Met-RANTES and particularly amino-oxypentane (AOP)-

RANTES, have been shown to act as antagonists or partial agonists of RANTES and to suppress HIV infection potently [21-23]. Chemically synthesized inhibitors, such as TAK-779, which can block the binding of HIV to CCR5, have also been produced and have genuine potential to become antiviral drugs [24]. However, a major obstacle to the development of these compounds as anti-retroviral agents has been the observation that, although RANTES can potently suppress HIV infection at low concentrations, it can actually enhance HIV infection, especially high concentrations [25][26]. The contrasting effects of RANTES on HIV replication *in vitro* are further illustrated by two recent studies describing opposing effects of single nucleotide polymorphisms in the RANTES promoter that are associated with increased production of the chemokine. In one study, the high-production genotype was associated with delayed disease progression in HIV-infected Japanese donors [27]. However, in another, more recent study, the same polymorphism was shown to increase susceptibility to HIV infection by two fold in the American multicenter AIDS cohort study (MACS) cohort while delaying disease progression by approximately 40% in those already infected. Thus, even at the genetic level, the role of RANTES in HIV disease susceptibility and progression is pleomorphic [28].

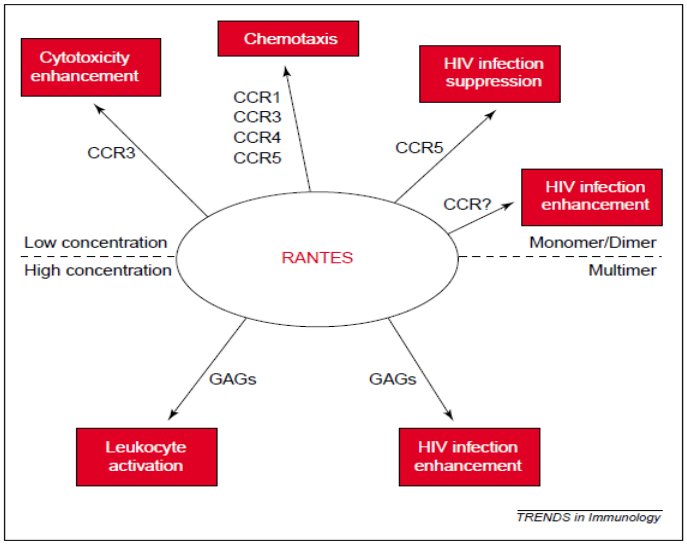

Fig 5

The diverse effects of RANTES in vitro. The effects of RANTES can be separated into two groups according to its concentration. At low concentrations, RANTES acts in a monomeric (or dimeric) form directly on its specific chemokine receptors; at high concentrations, it self-aggregates and acts through interactions with cell-surface glycosaminoglycans. Abbreviation: GAG, glycosaminoglycans.

Conclusion & Future research directions:

It is essential to define the precise *in vivo* role of RANTES. An invaluable model system would be the generation of transgenic mice expressing a non-aggregating (E66S) form of RANTES in place of the wild-type molecule. Although much less is known about murine RANTES biology, it seems highly likely that human (h) and mouse (m) RANTES share similar properties. The two proteins have considerable homology in both sequence and structure (Fig 6): they share 83% sequence identity and the Glu residues involved in hRANTES aggregation are conserved in mRANTES. Furthermore, high concentrations of hRANTES can directly activate mouse lymphocytes. The production

and study of mRANTES E66S mice would provide a setting in which to address definitively the requirement for aggregation for RANTES activity *in vivo*, and to examine its role in a range of inflammatory disorders.

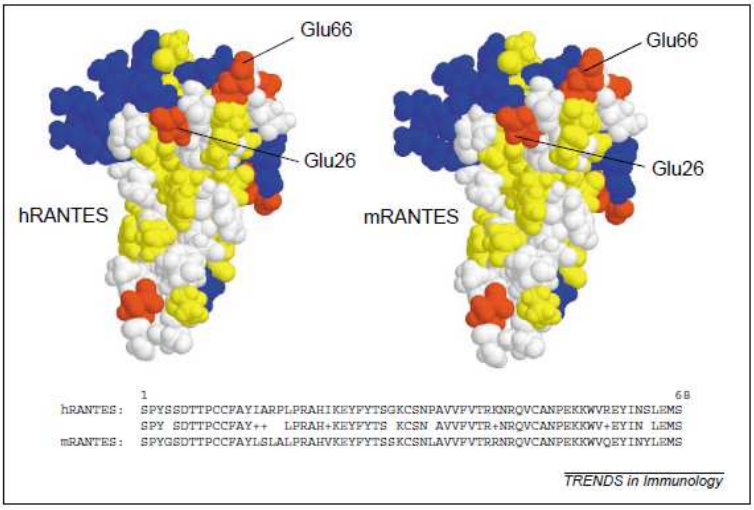

Fig 6

Human (h) RANTES and mouse (m) RANTES are highly homologous proteins. Positively charged residues are colored in blue, negatively charged residues are in red and hydrophobic residues are in yellow. The two proteins share 83% identity and show 91% homology for their charged residues. Their structures are nearly identical. The residues involved in hRANTES self aggregation are conserved in mRANTES, suggesting they would have similar aggregation properties.

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