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LOW-MOLECULAR-WEIGHT ANTI-HIV-1 PEPTIDES FROM THE AMINO-TERMINAL SEQUENCE OF RANTES: POSSIBLE LEAD COMPOUNDS FOR CORECEPTOR-DIRECTED ANTI-HIV-1 AGENTS

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Abstract A series of small peptides corresponding to the amino-terminal sequence of RANTES were synthesized, and their anti-HIV-1 activity was evaluated. Pentapeptides, H-(^{10}Ala-RANTES 6-10)-OH and Ac-(^{10}Ala-RANTES 6-10)-NH_2, were the smallest anti-HIV-1 peptides so far developed, and would be potentially important lead compounds for coreceptor-directed anti-HIV-1 drugs.

RANTES (regulated upon activation, normal T-cell expressed and secreted), one of the CC-chemokines, is a chemotactic and activating agent for a variety of leukocytes including T-lymphocytes.[1] Recently, it has been reported that RANTES as well as macrophage inflammatory protein (MIP)-1α and MIP-1β inhibits infection with human immunodeficiency virus type-1 (HIV-1) *in vitro* by interacting with CC-chemokine receptor-5 (CCR-5), a coreceptor for macrophage-tropic (M-tropic) HIV-1.[2-4] Furthermore, CC-chemokine antagonists, RANTES 9-68 and aminooxypentane (AOP)-RANTES, have been found to show high anti-HIV-1 activity.[5,6] These findings suggest the possibility of effective treatment of HIV-1 infected individuals by blocking coreceptors with low-molecular-weight ligands. Thus our attention has been focused on the structural requirements for the anti-HIV-1 activity of chemokines to develop a novel class of anti-HIV-1 agents.

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1                   20                  40                  60
.                   .                   .                   .
SPYSSDTTPCCFAYIARPLPRAHIKEYFYTSGKCSNPAVVFVTRKNRQVCANPEKKWVREYINSLEMS
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Fig. 1 *Amino acid sequence of human RANTES*

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As shown in Fig. 1, RANTES consists of 68 amino acid residues including four Cys residues, the first two being located adjacently to form a unique sequence of the CC-chemokine family, Cys-Cys. The amino-terminal region before the first disulfide bridge was known to be critical for chemotactic activity.[7] Recently, we have reported that this region is important also for anti-HIV-1 activity of RANTES.[8] An acetylated decapeptide-amide having the amino acid sequence of the positions 1-10, where the original Cys at position 10 is replaced with Ala [Ac-(^{10}Ala-RANTES 1-10)-NH_2], has been shown therein to exhibit potent anti-HIV-1 activity.[8] The result prompted us to carry out a screening study for shorter analogues to discuss the minimum structural requirements for the anti-HIV-1 activity. This communication deals with the synthesis and anti-HIV-1 activity of a series of small peptides relating to Ac-(^{10}Ala-RANTES 1-10)-NH_2.

H-(^{10}Ala-RANTES 1-10)-OH (**1**), H-(^{10}Ala-RANTES 2-10)-OH (**2**), H-(^{10}Ala-RANTES 3-10)-OH (**3**), H-(^{10}Ala-RANTES 4-10)-OH (**4**), H-(^{10}Ala-RANTES 5-10)-OH (**5**), H-(^{10}Ala-RANTES 6-10)-OH (**6**), and H-(^{10}Ala-RANTES 7-10)-OH (**7**) were synthesized by the solution method based on Boc-chemistry.[9,10] Their anti-HIV-1 activity was evaluated using phytohemagglutinin-activated peripheral blood mononuclear cells and HIV-$1_{JR\text{-}CSF}$ virus,[11] and is shown in Table 1. Surprisingly, H-(^{10}Ala-RANTES 1-10)-OH **1** showed no appreciable anti-HIV-1 activity even at 100 nM, despite the 51 % inhibition by Ac-(^{10}Ala-RANTES 1-10)-NH_2 at 10 nM.[8] This indicates that both or either of the amino-terminal acetyl and carboxy-terminal amide groups contribute to the anti-HIV-1 activity of Ac-(^{10}Ala-RANTES 1-10)-NH_2. However, shorter analogues of **1**, *i.e.*, peptides **2**, **3**, **4**, **5**, and **6**, showed anti-HIV-1 activity. Tetrapeptide **7** was not active at the concentrations examined. Pentapeptide **6**, Asp-Thr-Thr-Pro-Ala (DTTPA), was thus the shortest anti-HIV-1 peptide so far examined, and it was therefore supposed to contain minimum structural requirements for the anti-HIV-1 effect of RANTES.

Table 1 *Anti-HIV-1 activity of synthetic peptides*

		% inhibition[a]	
		10 nM	100 nM
SPYSSDTTPA	1	0	0
PYSSDTTPA	2	24	39
YSSDTTPA	3	41	34
SSDTTPA	4	22	22
SDTTPA	5	9	4
DTTPA	6	18	29
TTPA	7	0	0
Ac-DTTPA-NH_2	8	43	46
Ac-SPYSSDTTPA-NH_2		51	69
rRANTES		53	95

a) Phytohemagglutinin-activated peripheral blood mononuclear cells were infected with macrophage-tropic HIV-1 virus (JR_{CSF}) and cultured in the presence or the absence of synthetic peptides. After 7 days, the amount of soluble HIV-1 p24 in each culture supernatant was determined by ELISA. Details of the anti-HIV-1 assay were described in a previous paper.[11] The anti-HIV-1 activity of peptides was represented as % decrement of p24 from the amount in the absence of peptides.

Low-molecular-weight anti-HIV-1 peptides obtained here may be useful for the development of coreceptor-directed anti-HIV-1 agents, and structure modification of these anti-HIV-1 peptides, particularly the smallest **6**, would be a promising approach. As a preliminary study, Ac-DTTPA-NH_2 (**8**), in which both termini had been blocked, was synthesized by Fmoc-based solid-phase synthesis,[12,13] and its anti-HIV-1 activity was assayed. The resulting **8** exhibited a somewhat higher activity than the parent **6** as shown in Table 1. Some appropriate modifications of the termini of **6** are, therefore, expected to result in further improved activity, as implied by a marked difference in the activity between H-(^{10}Ala-RANTES 1-10)-OH **1** and Ac-(^{10}Ala-RANTES 1-10)-NH_2.

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                    1                   20
(a)  RANTES   -SPYSSDT-TPC-CFAYIARPLP···
     MIP-1α   -ASLAADTPTAC-CFSYTSRQIP···
     MIP-1β   -APMGSDPPTAC-CFSYTARKLP···

(b)  MCP-1    -QPDAINAPVTC-CYNFTNRKIS···
     MCP-2    AQPDSVSIPITC-CFNVINRKIP···
     MCP-3    -QPVGINTSTTC-CYRFIN-KIP···

(c)  SDF-1    ---KPVSLSYRCPCRFFESHVAR···
     IL-8     AVLPRSAKELRCQCIKTYSKPFH···
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Fig. 2 *Amino acid sequences of amino-terminal regions of (a) CC-chemokines, which have anti-HIV-1 activity, (b) some CC-chemokines, which do not have anti-HIV-1 activity, and (c) CXC-chemokines. The DTTPC and analogous sequences are highlighted with underlines. The numbering at the top relates to that of RANTES.*

The amino acid sequences of the amino-terminal region of various chemokines are listed in Fig. 2. In anti-HIV-1 chemokines targeting CCR-5 such as RANTES, MIP-1α, and MIP-1β, amino acid sequences analogous to the minimun structure for anti-HIV-1 activity found here, DTTPA (or DTTPC in the original sequence), are observed. On the contrary, such sequences are not observed in other CC-chemokines and CXC-chemokines. This suggests that the DTTPC regions in these CC-chemokines play a crucial role in anti-HIV-1 effect and binding with CCR-5. Previously, the amino-terminal region at the positions 1-10 of monocyte chemotactic protein (MCP)-1, a member of CC-chemokines having no anti-HIV-1 activity, was shown to be essential for chemoatractant activity; the positions 1-6 and 7-10 associate with receptor-activation and receptor-binding, respectively.[14] Based on the close resemblance of three dimensional structures of various chemokines,[15] the receptor-binding and receptor-activating domains of RANTES could be speculated to distribute in a similar fashion to those of MCP-1, and the DTTPC regions in anti-HIV-1 CC-chemokines may be serving as a receptor-binding domain. The significant anti-HIV-1 activity of pentapeptides **6** and **8** suggests that the DTTPC region of RANTES and the coreceptor-binding component of HIV-1 compete for the same binding site on CCR-5. Recently, the monoclonal antibody 2D7, which recognizes the second extracellular loop of CCR-5, was reported to block the binding of RANTES with CCR-5 and the infectivity of several M-tropic and dual-tropic HIV-1 strains.[16] This clearly indicates that the RANTES-binding site on CCR-5 is, at least partly, overlapping with the virus-binding site on CCR-5, which reasonably supports our speculation. Furthermore, 2D7 was shown to

inhibit the chemotactic activity of RANTES,[16] and thus the responsible region for chemotaxis on CCR-5 would be close to the binding site of 2D7, which HIV-1 also recognizes. The avtive domains for chemotaxis and anti-HIV-1 effect would be, then, close to each other, and this is consistent with our finding that the DTTPC region, which is located in the active region for chemotaxis,[7] contains minimum structural requirements for anti-HIV-1 activity of RANTES.

In conclusions, some low-molecular-weight anti-HIV-1 peptides were found from the amino-terminal structure of RANTES. Particularly, DTTPA **6** and Ac-DTTPA-NH_2 **8** are the smallest chemokine derivatives having anti-HIV-1 activity at present. Although they are somewhat less potent than RANTES, peptides **6** and **8** may possibly be important lead compounds for coreceptor-directed anti-HIV-1 agents. The fact that DTTPC and analogous sequences are common only in anti-HIV-1 CC-chemokines also supports the significance of the sequence DTTPC. These results provide us some structural bases for developing coreceptor-directed anti-HIV-1 agents. Further structure-activity relationship study is now under way.

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