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Extracellular HIV Tat and Tat cysteine rich peptide increase CCR5 expression in monocytes

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Abstract: In our previous work we reported that HIV Tat and 6 cysteine rich peptides of Tat induce tumor necrosis factor-related apoptosis-induced ligand (TRAIL) in human monocytes (Yang *et al*., 2003). Here our results showed that HIV Tat and Tat cysteine rich peptide increase CCR5 expression in human monocytes, and this activity is inhibited by rabbit anti-Tat. Boiled Tat does not increase CCR5 expression in monocytes. These results provide insight into a new mechanism by which HIV Tat plays a key role in the pathogenesis of HIV-1 infection.

Key words: HIV Tat, Tat cysteine rich peptide, CCR5, Monocytes
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INTRODUCTION

The human immunodeficiency virus-type 1 (HIV-1) Tat protein is essential for HIV-1 transcription and replication, and also modulates the expression of genes responsible for cell survival and proliferation (Jons, 1993). Tat is also a secreted protein that can be detected in sera from HIV-infected individuals and that binds to cell surfaces through electrostatic interactions, chemokine receptors, or cell surface integrins and activates various interacellular signal transduction pathways (Barillari *et al*., 1993). Cells treated with Tat showed increased expression of chemokine receptors CCR5 and CXCR4 (Huang *et al*., 1998), overproduction of Interleukine-2 (IL-2) (Westendorp *et al*., 1994), Interleukine-8 (IL-8) (Ott *et al*., 1998) and enhanced expression of TNF-α related apoptosis induced ligand (TRAIL) (Yang *et al*., 2003) on monocyte/macrophages that could cause cell death in cultured peripheral blood mononuclear cells (PBMC). Tat protein can also be taken up by infected and uninfected PBMC, thus inducing biological effects through an autocrine/paracrine mechanism (Zauli *et al*., 1995). HIV Tat contains several functional subdomains, the first 13 amino acids (N-terminal) with amphipathic characteristics. Cysteines rich domain (aa 22–37), and the core region (1 to 48) together constitute the minimal activation domain for transcription in vitro. The cysteine rich subdomains are highly conserved between different isolates of HIV-1s (Jeang *et al*., 1999). The cysteine rich domain mediates the pathogenesis of HIV infection in monocytes in vitro (Tikhonov *et al*., 2003), and is also involved in monocyte chemotaxis (Boykins *et al*., 1999; Albini *et al*., 1998).

The role of chemokines in regulating the progression of HIV infection has been identified by the ability of chemokines receptor of function as co-receptors that mediate HIV entry (Alkhatib *et al*., 1996; Choe *et al*., 1996). This study indicates that HIV Tat and cysteine rich peptide of Tat up-regulate CCR5 expression in monocytes, and that polyclonal rabbit

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anti-Tat inhibits CCR5 expression.

MATERIALS AND METHODS

Reagents

HIV Tat protein, Tat-86, with a C-terminal truncation of 15 amino acids was obtained from the National Institute of Health AIDS Research and Reference Reagent Program (ARRRP). FITC-anti-CD4, phycoerythrin (PE)-anti-human $CD8^+$, FITC-anti-CD14, PE-anti-CCR5 (Pharmingen, San Diago, CA) were used for Flow Cytometry. The Tat peptides were as follows, with the included amino acid residues indicated in the subscript: $Tat_{1–20}$ (Peptide 1) is MEPVDPRLEPWKHPGSQPKT; $Tat_{16–35}$ (Peptide 8) is SQPKTACTNCYCKKCCFHCQ; $Tat_{scramble}$ (Peptide 12, a scramble of peptide 8) is TCCQKNKCPTKHQCCFSAYC; $Tat_{31–50}$ (Peptide 13) is CFHCQVCFMTKALGISYGRK; $Tat_{46–65}$ (Peptide 19) is SYGRKKRRQRRRAHQDSQTH; $Tat_{61–80}$ (Peptide 25) is NSQTHQASLSKQPTSQSR GD; $Tat_{76–95}$ (Peptide 31) is QSRGDPTGPKESKKK VERET.

Cells and culture

Human monocytes were isolated from PBMC of healthy donors by adherence to plastic (i.e., they were incubated for 2 h in T125 flasks in RPMI 1640 medium containing 10% heat-inactivated AB serum). Fluorescence-activated cell sorter analysis demonstrated that more than 98% of the cells were $CD14^+$ monocytes of high purity. $CD4^+$ lymphocytes were purified by negative-selection on affinity columns (R & D systems, Minneapolis, MN). The purity of recovered cells was more than 90%, with no detectable crossing-contamination by $CD8^+$ cells. $CD4^+$ cells were incubated with 10% heat-inactivated FCS, 2 mmol/L L-glutamine, 100 U penicillin/ml, 100 g streptomycin/ml, and 50 IU interleukin-2/ml (RO 23-6019; Hoffmann-La Roche, Nutley, NJ).

Flow Cytometry

Monocytes were detached with 0.5 mmol/L EDTA/PBS and washed with PBS. 5×10^5 monocytes were treated with HIV Tat or Tat peptide, then FITC-conjugated anti-CD14 (a monocyte marker) and PE-conjugated anti-CCR5 were added for 30 min at 4 °C. Cells were fixed in 2% paraformaldehyde and analyzed by Flow Cytometry. Gated acquisition of monocytes (10000 events) was performed based on forward- and side-scatter parameters.

RESULTS

HIV Tat up-regulates CCR5 expression on monocytes

Human monocytes incubated with extracellular HIV Tat for 5 d express more cell-surface CCR5, as detected by Flow Cytometry, than monocytes incubated without Tat (Fig.1). In a dose-dependent manner, HIV Tat stimulation caused a gradual increase in CCR5 expression that reached maximal levels by day 5. One hundred nmol/ml was the most appropriate concentration, as indicated in Fig.2. To further determine the specificity of HIV Tat activity, we mixed polyclonal rabbit anti-Tat with HIV Tat for 30 min, then added the mixture to monocytes, and observed that expression of CCR5 was inhibited by the rabbit anti-Tat.

In order to exclude lipopolysacride (LPS) contamination, we also used boiled Tat (100 °C, 10 min) as a control, as indicated in Fig.3. Boiled Tat does not increase CCR5 expression in monocytes.

Cysteine rich peptide of Tat is responsible for increasing CCR5 expression on monocytes

In order to determine which portion of Tat was responsible for up-regulation of CCR5 we incubated monocytes with different peptides covering six overlapping regions of the Tat molecule. Only peptide 8, the cysteine rich peptide, is responsible for CCR5 induction, the other peptides do not up-regulate CCR5 expression (Fig.4).

DISCUSSION

In this study, we demonstrated that extracelluar Tat and Tat cysteine rich peptide 8 dramatically increase HIV co-receptor CCR5 expression in monocytes. Tat and Tat peptide 8 increase CCR5 expression in monocytes. Polyclonal rabbit anti-Tat inhibits this activity.

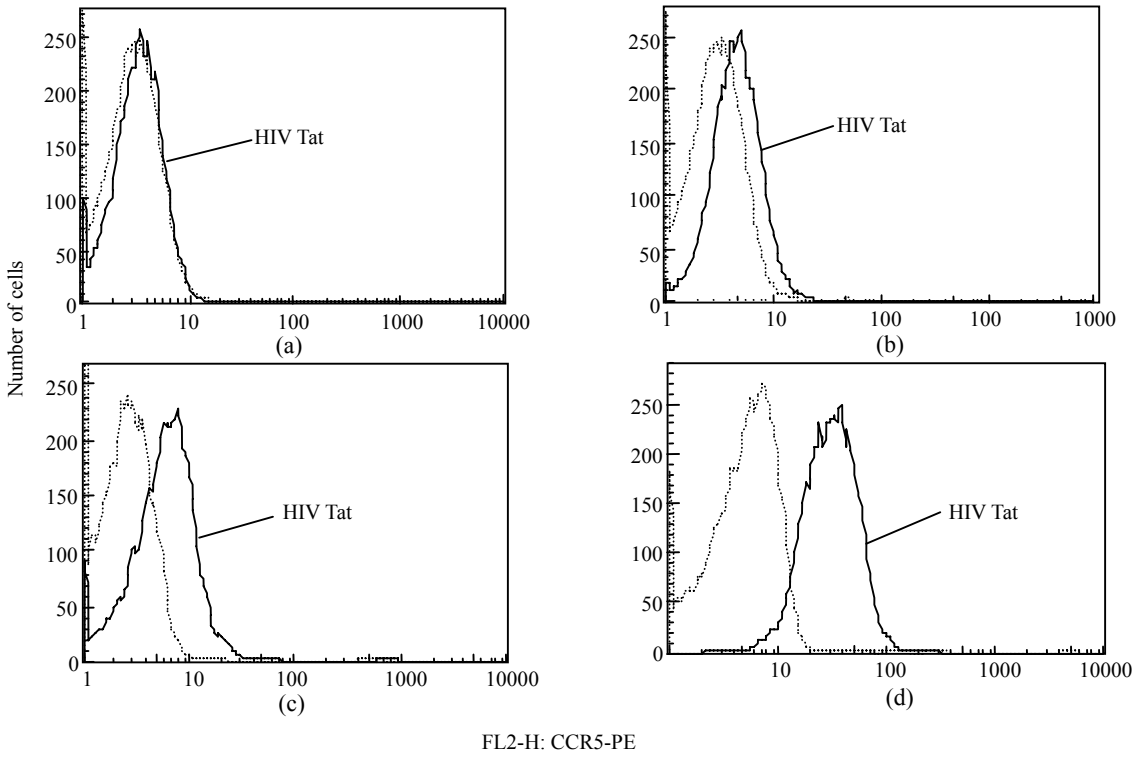

Fig.1 HIV Tat increase CCR5 expression in monocytes. One million monocytes were cultured with 100 nmol/L HIV Tat, at day 0 (a), 1 (b), 3 (c), 5 (d), collected cells and detected CCR5 expression in monocytes using Flow Cytometry analysis. Results shown are representative of three independent experiments. Dotted lines represent unstimulated control cells

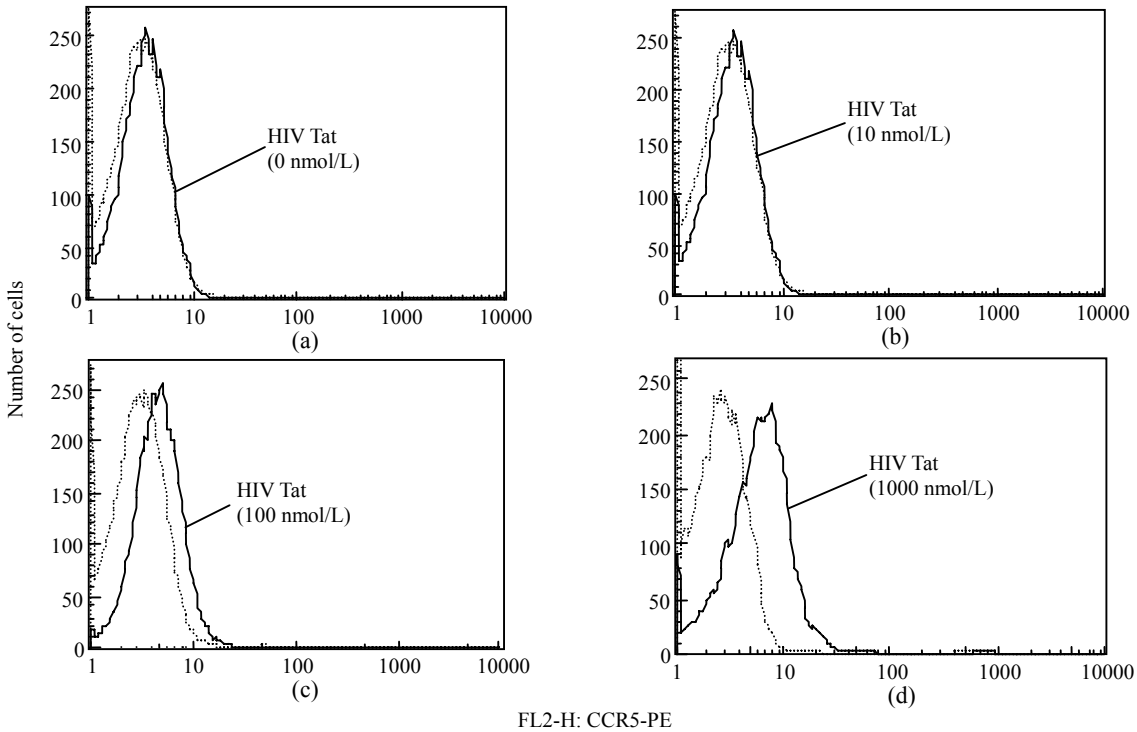

Fig.2 HIV Tat increases CCR5 expression in monocytes in dose dependent manner. One million monocytes were cultured with [10 nmol/L (b), 100 nmol/L (c), 1000 nmol/L (d)] or without Tat (a) for 5 d, collected cells and detected CCR5 using Flow Cytometry. Results shown are representative of three independent experiments. Dotted lines represent unstimulated control cells

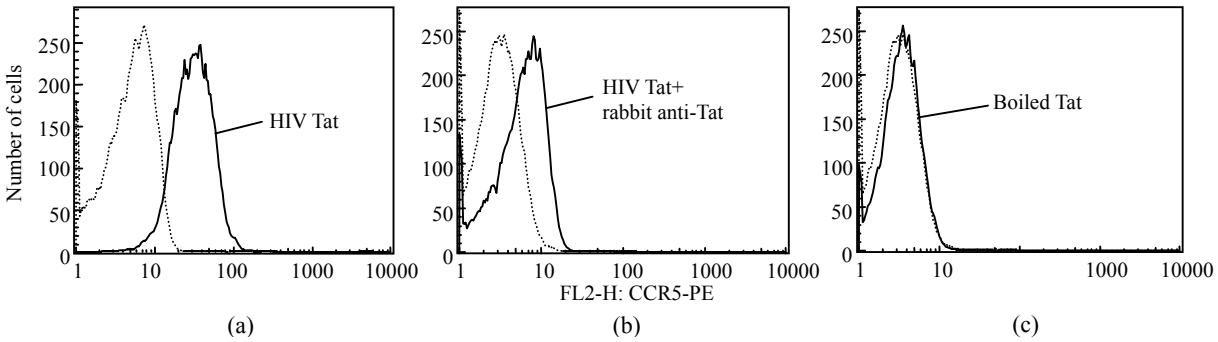

Fig.3 HIV Tat specifically increase CCR5 expression in monocytes. HIV Tat alone (a), HIV Tat+rabbit anti-Tat (b), boiled Tat (c) co-cultured with monocytes for 5 d, CCR5 expression was analyzed by Flow Cytometry. Results shown are representative of three independent experiments. Dotted lines represent unstimulated control cells

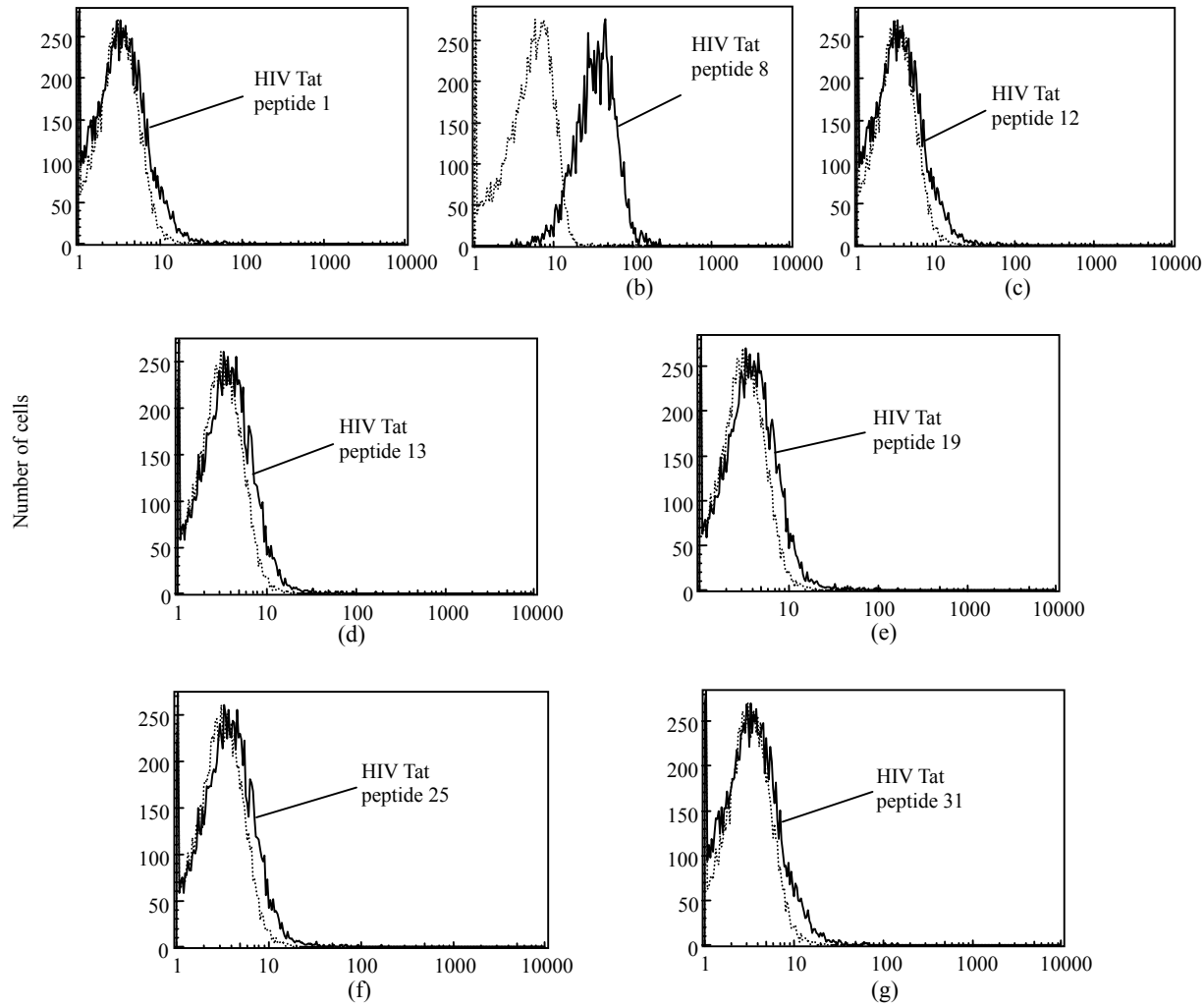

Fig.4 HIV Tat peptide 8 increase CCR5 expression in monocytes. Monocytes were cultured with Tat peptide 1 (a), 8 (b), 12 (c), 13 (d), 19 (e), 25 (f), 31 (g) as indicated in methods and materials, for 5 d and CCR5 expression was analyzed by Flow Cytometry. Results shown are representative of three independent experiment. Dotted lines represent unstimulated control cells

CCR5 is a key co-receptor in HIV infection, especially in macrophage tropic HIV infection. Our results indicated that Tat dramatically induced CCR5 expression in monocytes. These results are complementary to those reported by Boykins *et al*.(1999), who showed that HIV Tat and cyteine rich Tat peptide increase NF-kB and HIV-LTR transactivation in monocytes, corresponding to p24 levels in HIV infected monocytes.

Detectable levels of Tat had been reported in HIV-infected patients (Westendorp *et al*., 1995), which suggested the presence of extracellular HIV Tat protein in certain phases of HIV infection. It has also been shown that high levels of anti-Tat Abs are directly related to low viral load in seropositive non-progression patients. Therefore, a strategy targeting required sites in Tat might provide a new therapeutic modality to reduce disease progression in HIV-infected patients.

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