Published in final edited form as:
AIDS. 2004 May 21; 18(8): 1217–1218.

Alpha-defensins inhibit HIV infection of macrophages through upregulation of CC-chemokines

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The possible involvement of α-defensins in CD8 T-cell-mediated anti-HIV activities has been the subject of recent investigations [1–3]. HIV host defence mechanisms are partly mediated by CD8 T-cell non-cytotoxic antiviral responses [4]. Walker *et al*. [5] first demonstrated that this anti-HIV activity involves a soluble factor(s) designated as CD8 cell antiviral factor (CAF) whose identity remains unknown [4]. Zhang *et al*. [1] proposed that α-defensins are produced by CD8 T cells and contribute to CAF-mediated anti-HIV activities. In contrast, the recent studies by Mackewicz *et al*. [2] and Chang *et al*. [3] demonstrated that the α-defensins are not produced by CD8 T cells but unexpectedly were found to be expressed by monocytes [2].

As CAF-mediated anti-HIV activity is also observed for macrophages [6,7] and monocytes express α-defensins [2], we investigated the capacity of α-defensins to suppress HIV infection of macrophages. The addition of α-defensins to peripheral blood monocyte-derived macrophage cultures markedly suppressed HIV Bal replication (Fig. 1a) [8,9]. In order to determine the mechanism(s) responsible for α-defensin-mediated HIV inhibition in macrophages, we investigated whether α-defensins regulate the expression of CC-chemokines. CC-chemokines [macrophage inflammatory protein (MIP)-1α, MIP-1β and Rantes] inhibit infection by competing with HIV M-tropic strains for the CCR5 receptor on macrophages [10,11]. Our experiments demonstrated that α-defensins dramatically enhance expression (as much as a 25-fold increase) of MIP-1α and MIP-1β messenger RNA in macrophages (Fig. 1b) [12]. This increased CC-chemokine gene expression by α-defensins was further confirmed by the demonstration of increased production (as much as a 57-fold increase) of MIP-1α and MIP-1β proteins in α-defensin-treated macrophage cultures (Fig. 1c). In addition, the antibodies to CC-chemokines completely abrogated α-defensin-mediated HIV inhibition in macrophages (Fig. 1d). Our data, therefore, indicate that the α-defensin-mediated inhibition of HIV infection of macrophages is mediated through the upregulation of CC-chemokines. This pathway is distinct from the anti-HIV activity of CAF in macrophages, because CC-chemokines are not responsible for the ability of CAF to suppress HIV infection of these cells [6,7].

The biological interaction of defensins with chemokines and chemokine receptors has been documented. Defensins functionally overlap with chemokines in microbicidal activity [13]. The treatment of dendritic cells with β-defensin-2 upregulated the expression of CC-chemokines (MIP-1α and MIP-1β) and down-regulated CCR5 expression [14]. By utilizing

chemokine receptors on immune cells, defensins may contribute to the regulation of host adaptive immunity against microbial invasion [15]. Taken together, our data provide evidence that α-defensins could play a role in host defence against HIV infection of macrophages. The biological interaction of α-defensins with CC-chemokines may constitute a unique mechanism of innate immunity against HIV disease.

Acknowledgments

Sponsorship: This work was supported by grants from the National Institutes of Health (DA12815 and DA16022 to W.Z.H., MH49981 and AA13547 to S.D.D.).

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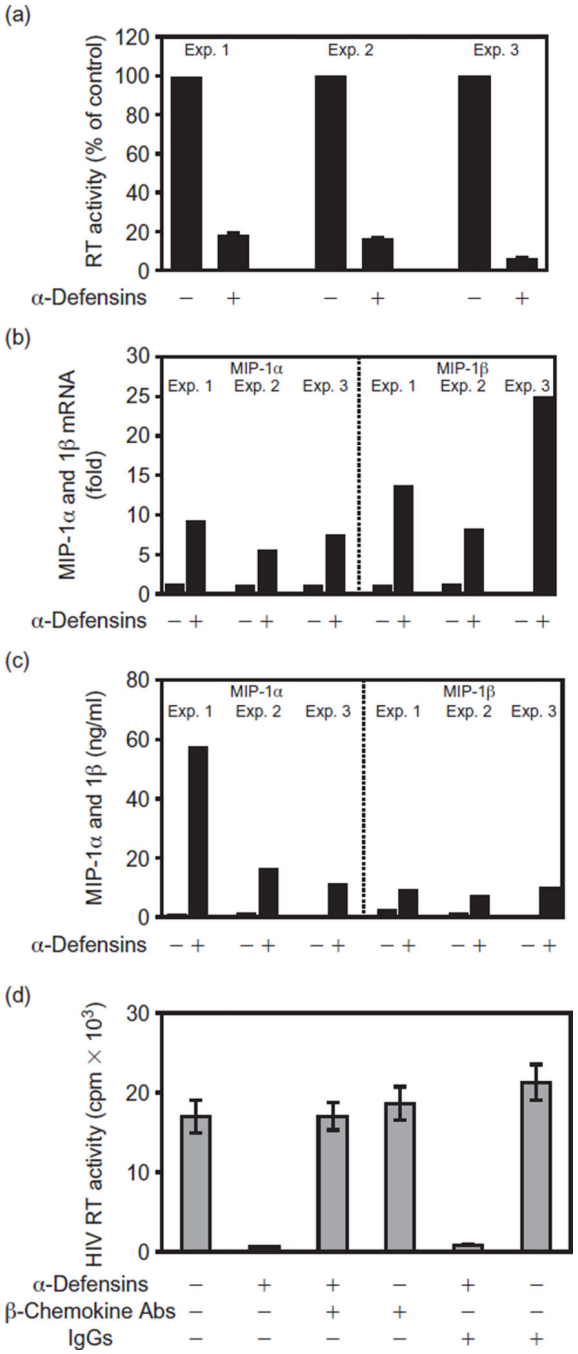

Fig. 1. Effect of α-defensins on HIV infection and β-chemokine expression in macrophages

Monocytes were purified from peripheral blood of three healthy HIV-negative adult donors according to our previously described techniques and were maintained as monocyte-derived macrophages [8]. Monocytes (> 98% purity) were plated in 48-well culture plates at a density of 5×10^5 cells/well in Dulbecco's modified essential medium containing 10% fetal calf serum. (a) Macrophages maintained for 7 days were preincubated with or without α-defensins (25 μM, hNP-1 and hNP-2; Chemi-Con International, Inc., Temecula, CA, USA) for 24 h and were then infected with HIV Bal strain. HIV replication in infected macrophage

cultures was analysed by measuring reverse transcriptase (RT) activity in culture supernatants [9] at day 8 post-infection and was expressed as a percentage of control (infected and untreated macrophage cultures), which was defined as 100%. (b) Macrophages were incubated with or without α-defensins (25 μM) for 3 h and total RNA iso-isolated from the cells was subjected to real-time reverse trancriptase–polymerase chain reaction [12] for quantification of macrophage inflammatory protein (MIP)-1α and MIP-1β messenger RNA. (c) Macrophages were incubated with or without α-defensins for 24 h, the culture supernatants were collected for CC-chemokine production using enzyme-linked immunosorbent assay kits (Endogen, Inc., Cambridge, MA, USA). (d) Macrophages were incubated with or without α-defensins or goat neutralizing polyclonal antibodies (25 μg/ml each) to human CC-chemokines (MIP-1α, MIP-1β and regulated upon activation: normal T cell expressed/secreted [Rants]; R&D Systems, Minneapolis, MN, USA) and goat IgG (control antibody; 75 μg/ml) for 24 h and were then infected with HIV Bal strain. HIV reverse transcriptase activity in the culture supernatants was measured at day 8 post-infection.