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CD40 Ligand Activity is Associated with Interleukin-16 and Modulated by Caspase-3

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Abstract

This investigation demonstrates that CD40 ligand (CD40L) effects on SIVmac replication involve active caspase-3 and interleukin-16 (IL-16). Caspase-3-specific-inhibition reverses the CD40L-induced SIVmac suppression. These data support our hypothesis that post-translational processing of proIL-16 to IL-16 is necessary for inhibition of SIVmac replication in rhesus macaque T-cells.

Keywords: SIV; Rhesus macaque; T cells; Interleukin-16

Introduction

Once thought to contribute to the ability of the elusive $CD8^+$ cell antiviral factor to control HIV and SIV replication, interleukin-16 [IL-16] has the ability to inhibit retroviral replication [1]. IL-16 and other T cell-derived soluble factors have the ability to inhibit retroviral replication in human and animal systems [2,3]. CD40 receptor, primarily expressed on B cells, activates caspase-3 [4]. Caspase-3 activation is critical to IL-16 bioactivity. We have previously reported that CD40L has IL-16-dependent antiviral effects [5]. Taken together, the study of the interaction of CD40 receptor, CD40L, caspase-3, and bioactive IL-16 was investigated to characterize CD40L's IL-16-related antiviral mechanism.

Materials and Methods

Animals and Cells

Peripheral Blood Mononuclear Cells (PBMCs) were obtained from the peripheral blood of four SIV negative rhesus macaques (*Macaca mulatta*) housed at the Yerkes Regional Primate Research Center, Emory University and cared for according to the guidelines of the American Association for the Accrediting of Laboratory Animal Care. Enriched CD4 populations were isolated with CD4-conjugated magnetic microbeads. Bead-isolated cell populations (typically 90-95% $CD4^+$ T cells by FACS) were detached from microbeads and cultured in complete media.

Cell culture and Viral Replication

$CD4^+$ T cells ($5x10^5$ cells) stimulated for 48 h with PHA (5 µg/ml), CD40L (50 µg/ml) and CD40L (50 µg/ml) + DEVD-fluoromethyl ketone (DEVD-fmk, 100 µM). Cultures were infected with SIVmac239 (0.01, multiplicity of infection). At 10 days' post infection, supernatant p27 concentrations were determined by antigen capture assay [3].

Caspase-3 Activity

To determine the levels of caspase-3 activation coincident with CD40L inhibition of viral replication, T-cell suspensions were pelleted, washed in excess PBS, and lysed in 0.1 ml sterile filtered lysis buffer [10 Tris-HCI (pH 7.5), 10 mM NaH_2PO_4/ $NaHPO_4$ (pH 7.5), 130 mM NaC1, 1% Triton®-X-100 NaPPi]. Cell lysate was added to reaction buffer [20 mM HEPES (pH 7.5), 10% glycerol, 2 mM DTT] containing 5 µg of the fluorogenic substrate N-Acetyl-Asp-Glu-Val-Asp AMC (Ac-DEVD-AMC) in a 96-well flat-bottom plate and incubated at 37 ^{0}C. Following 1 h incubation, the plates were read in a spectrofluorometric plate reader (excitation wavelength, 380 nm; emission wavelength, 460 nm). Caspase-3 activity was evaluated by and expressed as relative AMC fluorescence units (rfu).

Results

The effect of CD40L on SIVmac viral replication was further studied in PHA-activated (control) cultures produced SIV p27 antigen (7949±208 pg/ml) while, PHA+CD40L treated cultures

demonstrated the typical 70% viral antigen inhibition (2290±94 pg/ml). However, the addition of DEVD-fmk to PHA+CD40L-treated cultures significantly increased SIV antigen production (8058±54 pg/ml; $p<0.05$), Figure 1A, open bars). The basal caspase-3 activity (measuring the appearance of the fluorescent (AMC) cleavage product) of untreated cells (97±2 rfu) was significantly increased after activation with PHA/CD40L (596±192 rfu, Figure 1A, solid bars). Caspase-3 activity (<50 rfu) was not observed in DEVD-fmk treated cultures.

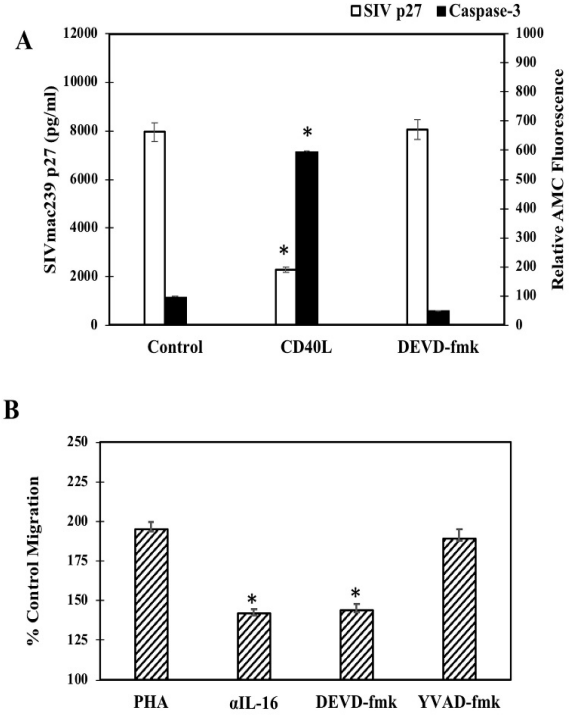

Figure 1: Viral inhibition and Caspase-3 Activation.

(A): CD4+ T cells were activated with PHA (control), PHA + CD40L (CD40L), or PHA + CD40L + DEVD-fmk (DEVD-fmk) for 2 days followed by SIVmac239 infection. Viral antigen production (open bars) is reported as mean+SEM. (A) Lysates prepared from rhesus CD4+ T cells treated with PHA (control), PHA + CD40L, or PHA + CD40L + DEVD-fmk (DEVD-fmk) were incubated with DEVD-AMC and liberated AMC was quantitated by fluorescence (solid bars). The asterisks designate values that are significantly different from control values ($p<0.05$). **(B):** T-cell chemotaxis in response to PHA-stimulated rhesus CD4+ T cell supernatants. Migration indices are represented for supernatants left untreated and neutralized with anti-IL-16 mAb. Caspase-related chemotaxis was demonstrated by pre-incubation of rhesus T cells with DEVD or YVAD. These values are compared to spontaneous migration of controls (represented by 100%). Values represent arithmetic means (±SD) from conditions run in triplicate.

To determine if CD40L stimulated release of bioactive IL-16, we assessed IL-16 dependent lymphocyte migration using a chemotaxis model [10]. No migration was observed in untreated cultures. However, supernatants from PHA-stimulated rhesus cultures induced 195±5% ($p<0.05$) of background migration (Figure 1B). IL-16 specific migration was partially neutralized by excess α-IL-16 mAb (clone 14.1) with only 142±3% migration observed. Caspase-3 inhibition also diminished migration (144±4%) migration was observed in supernatants from cultures pretreated with caspase-3 inhibitor (DEVD).

Discussion

Herein, we demonstrate that PHA/CD40L co-stimulation induces caspase-3 activation, produces bioactive IL-16 release as evidenced by chemotaxis and inhibits SIVmac replication. Further, we show evidence that caspase-3 inhibition reverses all of these observed effects. CD40L-mediated viral suppression mimics IL-16 inhibition of SIVmac239 and SIVmac251 [3]. Our results suggest that neither viral suppression nor increased caspase-3 activation following PHA/CD40L incubation is likely due to this level of CD40 receptor expression. We observe this response in the absence of CD40 receptor mRNA or cell expression (data not shown). These data suggest an unidentified signaling/mechanism for CD40L-associated cellular responses in $CD4^+$ T cells. Monomeric and multimeric forms of CD40L are present in plasma of HIV infected human subjects with differential effects in vivo [6].

The role for caspase-3 in the emergence of bioactive human IL-16 has been established [7], however, such a role has not been set forth in an animal model. We demonstrate caspase activation of bioactive IL-16. Although caspases play a critical role in mediating apoptosis in many different cell types, T cells maintain high levels of activated caspases without evidence of apoptosis [8]. Yaftian, et al. [9] report CD40 ligand induction of caspase-mediated apoptosis. Therefore, caspase-3 induction could have divergent effects that lead to apoptosis and/or to the activation of pro-IL-16.

Conclusion

This study corroborates previous findings with the involvement of caspase-3 activation in the observation of rhesus IL-16-specific bioactivity as determined by migration of human T-cells [10]. ß-chemokines are also released after PHA stimulation and residual migration following antibody neutralization and caspase-3 inhibition is likely due to other chemokines released upon stimulation [2]. It is also well established that ß-chemokines have no inhibitory effect on SIVmac239 or other T-tropic immunodeficiency viruses and therefore do not contribute to the observed antiviral effect in this experimental system [2].

Others have identified herpes simplex virus 1-related

antiviral activities for CD40L but only in stably CD40 receptor transfected cells [11]. To date, the correlation between CD40L-mediated caspase-3 activation and inhibition of SIVmac replication is unreported in the literature and suggests an IL-16-directed effect. Others [12] report an immunosuppressive effect by CD40L in HIV-infected T cells but not associated with viral load. Previous investigations of IL-16 protein secretion and mRNA expression as a result of CD40L incubation demonstrated no increases in secretion [5]. Concrete evidence of any intracellular biologic effects for IL-16 have not been demonstrated. IL-16-rich exosomes are released in HIV infected and viremic individuals [13], however, these membranous vesicles are associated with activating latently HIV infected cells not viral inhibition [14,15]. While the physiologic relevance of these activities in vivo remains unclear, these data clearly suggest that further investigation into caspase-3 activation and its role in modulating the virologic functions of IL-16 is warranted.

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