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Extracellular HIV-1 Nef protein modulates lytic activity and proliferation of human CD8$^+$ T lymphocytes

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Abstract

The effect of extracellular HIV Nef (*ex*Nef) protein on the induction of lytic activity and proliferation of CD8$^+$T lymphocytes from 18 donors was studied. At 10 ng/ml, *ex*Nef-induced a 2- to 8-fold enhancement of basal lytic activity in cells from all donors in an allogeneic induction assay, whereas it was ineffective at 100 ng/ml. The extent of enhancement was inversely correlated with the basal level of lytic activity without *ex*Nef. Only in combination with PHA did both *ex*Nef concentrations stimulate proliferation, and in a manner inversely related to the effect of PHA alone. Thus, concentrations of *ex*Nef commonly found in sera of HIV-infected patients were found to modulate the induction of lytic activity and proliferation of CD8$^+$ T lymphocytes *in vitro*, to an extent strongly dependent on the quite variable responsiveness of each donor. These findings point to Nef as a potential agent for modulating CD8$^+$ T cell function in pathogenesis and therapy.

Keywords: HIV Nef; CD8$^+$ T lymphocyte; Lytic activity; Proliferation

1. Introduction

Nef, a HIV-1 accessory protein (27 kDa) expressed in the cytoplasm and membrane of infected cells [1], has been involved in multiple processes that favor viral replication, immune evasion of infected cells, and alteration of immunological functions [2,3].

Nef can be released by infected cells and is found in cell-culture supernatants and in the serum of most HIV-1-infected patients at concentrations of 5–10 ng/ml [4–6]. The extracellular form of Nef (*ex*Nef) can promote a variety of effects: induction of proliferation [7], apoptosis of non-infected CD4$^+$ T lymphocytes [8], leukocyte recruitment into the central nervous system [9], inhibition of antibody secretion in B lymphocytes [10] and impairment of dendritic cells function [11].

CD8$^+$ T cells are critical for the containment of infected and tumor cells. Few studies have addressed the effect of *ex*Nef on the function of human CD8$^+$ T lymphocytes [12,13]. Herein, the effect of *ex*Nef on induction of lytic activity and proliferation of CD8$^+$ T lymphocytes from eighteen healthy donors was studied. Lytic activity was measured after alloreactive priming of CD8$^+$ T cells by mature dendritic cells (mDC). In later, parallel experiments, the proliferative capacity of purified, unprimed CD8$^+$ T cells after PHA-induced activation was determined in the presence or absence of *ex*Nef. In addition, the relationship between lytic activity and proliferation was explored. Results suggest that *ex*Nef may modulate the cytotoxic immune response during HIV-1 infection and may be of aid as a therapeutic agent in other pathologies involving CD8$^+$ T lymphocyte function.

2. Materials and methods

2.1. Culture medium and reagents

Human rIL-2, rIL-4, and rGM-CSF were purchased from Peprotech (Rocky Hill, NJ), LPS and PHA from

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Sigma (St. Louis, MO), and soluble recombinant HIV-1 IIIB Nef protein, from International Immuno-Diagnostics (Foster City, CA). The Nef protein stock was free of endotoxin (<0.01 U/ml) as determined by the *Limulus* amebocyte lysate test (Cambrex Bioscience, Walkersvilles, MD).

2.2. Design

Eighteen donors of $CD8^+$ T cells, 15 males and three females, and six male donors of monocytes, were randomly divided into six experimental groups, each comprised of three donors of $CD8^+$ T cells and one donor of monocytes. All were healthy individuals with ages ranging from 19 to 42 years. The $CD8^+$ T lymphocytes from each donor were split into two parts, one part was used in the induction of lytic activity and the other, in the proliferation assay.

2.3. Monocyte separation and differentiation into mature dendritic cells

Peripheral blood mononuclear cells (PBMC) were isolated from the buffy coat layers from blood of healthy donors, obtained from the Central Blood Bank at Centro Medico Nacional Siglo XXI, México D.F., by Ficoll-Hypaque (Cambrex Bioscience, Walkersvilles, MD) density centrifugation. Subsequently, $CD14^+$ cells were separated by positive selection by using magnetic cell sorting (MACS; Miltenyi Biotec, Bergish Gladbach, Germany) [14] and cultured at 10^6 cells/ml in polystyrene 6-well plates (Costar Corp., Cambridge, MA) in RPMI-10 (RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 μg/ml streptomycin and 100 U/ml penicillin) containing GM-CSF (1000 U/ml) and IL-4 (15 ng/ml). Every two days, half the medium was removed and renewed with fresh medium containing IL-4 and GM-CSF. At Day 4 of culture, half the cells were frozen at −70 °C until their use in a cytotoxicity assay. To induce mDC, the remaining cells were harvested at Day 6 of culture, and replated at 0.5×10^6 cells/ml in RPMI-10 containing GM-CSF and IL-4, now with added LPS (200 ng/ml), and incubated for an additional 24 h [14].

2.4. $CD8^+$ T cell separation and induction of CTL

$CD8^+$ T cells were separated from PBMC by using magnetic cell sorting (MACS; Miltenyi Biotec, Bergish Gladbach, Germany) [15]. Purity of the $CD8^+$ T cell suspension was greater than 98% by flow cytometry. $CD8^+$ T cells were co-cultured with allogeneic mDC in RPMI-10 at a co-culture ratio of 10:1. *ex*Nef at final concentration of 10 ng/ml or 100 ng/ml was added at the start of co-culture. Human rIL-2 (20 IU/ml) was added on Day 2. Co-cultures were maintained for five days at 37 °C and 5% CO_2 in a humidified atmosphere. CTL were then collected for use.

2.5. $CD8^+$ T cell proliferation assay

Proliferation assay was performed as described [15]. Briefly, purified $CD8^+$ T lymphocytes were adjusted to 1×10^6 cells/ml in RPMI-10 and 100 μL was transferred into the wells of 96-well plates. As stimulator cells, 100 μL of autologous, irradiated (3000 rad) PBMC at the same concentration was added to each well. Cells were activated by using 5 μg/ml of either PHA-L or PHA-L plus *ex*Nef at final concentration of 10 ng/ml or 100 ng/ml. After 72-h in co-culture, ^{3}H-thymidine (Amersham) was added. Cells were harvested 18 h later and the ^{3}H-thymidine incorporation was assessed by liquid scintillation spectroscopy in a Betaplate counter (Wallac, Turku, Finland). The effect of *ex*Nef was expressed as the *ex*Nef-stimulation index, calculated as follows: $cpm_{(exNef+PHA)}/cpm_{PHA}$.

2.6. ^{51}Cr-release cytotoxicity assay

One day prior to the cytotoxic assay, mDC were incubated with sterile sodium ^{51}chromate overnight (Amersham) in RPMI 1640 medium supplemented with 100 μg/ml streptomycin, 100 U/ml penicillin, and 30% heat-inactivated FBS (Patrizia Comoli, personal communication). Then, the mDC were washed three times with RPMI-10 and plated at 5×10^3 cells/well in the same medium. CTL were added, at an effector/target (E/T) ratio of 40:1, to round-bottomed plates and incubated at 37 ° C and 5% CO_2 in a humidified atmosphere. After 5–6 h in co-culture, ^{51}Cr release was measured in supernatants by using the Betaplate counter. The percentage of lytic activity was calculated as follows: ((experimental release cpm − spontaneous release cpm)/(total release cpm − spontaneous release cpm)) × 100. Changes in values of lytic activity related to *ex*Nef 10 ng/ml were expressed as *ex*Nef-enhancing index, as follows: *ex*Nef 10 ng/ml lytic activity/basal lytic activity.

2.7. Statistical analysis

Statistical analysis was performed by using the Graphpad Prism 3.03 (Graphpad Software, San Diego, CA, USA). Analysis of variance (ANOVA) was used for the between-groups comparisons of mean lytic activity. Tukey tests were used to compare the between-individuals means. Non-linear regression analysis was used to examine the association between the cpm obtained from PHA-stimulated cells and basal lytic activity, and also between basal lytic activity from non-treated controls and *ex*Nef-enhancing index obtained from *ex*Nef-treated cells.

Schwar's Bayesian clustering criterion was used to perform a two-step cluster analysis on the parameter *P* (the product of the basal lytic activity and proliferation values) from all donors by using the SPSS for Windows, release 10.1.3 (SPSS, Chicago, IL). A *t*-test was applied to the two clusters derived from this analysis.