

Effects On Human B Lymphocytes of HIV-1-associated Host CD40 Ligand

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ABSTRACT

Background: Several B-cell abnormalities are seen following infection with HIV-1. However, the exact mechanisms of B-cell dysfunction during HIV-1 infection are poorly understood. Having recently shown that CD40L, the ligand for CD40, is inserted within budding HIV-1 particles, we hypothesized that the contact between virus-anchored host CD40L and CD40 on the surface of B lymphocytes might result in activation of this cell type. Moreover, we wondered whether this additional interaction between HIV-1 and B cells might facilitate an eventual virus transfer to more susceptible target cells such as autologous CD4+ T lymphocytes.

Methods: Autologous B cells and CD4+ T lymphocytes were isolated from human tonsils. Isogenic virions either lacking (NL4.3/null) or bearing CD40L (NL4.3/CD40L) were produced by transient transfection of 293T cells. Virus preparations were incubated first with purified B cells before performing virus binding, electrophoretic mobility shift and transmission assays.

Results: Attachment of NL4.3/CD40L virions to B lymphocytes was augmented compared to isogenic NL4.3/null viruses. Moreover, NL4.3 particles bearing CD40L, but not viruses lacking host CD40L, induced the nuclear translocation of NF- κ B in B lymphocytes. The presence of host-derived CD40L within HIV-1 resulted in an enhanced infection of CD4+ T cells *in trans* upon co-culture with B lymphocytes.

Conclusions: Altogether the data gathered from this series of investigations suggest that incorporation of host-encoded CD40L in HIV-1 might play a role in the described B-cell abnormalities seen in infected individuals.

INTRODUCTION

B lymphocytes abnormalities are commonly observed in HIV-associated infection. They appear before dysregulation of the T CD4+ lymphocytes pool (Miedema *et al.* 1988; Terpstra *et al.* 1989), are associated to high viremia (Moir *et al.* 2001) and are reflected by lymphomas, polyclonal activity and hypergammaglobulinemia (Lane *et al.* 1983; Przybylski *et al.* 1996; Ng and McGrath 1998; Gaidano *et al.* 1998). Because NF- κ B is translocated to the nucleus upon ligation of CD40 in B cell lymphomas (Pham *et al.* 2002) and because budding HIV-1 particles incorporate CD40L (Martin and Tremblay 2004), we wondered if the ligation of CD40 on the surface of B lymphocytes by host-derived CD40L carried by HIV-1 particles could lead to such activation of B cells. Moreover, as B lymphocytes are in continuous circulation between the periphery and lymphoid tissues, we hypothesized that HIV-1 particles linked to B lymphocytes by a CD40/CD40L interaction could be transmitted to HIV-1-targeted types of cells such as CD4+ T lymphocytes.

CONCLUSION

The acquisition of the host molecule CD40L by HIV-1 leads to a better attachment of viral particles to B lymphocytes, activation of these cells by NF- κ B and transfer to T CD4+ lymphocytes which become infected.

RESULTS

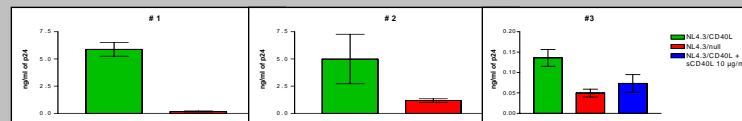


Figure 1. Compared to NL4.3/null, NL4.3/CD40L virions bind more to B lymphocytes from human tonsils and this interaction is specific. 293T cells were either transfected with pNL4.3 (NL4.3/null) or cotransfected with pNL4.3 and pcDNA3.1CD40L expression vector (NL4.3/CD40L). B lymphocytes isolated from tonsillar tissues and HIV-1 preparations were incubated (350 ng/3.5 x 10⁶ cells) with or without sCD40L (10 μ g/ml) for 1 h at 37°C, washed, lysed and tested for the p24 content. The data shown represent the mean \pm standard deviations of triplicate samples in three independent experiments (#1, #2, and #3) performed with tonsils from different donors and various HIV-1 stocks.

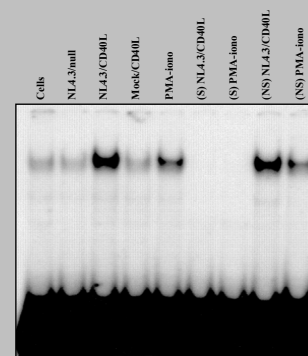


Figure 2. Activation-induced nuclear translocation of NF- κ B by NL4.3/CD40L virions, but not NL4.3/null, in B lymphocytes from human tonsils.

Cells were either left untreated or were treated with NL4.3/null (1050 ng/3.5 x 10⁶ cells), NL4.3/CD40L (1050 ng/3.5 x 10⁶ cells), Mock/CD40L or PMA-ino for 30 min at 37°C. Nuclear extracts were then incubated with an NF- κ B-labeled probe and analyzed by electrophoretic mobility shift assay on a 4% native polyacrylamide gel. Specific (S) and nonspecific (NS) competitions were performed. The data shown are representative of three independent experiments performed with tonsils from different donors and various HIV-1 stocks. Stimulation of 1 h, ratios of 350 ng/3.5 x 10⁶ and 700 ng/3.5 x 10⁶ cells were also tested and gave similar results (data not shown). An SP1-labeled probe has also been used to test unspecific binding of nuclear content to the probe with no signal detected (data not shown).

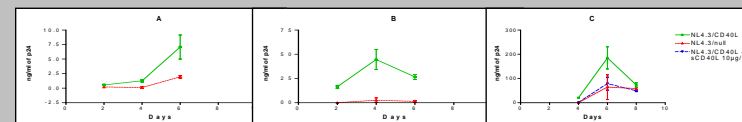


Figure 3. Compared to NL4.3/null, NL4.3/CD40L virions interacted specifically with CD40 on B lymphocytes from human tonsils and enhanced infection of autologous T CD4+ lymphocytes *in trans*. B lymphocytes and HIV-1 preparations were incubated (10 ng/1.5 x 10⁵ cells) with or without sCD40L (10 μ g/ml) for 1 h at 37°C, washed and then cocultured with autologous PHA-IL-2-stimulated T CD4+ lymphocytes (1 x 10⁵). The supernatants were harvested at day 2, 4, 6 or 8 post-infection to measure the p24 content. The data shown represent the mean \pm standard deviations of triplicate samples in three independent experiments (A, B, and C) performed with tonsils from different donors and various HIV-1 stocks.



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