CIB1 and CIB2 are HIV-1 helper factors and their modulation influences envelope-mediated viral entry

Ana Godinho-Santos[1,2], Allan J. Hance[2,3], João Gonçalves[1] and Fabrizio Mammano[2,3]

1 – iMed.ULisboa, Faculty of Pharmacy, University of Lisbon, Portugal;
2 – INSERM, U941, Paris, F-75010, France; 3 – Univ Paris Diderot, Sorbonne Paris Cité, F-75475, Paris, France

ABSTRACT

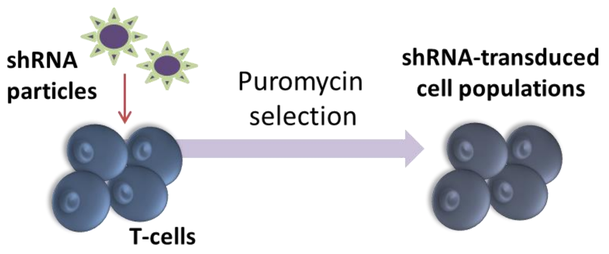
Human Immunodeficiency Virus type 1 (HIV-1) relies on the host cell machinery to complete its replication cycle. Several HIV-1 helper factors have been identified, contributing to a better understanding of host-HIV interactions and providing potential novel antiviral targets. As the characterization for many of these proteins is still limited, we aimed to depict the contribution of CIB2, previously identified in two screening for helper factors, as well as the potential contribution of its homolog, CIB1. Knockdown of either CIB1 or CIB2 in shRNA-transduced cell populations strongly impaired viral replication in target cells, recognizing these proteins as non-redundant HIV-1 helper factors. Also, a single-cycle assay demonstrated that normal levels of CIB1 and CIB2 are required for HIV-1 envelope-mediated entry process. In fact, the infectivity of both X4 and R5 viral strains was significantly reduced upon reduction of the espression of CIB1 and CIB2. Moreover, CIB1- and CIB2-knockdown populations displayed reduced viral fusion efficiency when compared to untransduced population, showing cell-free virus entry impairment. Furthermore, virus-transfer through cell-cell contacts was decreased after co-culture of infected donor cells with CIB1- and CIB2-knockdown target cells. Flow cytometry showed that surface expression of some key players of both routes of viral entry was altered in CIBs-depleted populations, namely co-receptor CXCR4 and integrin α4β7. Taken together, these studies revealed for the first time that CIB1 is a HIV-1 helper factor along with CIB2, and suggest that both CIB1 and CIB2 facilitate HIV-1 entry in natural target cells possibly through modulation of CXCR4 and α4β7.

BACKGROUND

Although numerous studies have explored HIV-1 helper factors, the characterization of the identified putative co-factors is still scarce. For this reason, we aimed to characterize the contribution of a previously identified helper factor, CIB2 (Rato et al., 2010). As a complement to this characterization, we aimed to assess whether CIB1, a member of the protein family of CIB2, is also required for efficient HIV replication. Since CIB1 and CIB2 interact with integrins and both are involved in the regulation of calcium signaling, we inquired if these proteins participated in virus entry. There are two main pathways of HIV infection of target cells: cell-free entry and direct cell-cell spread (Martin and Sattentau 2009).

EXPERIMENTAL DESIGN

Primary CD4+ T cells were transduced with lentiviral-based vectors that express RNA-inducing shRNAs for each of gene and challenged gradually with increasing concentrations of puromycin until 2 µg/mL. The shRNA-transduced cell populations were expanded and cultured in medium supplemented with puromycin and analyzed for their knockdown efficiency and cellular viability before performing the following assays.



CONCLUSIONS

CIB2 was confirmed to be helper factor in **primary CD4+ T cells.**

Identification of **CIB1 as HIV-1 helper factor** in primary cells.

Normal levels of CIB1 and CIB2 are required for HIV-1 entry by both X4- and R5-Env-pseudotyped particles.

CIB1 and CIB2 facilitate **HIV-1 entry** in natural target cells – both by cell-free and cell-to-cell transmission.

Surface expression of **co-receptor CXCR4** and **integrin α4β7** was altered in CIBs-depleted populations.

This work brings new insights for the complex interaction between HIV and its cellular host

ACKNOWLEDGEMENTS

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RESULTS

CIBs knockdown affects HIV-1 replication in primary CD4+ T cells

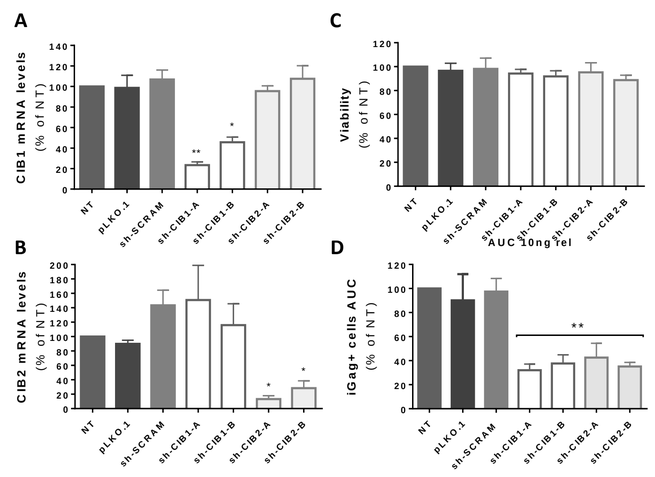

Figure 1. Panels A and B. Total RNA was extracted and cDNA was synthesized for quantification by real-time PCR mRNA levels for CIB1 (A) or CIB2 (B) are relative to non-transduced (NT) primary CD4+ T cells after normalization to GAPDH. **C.** Viability of shRNA populations measured by MTT after cell expansion. **Panels D and E.** Viral replication was measured by percentage of intracellular Gag positive cells stained with anti-Gag-PE by flow cytometry on days 2 and 3 after infection with HIV-1_{NL43}. Replication kinetics of HIV-1 in the different populations is shown in panel E, and in the panel D is represented virus infectivity based on the area under the curve (AUC) from replication kinetics. Mean ± SEM of 4 or more independent transductions.*P<0.05, **P<0.01 versus shSCRAM (Mann Whitney test).

CIBs depletion in primary CD4+ T cells affects HIV in an Env-specific way

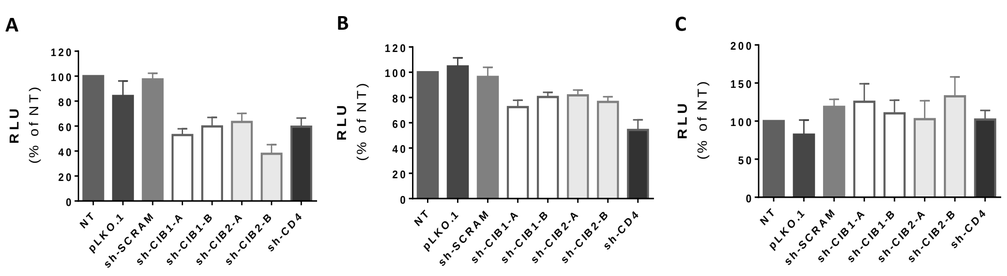

Figure 2. shRNA cell populations were challenged with HIV-1 particles carrying the luciferase gene in place of *nef* and pseudotyped with X4-HIV-1 (A) or R5-HIV-1 envelopes (B) or VSVg envelope (C), and 48h later the luciferase activity in target cells was measured. Values represent mean ± SEM of 5 or more independent transductions and are relative to non-transduced (NT) cells. *P<0.05, **P<0.01, ***P<0.005 versus shSCRAM (Mann Whitney test).

CIBs knockdown in primary CD4+ T cells influences cell-free virus entry

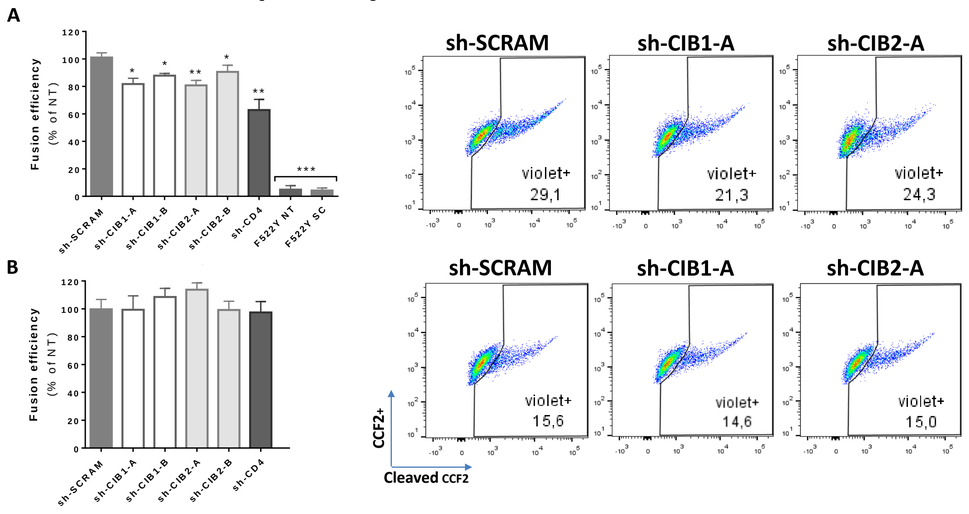

Figure 3. shRNA cell populations were exposed to HIV-1 particles (200 ng $p24^{CA}$) carrying the BlaM-Vpr fusion protein and pseudotyped with HIV-1 (A) or VSVg (B) envelopes, and cells were incubated with CCF2 substrate after 4 hours of challenge (Cavrois, De Noronha, Greene, 2002). Fusion efficiency was measured by flow cytometry using a violet laser for excitation of cleaved CCF2. Values represent mean ± SEM of 5 independent transductions and are relative to non-transduced cells (NT). *P<0.05, **P<0.01, ***P<0.005 versus shSCRAM (Mann Whitney test).

Cell-cell transfer is modulated in shRNA-transduced cell populations

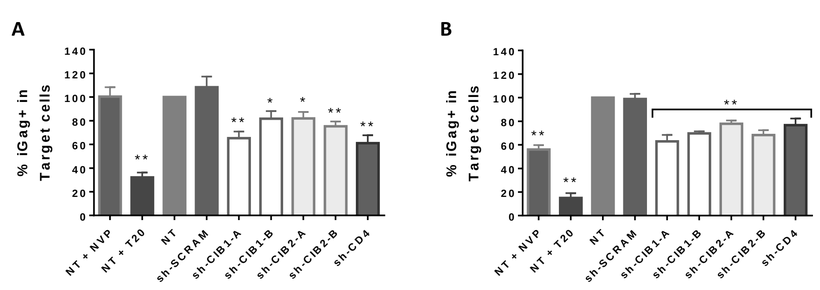

Figure 4. Cell-to-cell viral transfer was measured by a flow cytometry-based assay (Sourisseau *et al*., 2007). Non-transduced primary CD4+ T lymphocytes were infected with HIV-1NL43 and used as donor cells. Donor cells were labeled with CFSE dye to allow distinguishing them from target cells. shRNA target cell populations were then cocultured with CFSE-labeled donor cells. The appearance of Gag+ target cells was measured after 4h (A) or 24h (B). Values represent mean ± SEM of 3 independent transductions.. .*P<0.05, **P<0.01 versus shSCRAM (Mann Whitney test).

Characterization of the surface expression level of relevant cellular factors in sh-CIBs populations

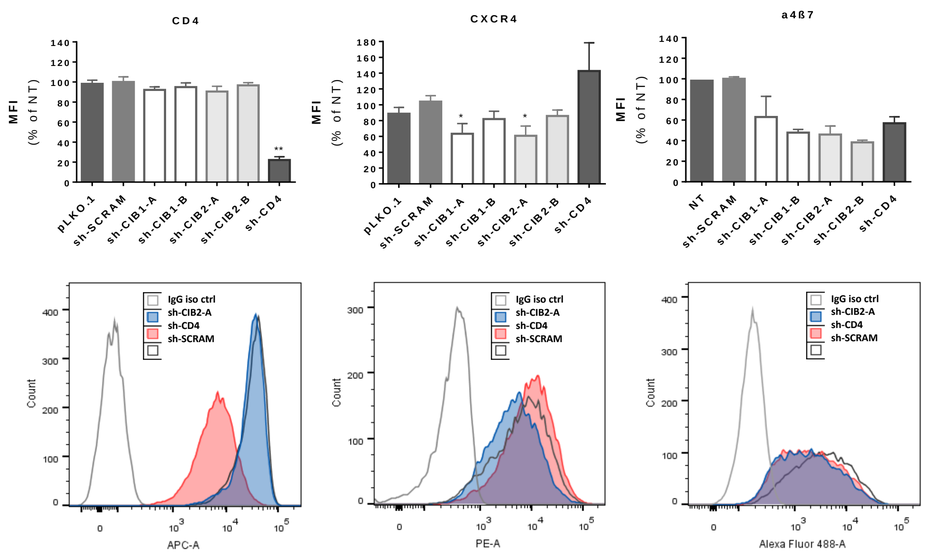

Figure 5. Surface expression levels of key players involved in HIV-1 entry was measured by flow cytometry after staining shRNA cell populations with different antibodies against CD4 (n=4), CXCR4 (n=4) and α4β7 (n=2). Values represent mean ± SD of 2 or 4 independent transductions and are relative to NT cells. .*P<0.05, **P<0.01 versus shSCRAM (Kruskal-Wallis test).