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Running title: IgG opsonization impedes HIV-CTL priming

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Abstract

Background: Control of HIV is suggested to depend on potent effector functions of the virus-specific $CD8^+$ T cell response. Antigen opsonization can modulate the capture of antigen, its presentation and the priming of specific $CD8^+$ T cell responses.

Objective: We have previously shown, that opsonization of retroviruses acts as endogenous adjuvant for DC-mediated induction of specific CTLs. However, in some HIV-positive individuals, high levels of antibodies and low levels of complement fragments coat the HIV surface.

Methods: Therefore, we analyzed the impact of IgG-opsonization on the antigen-presenting capacity of DC by $CD8^+$ T cell proliferation assays following repeated prime-boosting, by measuring the antiviral activity against HIV-infected autologous $CD4^+$ T cells, and by IFN-γ secretion from HIV-specific CTL clones.

Results: We find that DC exposed to IgG-opsonized HIV significantly decreased the HIV-specific $CD8^+$ T cell response compared to the earlier described efficient $CD8^+$ T cell activation induced by DC loaded with complement-opsonized HIV. DC exposed to HIV bearing high surface IgG levels, following incubation in plasma from HIV-infected individuals, acted as weak stimulators for HIV-specific CTL clones. In contrast, HIV opsonized with plasma from patients exhibiting high C and low IgG deposition on the viral surface favored significantly higher activation of HIV-specific $CD8^+$ T cell clones.

Conclusion: Our *ex vivo* and *in vitro* observations provide the first evidence that IgG-opsonization of HIV is associated with a decreased CTL-stimulatory capacity of DC.

Key Messages

- Plasma from HIV positive individuals with high complement activity mediates high CTL-induction.
- First *ex vivo* and *in vitro* evidence for decreased CTL-stimulatory capacity of DC exposed to Ab HIV.
- Implication that strong Ab response after vaccination contribute to weaken CTL by DC modulation.

Capsule Summary

Our results are relevant regarding HIV vaccination strategies as they suggest that strong, transient Ab responses after vaccination might contribute to weaken CTL-induction by modulation of DC function as observed in our study.

Keywords: HIV, IgG, opsonization, DC, CTLs

Abbreviations:

HIV - human immunodeficiency virus

DC – dendritic cell, iDC – immature dendritic cell, mDC – mature dendritic cell

CTL – cytotoxic T lymphocyte

IgG – immunoglobulin G

(n)Ab – (neutralizing) antibodies

C – complement

SEB – staphylococcal enterotoxin B

Introduction

Appearance of HIV-specific CTLs correlates with declining viremia during the acute phase of infection. During the chronic phase of infection, HIV-CTL are exhausted and are not able to efficiently control infection [1-5]. However, some individuals who commonly have viral loads below standard detection levels without undergoing antiretroviral therapy (HIV controllers), possess efficient $CD8^+$ T cells able to proliferate, produce multiple cytokines and upregulate cytolytic molecules in response to antigen stimulation and endowed with a strong capacity to eliminate infected $CD4^+$ T cells [6-10].

HIV-1 initiates immediate responses of the immune system upon crossing mucosal surfaces. There, the complement (C) system constitutes a first line of defense against the virus. Recently, we illustrated an important role for C-opsonization of retroviruses as an endogenous adjuvant for DC-mediated CTL-induction [11]. The first antibody responses to HIV-1 envelope glycoproteins gp41 and gp120 appear in plasma two weeks and four weeks post infection, respectively. These antibodies were demonstrated to have only modest impact on viral load [12-14]. Nevertheless, HIV is opsonized by antibodies and complement fragments after appearance of the first antibodies. After months of infection, neutralizing antibodies (nAbs) appear, becoming increasingly potent in some individuals to develop broadly neutralizing capacity [15, 16]. Whether HIV-1 nAbs confer viral control in HIV long term non-progressors (LTNP) or elite HIV controllers is not clear [17-19]. Independent of neutralizing capacity, binding of specific antibodies to the HIV surface increases and results in either amplification of C activation and deposition on the HIV surface (HIV-C), or enhanced interaction of HIV-bound antibodies (HIV-Ig) with Fc receptors expressed on hematopoetic cells. Immune complexes containing infectious viral particles have been identified in blood and on lymphoid follicles of HIV-positive individuals [20, 21]. Previously, we have shown that a differential opsonization pattern results in differential outcomes in DC infection, viral

integration and long-term transmission to T cells [22, 23]. Moreover, upon retroviral infections, complement opsonization seems to favor effective CTL priming by DC [11]. Thus, the opsonization pattern of HIV-1 does not merely result in differential DC infection and integration, but it also has profound consequences on the antigen-presenting capacity of DC *in vitro* and *in vivo.*

Here we sought to understand whether DC exposed to IgG-opsonized HIV are comparable in CTL-stimulatory capacity to HIV-C-DC. Using *in vitro* priming experiments, we find that DC exposed to IgG-opsonized HIV significantly decreased the HIV-specific $CD8^+$ T cell response compared to the efficient HIV-C-DC-induced $CD8^+$ T cell activation described earlier [11]. DC exposed to HIV bearing high surface IgG levels, following incubation in plasma from HIV-infected individuals, acted as weak stimulators for HIV-specific CTL clones. In contrast, HIV opsonized with plasma from patients exhibiting high C and low IgG deposition on the viral surface favored significantly higher activation of HIV-specific $CD8^+$ T cell clones. Our *ex vivo* and *in vitro* observations provide the first evidence that IgG-opsonization of HIV is associated with a decreased CTL-stimulatory capacity of DC.

Methods

Samples

Plasma samples were obtained from 35 HIV infected individuals recruited among patients followed-up in CHU St Louis, Hopital Europeen Georges Pompidou and CHU de Bicetre in France. All the subjects gave their informed consent to participate in the study. The Ethics review committee CPP (Comité de protection des personnes) Ile de France VII and the Clinic Research Committee of Institut Pasteur approved the studies performed.

A written informed consent was also obtained from the participating blood donors by the Central Institute for Blood Transfusion & Immunological Department, Innsbruck, Austria, to isolate monocytes and naive $CD4^+$ and $CD8^+$ T cells from the blood packs.

Generation of primary human monocyte-derived DCs and isolation of human $CD4^+$ and $CD8^+$ T cells

Monocytes were isolated from blood of normal healthy donors by using human CD14 MicroBeads (Miltenyi Biotec), according to the manufacturer's instructions. DCs were generated and analyzed as described [11, 23]. Subsequently, $CD4^+$ (>95% purity) and $CD8^+$ (>94% purity) T cells were bead-purified and used with autologous DC's for the *in vitro* experiments.

Opsonization of HIV-1

Purified R5-tropic HIV (BaL, 92UG037) was incubated for 1 hr at 37°C with normal human serum (NHS) as complement (C) source (HIV-C) or commercially available human C serum (Quidel) (HIV-C (human C serum, Quidel)) in a 1:10 dilution, with IgGs (50 μg/ml; Centre

for AIDS Reagents) to obtain IgG-opsonized virus (HIV-Ig), or a combination of both (HIV-CIg). As negative control the virus was incubated under the same conditions in medium or heat-inactivated serum (HIV). For experiments using plasma samples from HIV infected individuals, HIV (BaL and 92UG037, concentration >1 μg/ml) was incubated under above mentioned conditions using the plasma in a 1:10 dilution. Subsequent to opsonization, the virus was washed, pelleted by ultracentrifugation (14000 rpm/90 min/4°C) and re-suspended in 100 μl RPMI medium without supplements. The opsonization pattern was determined by virus capture assay (VCA) as described [23] using anti-human C3c/C3d-, IgG- or mouse IgG Abs as control for background binding. The coated VCA plates were incubated overnight with the differentially opsonized virus preparations (2.5 ng p24/well) at 4°C and washed 5 times using RPMI medium to remove unbound virus. Bound virus was lysed (2% Igepal) and transferred to a pre-coated p24 ELISA plate [24].

Prime-boost experiments

The *in vitro* generated $CD8^+$ T cells were analyzed for expansion by FACS analyses, and functionality (antiviral assay) as described [11], but detailed description of the *in vitro* $CD8^+$ T cell generation and antiviral activity testing is additionally provided below.

In vitro generation of HIV-specific $CD8^+$ T cells

Day 5 iDC were stimulated with a cytokine-cocktail (IL-1β, IL-6, PGE_2, TNF-α, IL-4, GM-CSF) for 24 hrs and then 10^4 cells/100 μl were transferred into 96-well plates. DC from all donors were loaded with 25 ng p24/ml non- (HIV), complement- (HIV-C), complement plus antibody- (HIV-CIg) or antibody-opsonized (HIV-Ig) HIV strains (R5-, R5X4- and X4-tropic) for 3 hrs. DC were exposed to 1 μg/ml of the superantigen Staphylococcal Enterotoxin B

(SEB, Sigma) for the same time-period as a positive control for T cell proliferation. As additional controls iDC and/or cytokine cocktail-stimulated DCs (ccDC) were included in the experimental settings. All tests were performed in triplicates. The cells were thoroughly washed and non-stimulated, naïve $CD8^+$ T cells were added to the primed DC at a ratio 3:1. 25 U/ml IL-2 was added after 4 days and after another 4 days the T cells were again boosted with loaded DCs. This procedure was repeated 3 times and the $CD8^+$ cells were analyzed for expansion, IFN-γ secretion, and functionality. HIV-induced proliferation of T cells was analyzed by FACS. Proliferated T cells were expanded with the CD3/CD28 T cell expander (Dynal) according to the manufacturer after the third priming with loaded DCs to perform tests for the antiviral activity of the *in vitro* generated HIV-specific CTLs.

Anti-HIV-activity of in vitro generated cytotoxic T cells

To further determine the functionality of the *in vitro* generated HIV-specific CTLs, frozen autologous $CD4^+$ T cells were thawed, washed, and stimulated with CD3/CD28 beads (Dynal) in presence of IL-2 (100 U/ml). Pre-stimulation of the $CD4^+$ T cells with CD3/CD28 was necessary to induce the up-regulation of CCR5. The *in vitro* primed and expanded CTLs were added to over night HIV-incubated $CD4^+$ T cells. A reduction in virus production assessed by p24 ELISA on several days post addition of the expanded CTLs indicated an indirect confirmation of their functionality. By using this assay we were in addition able to circumvent the problem of the unknown HLA-type of the majority of the donors. This indirect confirmation of the functionality was performed with expanded CTLs from seven donors primed and boosted with HIV-exposed DCs. Both methods (*in vitro* generation and antiviral assay) were performed exactly as described in Banki et al., 2010 [11].

HIV-specific CTLs and Elispot analyses

SL9 clone 2, an HIV-specific $CD8^+$ CTL clone, was derived from an HIV-infected patient and recognizes the well-characterized immunodominant epitope of Gag p17 SLYNTVATL (SL9) presented by HLA-A*020 [25, 26]. Loaded DC were co-cultured overnight with $CD8^+$ T cell clones (ranging from 2500 to 10000 SL9 clone 2/well). As positive controls, stimulators (i.e. DC) were incubated with 1 μg/ml of cognate peptide (SLYNTVATL, SL9) before washing and addition of the HIV-specific CTL clones overnight. The next day, IFN-□ production was monitored in an Elispot assay as described [25]. All Abs used for the IFN-□□Elispot were from Mabtech.

Immunofluorescence and confocal microscopy

For intracellular HIV localization iDC were exposed to differentially opsonized HIV (250 ng of p24/2.5 × 10^5 cells) overnight. After washing, cells were left to adhere on glass coverslips for 1 h at 37°C. Cells were then fixed and permeabilized 20 min at 4°C in BD Cytofix/Cytoperm (BD Biosciences) and washed with BD Perm/Wash™ buffer containing human IgG (20 μg/condition). Triple labeling of cells was done as follows: iDC pulsed with HIV-1 were stained with primary Abs against HLA-ABC (BD Biosciences), CD63 (monoclonal (1B5)), and LAMP-1 (polyclonal Ab). After extensive washing, cells were then stained with secondary donkey anti-mouse and anti-rabbit Abs coupled to rhodamine (Jackson ImmunoResearch Laboratories). To avoid unspecific labeling, cells were incubated 20 min at room temperature in PBS containing BSA, saponin, and mouse serum (0.5 mg/ml). Finally, HIV-1-$p24^{gag}$ was detected using a monoclonal anti-HIV-1-$p24^{gag}$ (KC57) coupled to FITC (Beckman-Coulter). Confocal laser scanning microscopy was performed with an LSM 510 microscope (Zeiss). Images were then processed using Photoshop. Quantifications of

colocalization were performed using the Metamorph software (Universal Imaging) on an average of 25–30 cells for each condition as previously described [27].

Statistical analysis

Differences were analyzed by the GraphPad prism software using the unpaired student's t-test (2-tailed) for comparisons between two groups. For multiparameter comparisons, values from the experiments were logarithmically transformed and analysed using ANOVA and Bonferroni´s multiple comparison test for selected groups.

Results

IgG-opsonization of HIV interferes with DC-mediated CD8$^+$ T cell proliferation.

To characterize the IgG-mediated effect on the CD8$^+$-stimulatory capacity of HIV-loaded DC, we differentially opsonized live virus using a commercially available IgG pool (HIV-Ig [Centre for AIDS reagents]) and commercially available serum (HIV-C [human C serum, Quidel]) and applied the preparations in prime-boost experiments with cells from healthy donors. In accordance to our previous results [11], DC exposed to HIV-C triggered significantly more CD8$^+$ T cell proliferation than those loaded with non-opsonized HIV ($p=0.0058$, Fig. 1A). In contrast, DC loaded with IgG-opsonized HIV stimulated CD8$^+$ T cell expansion much less than HIV-C loaded DC ($p=0.0111$, Fig. 1), The CD8$^+$ T cell-stimulatory capacity of DC loaded with HIV opsonized in the presence of C and IgGs (HIV-CIg) was intermediate and not significantly different from HIV-C or HIV-Ig (Fig. 1). Staphylococcus enterotoxin B (SEB)-stimulated CD8$^+$ T cell proliferation served as positive control, and expansion by cytokine-loaded DC and iDC as negative controls (Fig. 1, SEB, mDC, iDC). Figure 1A illustrates the average expansion of CD8$^+$ T cells from 5 donors and two different virus strains (BaL, 92UG037) compared to the positive control (SEB-DC-activated CD8$^+$ T cells). Thus, results for each sample (SEB, mDC, iDC, HIV, HIV-C, HIV-CIg, HIV-Ig) were confirmed 10 times. To further confirm the T cell expansion, CD8$^+$ T cells from 3 donors were labeled with CFSE prior the first priming with loaded DCs. Expanded CD8$^+$ T cells were measured as CFSE^{low} population after the third boost and confirmed the data above (Suppl. Fig. 1).

HIV-Ig-DC-primed CD8$^+$ T cells elicit weaker anti-HIV activity than HIV-C-DC-primed CD8$^+$ T cells.

It has been suggested that the efficiency of HIV-specific $CD8^+$ T cells is well illustrated by the capacity of these cells to suppress HIV-1 infection in co-cultures with infected $CD4^+$ T cells [8]. To place the above finding in the context of $CD8^+$ T cell-mediated control of virally infected $CD4^+$ T cells, we designed a co-culture experiment using our HIV-C- or HIV-Ig-loaded DC cells. HIV-Ig-DC-primed $CD8^+$ T cells elicited a weak but non-significant decrease in p24 production of infected, autologous $CD4^+$ T cells compared to SEB-DC-primed $CD8^+$ T cells (Fig. 2). In contrast, HIV-C-DC-primed $CD8^+$ T cells exerted a strong anti-HIV-activity against HIV-infected $CD4^+$ T cells (Fig. 2). Additionally, HIV-CIg-DC-$CD8^+$ T cells partially blocked infection in these co-cultures compared to HIV-Ig-DC-$CD8^+$ T cells ($p = 0.0139$, Fig. 2), but significantly weaker than HIV-C-DC-$CD8^+$ T cells ($p = 0.0024$, Fig. 2). These results suggest that the presence of Ig on the surface of viral particles counterbalances the positive effect of complement opsonization on the CTL-stimulatory capacity of DC. The assays were confirmed with cells isolated from 7 different donors using R5-, X4- and R5X4-tropic viral isolates or SEB for DC exposure. Thereby, the results were proved more than 20 times in triplicates for the various groups.

HIV-specific CTL clones are activated to a significantly lower degree by DC exposed to HIV, which was coated with a high IgG/C ratio.

We then evaluated the effect of HIV-opsonization using plasma from HIV-infected patients with different ratios of Ig and C on the ability of DC to induce CTL responses. We first screened the degree of complement activation or antibody binding to HIV-BaL *ex vivo* using plasma from 35 HIV-positive individuals and 10 healthy individuals. The IgG and C3-deposition on the viral surface was analyzed by a virus capture assay (VCA) as described [11]. As expected, HIV-BaL was covered with significantly more IgG ($p<0.0001$) and C3 fragments ($p=0.0016$) using plasma from 35 HIV-positive individuals compared to 10 healthy

controls (Fig. 3). Background IgG levels were measured on the surface of HIV-BaL, which was opsonized using plasma from healthy donors (Fig. 3, IgG VCA, red quadrants), HIV-C (human C serum, Quidel) (n=2) (Fig. 3, IgG VCA, green triangle) or the non-opsonized control (n=2) (Fig. 3, IgG VCA, blue triangle), thus excluding the presence of natural Abs on the surface of plasma or NHS-incubated virus preparations. Complement activation was also significantly higher in HIV-positive (n=35) compared to healthy (n=10) individuals (p=0.0016) (Fig. 3, C3 VCA, yellow circles, red quadrants). Non-opsonized control and HIV-C (human C serum, Quidel) preparations were added to each analysis as controls for functionality of the VCA. The VCA was performed using all plasma samples (35 HIV^+, 10 healthy) and 2 different virus preparations (BaL, 92UG037).

We selected HIV preparations opsonized with plasma from HIV-infected individuals, which displayed a significantly higher IgG (Fig. 4, blue bars) to C (Fig. 4, red bars) ratio (Fig. 4, $p = 0.0046$) or vice versa (Fig. 4, $p = 0.0025$) to investigate the differential functional impact of complement- or IgG-opsonization on the antigen-presenting capacity of DC to HIV-specific $CD8^+$ T cell clones. Fig. 4 shows a representative VCA for HIV-C high or HIV-Ig high samples. Differences between HIV-C and HIV-Ig binding to the virus capture plate might be explained by diverse avidity of the Abs used in the VCA (mAb against human C3c or IgG). Nevertheless, the binding of the differentially opsonized HIV preparations is due to specific interactions with the various receptors expressed on DC as shown by experiments using blocking DC-SIGN, CR3 or CD32 Abs (not shown). Additionally, the amounts of IgGs or C3c bound on the surface of HIV after opsonization with the *ex vivo* plasma samples did not differ significantly (Suppl. Fig. 2).

DC were exposed to the HIV-C high or HIV-Ig high preparations, washed, and HLA-matched HIV-specific $CD8^+$ T cell clones were added at a 10:1 E:T ratio. The activation of the specific clones was measured by IFNγ Elispot. We found that significantly less IFN-γ spots were

induced when using HIV-Ig high-DC for stimulation compared to DC exposed to HIV-C high ($p \leq 0.0001$, Fig. 5) or HIV-C (human C serum, Quidel) ($p \leq 0.001$, Fig. 5). As positive controls for IFNγ induction from HIV-specific $CD8^+$ T cells, peptide (SLYNTVATL)-loaded DC (pepDC) and DC exposed to human complement serum (HIV-C (human C serum, Quidel)), as negative controls immature DC (iDC), DC exposed to non-opsonized HIV (HIV) and DC loaded with IgG-opsonized HIV (MRC IgG-pool) were used. The results were verified with 10 preparations for each group (pepDC, iDC, HIV, HIV-C, HIV-Ig, HIV-C high, HIV-Ig high) in triplicates.

In contrast to IgG-opsonized HIV, complement-coated virus co-localizes with HLA-ABC in DCs.

To explain the different CTL-stimulatory capacity of C- and IgG-loaded DC, we performed confocal microscopic analyses using differentially loaded DC (HIV-C, HIV-CIg, HIV-Ig) and stained the cells with antibodies against various endo- and lysosomal markers or HLA-ABC and HIV-Gag (KC57-FITC). While after incubation with HIV-C, where HIV Gag co-localized with HLA-ABC in more than 90% of the investigated DCs (Fig. 6A, left panel and Fig. 6B), a significantly lower co-localization (31.5%) was observed after overnight incubation with HIV-Ig in DCs (Fig. 6A, right panel and Fig. 6B). The co-localization of HIV and HLA-ABC in HIV-CIg-incubated DCs was intermediate (Fig. 6A, middle panel and Fig. 6B). A significantly lower co-localization of HLA-ABC and HIV-p24 was observed in HIV-Ig-incubated DCs compared to HIV-C- and HIV-CIg-exposed cells ($p \leq 0.0001$).

No increased co-localization of all different virus preparations (HIV, HIV-C, HIV-CIg (not shown), HIV-Ig) with late endosomes/multivesicular bodies (marker: CD63) or lysosomal compartments (marker: Lamp-1) was detected (Fig. 7). All confocal microscopic analyses

were performed using differentially opsonized 92UG037, 93BR020, and NL4-3. Quantifications of colocalization were performed using the Metamorph software (Universal Imaging) on an average of 20–25 cells for each condition as previously described [27].

Discussion

The *ex vivo* and *in vitro* data presented in this study using plasma from HIV-infected individuals confirm our findings that complement acted as an endogenous adjuvant for DC-mediated induction of retroviral CTLs *in vitro* and *in vivo* [11], while we could show here that Ab coating of the viral surface significantly decreased the DC-mediated CTL activation. Investigations of DC interactions with differentially opsonized HIV are important, since *in vivo* HIV is found opsonized with complement fragments and specific IgGs in all compartments tested so far, i.e. plasma, seminal fluid [28], or HIV stripped from tonsillar tissue [29]. It has been shown that targeting Ag to FcγR on DC increased Ab responses to soluble Ags and to tumor cells [30]. *In vitro* studies involving FcγR uptake through the endosomal system demonstrated that, besides enhancing MHC class II antigenic processing [30-32] and presentation, this uptake provided access to the cytosolic or endogenous pathway [33-35]. A different co-localization of HIV-C or HIV-Ig with HLA class I molecules or the degradation of HIV-Ig in lysosomal compartments may constitute mechanisms explaining the weaker $CD8^+$ T cell stimulation of DC exposed to to HIV-Ig. We now detected that when tracking HIV-C or HIV-Ig preparations and HLA-ABC intracellularly, the magnitude of Gag co-localization of HIV-C with HLA-class I was significantly higher compared to HIV-Ig-incubated cells. Co-localization of both, HIV-C and HIV-Ig, with other cellular markers such as CD63 (marker of late endosomes/multi vesicular bodies), HLA-DM, or Lamp-1 (marker of lysosomes) was marginal, while the tetraspanin-rich CD81-containing compartment was targeted by HIV-C and HIV-Ig in DCs as previously shown [23].

Efficient early $CD8^+$ T cell responses are critical in controlling HIV-1 replication and their determinant role in HIV-1 control is further supported by association of certain HLA class I alleles and an improved disease progression [36-38]. In view of our observations, we are proposing that during acute viral infection $CD8^+$ T cells are efficiently induced by DC,

probably due to (i) enhanced infection of DC by HIV-C [23] and presentation of endogenously synthesized viral Ags processed through HLA-ABC and (ii) more efficient cross-presentation from incoming complement-opsonized virus.

The novel modulatory effects of the HIV-opsonization state on the CTL-activating capacity of DC described herein call for further investigation, since current HIV vaccination strategies aim in developing a vaccine that efficiently induces both, a cellular as well as humoral immune response [39-41]. Thus far, disappointing results have been achieved in clinical trials targeting either cellular immunity [42, 43] or humoral immunity [44, 45]. The RV144 AIDS vaccine trial in Thailand [46] evoked strong, but transient Env-specific $CD4^+$ T cell and Ab responses, but only weak HIV-specific $CD8^+$ T cell responses [40, 47]. Our results are highly relevant in this regard as they suggest that strong, transient Ab responses after vaccination might contribute to weaken CTL-induction by modulation of DC function as uncovered in this *ex vivo* and *in vitro* study and thereby might play a critical role for future vaccination strategies.

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Conflict of Interest Statement: The authors declare not to have any conflict of interest.

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Figure Legends

Figure 1 $CD8^+$ T cell proliferation induced by differentially loaded DC. $CD8^+$ T cell expansion by DC loaded with HIV-C was 7-fold higher compared to HIV-Ig-DC ($p \leq 0.05$) or HIV-DC ($p \leq 0.05$). SEB-stimulated $CD8^+$ T cell proliferation served as positive control and was set 100, mDC and iDC as negative controls. The $CD8^+$ T cell-stimulatory capacity of DC loaded with HIV-CIg was intermediate. **63 words**

Figure 2 Antiviral effects of *in vitro* generated HIV-specific CTLs. HIV-C-DC-primed CTLs significantly ($p \leq 0.001$) blocked the virus production in $CD4^+/CD8^+$ T cell co-cultures compared to negative controls (w/o $CD8^+$ T cells, SEB), or HIV-Ig-DC-primed CTLs. Inhibition by HIV-CIg- and HIV-DC-primed $CD8^+$ T cells was intermediate. Percentages of infection compared to infected CD4+ T cells are shown. **56 words**

Figure 3 VCA of HIV-BaL opsonized with plasma from HIV-positive and healthy individuals. The left panel shows IgG-coating of HIV opsonized with plasma from 35 HIV-positive (HIV^+ individuals) or 10 healthy controls, the right panel C3-deposition. The VCA using the above mentioned plasma samples was repeated three times using BaL (2x) or 92UG037 (1x). **52 words**

Figure 4 Selection of HIV-C high or HIV-Ig high samples for Elispot analyses. C3 fragments and HIV-specific Abs on the surface of HIV-BaL were analyzed by VCA following opsonization of the virus using plasma from HIV^+ individuals. Examples from 3 individuals

displaying either high C/low Ig (HIV-C high) or low C/high Ig (HIV-Ig high) are shown in this graph. **57 words**

Figure 5 Elispot analyses using HIV-C high or HIV-Ig high virus preparations for DC exposure. Induction of IFNγ by HIV-C-high exposed DC was significantly stronger than that of HIV-Ig-high-DC ($p \leq 0.0001$). As positive controls pepDC and HIV-C (human C serum, Quidel) were used, as negative controls iDC, HIV- and HIV-Ig-loaded DC (HIV, HIV-Ig (MRC IgG-pool)). **52 words**

Figure 6A/B Intracellular localization of differentially opsonized HIV in DC. HIV-C (green) co-localizes with HLA-ABC (red) in DC (A, left panel) to 94.6% (B), while HIV-Ig (green) only shows minor co-localization (31.5%) (B) with the MHC class I molecule (A, right panel). The HIV-CIg preparation showed intermediate co-localization (A, middle panel) (77.7%) (B). Cells were permeabilized and stained using labelled anti-human HLA-ABC mAb and anti-HIV $p24^{Gag}$. **64 words**

Fig. 7 Endosomal characterization of intracellular HIV localization in DC. To illustrate, if the enhanced CTL-stimulatory capacity is due to enhanced degradation or multi-vesicular body co-localization, we stained HIV-, HIV-C- and HIV-Ig-incubated DCs with HIV-1-$p24^{gag}$ and CD63 or Lamp-1, respectively. No significant differences and only marginal co-localization with these markers were observable as depicted in this histogram plot. **56 words**

Supplementary Figure Legends

Supplementary Figure 1 DC-mediated $CD8^+$ T cell expansion measured by CFSE-labeling. HIV-IgDC-mediated $CD8^+$ T cell proliferation compared to HIV-C-DC-$CD8^+$ T cells was detected by pre-staining T cells with 2.5 µM CFSE. Mean percentages of $CFSE^-$ [proliferated]/$CD8^+$ T cells of SEB-, HIV-, HIV-C- or HIV-Ig-DC-primed $CD8^+$ T cells from six experiments using 92UG037 and 92BR030 are shown. Data were analyzed by ANOVA and subsequently Bonferroni. **61 words**

Supplementary Figure 2 VCA of HIV-BaL opsonized with plasma from HIV-positive individuals. IgG- and C3-deposition on HIV opsonized with 35 plasma samples from HIV-positive individuals were analyzed by VCA. Differences in IgG- or C-amounts bound to the viral surface were analyzed by an unpaired Student´s t test (2-tailed) and were shown to be non-significant. **51 words**

Fig. 1

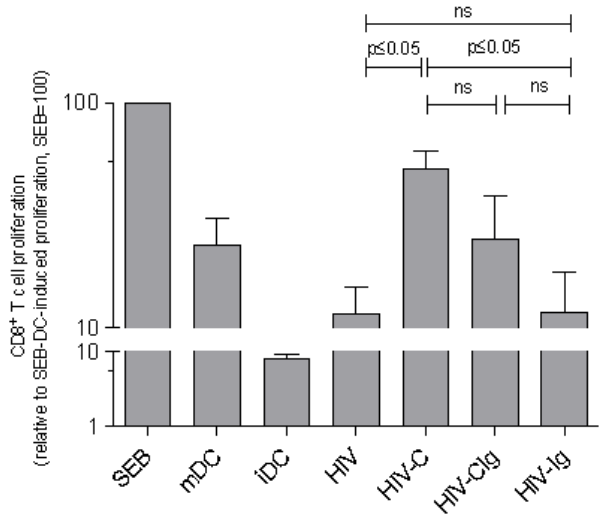

ns
p≤0.05
p≤0.05
ns
ns
100
10
10
1
CD8+ T cell proliferation
(relative to SEB-DC-induced proliferation, SEB=100)
SEB
mDC
iDC
HIV
HIV-C
HIV-Clg
HIV-Ig

Fig. 2

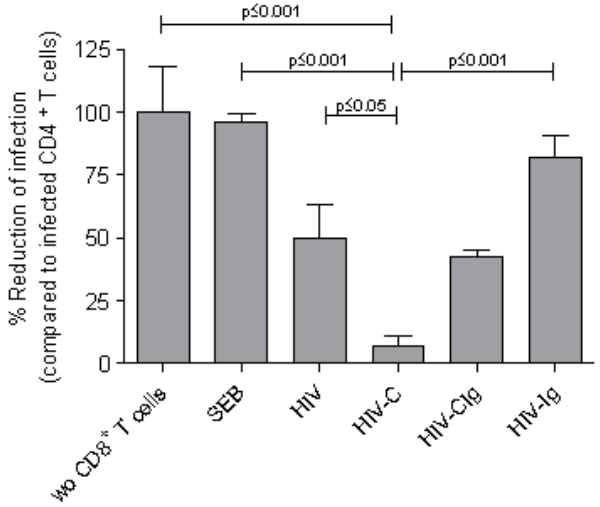

p≤0.001
p≤0.001
p≤0.001
p≤0.05
125
100
75
50
25
0
% Reduction of infection
(compared to infected CD4 + T cells)
w/o CD8+ T cells
SEB
HIV
HIV-C
HIV-Clg
HIV-Ig

Fig. 3

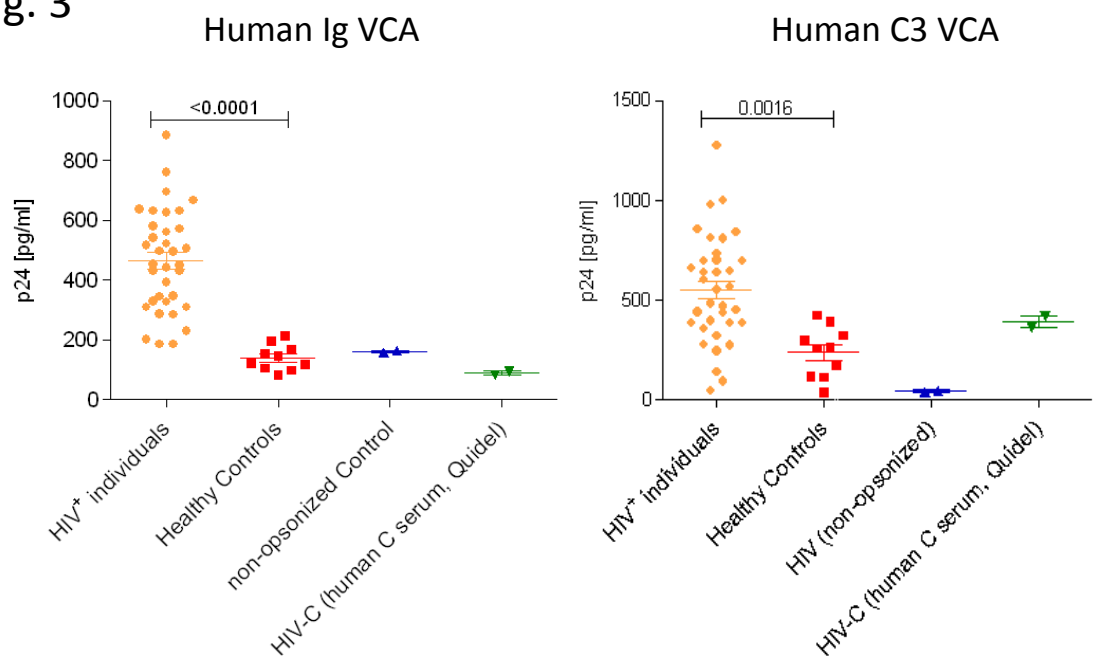

Human Ig VCA
Human C3 VCA
p24 [pg/ml]
<0.0001
0.0016
1000
800
600
400
200
0
1500
1000
500
HIV+ individuals
Healthy Controls
non-opsonized Control
HIV-C (human C serum, Quidel)
HIV (non-opsonized)

Fig. 4

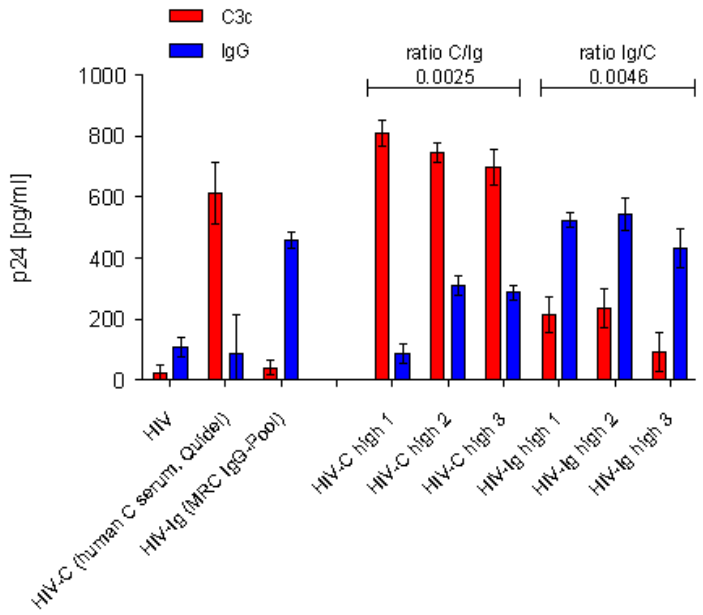

C3c
IgG
ratio C/Ig
0.0025
ratio Ig/C
0.0046
p24 [pg/ml]
1000
800
600
400
200
0
HIV
HIV-C (human C serum, Quidel)
HIV-Ig (MRC IgG-Pool)
HIV-C high 1
HIV-C high 2
HIV-C high 3
HIV-Ig high 1
HIV-Ig high 2
HIV-Ig high 3

Fig. 5

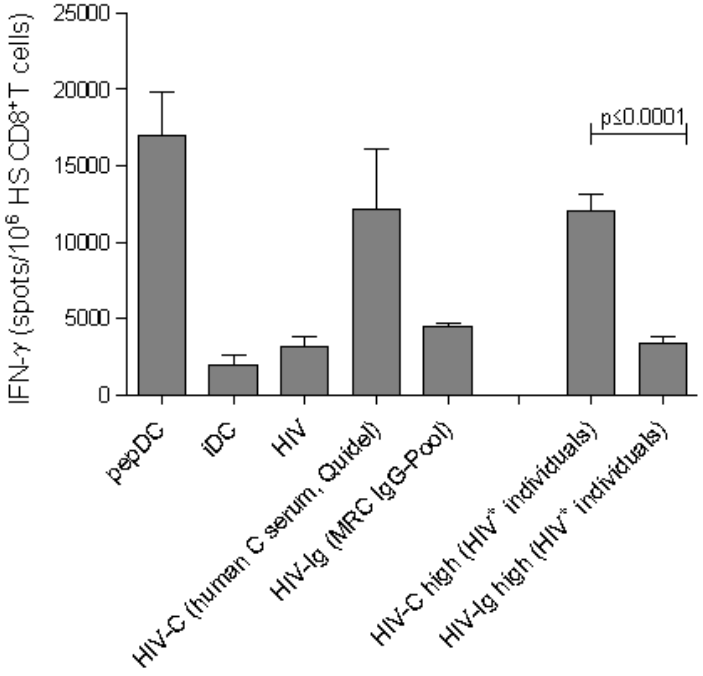

IFN-γ (spots/10⁶ HS CD8⁺T cells)
25000
20000
15000
10000
5000
0
p≤0.0001
pepDC
iDC
HIV
HIV-C (human C serum, Quidel)
HIV-Ig (MRC IgG-Pool)
HIV-C high (HIV⁺ individuals)
HIV-Ig high (HIV⁺ individuals)

Fig. 6A

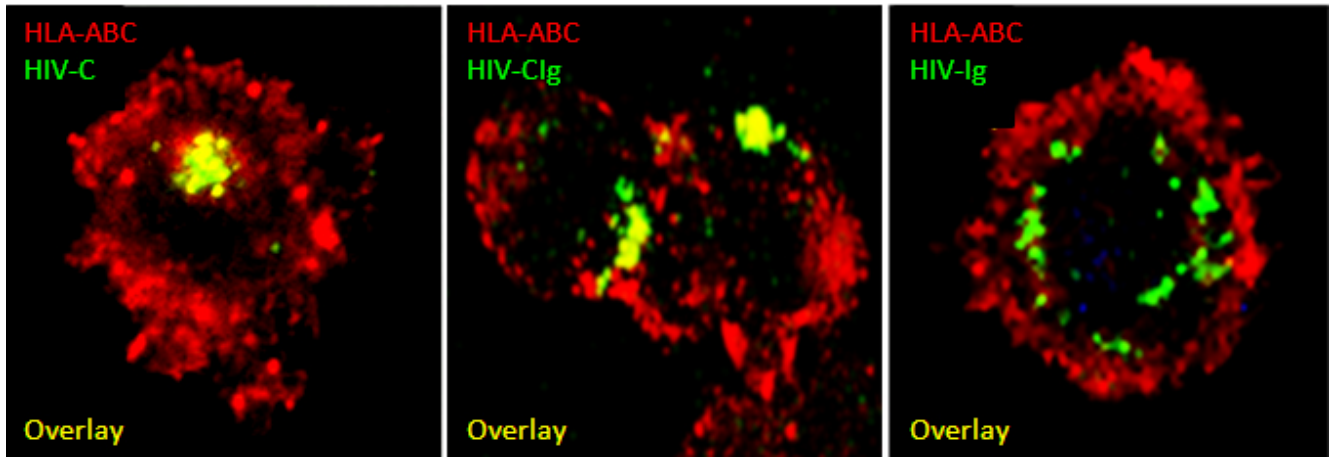


Fig. 6B

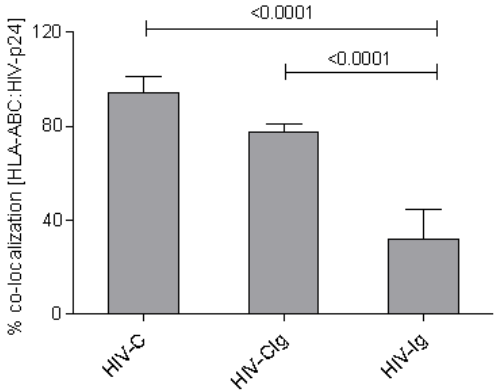
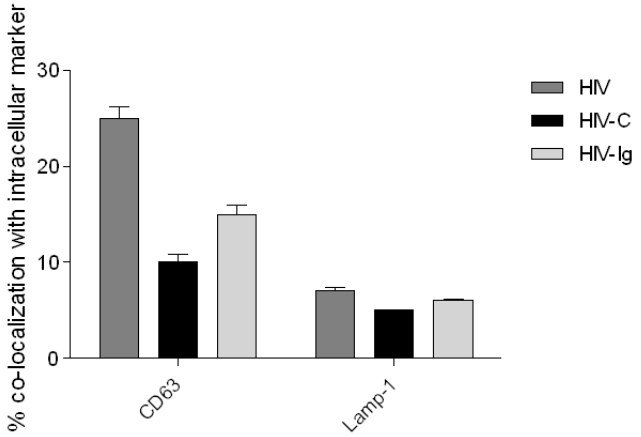
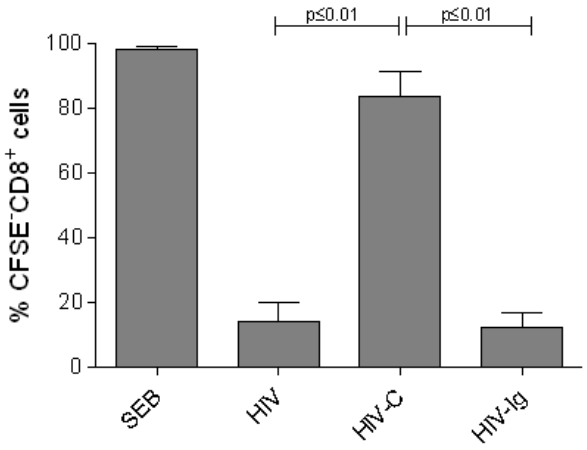


Fig. 7



Suppl.Fig. 1



Suppl.Fig. 2