Published in final edited form as:

J Allergy Clin Immunol. 2012 December ; 130(6): 1368–74.e2. doi:10.1016/j.jaci.2012.08.025.

Antibodies attenuate the capacity of dendritic cells to stimulate HIV-specific cytotoxic T lymphocytes

Wilfried Posch, PhD^a, Sylvain Cardinaud, PhD^b, Chiraz Hamimi, MSc^c, Adam Fletcher, PhD^d, Annelies Mühlbacher, MD^e, Klaus Loacker, PhD^e, Paul Eichberger, PhD^f, Manfred P. Dierich, MD^a, Gianfranco Pancino, MD, PhD^c, Cornelia Lass-Flörl, MD^a, Arnaud Moris, PhD^b, Asier Saez-Cirion, PhD^c, and Doris Wilflingseder, PhD^a

^aDivision of Hygiene and Medical Microbiology, Innsbruck Medical University

^bINSERM UMRS-945, Infection and Immunity, Hôpital La Pitié-Salpêtrière, Université Pierre et Marie Curie (Paris-6), Paris

^cUnité de Régulation des Infections Rétrovirales, Institut Pasteur, Paris

^dMRC/UCL Centre for Medical Molecular Virology, Division of Infection and Immunity, University College London

^eCentral Institute for Blood Transfusion & Immunological Department, Innsbruck Medical University

^fDepartment of Therapeutic Radiology and Oncology, Innsbruck Medical University

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 an endogenous adjuvant for dendritic cell (DC)–mediated induction of specific cytotoxic T lymphocytes (CTLs). However, in some HIV-positive subjects, high levels of antibodies and low levels of complement fragments coat the HIV surface.

Methods—Therefore we analyzed the effect of IgG opsonization on the antigen-presenting capacity of DCs by using CD8⁺ T-cell proliferation assays after repeated prime boosting, by measuring the antiviral activity against HIV-infected autologous CD4⁺ T cells, and by determining IFN- γ secretion from HIV-specific CTL clones.

Corresponding author: Doris Wilflingseder, PhD, Division of Hygiene and Medical Microbiology, Innsbruck Medical University, Fritz-Pregl-Str. 3, A-6020 Innsbruck, Austria. doris.wilflingseder@i-med.ac.at.

Disclosure of potential conflict of interest: W. Posch has received research support from the Tyrolean Science Fund and the Austrian National Bank Fund. S. Cardinaud has received research support from Sidaction. C. Lass-Flörl has received consultancy fees from Gilead Sciences, MSD, Pfizer, and Schering-Plough; has received research support from Astellas Pharma, Gilead Sciences, and Pfizer; has received lecture fees from Astellas Pharma, Pfizer, and MSD; and has received travel expenses from Gilead Sciences, MSD, Pfizer, and Astellas Pharma. A. Moris has received research support from Sidaction/ANRS. A. Saez-Cirion has received lecture fees from MSF. D. Wilflingseder has received research support from the Austrian Science Fund. The rest of the authors declare that they have no relevant conflicts of interest.

Results—We find that DCs exposed to IgG-opsonized HIV significantly decreased the HIV-specific CD8⁺ T-cell response compared with the earlier described efficient CD8⁺ T-cell activation induced by DCs loaded with complement-opsonized HIV. DCs exposed to HIV bearing high surface IgG levels after incubation in plasma from HIV-infected subjects acted as weak stimulators for HIV-specific CTL clones. In contrast, HIV opsonized with plasma from patients exhibiting high complement 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 DCs.

Keywords

HIV; IgG; opsonization; dendritic cells; cytotoxic T lymphocytes

Appearance of HIV-specific cytotoxic T lymphocytes (CTLs) correlates with decreasing viremia during the acute phase of infection. During the chronic phase of infection, HIV CTLs are exhausted and not able to efficiently control infection.¹⁻⁵ However, some patients who commonly have viral loads of less than 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 on crossing mucosal surfaces. There the complement system constitutes a first line of defense against the virus. Recently, we illustrated an important role for complement opsonization of retroviruses as an endogenous adjuvant for dendritic cell (DC)–mediated CTL induction.¹¹ The first antibody responses to the HIV-1 envelope glycoproteins gp41 and gp120 appear in plasma 2 and 4 weeks after infection, respectively. These antibodies were demonstrated to have only a modest effect 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 appear, becoming increasingly potent in some patients to develop broadly neutralizing capacity.^{15,16} Whether HIV-1 neutralizing antibodies confer viral control in HIV long-term nonprogressors 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 complement activation and deposition on the HIV surface (HIV-C) or enhanced interaction of HIV-bound antibodies (HIV-Ig) with Fc receptors expressed on hematopoietic cells. Immune complexes containing infectious viral particles have been identified in blood and on lymphoid follicles of HIV-positive patients.^{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, on retroviral infections, complement opsonization seems to favor effective CTL priming by DCs.¹¹ Thus the opsonization pattern of HIV-1 does not merely result in differential DC infection and integration but also has profound consequences on the antigen-presenting capacity of DCs *in vitro* and *in vivo*.

Here we sought to understand whether DCs exposed to IgG-opsonized HIV are comparable in terms of CTL-stimulatory capacity with HIV-C DCs. Using *in vitro* priming experiments, we find that DCs exposed to IgG-opsonized HIV significantly decreased the HIV-specific CD8⁺ T-cell response compared with the efficient HIV-C DC-induced CD8⁺ T-cell activation described earlier.¹¹ DCs exposed to HIV bearing high surface IgG levels after incubation in plasma from HIV-infected patients acted as weak stimulators for HIV-specific CTL clones. In contrast, HIV opsonized with plasma from patients exhibiting high complement 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 DCs.

METHODS

Samples

Plasma samples were obtained from 35 HIV-infected patients recruited from patients followed up in CHU St Louis, Hopital Europeen Georges Pompidou, and CHU de Bicetre in France. All the subjects provided 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.

Written informed consent was also obtained from the participating blood donors by the Central Institute for Blood Transfusion and the 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 the blood of healthy donors by using human CD14 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the manufacturer's instructions. DCs were generated and analyzed as described previously.^{11,23} Subsequently, CD4⁺ (>95% purity) and CD8⁺ (>94% purity) T cells were bead purified and used with autologous DCs for the *in vitro* experiments.

Opsonization of HIV-1

Purified R5-tropic HIV (BaL, 92UG037) was incubated for 1 hour at 37°C with normal human serum as a complement source (HIV-C) or commercially available human complement serum HIV-C [human C serum; Quidel, San Diego, Calif]) 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 a negative control, the virus was incubated under the same conditions in medium or heat-inactivated serum (HIV). For some experiments, differentially opsonized R5X4-tropic (93BR020) or X4-tropic (NL4-3) HIV was used also. For experiments using plasma samples from HIV-infected patients, HIV (BaL and 92UG037; concentration, >1 µg/mL) was incubated under the abovementioned conditions by using the plasma in a 1:10 dilution. Subsequent to opsonization, the virus was washed, placed into pellets by means of ultracentrifugation (14,000 rpm for 90 minutes at 4°C), and

resuspended in 100 μ L of RPMI medium without supplements. The opsonization pattern was determined by using a virus-capture assay (VCA), as previously described,²³ with anti-human C3c/C3d, IgG, or mouse IgG antibodies as control for background binding. The coated VCA plates were incubated overnight with the differentially opsonized viral preparations (2.5 ng of p24 per well) at 4°C and washed 5 times with RPMI medium to remove unbound virus. Bound virus was lysed (2% Igepal) and transferred to a precoated p24 ELISA plate²⁴ to confirm the opsonization pattern.

Prime boost experiments

The *in vitro*-generated CD8⁺ T cells were analyzed for expansion by using fluorescence-activated cell sorting analyses and for functionality by using an antiviral assay, as previously described,¹¹ but a detailed description of the *in vitro* CD8⁺ T-cell generation and antiviral activity testing is provided below.

In vitro generation of HIV-specific CD8⁺ T cells

Day 5 immature dendritic cells (iDCs) were stimulated with a cytokine cocktail (IL-1 β , IL-6, prostaglandin E₂, TNF- α , IL-4, and GM-CSF) for 24 hours, and then 10⁴ cells/100 μ L were transferred into 96-well plates. DCs from all donors were loaded with 25 ng of p24 per milliliter of nonopsonized (HIV), complement-opsonized (HIV-C), complement plus antibody-opsonized (HIV-CIg), or antibody-opsonized (HIV-Ig) HIV strains (R5-, R5X4-, and X4-tropic) for 3 hours. DCs were exposed to 1 μ g/mL of the superantigen staphylococcal enterotoxin B (SEB; Sigma, St Louis, Mo) for the same time period as a positive control for T-cell proliferation. As additional controls, iDCs, cytokine cocktail-stimulated DCs, or both were included in the experimental settings. All tests were performed in triplicates. The cells were thoroughly washed, and nonstimulated naive CD8⁺ T cells were added to the primed DCs at a 3:1 ratio. Twenty-five units per milliliter of 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, specificity, and functionality. HIV-induced proliferation of T cells was analyzed by using fluorescence-activated cell sorting. Proliferated T cells were expanded with the CD3/CD28 T-cell expander (Life Technologies, Carlsbad, Calif), according to the manufacturer's instructions, after the third priming with loaded DCs to perform antiviral activity tests of the *in vitro*-generated HIV-specific CTLs.

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

We measured the antiviral activity of CTLs primed with the different conditioned DCs on infected CD4⁺ T cells. This assay measures the elimination of infected cells^{8,11} and is currently considered one of the best correlates to assess the ability of CTLs to control infection. For this, frozen autologous CD4⁺ T cells were thawed, washed, and stimulated with CD3/CD28 beads (Life Technologies) in the presence of IL-2 (100 U/mL). Prestimulation of the CD4⁺ T cells with CD3/CD28 was necessary to induce upregulation of CCR5. The *in vitro* primed and expanded CTLs were added to overnight HIV-incubated CD4⁺ T cells. A reduction in viral production assessed by using the p24 ELISA on several days after addition of the expanded CTLs indicated a confirmation of their functionality. Data were reproduced with expanded CTLs from 7 donors primed and boosted with

different conditioned DCs. Both methods (*in vitro* generation and antiviral assay) were performed exactly as described by Banki et al.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 DCs were cocultured overnight with CD8⁺ T-cell clones (range, 2,500-10,000 SL9 clone 2 per well). As positive controls, stimulators (ie, DCs) were incubated with 1 µg/mL cognate peptide (SLYNTVATL, SL9) before washing and addition of the HIV-specific CTL clones overnight. The next day, IFN-γ production was monitored in an ELISpot assay, as previously described.²⁵ All antibodies used for the IFN-γ ELISpot were from Mabtech (Nacka Strand, Sweden).

Immunofluorescence and confocal microscopy

For intracellular HIV localization, iDCs were exposed to differentially opsonized HIV (250 ng of p24/2.5 × 10⁵ cells) overnight. After washing, cells were left to adhere on glass cover slips for 1 hour at 37°C. Cells were then fixed and permeabilized for 20 minutes at 4°C in BD Cytotfix/Cytoperm (BD Biosciences, San Jose, Calif) and washed with BD Perm/Wash buffer containing human IgG (20 µg per condition). Triple labeling of cells was done as follows: iDCs pulsed with HIV-1 were stained with primary antibodies against HLA-ABC (BD Biosciences), CD63 (mAb 1B5), and LAMP-1 (polyclonal antibody). After extensive washing, cells were then stained with secondary donkey anti-mouse and anti-rabbit antibodies coupled to rhodamine (Jackson ImmunoResearch Laboratories, West Grove, Pa). Cells were incubated for 20 minutes at room temperature in PBS containing BSA, saponin, and mouse serum (0.5 mg/mL) to avoid unspecific labeling. Finally, HIV-1-p24^{gag} was detected by using a monoclonal anti-HIV-1-p24^{gag} (KC57) coupled to fluorescein isothiocyanate (Beckman Coulter, Fullerton, Calif). Confocal laser scanning microscopy was performed with an LSM 510 microscope (Carl Zeiss, Oberkochen, Germany). Images were then processed with Photoshop (Adobe, San Jose, Calif). Quantifications of colocalization were performed by using the Metamorph software (Universal Imaging, Molecular Devices, Sunnyvale, Calif) on an average of 25 to 30 cells for each condition, as previously described.²⁷

Statistical analysis

Differences were analyzed by using GraphPad Prism software (GraphPad Software, La Jolla, Calif) with the unpaired Student *t* test (2-tailed) for comparisons between 2 groups. For multiparameter comparisons, values from the experiments were logarithmically transformed and analyzed by using 1-way ANOVA and the Bonferroni 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 DCs, we differentially opsonized live virus by 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 with our previous results,¹¹ DCs exposed to HIV-C triggered significantly more CD8⁺ T-cell proliferation than those loaded with nonopsonized HIV ($P < .05$, Fig 1). In contrast, DCs loaded with IgG-opsonized HIV stimulated CD8⁺ T-cell expansion much less than HIV-C-loaded DCs ($P < .05$, Fig 1). The CD8⁺ T cell-stimulatory capacity of DCs loaded with HIV opsonized in the presence of complement and IgGs (HIV-CIg) was intermediate and not significantly different from that of HIV-C or HIV-Ig (Fig 1). SEB-stimulated CD8⁺ T-cell proliferation served as a positive control, and expansion by cytokine-loaded DCs and iDCs served as negative controls (Fig 1, SEB, mature DCs, and iDCs). Fig 1 shows the average expansion of CD8⁺ T cells from 5 donors and 2 different viral strains (BaL and 92UG037) compared with the positive control (SEB DC-activated CD8⁺ T cells). Thus results for each sample (SEB, mature DCs, iDCs, HIV, HIV-C, HIV-CIg, and HIV-Ig) were confirmed 10 times. CD8⁺ T cells from 3 donors were labeled with carboxyfluorescein succinimidyl ester before the first priming with loaded DCs to further verify the T-cell expansion. Expanded CD8⁺ T cells were measured as a carboxyfluorescein succinimidyl ester-low population after the third boost, which confirmed the data above because HIV-C-exposed DCs caused significantly higher ($P < .01$) CD8⁺ T-cell proliferation than HIV-loaded or HIV-Ig-loaded DCs (see Fig E1).

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 cocultures 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 coculture experiment using our HIV-C- or HIV-Ig-loaded DCs. HIV-Ig DC-primed CD8⁺ T cells elicited a weak but nonsignificant decrease in p24 production of infected autologous CD4⁺ T cells compared with 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 ($P < .001$, Fig 2). Additionally, HIV-CIg DC-primed CD8⁺ T cells partially blocked infection in these cocultures compared with HIV-Ig DC-primed CD8⁺ T cells (Fig 2) but at a weaker level than HIV-C DC-primed CD8⁺ T cells (Fig 2). These results suggest that the presence of immunoglobulin on the surface of viral particles counterbalances the positive effect of complement opsonization on the CTL-stimulatory capacity of DCs. The assays were confirmed with cells isolated from 7 different donors by using R5-, X4-, and R5X4-tropic viral isolates or SEB for DC exposure. 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 DCs exposed to HIV, which was coated with a high IgG/complement ratio

We then evaluated the effect of HIV opsonization using plasma from HIV-infected patients with different ratios of immunoglobulin and complement on the ability of DCs to induce CTL responses. We first screened the degree of complement activation or antibody binding to HIV-BaL *ex vivo* by using plasma from 35 HIV-positive patients and 10 healthy subjects. The IgG and C3 deposition on the viral surface was analyzed by using a VCA, as previously

described.¹¹ As expected, HIV-BaL was covered with significantly more IgG ($P < .0001$) and C3 fragments ($P = .0016$) using plasma from 35 HIV-positive patients compared with plasma from 10 healthy control subjects (Fig 3). Background IgG levels were measured on the surface of HIV-BaL, which was opsonized by 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 nonopsonized control ($n = 2$; Fig 3, IgG VCA, *blue triangle*), thus excluding the presence of natural antibodies on the surface of plasma or normal human serum-incubated viral preparations. Complement activation was also significantly higher in HIV-positive patients ($n = 35$) compared with healthy subjects ($n = 10$, $P = .0016$; Fig 3, C3 VCA, *yellow circles, red quadrants*). Nonopsonized control and HIV-C (human C serum, Quidel) preparations were added to each analysis as controls for the functionality of the VCA. The VCA was performed by using all plasma samples (35 HIV-positive patients and 10 healthy subjects) and 2 different viral preparations (BaL [2×] and 92UG037 [1×]).

We selected HIV preparations opsonized with plasma from HIV-infected patients, which displayed a significantly higher IgG (Fig 4, *blue bars*) to complement (Fig 4, *red bars*) ratio (Fig 4, $P = .0046$) or *vice versa* (Fig 4, $P = .0025$) to investigate the differential functional effect of complement or IgG opsonization on the antigen-presenting capacity of DCs 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 the diverse avidity of the antibodies used in the VCA (mAb against human C3c or IgG). Nevertheless, binding of the differentially opsonized HIV preparations is due to specific interactions with the various receptors expressed on DCs, as shown by experiments using blocking DC-SIGN, CR3, or CD32 antibodies (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 ($P = .1122$, see Fig E2).

DCs were exposed to the HIV-C–high or HIV-Ig–high preparations and washed, and HLA-matched HIV-specific CD8⁺ T-cell clones were added at a 10:1 effector to target ratio. The activation of the specific clones was measured by using the IFN- γ ELISpot. We found that significantly less IFN- γ spots were induced when using HIV-Ig–high DCs for stimulation compared with use of DCs exposed to HIV-C–high ($P < .0001$, Fig 5) or HIV-C (human C serum, Quidel; $P < .001$, Fig 5) conditions. Peptide (SLYNTVATL)–loaded DCs, and DCs exposed to human complement serum (HIV-C [human C serum, Quidel]) were used as positive controls for IFN- γ induction from HIV-specific CD8⁺ T cells, and iDCs, DCs exposed to nonopsonized HIV (HIV), and DCs loaded with IgG-opsonized HIV (MRC IgG-pool) were used as negative controls. The results were verified with 10 preparations for each group (peptide [SLYNTVATL]–loaded DCs, iDC, HIV, HIV-C, HIV-Ig, HIV-C high, and HIV-Ig high) in triplicates.

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

To explain the different CTL-stimulatory capacity of complement- and IgG-loaded DCs, we performed confocal microscopic analyses using differentially loaded DCs (HIV-C, HIV-CIg, and HIV-Ig) and stained the cells with antibodies against various endosomal and lysosomal

markers or HLA-ABC and HIV-Gag (KC57–fluorescein isothiocyanate). After incubation with HIV-C, where HIV-Gag colocalized with HLA-ABC in more than 90% of the investigated DCs (Fig 6, *A, left panel*, and Fig 6, *B*), a significantly lower colocalization (31.5%) was observed after overnight incubation with HIV-Ig in DCs (Fig 6, *A, right panel*, and Fig 6, *B*). The colocalization of HIV and HLA-ABC in HIV-CIg–incubated DCs was intermediate (Fig 6, *A, middle panel*, and Fig 6, *B*). A significantly lower colocalization of HLA-ABC and HIV-p24 was observed in HIV-Ig–incubated DCs compared with that seen in HIV-C– and HIV-CIg–exposed cells ($P < .0001$).

No increased colocalization of the different viral preparations (HIV, HIV-C, HIV-CIg [not shown], and 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 with differentially opsonized 92UG037, 93BR020, and NL4-3. Quantifications of colocalization were performed with Metamorph software (Universal Imaging) on an average of 20 to 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 patients confirm our findings that complement acted as an endogenous adjuvant for DC-mediated induction of retroviral CTLs *in vitro* and *in vivo*,¹¹ whereas we could show here that antibody coating of the viral surface significantly decreased the DC-mediated CTL activation. Investigations of DC interactions with differentially opsonized HIVs are important because *in vivo* HIV is found opsonized with complement fragments and specific IgGs in all compartments tested thus far (ie, plasma, seminal fluid,²⁸ or HIV stripped from tonsillar tissue²⁹). It has been shown that targeting antigen to Fcγ receptor on DCs increased antibody responses to soluble antigens and tumor cells.³⁰ *In vitro* studies involving Fcγ receptor uptake through the endosomal system demonstrated that, in addition to enhancing MHC class II antigenic processing^{30–32} and presentation, this uptake provided access to the cytosolic or endogenous pathway.^{33–35} A different colocalization of HIV-C or HIV-Ig with HLA class I molecules or the degradation of HIV-Ig in lysosomal compartments might constitute mechanisms explaining the weaker CD8⁺ T-cell stimulation of DCs exposed to HIV-Ig. We detected that when tracking HIV-C or HIV-Ig preparations and HLA-ABC intracellularly, the magnitude of HIV-Gag colocalization of HIV-C with HLA class I was significantly higher compared with that seen in HIV-Ig–incubated cells. Colocalization of both HIV-C and HIV-Ig with other cellular markers, such as CD63 (marker of late endosomes/multivesicular bodies), HLADM, or Lamp-1 (marker of lysosomes), was marginal, whereas 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 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 DCs, probably because of (1) enhanced infection of DCs by HIV-C²³ and presentation of endogenously

synthesized viral antigens processed through HLA-ABC and (2) more efficient cross-presentation from incoming complement-opsonized virus.

The novel modulatory effects of the HIV opsonization state on the CTL-activating capacity of DCs described herein call for further investigation because current HIV vaccination strategies aim at developing a vaccine that efficiently induces both a cellular and 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 Thailand⁴⁶ evoked strong but transient Env-specific CD4⁺ T-cell and antibody responses but only weak HIV-specific CD8⁺ T-cell responses.^{40,47} Our results are highly relevant in this regard because they suggest that strong transient antibody responses after vaccination might contribute to weakening of CTL induction through 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Professor Olivier Lambotte, Professor Laurence Weiss, Dr Caroline Lascoux, Dr Olivier Taulera, Katia Bourdic, Jeannine Delgado, Maria Manea, and all the other physicians and nurses who cared for the patients whose samples were included in the study. Additionally, we want to thank Polymun Scientific, Donaustrasse 99, Klosterneuburg, Austria, who provided all reagents for p24 ELISA. The reagents ARP118 (HIV-BaL), ARP513 (HIV-Ig pool), and ARP177.8 (HIV-92UG037) were obtained from the Centre for AIDS Reagents, NIBSC HPA UK, which is supported by the EC FP6/7 Europrise Network of Excellence, the NGIN consortia, and the Bill and Melinda Gates GHRC-CAVD Project, and were donated by Dr S. Gartner, Dr M. Popovic, Dr R. Gallo (Courtesy of the NIH AIDS Research and Reference Reagent Program [BaL]), Dr B. Wahren (the Swedish Institute for Infectious Disease Control, Stockholm, Sweden [HIV-Ig pool]), and the World Health Organization UN AIDS Network for HIV isolation and characterization (92UG037).

Abbreviations used

CTL	Cytotoxic T lymphocyte
DC	Dendritic cell
HIV-C	HIV surface coated with complement fragments following opsonization
HIV-Ig	HIV surface coated with specific antibodies following opsonization
iDC	Immature dendritic cell
SEB	Staphylococcal enterotoxin B
VCA	Virus-capture assay

REFERENCES

1. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J Virol*. 1994; 68:4650–5. [PubMed: 8207839]

2. Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *J Virol.* 1994; 68:6103–10. [PubMed: 8057491]
3. McMichael AJ, Rowland-Jones SL. Cellular immune responses to HIV. *Nature.* 2001; 410:980–7. [PubMed: 11309628]
4. Freeman GJ, Wherry EJ, Ahmed R, Sharpe AH. Reinvigorating exhausted HIV-specific T cells via PD-1-PD-1 ligand blockade. *J Exp Med.* 2006; 203:2223–7. [PubMed: 17000870]
5. Lewis MJ, Dagarag M, Khan B, Ali A, Yang OO. Partial escape of HIV-1 from cytotoxic T lymphocytes during chronic infection. *J Virol.* 2012; 86:7459–63. [PubMed: 22553321]
6. Saez-Cirion A, Pancino G, Sinet M, Venet A, Lambotte O. HIV controllers: how do they tame the virus? *Trends Immunol.* 2007; 28:532–40. [PubMed: 17981085]
7. Lambotte O, Boufassa F, Madec Y, Nguyen A, Goujard C, Meyer L, et al. HIV controllers: a homogeneous group of HIV-1-infected patients with spontaneous control of viral replication. *Clin Infect Dis.* 2005; 41:1053–6. [PubMed: 16142675]
8. Saez-Cirion A, Lacabaratz C, Lambotte O, Versmisse P, Urrutia A, Boufassa A, et al. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. *Proc Natl Acad Sci U S A.* 2007; 104:6776–81. [PubMed: 17428922]
9. Migueles SA, Osborne CM, Royce C, Compton AA, Joshi RP, Weeks KA, et al. Lytic granule loading of CD81 T cells is required for HIV-infected cell elimination associated with immune control. *Immunity.* 2008; 29:1009–21. [PubMed: 19062316]
10. Miura T, Brumme ZL, Brockman MA, Rosato P, Sela J, Brumme CJ, et al. Impaired replication capacity of acute/early viruses in persons who become HIV controllers. *J Virol.* 2010; 84:7581–91. [PubMed: 20504921]
11. Banki Z, Posch W, Ejaz A, Oberhauser V, Willey S, Gasner C, et al. Complement as an endogenous adjuvant for dendritic cell-mediated induction of retrovirus-specific CTLs. *PLoS Pathog.* 2010; 6:e1000891. [PubMed: 20442876]
12. Tomaras GD, Yates NL, Liu P, Qin L, Fouda GG, Chavez LL, et al. Initial B-cell responses to transmitted human immunodeficiency virus type 1: virion-binding immunoglobulin M (IgM) and IgG antibodies followed by plasma anti-gp41 antibodies with ineffective control of initial viremia. *J Virol.* 2008; 82:12449–63. [PubMed: 18842730]
13. Haynes BF, Moody MA, Liao HX, Verkoczy L, Tomaras GD. B cell responses to HIV-1 infection and vaccination: pathways to preventing infection. *Trends Mol Med.* 2011; 17:108–16. [PubMed: 21112250]
14. Bar KJ, Li H, Chamberland A, Tremblay C, Routy JP, Grayson T, et al. Wide variation in the multiplicity of HIV-1 infection among injection drug users. *J Virol.* 2010; 84:6241–7. [PubMed: 20375173]
15. Moog C, Fleury HJ, Pellegrin I, Kirn A, Aubertin AM. Autologous and heterologous neutralizing antibody responses following initial seroconversion in human immunodeficiency virus type 1-infected individuals. *J Virol.* 1997; 71:3734–41. [PubMed: 9094648]
16. Gray ES, Moody MA, Wibmer CK, Chen X, Marshall D, Amos J, et al. Isolation of a monoclonal antibody that targets the alpha-2 helix of gp120 and represents the initial autologous neutralizing-antibody response in an HIV-1 subtype C-infected individual. *J Virol.* 2011; 85:7719–29. [PubMed: 21613396]
17. Cao Y, Qin L, Zhang L, Safrit J, Ho DD. Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. *N Engl J Med.* 1995; 332:201–8. [PubMed: 7808485]
18. Montefiori DC, Pantaleo G, Fink LM, Zhou JT, Zhou JY, Bilska M, et al. Neutralizing and infection-enhancing antibody responses to human immunodeficiency virus type 1 in long-term nonprogressors. *J Infect Dis.* 1996; 173:60–7. [PubMed: 8537683]
19. Lambotte O, Ferrari G, Moog C, Yates NL, Liao HX, Parks RJ, et al. Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers. *AIDS.* 2009; 23:897–906. [PubMed: 19414990]

20. Heath SL, Tew JG, Szakal AK, Burton GF. Follicular dendritic cells and human immunodeficiency virus infectivity. *Nature*. 1995; 377:740–4. [PubMed: 7477265]
21. Jakubik JJ, Saifuddin M, Takefman DM, Spear GT. Immune complexes containing human immunodeficiency virus type 1 primary isolates bind to lymphoid tissue B lymphocytes and are infectious for T lymphocytes. *J Virol*. 2000; 74:552–5. [PubMed: 10590148]
22. Holl V, Peressin M, Decoville T, Schmidt S, Zolla-Pazner S, Aubertin AM, et al. Non-neutralizing antibodies are able to inhibit human immunodeficiency virus type 1 replication in macrophages and immature dendritic cells. *J Virol*. 2006; 80:6177–81. [PubMed: 16731957]
23. Wilflingseder D, Banki Z, Garcia E, Pruenster M, Pfister G, Muellauer B, et al. IgG opsonization of HIV impedes provirus formation in and infection of dendritic cells and subsequent long-term transfer to T cells. *J Immunol*. 2007; 178:7840–8. [PubMed: 17548622]
24. Purtscher M, Trkola A, Gruber G, Burchacher A, Predl R, Steindl F, et al. A broadly neutralizing human monoclonal antibody against gp41 of human immunodeficiency virus type 1. *AIDS Res Hum Retroviruses*. 1994; 10:1651–8. [PubMed: 7888224]
25. Casartelli N, Guivel-Benhassine F, Bouziat R, Brandler S, Schwartz O, Moris A. The antiviral factor APOBEC3G improves CTL recognition of cultured HIV-infected T cells. *J Exp Med*. 2010; 207:39–49. [PubMed: 20038599]
26. Moris A, Nobile C, Buseyne F, Porrot F, Abastado JP, Schwartz O. DC-SIGN promotes exogenous MHC-I-restricted HIV-1 antigen presentation. *Blood*. 2004; 103:2648–54. [PubMed: 14576049]
27. Garcia E, Pion M, Pelchen-Matthews A, Collinson L, Arrighi JF, Blot G, et al. HIV-1 trafficking to the dendritic cell-T-cell infectious synapse uses a pathway of tetraspanin sorting to the immunological synapse. *Traffic*. 2005; 6:488–501. [PubMed: 15882445]
28. Bouhlal H, Chomont N, Haeffner-Cavaillon N, Kazatchkine MD, Belec L, Hocini H. Opsonization of HIV-1 by semen complement enhances infection of human epithelial cells. *J Immunol*. 2002; 169:3301–6. [PubMed: 12218150]
29. Banki Z, Kacani L, Rusert P, Pruenster M, Wilflingseder D, Falkensammer B, et al. Complement dependent trapping of infectious HIV in human lymphoid tissues. *AIDS*. 2005; 19:481–6. [PubMed: 15764853]
30. Heyman B. The immune complex: possible ways of regulating the antibody response. *Immunol Today*. 1990; 11:310–3. [PubMed: 2206276]
31. Tanaka Y, Koido S, Ohana M, Liu C, Gong J. Induction of impaired antitumor immunity by fusion of MHC class II-deficient dendritic cells with tumor cells. *J Immunol*. 2005; 174:1274–80. [PubMed: 15661883]
32. Turley SJ, Inaba K, Garrett WS, Ebersold M, Unternaehrer J, Steinman RM, et al. Transport of peptide-MHC class II complexes in developing dendritic cells. *Science*. 2000; 288:522–7. [PubMed: 10775112]
33. Lutz MB, Kukutsch N, Ogilvie AL, Rössner S, Koch F, Romani N, et al. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J Immunol Methods*. 1999; 223:77–92. [PubMed: 10037236]
34. Rafiq K, Bergtold A, Clynes R. Immune complex-mediated antigen presentation induces tumor immunity. *J Clin Invest*. 2002; 110:71–9. [PubMed: 12093890]
35. Regnault A, Lankar D, Lacabanne V, Rodriguez A, Théry C, Rescigno M, et al. Fcγ receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization. *J Exp Med*. 1999; 189:371–80. [PubMed: 9892619]
36. Carrington M, O'Brien SJ. The influence of HLA genotype on AIDS. *Annu Rev Med*. 2003; 54:535–51. [PubMed: 12525683]
37. Goulder PJ, Watkins DI. Impact of MHC class I diversity on immune control of immunodeficiency virus replication. *Nat Rev Immunol*. 2008; 8:619–30. [PubMed: 18617886]
38. Streeck H, Jolin JS, Qi Y, Yassine-Diab B, Johnson RC, Kwon DS, et al. Human immunodeficiency virus type 1-specific CD8+ T-cell responses during primary infection are major determinants of the viral set point and loss of CD4+ T cells. *J Virol*. 2009; 83:7641–8. [PubMed: 19458000]

39. Andrieu JM, Lu W. A dendritic cell-based vaccine for treating HIV infection: background and preliminary results. *J Intern Med*. 2007; 261:123–31. [PubMed: 17241177]
40. McElrath MJ, Haynes BF. Induction of immunity to human immunodeficiency virus type-1 by vaccination. *Immunity*. 2010; 33:542–54. [PubMed: 21029964]
41. Picker LJ, Hansen SG, Lifson JD. New paradigms for HIV/AIDS vaccine development. *Annu Rev Med*. 2012; 63:95–111. [PubMed: 21942424]
42. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet*. 2008; 372:1881–93. [PubMed: 19012954]
43. McElrath MJ, De Rosa SC, Moodie Z, Dubey S, Kierstead L, Janes H, et al. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. *Lancet*. 2008; 372:1894–905. [PubMed: 19012957]
44. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. *J Infect Dis*. 2005; 191:654–65. [PubMed: 15688278]
45. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, van Griensven F, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. *J Infect Dis*. 2006; 194:1661–71. [PubMed: 17109337]
46. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med*. 2009; 361:2209–20. [PubMed: 19843557]
47. McElrath MJ. Immune responses to HIV vaccines and potential impact on control of acute HIV-1 infection. *J Infect Dis*. 2010; 202(suppl 2):S323–6. [PubMed: 20846040]

Key messages

- Plasma from HIV-positive patients with high complement activity mediates high CTL induction.
- We obtained the first *ex vivo* and *in vitro* evidence for decreased CTL-stimulatory capacity of DCs exposed to antibody HIV.
- There is an implication that a strong antibody response after vaccination contributes to weakening CTL by means of DC modulation.

FIG 1. CD8⁺ T-cell proliferation induced by differentially loaded DCs.

CD8⁺ T-cell expansion by DCs loaded with HIV-C was approximately 7-fold higher compared with that seen in HIV-Ig-loaded DCs ($P < .05$) or HIV-loaded DCs ($P < .05$). SEB-stimulated CD8⁺ T-cell proliferation served as a positive control and was set at 100, and mature DCs (*mDC*) and iDCs were used as negative controls. The CD8⁺ T cell-stimulatory capacity of DCs loaded with HIV-CIg was intermediate. *ns*, Not significant.

FIG 2. Antiviral effects of *in vitro*-generated HIV-specific CTLs.

HIV-C DC-primed CTLs significantly ($P < .001$) blocked viral production in CD4⁺/CD8⁺ T-cell cocultures compared with negative controls (*wo CD8⁺ T cells* and SEB), or HIV-Ig DC-primed CTLs. Inhibition by HIV-CIg- and HIV DC-primed CD8⁺ T cells was intermediate. Percentages of infection compared with infected CD4⁺ T cells alone (*wo CD8⁺ T cells*, 100%) are shown.

FIG 3. VCA of HIV-BaL opsonized with plasma from HIV-positive and healthy subjects. The *left panel* shows IgG coating of HIV opsonized with plasma from 35 HIV-positive patients or 10 healthy control subjects, and the *right panel* shows C3 deposition. The VCA with the abovementioned plasma samples was repeated 3 times with BaL (2×) or 92UG037 (1×).

FIG 4. Selection of HIV-C-high or HIV-Ig-high samples for ELISpot analyses.

C3 fragments and HIV-specific antibodies on the surface of HIV-BaL were analyzed by using the VCA after opsonization of the virus with plasma from HIV-positive patients. Examples from 3 subjects displaying either high complement/low immunoglobulin (*HIV-C high*) or low complement/high immunoglobulin (*HIV-Ig high*) are shown.

FIG 5. ELISpot analyses with HIV-C-high or HIV-Ig-high viral preparations for DC exposure. Induction of IFN- γ by HIV-C-high DCs was significantly stronger than that of HIV-Ig-high DCs ($P < .0001$). As positive controls, peptide (SLYNTVATL)-loaded DCs (*pepDC*) and HIV-C (human C serum, Quidel)-loaded DCs were used, and as negative controls, iDCs and HIV-loaded and HIV-Ig-loaded DCs (HIV, HIV-Ig [MRC IgG-pool]) were used.

FIG 6. Intracellular localization of differentially opsonized HIV in DCs.

HIV-C (*green*) colocalizes with HLA-ABC (*red*) in DCs (**A**, *left panel*) to 94.6% (**B**), whereas HIV-Ig (*green*) only shows minor colocalization (31.5%, Fig 6, *B*) with the MHC class I molecule (Fig 6, *A*, *right panel*). The HIV-CIg preparation showed intermediate colocalization (Fig 6, *A*, *middle panel*; 77.7%; Fig 6, *B*). Cells were permeabilized and stained with labeled anti-human HLA-ABC mAb and anti-HIV p24^{Gag}.

FIG 7. Endosomal characterization of intracellular HIV localization in DCs.

To illustrate, if the enhanced CTL-stimulatory capacity is due to enhanced degradation or multivesicular body colocalization, 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 colocalization with these markers were observable, as depicted in this histogram plot.