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Early on in human immunodeficiency virus (HIV) type 1 infection, gut T-helper (Th) 17 cells are massively depleted leading eventually to compromised intestinal barrier function and excessive immune activation. In contrast, the functional Th17 cell compartment of the gut is well-maintained in nonpathogenic simian immunodeficiency virus infection as well as HIV-1 long-term nonprogressors. Here, we show that dendritic cells (DCs) loaded with HIV-1 bearing high surface complement levels after incubation in plasma from HIV-infected individuals secreted significantly higher concentrations of Th17-polarizing cytokines than DCs exposed to nonopsonized HIV-1. The enhanced Th17-polarizing capacity of in vitro–generated and BDCA-1$^+$ DCs directly isolated from blood was linked to activation of ERK. In addition, C3a produced from DCs exposed to complement-opsonized HIV was associated with the higher Th17 polarization. Our in vitro and ex vivo data, therefore, indicate that complement opsonization of HIV-1 strengthens DC-mediated antiviral immune functions by simultaneously triggering Th17 expansion and intrinsic C3 formation via DC activation.

Keywords. Th17; HIV-1; dendritic cells; opsonization; MAPK; complement.

Previous studies have observed massive depletions of gut T-helper (Th) Th17 cells during the early stages of human immunodeficiency virus (HIV) type 1 or simian immunodeficiency virus (SIV) infection, leading to compromised intestinal barrier function [1–4]. In HIV-1–infected patients, efficient CD4$^+$ T-cell restoration in gut-associated lymphoid tissue was associated with enhanced Th17 cell count and polyfunctional HIV-1-specific T-cell responses [5]. Th17 cells produce cytokines important in the maintenance of an intact epithelium and in host defenses against opportunistic pathogens [6]. The dendritic cell (DC)-secreted cytokines transforming growth factor (TGF) β, interleukin 1β (IL-1β), interleukin 6 (IL-6), and interleukin 23 (IL-23) are responsible for inducing and shaping Th17 cells [7–9]. IL-23 and IL-1β production by DCs depends on activation of mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinases (ERK), and treatment of DCs with an ERK inhibitor attenuated experimental autoimmune encephalomyelitis by suppressing IL-1β and IL-23 secretion [10, 11].

From various disease models including HIV-1 infection, it still remains unclear whether Th17 cells play a protective or harmful role during the immune responses [7, 12]. Th17 cells have been implicated in facilitating mother-to-child transmission of HIV-1, in enhanced susceptibility to HIV-1, in increased viral persistence,

and in amplified HIV transmission [13–16]. On the other hand, recent studies have demonstrated that Th17 cells play a protective role in HIV-1 pathogenesis and in antiretroviral therapy–induced immune reconstitution of the gut mucosa [17, 18]. These studies showed that the integrity of the gut mucosal barrier is damaged owing to loss of Th17 cells in HIV-1 infection, therefore making patients more susceptible to microbial translocation from the gut. Hartigan-O'Connor et al [19] showed that SIV replication in infected macaques depended on the size of the preexisting Th17 cell compartment. Animals with high Th17 cell counts in the blood and gut mucosa before infection showed lower set-point viral load. Furthermore, Ciccone et al [20] found that long-term non-progressors exhibit preserved gut mucosal CD4+ T-cell populations, including Th17 cells, along with intact expression of homing molecules on the CD4+ T-cell subsets.

In the current study, we found that using blood CD4+ T cells that complement opsonization of HIV-1, as predominantly found during the acute phase of infection and also in HIV-1–positive individuals [21], induces Th17 polarization through DC-mediated actions. These complement- and DC-mediated effects were not restricted to HIV, because opsonized beads and *Klebsiella* also induced a Th17-shaping cytokine profile in human and murine DCs. Our data suggest that complement deposition on HIV-1 not only induces an enhanced viral replication in DCs and a higher cytotoxic lymphocyte (CTL) activation via DCs, as illustrated earlier by us [22–24], but also modulates CD4+ T-cell differentiation.

METHODS

Ethics Statement – Blood Samples
For analyzing complement effects on cytokine secretion, HIV opsonized with ultracentrifuged plasma samples from 10 adult HIV-infected individuals was used (Supplementary Data). All subjects gave their written informed consent to participate in the French ANRS controller cohort study. The ethical review committee of Bicêtre Hospital (Comité de Protection des Personnes Ile de France VII, No. 05–22), and the Clinic Research Committee of Institut Pasteur approved the studies performed. Written informed consent was obtained from the participating blood donors by the Central Institute for Blood Transfusion and Immunological Department, Innsbruck, Austria.

Ethics Statement - Mice
Animal experiments were approved by the Committee on the Ethics of Animal Experiments of the Austrian Ministry (BMWF-66.011/0011-II/10b/2010). C57BL/6 wild-type male mice were kept on a standard rodent diet (C2010 Altromin).

DC Differentiation and CD4+ T-Cell Isolation
Human monocytes were isolated from whole blood by magnetic bead separation, and differentiated into immature DCs (iDCs),

which were analyzed as described in the Supplementary Data and elsewhere [22, 25]. Nonspecific DC maturation by microvesicle compounds of HIV-1 inocula [26] was excluded by fluorescence-activated cell sorting (FACS) analyses of characteristic DC activation markers (CD83, CD86, and CCR7) because non-opsonized HIV only caused low maturation (not shown). Naive CD4+CD45RA+ T cells were bead purified (>95% purity) from the remaining peripheral blood lymphocytes using the Human Naive CD4+ T-cell Enrichment Set–DM (BD Biosciences) according to the manufacturer and characterized for their naive phenotype by flow cytometry. After isolation and characterization, CD4+ T cells were stimulated with differentially loaded autologous DCs or supernatants. Blood BDCA-1+CD1c+ DCs were isolated using the Dendritic Cell Enrichment Set–DM (BD Biosciences), according to the manufacturer's instructions. Murine bone marrow DCs (bmDCs) were generated as described by Inaba et al [27], with slight modifications. Briefly, 2×10^6 bmDCs were cultivated in 10 mL of DC medium (Roswell Park Memorial Institute 1640 medium supplemented with 10% fetal calf serum, 2 mmol/L L-glutamine, 500 nmol/L 2-mercaptoethanol, 100 U/mL penicillin-streptomycin, and 1000 U/mL mouse granulocyte-macrophage colony-stimulating factor) for 3 days at 37°C. After that, 10 mL of fresh DC medium was added, and cells were cultured for another 3 days. On day 6, nonadherent cells were removed, washed, and cultivated in 20 mL of fresh DC medium. Nonadherent cells obtained after 8 days displayed a myeloid DC phenotype (>85% CD11c and >95% CD11b), as revealed by FACS analyses.

Virus Preparation and Opsonization
HIV-1 (92UG037, Ba-L, 92BR030) supernatants from infected peripheral blood mononuclear cell cultures were filtered through filters with 0.22-μm pores, concentrated by ultracentrifugation (20 000 rpm at 90 minutes and 4°C) and resuspended in Roswell Park Memorial Institute -1640 medium without any supplements. The virus concentration was determined by p24 enzyme-linked immunosorbent assay (ELISA) [28]. To obtain complement-opsonized HIV (HIV-C), the virus was incubated in commercially available human complement serum (Quidel) or plasma from HIV-positive individuals, while C3-deficient serum (Quidel) or plasma from healthy controls was used for the nonopsonized virus preparation (HIV). After opsonization, the virus preparations were thoroughly washed and resuspended in medium. The opsonization pattern of HIV-1 was determined by virus capture assay as described elsewhere [21, 24]. Briefly, plates were coated using mouse-anti-human C3c/C3d, anti-human immunoglobulin (Ig) G, or anti-mouse IgG antibodies. Plates coated with anti-human IgG served as a control for binding of natural antibodies to the HIV surface, with anti-mouse IgGs used as a negative control for background binding. Plates were incubated overnight with the virus preparations (2.5 ng of p24 per well) at 4°C and then washed

5 times to remove unbound virus. Bound virus was lysed (2% Igepal) and transferred to a precoated p24 ELISA [28] plate to measure the C3 or IgG deposition on the viral surface. Background binding to mouse IgGs was subtracted. Figure 1A depicts a representative example for a virus capture assay.

Opsonization of *Klebsiella*/Latex Beads

Klebsiella pneumoniae subsp. *pneumoniae* American Type Culture Collection 43 816 was heat inactivated for 30 minutes at 75°C. After that, 0.8-μm latex beads (Sigma-Aldrich) or *Klebsiella* were incubated with mouse or human serum for 2 hours at 37°C, and then the beads or bacteria were washed and resuspended in medium. Finally, human DCs or murine bmDCs (0.3×10^6 cells for each condition) were incubated for 6 hours at a 1:10 ratio with nonopsonized or C3-opsonized latex beads/bacteria to evaluate cytokine production from supernatants.

Microarray Analyses

Day 5 iDCs (0.5×10^6 cells for each condition) were exposed to lipopolysaccharide (LPS) (100 ng/mL; Sigma-Aldrich), HIV, or HIV-C (300 ng p24/mL each) or left untreated (iDC control) for 24 hours. All experimental parameters, protocols, and raw and transformed data were submitted to a public repository (ArrayExpress; E-MEXP-3706), and the procedure is described in the Supplementary Data. Whole Human Genome Microarray Kits 4 × 44 K (Agilent Technologies) were used to determine up- or down-modulated genes in treated versus untreated cells.

Real-time Reverse-Transcription Polymerase Chain Reaction

Cytokines (human and mouse IL-1B, IL-6, and IL-23A, and human type I interferon [IFN]) were analyzed by real-time reverse-transcription polymerase chain reaction (RT-PCR), as described elsewhere [29], using gene-specific primer/probe pairs (TaqMan Gene Expression Assays; Life Technologies). Glyceraldehyde 3-phosphate dehydrogenase (human) or hypoxanthine-guanine phosphoribosyltransferase (mouse) PCR using specific primer-probe pairs (Life Technologies) analyses served as internal controls to quantify the relative gene expression of target genes. The iScript One-Step RT-PCR Kit for Probes (BioRad) was used for target amplification, and assays were performed on the CFX96 real-time detection system (BioRad). The cycling conditions were as follows for all analyses: 10 minutes at 50°C (reverse transcriptase); 3 minutes at 95°C; 40 cycles: 5 seconds at 95°C and 15 seconds at 60°C. The results were analyzed using the gene expression software of the cycler (CFX Manager Software, ΔΔCt method) [30].

Cytokine and Anaphylatoxin Analyses by ELISA and Intracellular FACS

Protein levels of cytokines (human and murine IL-1β, IL-6, IL-23p19, interleukin 12p40 [IL-12p40]) or anaphylatoxins (human C3a and C5a) secreted by DCs were measured by ELISA (Biolegend; eBiosciences). For intracellular FACS

analyses, differentially loaded DCs were first stained for characteristic surface antigens (CD11b and CD11c), fixed with paraformaldehyde, and then permeabilized using Cytofix/Cytoperm (BD Biosciences), allowing anti-human cytokine monoclonal antibodies access to intracellular compartments. Cells were incubated with GolgiStop protein transport inhibitor (BD Biosciences) before beginning the staining procedure. All samples were measured using a BD FACSVerse system.

Functional Assay for Secreted Cytokines

T-cell receptor–independent differentiation of naive CD4^{+} T cells by cytokines has been reported elsewhere [31]. Therefore, to test the functionality of the Th17-polarizing effects, naive CD4^{+} T cells were incubated with supernatants of differentially treated DCs at 24 hours after incubation. CD4^{+} T cells were incubated for 5 days in the supernatants and then restimulated with phorbol myristate acetate/ionomycin for 5 hours in the presence of GolgiStop protein transport inhibitor. The percentages of T-helper subsets were measured by intracellular FACS analyses using anti-human monoclonal antibodies against IL-17A, IFN-γ, TGF-β, and IL-10 (BD Biosciences). In parallel, DCs and CD4^{+} T cells were cocultured to monitor the cell-cell–dependent differentiation of IL-17–secreting CD4^{+} T cells.

Western Blotting

The iDCs, in the presence or absence of mitogen-activated protein kinase1/2 inhibitor U0126 (5 μmol/L) or p38 MAPK inhibitor SB203580 (10 μmol/L), were incubated with HIV or HIV-C (25 ng p24/mL). Cell lysates were prepared, assayed on 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels, and transferred to polyvinylidene fluoride membranes. Blots were incubated with anti–phosphorylated ERK, or anti-ERK (Santa Cruz Biotechnology) and with horseradish peroxidase–conjugated secondary antibodies and developed using enhanced chemiluminescence.

Statistical Analyses

All values shown in the figures are expressed as means and standard deviations for a given number of determinations. For comparisons between 2 groups, statistical differences were analyzed using GraphPad prism software with the unpaired Student t test (2 tailed). Multiparameter comparisons were performed using 1-way analysis of variance and the Bonferroni posttest.

RESULTS

Th1/Th17-Inducing Cytokine Profile in Human DCs by Complement Opsonization of HIV

To determine the effect of HIV-1 complement opsonization, we performed microarray analyses according to the scheme in Supplementary Figure 1A. For these, the opsonization patterns of HIV preparations incubated with human complement serum (HIV-C) or C3-deficient serum as a control for nonopsonized
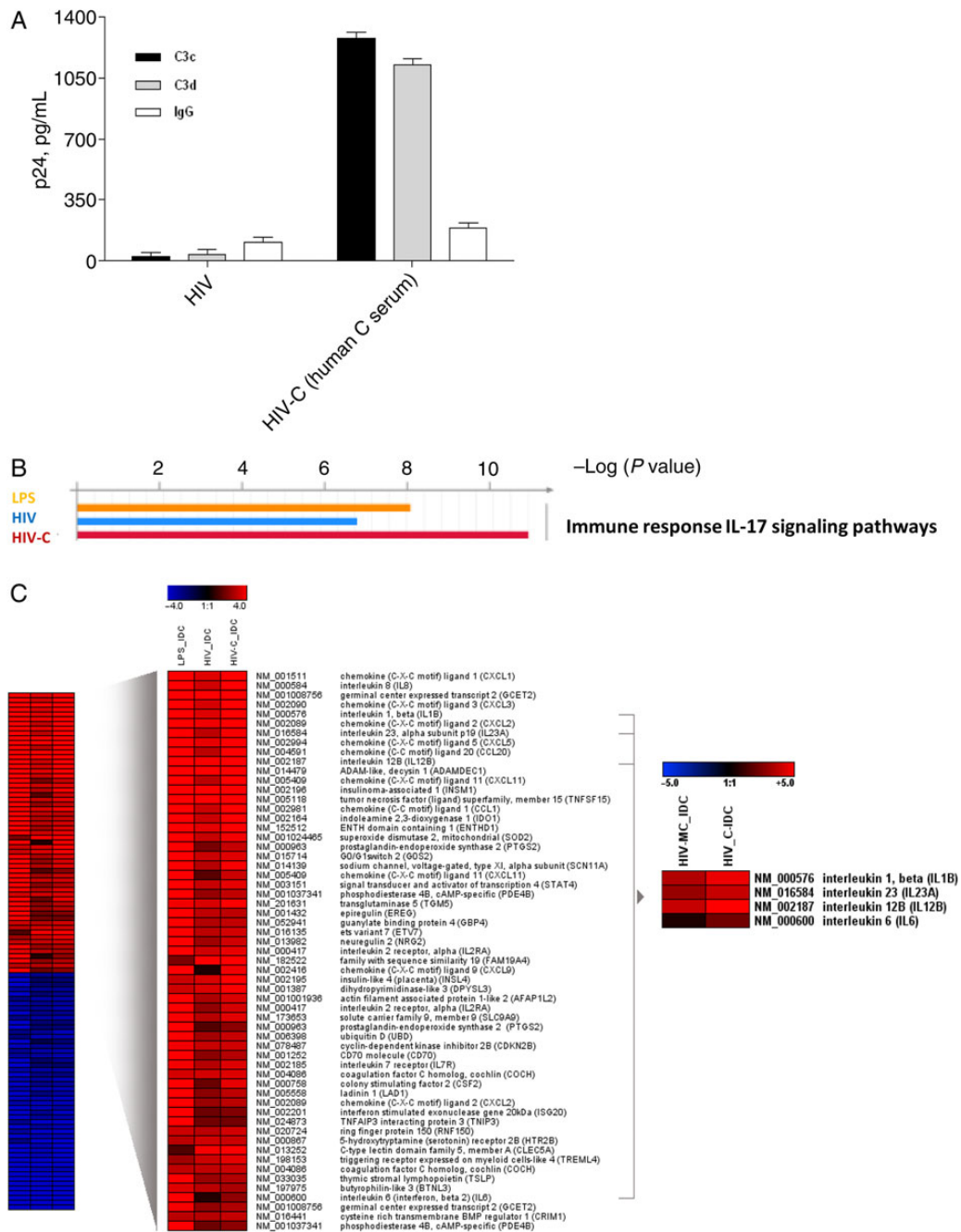
Figure 1. *A*, Characterization of the viral surface after opsonization. C3c, C3d, and immunoglobulin (Ig) G deposition on the human immunodeficiency virus (HIV) surface using C3-deficient serum (HIV) or human complement serum (HIV-C) was characterized by virus capture assay, as described in the Methods section. Although nonopsonized HIV did not bind to any of the coated antibodies (anti-human C3c or C3d, anti-human IgG), a strong binding of complement-opsonized HIV (HIV-C) to anti-human C3c and C3d was observed and only background binding to anti-human IgG. Background binding of the virus preparations to anti-mouse IgG antibodies was subtracted from C3c, C3d, and anti-human IgG values. A virus capture assay is routinely performed after HIV opsonization, and a representative graph is depicted. *B*, T-helper (Th) 17-inducing cytokine profile is mediated by HIV-C in dendritic cells (DCs). Enrichment analyses by MetaCore software after microarray analyses of DCs exposed to lipopolysaccharide (LPS), HIV, or HIV-C for 24 hours revealed that immune responses to interleukin 17 (IL-17) signaling pathways are mainly mediated by HIV-C and to a lower extent by LPS and HIV. *C*, For heat map visualization (*left*; values presented in log scale) most deregulated genes were presented, and for the sake of limitation in space the arbitrary stringent cutoff of 8-fold deregulation was used. Blue in the heat map represents expression values below and red expression above control values. All of these genes were significant in ≥1 of the 3 conditions (LPS, HIV, HIV-C), compared with untreated DCs, according to commonly used definitions of differentially expressed genes (≥2-fold changes and adjusted *P* value (false discovery rate) < .1). Among the highest up-regulated genes were the Th17-polarizing cytokines interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 12B (IL-12B), and interleukin 23A (IL-23A) (*right*). IL-1β, IL-12B, and IL-23A were up-regulated in DCs treated with HIV and HIV-C, whereas IL-6 was increased in HIV-C–treated DCs only. Microarrays were performed using DCs from 5 donors treated with LPS or R5-tropic strains 92UG037 or BaL.
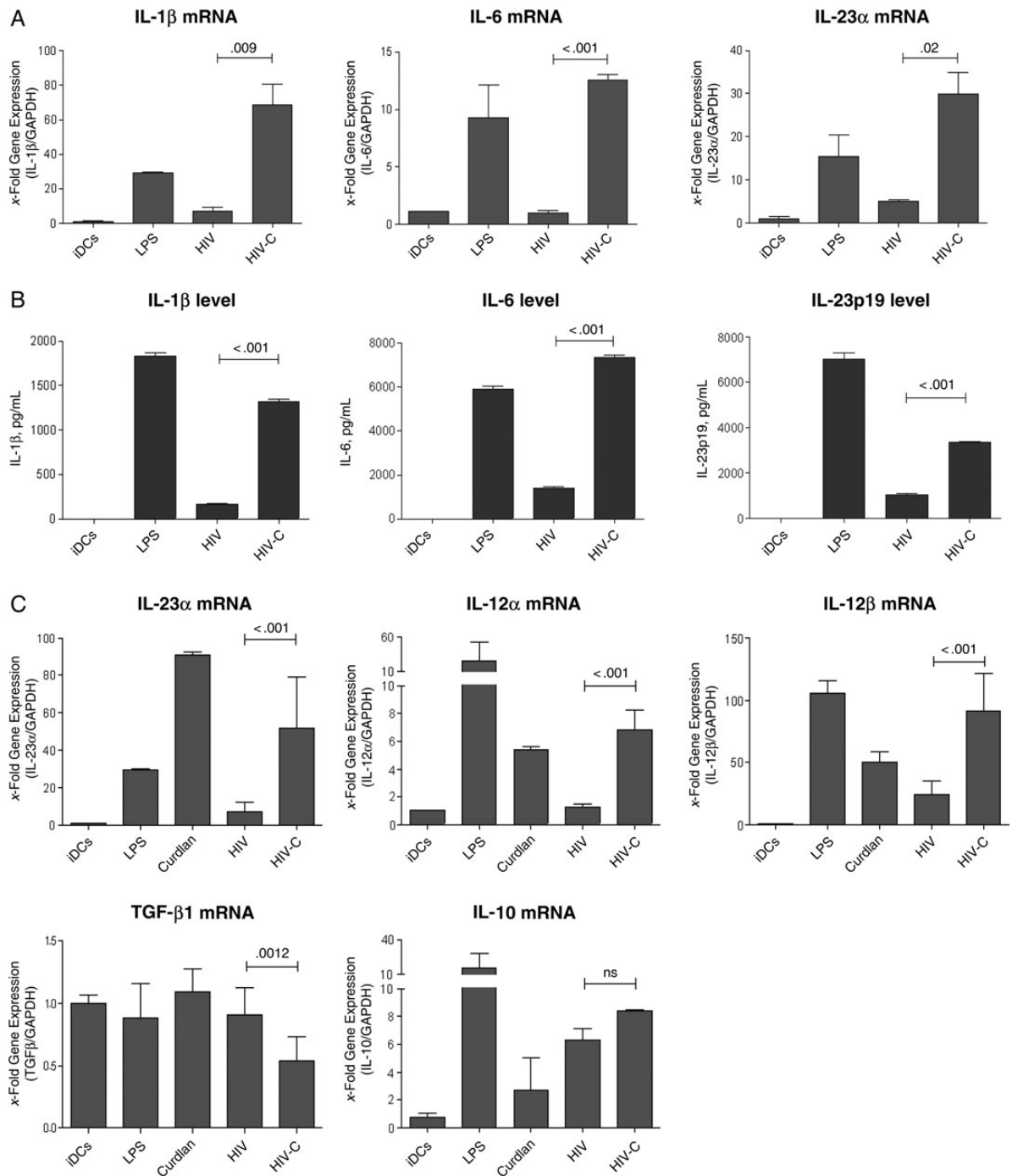
Figure 2. *A, B,* Complement-opsonized human immunodeficiency virus (HIV-C) up-regulates interleukin 1β (IL-1β), interleukin 6 (IL-6), and interleukin 23 (IL-23) messenger RNA (mRNA) (*A*) and protein levels (*B*) in dendritic cells (DCs). *A,* Compared with nonopsonized human immunodeficiency virus (HIV), HIV-C mediated significantly higher IL-1β (*P* = .009) (*left*), IL-6 (*P* < .001) (*middle*), and IL-23A (*P* = .02) (*right*) mRNA expression in DCs. The target genes were quantified relative to normalization of the reference gene glyceraldehyde 3-phosphate dehydrogenase and analyzed using the ΔΔCt method. Real-time quantification was repeated 4 times in triplicate for each cytokine, summarized, and evaluated for significant changes between the 2 groups (HIV and HIV-C) using the unpaired Student *t* test (2 tailed). *B,* DCs treated with HIV-C also secreted significantly higher amounts of IL-1β (*left*), IL-6 (*middle*), and IL-23p19 (*right*) (all *P* < .001) compared with those treated with HIV, as measured with enzyme-linked immunosorbent assay. Protein levels were evaluated for each cytokine from 4 donors, summarized and analyzed for significant differences between HIV- and HIV-C–incubated DCs using the unpaired Student *t* test (2 tailed); *P* values are shown above graphs. *C,* A T-helper (Th) 1/Th17–polarizing cytokine pattern is mediated in HIV-C–treated DCs. IL-23A (*upper left*), interleukin 12A (IL-12A) (*upper middle*), and IL-12B (*upper right*) are significantly up-regulated in HIV-C–treated DCs in contrast to HIV-treated or immature DCs (iDCs). Dependent on the cytokine analyzed, lipopolysaccharide (LPS) or curdlan served as a positive control. In contrast, transforming growth factor (TGF) β1 mRNA levels significantly lower in HIV-C–treated than in HIV-treated DCs (*lower left*), and interleukin 10 (IL-10) levels were similar (*lower right*). Triplicates of real-time reverse-transcription polymerase chain reaction values obtained from 4 donors are shown.
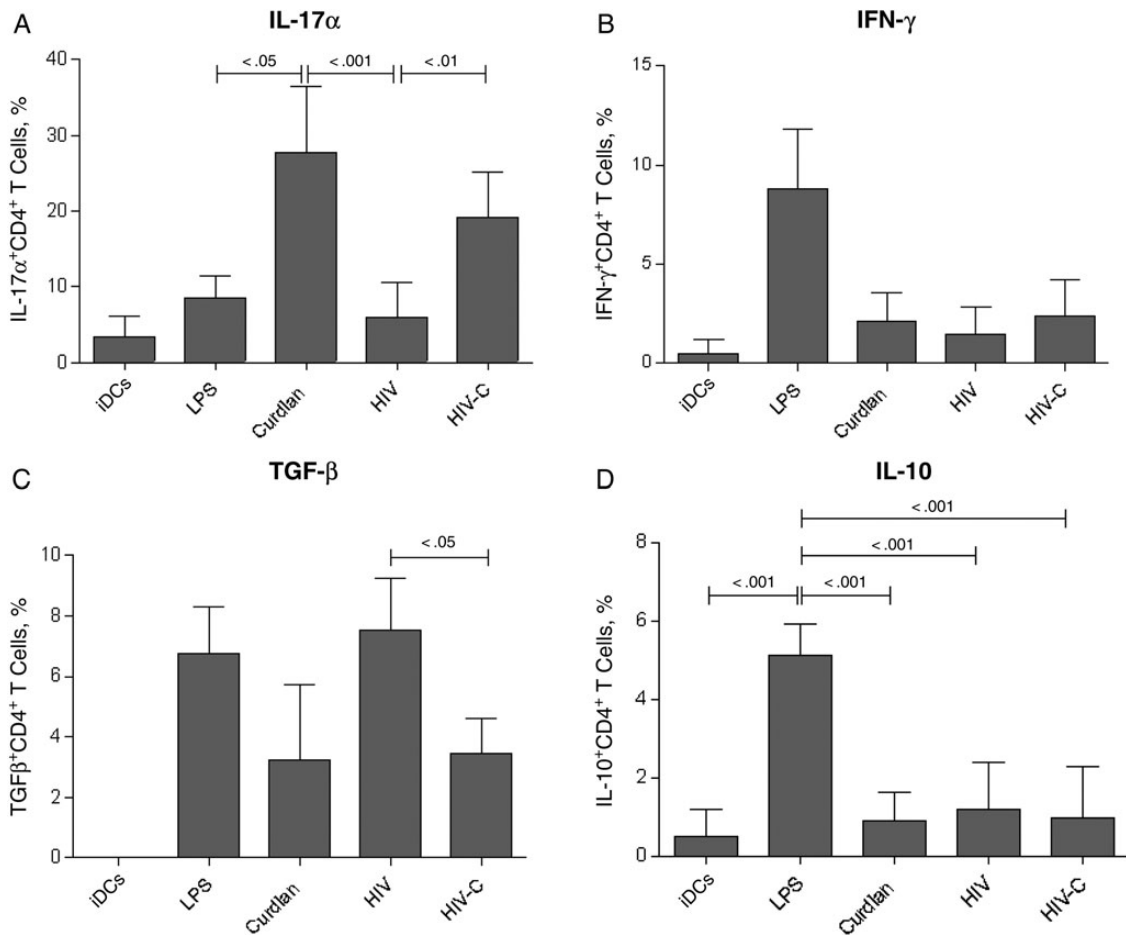
Figure 3. The T-helper (Th) 17–inducing cytokines secreted from complement-opsonized human immunodeficiency virus (HIV-C)–exposed dendritic cells (DCs) exhibit high polarization potential. *A,* The supernatants of curdlan- and HIV-C–treated DCs stimulated naive CD4+ T cells to differentiate into an interleukin 17a (IL-17a)+/CD4+ T cells at significantly higher levels than supernatants from immature DCs (iDCs) or lipopolysaccharide (LPS)- or human immunodeficiency virus (HIV)–treated DCs, as measured by intracellular fluorescence-activated cell sorting analyses. *B,* Similar percentages of interferon (IFN) γ+CD4+ T cells were detected after treatment with supernatants of HIV- or HIV-C–treated DCs, whereas LPS especially induced higher mean percentages of Th1 cells. *C,* Significantly lower amounts of transforming growth factor (TGF) β+CD4+ T cells were induced by supernatants from HIV-C–treated compared with HIV-treated DCs. Supernatants from LPS- and HIV-treated DCs mediated the highest percentages of regulatory T-cell generation in contrast to supernatants from curdlan- or HIV-C–treated DCs. iDC supernatants served as negative controls. *D,* Interleukin 10 (IL-10) induction was low under all tested conditions excluding supernatants from LPS-treated DCs. For all analyses, induction of naive CD4+ T-cell differentiation into Th17 cells was evaluated by the addition of variously treated DC supernatants (n = 7). Statistical analysis between groups was performed using 1-way analysis of variance with a Bonferroni posttest; *P* values are shown above graphs.

HIV (HIV) were analyzed by a virus capture assay, as described in "Methods" section and depicted in Figure 1*A.* In independent experiments, iDCs from 5 donors were loaded with HIV, HIV-C, or LPS or left untreated. LPS treatment served as positive control for DC maturation. Within each donor, all treated samples were normalized to untreated iDCs to exclude donor-specific variations. Pathway analyses for up- and down-regulated genes of DCs exposed to LPS, HIV, or HIV-C were obtained using MetaCore analyses software (GeneGo), and the major immune response stimulated in iDCs, due to HIV-C treatment, was dedicated to the Th1/Th17-inducing pathway (Figure 1*B*). As outlined in the Venn diagram (Supplementary Figure 1*B*)

and Figure 1*C*, HIV-C–exposed DCs showed the most prominent changes in expression of the Th1/Th17-activating cytokines IL-1β, IL-6, IL-12B, and IL-23, compared with DCs loaded with nonopsonized HIV (Figure 1*C*). No increase in IL-6 formation was measured in HIV-exposed DCs compared with iDCs, indicating that HIV-C exclusively triggers a Th17- or Th1/Th17-polarizing program in DCs.

Th17-Polarizing Cytokine Secretion by HIV-C–Treated DCs

Next, we investigated whether HIV-C induces a Th17-polarizing cytokine profile on the messenger RNA (mRNA) and protein level. In addition, we wanted to exclude the possibility that
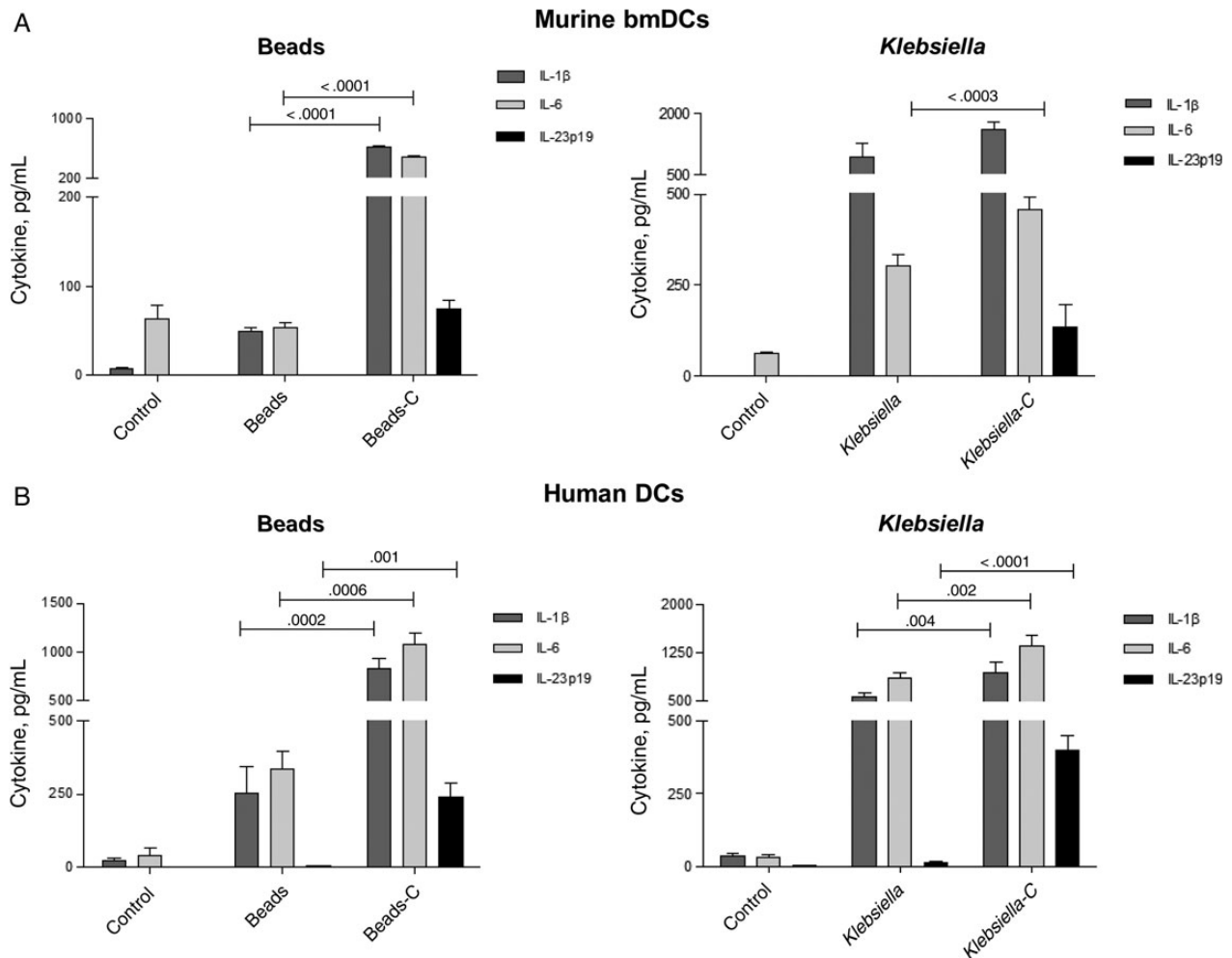
Figure 4. Complement-opsonized beads or *Klebsiella* also mediate secretion of T-helper 17–inducing cytokines in murine bone marrow dendritic cells (bmDCs) and human dendritic cells (DCs). *A*, Latex beads (*left*) or heat-inactivated *Klebsiella* (*right*), which were first opsonized with mouse complement serum, induced secretion of interleukin 1β (IL-1β) (*dark gray bars*), interleukin 6 (IL-6) (*light gray bars*), and interleukin 23p19 (IL-23p19) (*black bars*) by murine bmDCs. Nonopsonized beads led to low and nonopsonized bacteria to higher IL-1β and IL-6 production. Importantly, only complement-opsonized beads (beads-C) or bacteria (*Klebsiella*-C) induced high secretion of IL-1β, IL-6, and IL-23A in murine bmDCs compared with control cells. *B*, Human DCs were incubated with same amounts of beads and heat-inactivated *Klebsiella* previously opsonized with human complement serum. Moreover, higher production of IL-1β (*dark gray bars*) and IL-6 (*light gray bars*) were detected with *Klebsiella* (*right*) than with beads (*left*), but only C-opsonized beads and *Klebsiella* showed production of IL-23p19 (*black bars*) by DCs. Values represent means and standard deviations from 5 experiments performed in triplicate. Significant differences between groups were assessed by 1-way analysis of variance with a Bonferroni posttest; *P* values are shown above graphs.

complement opsonization induces an increased nonspecific cytokine formation in DCs in general. iDCs stimulated with HIV-C showed significantly increased IL-1β and IL-6 mRNA levels compared with HIV-exposed DCs (Figure 2*A*). We also found that DCs exposed to HIV-C showed a significantly higher expression of IL-23A than those exposed to HIV (Figure 2*A*), as observed here using microarray analyses (Figure 1). Furthermore, for the protein level, DCs stimulated with HIV-C secreted significantly higher amounts of IL-1β, IL-6, and IL-23p19 than HIV-treated DCs (Figure 2*B*).

Next we studied cytokine profiles related to the major T-helper subsets, and we assayed IL-12p35 (IL-12A), IL-12p40 (IL-12B), IL-23p19 (IL-23A), TGF-β, and IL-10 from human DCs stimulated with HIV or HIV-C. Beside LPS, which was used as positive control for DC activation in the microarrays, we included curdlan as a positive control for Th17-polarizing effects. We found that DCs exposed to HIV-C not only up-regulates mRNA expression of IL-23A compared with HIV but also increases IL-12A and IL-12B expression (Figure 2*C*). Simultaneously TGF-β transcript levels were significantly lower in HIV-C–treated DCs, and no significant change was observed in IL-10 mRNA expression between HIV-C– and HIV-treated DCs (Figure 2*C*). LPS-stimulated DCs preferentially up-regulated a Th1 or Th2-polarizing cytokine profile in DCs

(Figure 2C), whereas curdlan served as positive control for Th17-shaping cytokines (Figure 2C).

Th17 Polarization by HIV-C–Treated DCs

We were then interested in assessing the ability of supernatants released from HIV-C–treated DCs, to facilitate the differentiation of naive CD4$^+$ T cells into the various T-helper subsets. LPS-, curdlan-, HIV-, or HIV-C–treated human DC supernatants were added to autologous CD4$^+$ T cells. After incubation, the numbers of IL-17/IFN-γ/TGF-β– and IL-10–positive T cells were measured by intracellular FACS analyses. These revealed that significantly more CD4$^+$ T cells differentiated into IL-17^{high}/CD4^{high} T cells after incubation with supernatants from DCs exposed to HIV-C as compared with DCs loaded with nonopsonized HIV (Figure 3A). No significant differences in Th17 cell percentages were observable using supernatants from curdlan- or HIV-C–treated DCs (Figure 3A). IFN-γ$^+$ and IL-10$^+$/CD4$^+$ T cells were polarized at low numbers by both HIV- and HIV-C–treated DC supernatants (Figure 3B and 3D), whereas HIV-treated DC supernatants preferentially polarized TGF-β^{high}/CD4^{high} T cells (Figure 3C). LPS- or curdlan-exposed DC supernatants served as positive and iDCs as negative controls, respectively (Figure 3A–D). These data indicate that HIV-C induces stronger Th17 responses than HIV.

Th17-Polarizing Cytokine Production in Human and Murine DCs by Complement-Opsonized Beads or *Klebsiella*

To illustrate whether Th17 differentiation mediated by complement opsonization is restricted to retroviruses or also found associated with other immune challenges and species, we studied the effects of C-opsonized latex beads or *Klebsiella* in a mouse model. We found that IL-1β and IL-6, but not IL-23, were secreted at low levels by murine bmDCs exposed to beads or higher levels by *Klebsiella,* a gram-negative bacterium stimulating proinflammatory cytokines (Figure 4A). In contrast, C-opsonized beads (Figure 4A, left) or *Klebsiella* (Figure 4A, right) also mediated IL-23 release from murine bmDCs as well as higher secretion of IL-1β and IL-6. In addition, human DCs were treated with nonopsonized and opsonized beads (Figure 4B, left) and *Klebsiella* (Figure 4B, right). IL-1β and IL-6, but not IL-23p19, were secreted by DCs after stimulation with nonopsonized beads and *Klebsiella* (Figure 4B). Production of IL-1β, IL-6 and particularly of IL-23p19 by human DCs exposed to C-opsonized beads or *Klebsiella* was significantly higher (Figure 4B). Interestingly, IL-1β/IL-6/IL-23 production was higher in human DCs exposed to HIV-C than in C-opsonized *Klebsiella* or beads.

High Levels of Th17-Polarizing Cytokines Production by DCs Exposed to Samples From HIV-Positive Individuals

We previously demonstrated that the HIV-specific CTL response mediated by DCs depends on the proportion of complement fragments or IgGs deposited on the viral surface [21]. Here we investigated the effect of high and low complement deposition on induction of Th17-polarizing cytokines by DCs using plasma samples from HIV-positive individuals for in vitro opsonization. We chose 5 samples each displaying high or low complement deposition on the virus independent on viral load, CD4$^+$ T-cell count, or treatment of HIV-positive individuals (Figure 5A).

Cytokine ELISAs from supernatants (Figure 5B) and intracellular FACS analyses of DCs (Supplementary Figure 2) revealed that, besides curdlan, preparations with high complement deposition and HIV-C (human C serum; Quidel) also led to significantly higher protein levels of IL-1β, IL-6, and IL-23 (Figure 5B and Supplementary Figure 2). In contrast, preparations with low complement deposition and nonopsonized HIV (Figure 5B and Supplementary Figure 2) did not demonstrate such a strong induction of Th17-polarizing cytokines. We also performed IL-10 detection to confirm the specific effect by HIV-C. IL-10 is a known inhibitor of effector T-cell function and promoter of regulatory T-cell development [32]. With these analyses, we found that IL-10 was only produced at high levels by DCs exposed to LPS or to a lower amount to curdlan, whereas all HIV-C preparations mediated only secretion of background IL-10 levels (Figure 5B).

Enhanced ERK2 Phosphorylation in HIV-C–Treated DCs

Because IL-23 and IL-1β production by DCs was demonstrated to be mediated via ERK phosphorylation [10], we tested whether ERK1 and ERK2 are activated to higher levels by HIV-C in iDCs. We found that ERK2 particularly was phosphorylated to a higher degree in HIV-C–treated DCs compared with HIV-treated DCs or untreated controls (Figure 6A). Figure 6B summarizes the relative P-ERK2 protein levels from 5 donors. We here show enhanced ERK2 activation on stimulation of DCs with HIV-C.

Abrogation of Th17-Polarizing Cytokines in DCs by Blocking the ERK Path

We next analyzed cytokine production and transcript levels of monocyte-differentiated DCs (not shown) as well as BDCA-1$^+$ DCs (Figure 6C and Supplementary Figure 3) in the presence of the ERK and p38 MAPK path inhibitors. To exclude the possibility that DCs differentiated from monocytes behave differently from primary DCs, BDCA-1$^+$/CD1c$^+$ DCs directly isolated from blood were also analyzed. Pretreatment of both DC subtypes with U0126 completely abrogated IL-1β, IL-6, and IL-23 cytokine secretion (Figure 6C) and mRNA formation (Supplementary Figure 3) from HIV-C–treated DCs, whereas SB203580 showed similar protein (Figure 6C) and mRNA (Supplementary Figure 3) levels compared with control cells. ERK1/2 activation plays a crucial role in HIV-C–stimulated DCs and BDCA-1$^+$ DCs to produce the Th17-polarizing cytokines IL-1β, IL-6, and IL-23.

Elevated Levels of C3a by HIV-C–Treated DCs

Beside enhanced ERK phosphorylation, C3aR- and C5aR-mediated signaling was recently illustrated as vital components
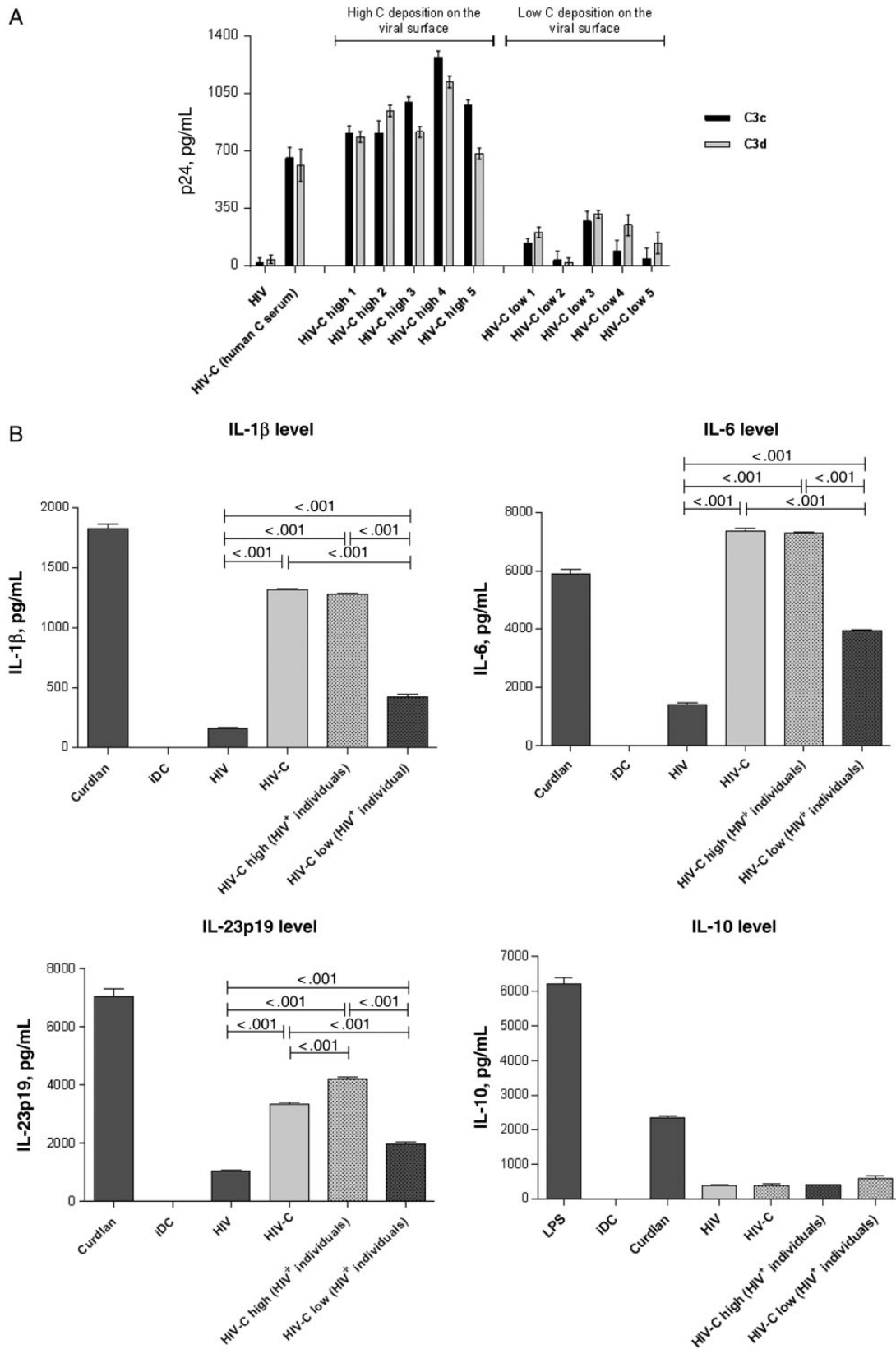
Figure 5. *A*, Plasma samples from human immunodeficiency virus (HIV)–positive individuals exhibit a diverse complement activation pattern. C3 fragments C3c and C3d on the surface of HIV-BaL were analyzed by virus capture assay after opsonization of the virus using plasma from HIV-positive individuals. Results from 5 individuals displaying either high (HIV-C high) or low (HIV-C low) complement deposition on the viral surface are shown. HIV opsonized with commercially available complement serum (HIV-C) served as a positive control and nonopsonized HIV as a negative control. *B*, HIV-C
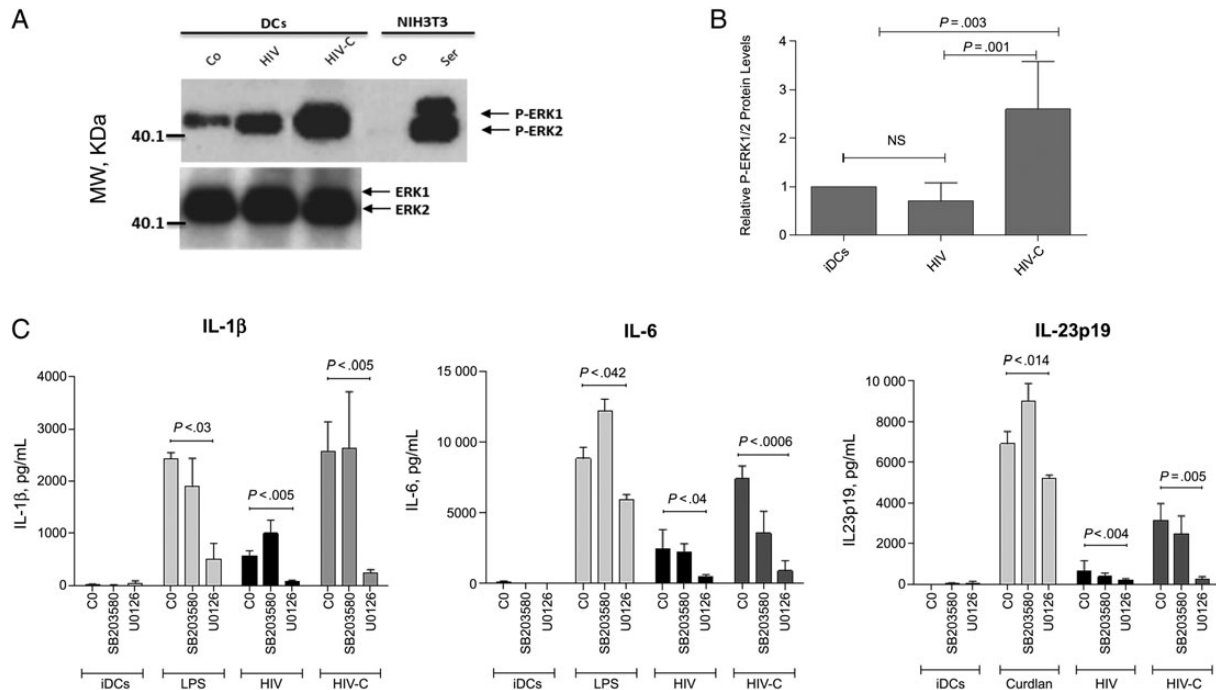
Figure 6. *A*, Exposure with complement-opsonized human immunodeficiency virus (HIV-C) induces a stronger extracellular signal-regulated kinases (ERK) phosphorylation in immature dendritic cells (iDCs) compared with human immunodeficiency (HIV). The iDCs stimulated with HIV-C for 15 minutes showed a stronger phosphorylation of ERK2 compared with untreated control cells or cells exposed to HIV (*lanes 1–3, upper panel*). As controls nonphosphorylated (*lane 4, upper panel*) and serum-stimulated (*lane 5, upper panel*) NIH3T3 cell extracts were loaded on the gel. In all figures same protein amounts were verified using the nonphosphorylated mouse-anti-human ERK1/2 antibody (*lower panel*). A representative blot from 1 donor is depicted. The results were confirmed with 3 distinct HIV-1 strains (BaL, 92UG037, and 92BR030) in 5 independent experiments. Co, non-phosphorylated control; P-ERK1, phosphorylated extracellular signal-regulated kinases 1; P-ERK-2, phosphorylated extracellular signal-regulated kinases 2; Ser, serum-stimulated; MW, molecular weight. *B*, summarizes relative P-ERK activation levels from 5 donors. *C*, Inhibition of the ERK pathway in BDCA-1+ dendritic cells (DCs) abrogates induction of T-helper (Th) 17–polarizing cytokines. A 30-minute pretreatment of freshly isolated BDCA-1+ DCs with 5 µmol/L mitogen-activated protein kinase (MEK1/2) inhibitor U0126 impaired the secretion of Th17-polarizing cytokines interleukin 1β (IL-1β) (*left*), interleukin 6 (IL-6) (*middle*), and interleukin 23p19 (IL-23p19) (*right*) from DCs. The decrease in cytokine production after incubation with U0126 was significant for all treatments (lipopolysaccharide [LPS], curdlan, HIV, and HIV-C) compared with the cocondition, as indicated in the figure. Inhibition of p38 mitogen-activated protein kinase using 10 µmol/L SB203580 had no significant effect on the cytokines. Data were confirmed in 3 independent experiments performed in duplicate; *P* values are shown above graphs.

of the Th1 and Th17 axis [33–35]. Therefore, we measured C3a and C5a concentrations in supernatants from LPS-, HIV- or HIV-C–treated DCs and untreated controls. We found that HIV-C caused significantly higher C3a levels in the supernatants than did HIV (Figure 7). C3a was not detected in supernatants from iDCs and LPS-treated DCs (Figure 7). C5a was undetectable in all samples.

DISCUSSION

Th17 cells are essential for intestinal epithelial integrity and preventing the invasion and translocation of specific gut bacteria in the surrounding tissue [3, 19, 36]. Lower viral loads before SIV infection were associated with high Th17 cell counts in the blood and gut mucosa [19]. In the current study, we showed

Figure 5 continued. high preparations mediate significantly higher secretion of interleukin 1β (IL-1β), interleukin 6 (IL-6), and interleukin 23p19 (IL-23p19) but not interleukin 10 (IL-10) from dendritic cells (DCs). IL-1β (*upper left*), IL-6 (*upper right*), and IL-23p19 (*lower left*) were secreted in significantly higher amounts (*P* < .001) by DCs stimulated with curdlan, HIV-C, and HIV-C high compared with HIV and HIV-C low. Lipopolysaccharide (LPS) treatment of DCs caused a high production of IL-10, compared with intermediate production for curdlan treatment. Immature DCs (iDCs) served as negative controls, and LPS- and curdlan-stimulated DCs as positive controls. Enzyme-linked immunosorbent assays were performed 3 times in triplicate using differentially opsonized HIV. Values from the experiments were analyzed using 1-way analysis of variance and Bonferroni multiple comparison test for selected groups; *P* values are shown above graphs.
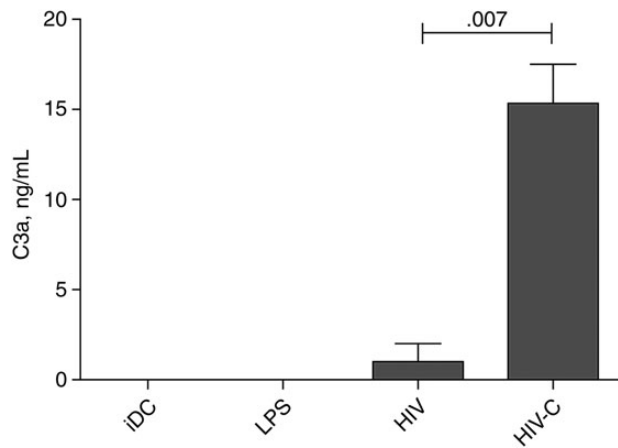
Figure 7. Elevated amounts of C3a are detected in supernatants of dendritic cells (DCs) treated with complement-opsonized human immunodeficiency virus (HIV-C). C3a generation was measured by enzyme-linked immunosorbent assay using supernatants of immature DCs (iDCs) or DCs exposed for 24 hours to lipopolysaccharide (LPS), human immunodeficiency virus (HIV), or HIV-C. These assays revealed that C3a generation was unique for DCs exposed to HIV-C, whereas no or very low amounts of this anaphylatoxin were generated when DCs were left untreated or stimulated with LPS or HIV. The experiments were repeated 3 times in duplicate; *P* value is shown above graph.

in vitro and ex vivo, using blood CD4$^+$ T cells, that complement opsonization of HIV-1 before DC exposure mediates polarization of Th17 cells, compared with DC incubation with HIV bearing low surface complement levels [21]. The enhanced Th17-polarizing mechanism is not limited to retroviruses but is also found on incubation with complement-opsonized latex beads or *Klebsiella*.

In previous in vitro and in vivo studies, we and others have shown that DCs exposed to complement-opsonized retroviruses strongly enhanced viral replication [23, 24, 37] and activation of CTLs [22]. In contrast, antibody coating of HIV-1 significantly attenuated CTL induction [21] and DC infection [24]. Here, we found that complement opsonization of HIV-1 together with DCs not only modifies CD8$^+$ T-cell priming [21, 22] but also affects induction of T-helper subsets. Immediate polarization of Th17 cells by complement- and DC-mediated actions might strengthen the immune defense against opportunistic pathogens especially during early HIV infection.

As seen in mice, complement components C3, factor B, and factor D were found to be locally produced by DCs after interaction with CD4$^+$ T cells [34]. This resulted in activation of C3 and C5 convertases and the generation of complement anaphylatoxins C3a and C5a. Binding of these fragments to C3aR or C5aR induced costimulatory and survival signals for effector T-cell responses [34, 35]. Weaver et al [38] demonstrated that C3aR and C5aR were responsible for the differentiation of CD4$^+$T cells into Th1 and Th17 cell subsets, because engagement of the cognate receptors caused production of IL-6, IL-12, and IL-23 by DCs. C3a was also shown by Asgari et al [33] to modulate IL-1β secretion in human monocytes, DCs, and macrophages and to induce Th17 responses. We found C3a to be locally produced by human DCs, which may contribute to the significantly higher polarization of IL-17-producing CD4$^+$ T cells.

It has been reported that a portion of the preexisting Th17 cell subset in gut tissue and plasma exerts a role on the viral set-point loads during early SIV infection [19]. In addition, long-term nonprogressors show intact Th17 cells in the gut mucosa as well as intact gut homing molecules on T-cell subsets in the peripheral blood [20]. Gresnigt et al [39] demonstrated that *Aspergillus*-induced Th1 and Th17 responses were significantly decreased by blocking CR3 but not by inhibiting dectin-1 or Toll-like receptor 2 [39]. Due to complement opsonization of HIV before DC exposure, CR3 and/or CR4 are initiating the herein demonstrated induction of Th17-polarizing cytokines, rather than C3aR. The interactions of DCs with HIV-C using plasma from HIV-infected individuals triggered the release of Th17-inducing cytokines IL-1β, IL-6, and IL-23 and efficient polarization of IL-17^{high}/CD4^{high} T cells. All other T-helper subsets were primed to equal or lower levels by HIV-C– compared with HIV-treated DC supernatants.

Activation of the MAPK ERK was essential for IL-23-dependent IL-17 production [10, 11]. We also found that a higher ERK phosphorylation was triggered in iDCs stimulated with HIV-C compared with the nonopsonized counterpart. Activation of ERK was essential for induction of Th17-polarizing cytokines in DCs, because inhibition of the ERK path completely abrogated HIV-C–mediated secretion of Th17-polarizing cytokines. SIV-infected asymptomatic sooty mangabeys exhibit an intact Th17 population in the gut without any microbial translocation despite high viral loads and severe gut CD4$^+$ T-cell destruction [1, 40]. Ciccone et al [20] also observed intact Th17 cell populations in the guts of long-term nonprogressors, comparable to findings HIV-uninfected controls. In this study, we found that complement opsonization of HIV promoted a significantly elevated polarization of Th17 cells from CD4$^+$ T cells and might thereby contribute to an intact gut Th17 compartment, which has to be further determined.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases online* (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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