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Viral Replication-Independent Blockade of Dendritic Cell Maturation and Interleukin-12 Production by Human Herpesvirus 6

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Human herpesvirus 6 (HHV-6) is a potentially immunosuppressive CD4^{+}-T-lymphotropic betaherpesvirus that causes severe human thymocyte depletion in heterochimeric SCID-hu *thy/liv* mice and has been implicated as a potential cofactor in the progression of AIDS. However, the mechanisms of HHV-6-mediated immunosuppression have not yet been fully elucidated. We investigated the phenotypic and functional alterations induced by HHV-6 on peripheral blood-derived human dendritic cells (DC). The infection of DC with HHV-6 A or B was nonproductive, as revealed by calibrated real-time PCR measuring the accumulation of viral genome equivalents over time. Nevertheless, preexposure to HHV-6 markedly impaired the maturation of DC driven by gamma interferon and lipopolysaccharide, as shown by the reduced surface expression of major histocompatibility complex class I molecules, HLA-DR, CD40, and CD80. Moreover, HHV-6, but not the closely related betaherpesvirus HHV-7, dramatically suppressed the secretion of interleukin-12 (IL-12) p70 by DC, while the production of other cytokines that influence DC maturation, i.e., IL-10 and tumor necrosis factor alpha, was not significantly modified. Likewise, the secretion of the CC chemokines macrophage inflammatory protein 1β and RANTES was unaltered. Functionally, a pretreatment with HHV-6 impaired the ability of DC to stimulate allogeneic T-cell proliferation. Altogether, these data identify interference with the functional maturation of DC as a potential mechanism of HHV-6-mediated immunosuppression.

Immune responses against invading microorganisms can be broadly divided into two major compartments, innate immunity and adaptive immunity, which differ markedly with regard to the mechanisms and specificities of molecular target recognition but also show areas of overlap in terms of regulatory cells and effector pathways. In this respect, a paradigmatic example is represented by dendritic cells (DC), which respond to pathogen-associated molecular patterns but also act as professional antigen-presenting cells (APC) for the antigen-specific activation of B and T lymphocytes (4). DC play a pivotal role in the orchestration of immune responses. They are involved in T-lymphocyte activation after antigen encounters and contribute to the polarization of immune responses towards the most suitable cytokine secretion phenotype (i.e., Th1 or Th2) for the control of specific microorganisms (4). The production of defined cytokines and the modulation of DC membrane markers are required for the development of such polarized responses. Modulation of the expression of several DC surface molecules (e.g., CD40, CD80, CD86, LFA-1, and major histocompatibility complex [MHC] class I and II molecules) influences the efficiency of subsequent T-cell responses; as for soluble factors, a critical element in the generation of effective antiviral immune responses is interleukin-12 (IL-12), a cytokine that drives the polarization of T lymphocytes towards a Th1 cytokine secretion pattern (12). In fact, Th1 cells, characterized by the selective production of gamma interferon (IFN-γ) and IL-2, play an important role in the generation of cytotoxic T lymphocytes, which effectively counteract intracellular microorganisms, including viruses. The essential role played by DC in the generation of effective immune responses makes them attractive targets for microbial strategies aimed at dysregulating host immune defenses. Thus, several viruses, including human herpesviruses (HHVs), have developed effective strategies for evading and subverting immune responses, which include targeting DC functions through the impairment of their maturation and cytokine production (1).

HHV-6, originally isolated from patients with lymphoproliferative diseases and immunosuppression (24), is classified within the *Betaherpetovirinae* subfamily, along with *Human cytomegalovirus* and HHV-7. HHV-6 is further classified into two variants, A and B, which show significant genetic, immunological, and biological divergence. HHV-6 B has been identified as the etiologic agent of exanthem subitum in infants (30), but the pathogenic role of HHV-6 A remains uncertain. Like other herpesviruses, HHV-6 is able to induce latent infections and may persist indefinitely in the host. In immunocompromised patients, HHV-6 reactivation or reinfection may cause severe opportunistic diseases, including encephalitis, pneumonitis, hepatitis, and retinitis, as well as bone marrow graft failure (8).

Multiple lines of clinical and experimental evidence suggest that HHV-6 may act as an immunosuppressive agent. In SCID-hu *thy/liv* mice engrafted with human thymic tissue, infection with either HHV-6 A or B results in vigorous virus replication and rapid destruction of the graft, with thymocyte depletion affecting all major intrathymic cell populations (11). Consistent

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with these experimental observations, sporadic cases of fatal immunosuppression associated with disseminated HHV-6 infection have been reported (16, 31). Moreover, for allogeneic stem cell transplant recipients, defective antigen-specific T-lymphocyte proliferation was documented during episodes of HHV-6 reactivation or reinfection (29). The mechanisms that underlie HHV-6-induced immunosuppression are still largely unknown. In vitro, HHV-6 shows a preferential tropism for $CD4^+$ T cells, in which it replicates most effectively and induces strong cytopathic effects (20). One important mechanism of HHV-6 pathogenesis is the engagement of the primary viral receptor, CD46 (25), a complement-regulatory cell surface molecule that provides a key link between innate and adaptive immune responses. Recently, we have shown that exposure to HHV-6 results in a dramatic inhibition of IL-12 p70 production by differentiated human macrophages in the absence of a productive viral infection (27), a phenomenon that is likely mediated by CD46 engagement. Other mechanisms of immune dysregulation by HHV-6 include defective antigen presentation by DC (13) and aberrant cytokine production by peripheral blood mononuclear cells, such as an increased secretion of IL-1β, tumor necrosis factor alpha (TNF-α), and IL-10 (2, 9) and a decreased secretion of IL-2 associated with diminished cellular proliferation (10).

For the present study, we investigated the effects of HHV-6 on the maturation and function of DC, which were studied as a model of professional APC. Although HHV-6 was unable to actively replicate in these cells, it prevented their full maturation and caused a dramatic and selective blockade of IL-12 p70 secretion. Such defects had a functional correlate, as HHV-6-treated DC showed a reduced ability to stimulate allogeneic T-cell proliferation. These data illustrate a novel mechanism of HHV-6-mediated immunosuppression.

MATERIALS AND METHODS

DC purification and differentiation. Peripheral blood mononuclear cells were generated by Ficoll-Paque gradient purification (Pharmacia Biotech AB, Uppsala, Sweden) from concentrated white blood cells of healthy blood donors. The lymphocyte-rich layer was harvested and washed three times with cold phosphate-buffered saline (PBS). After the final wash, the cell pellet was resuspended in 20 ml of isosmotic RPMI containing 10% fetal calf serum (Bio-Whittaker Europe, Verviers, Belgium). Five milliliters of the cell solution was layered over 5 ml of a 46% Percoll solution in a 15-ml polystyrene tube (Pharmacia Biotech AB). The Percoll gradients were spun at $550 \times g$ for 30 min at 4°C with the brake turned off. The monocyte layer was harvested and washed three times with cold PBS. After the last wash, the cells were resuspended in complete Dulbecco's modified Eagle's medium, counted, and diluted to 0.5×10^6 cells/ml with Dulbecco's modified Eagle's medium. Cells (1×10^6 to 3×10^6) were plated in six-well polystyrene plates for 1 h at 37°C to allow for adherence. After 1 h, the plates were washed three times with warm PBS to remove nonadherent cells. Finally, 2 ml of complete RPMI containing 500 U of IL-4/ml and 50 ng of granulocyte-macrophage colony-stimulating factor (Pharmacia Biotech AB)/ml was added to each well. Every 2 to 3 days for 5 to 7 days, 1 ml of medium was replaced with fresh medium and cytokines. At the end of the differentiation period, the cells were harvested and the DC phenotype was confirmed by fluorocytometric analysis. Altogether, residual contamination by non-DC (i.e., $CD14^+$ mononuclear phagocytes, $CD3^+$ T cells, $CD19^+$ B cells, or $CD56^+$ NK cells) was consistently lower than 5%.

For DC differentiation, the cells were treated first with 500 U of IFN-γ (CABRU SAS, Milan, Italy)/ml for 17 h and then with 1 μg of lipopolysaccharide (LPS; Sigma, St. Louis, Mo.)/ml for 24 h. Cell-free culture supernatants were then harvested for the measurement of cytokines and chemokines by enzyme-linked immunosorbent assays (ELISAs), whereas the cells were used for fluorocytometric analyses of surface marker expression.

Viral stocks and inactivation with UV light. The viral strains used for this study were HHV-6 A strain GS (24) and HHV-B strain PL-1 (11). Viral stocks were generated by expansion in cord blood mononuclear cells (CBMC) that had been previously activated for 3 days with 5 μg of phytohemagglutinin (PHA; Sigma)/ml. Briefly, 5×10^6 activated CBMC were pelleted in a 50-ml polystyrene tube at $300 \times g$ for 10 min. The cells were infected at a multiplicity of infection (MOI) of about 0.2 (i.e., 2 infectious units per 10 cells) for 2 to 4 h at 37°C. The cultures were then diluted to 10^6 cells/ml with RPMI containing 10% fetal calf serum and were incubated for 7 days. Culture supernatants were collected on day 7 or when the majority of the cells showed morphological signs of infection. Infectious supernatants were clarified by spinning at $300 \times g$ for 10 min and then stored at −80°C until use. For HHV-6 inactivation by UV light, infectious supernatants were exposed to UV light for 5 min at a distance from the source of 20 cm and then kept on ice until use. HHV-7 infectious supernatants (strain AL) (26) were produced by CD8-depleted, PHA-activated CBMC under similar conditions as those used to grow HHV-6, with the exception that recombinant human IL-2 (Chiron, Amsterdam, The Netherlands) was added at 10 U/ml. All reagents, including viral supernatants, were tested for endotoxin contamination by the use of the Pyrogen Plus detection kit (BioWhittaker) with a lower detection limit of 0.125 EU/ml.

Virion enrichment by ultracentrifugation and infection of DC with HHV-6 or HHV-7. An HHV-6 (A or B) or HHV-7 infectious supernatant was layered over a 20% sucrose cushion and spun at $36,000 \times g$ for 1 h in a type 50 Ti rotor in an L-80 ultracentrifuge (Beckman Instruments, Inc., Palo Alto, Calif.). The pellet was then resuspended in complete RPMI to generate a 23× concentrated stock of each virus.

For infections, DC were plated at 10^6 cells/ml in 12-well polystyrene plates in complete RPMI containing 500 U of IL-4/ml and 50 ng of granulocyte-macrophage colony-stimulating factor/ml. Ultracentrifugation-purified HHV-6 and HHV-7 were diluted 23 times in complete medium and added to the cultures at an approximate MOI of 1. The cells were cultured in the presence of virus for 17 h, after which they were washed and induced to mature by the use of IFN-γ and LPS.

Quantification of HHV-6 DNA by real-time PCR. HHV-6 replication, measured as the accumulation of intracellular viral genome equivalents over time, was assessed by quantitative PCR for HHV-6 DNA, which was based on TaqMan technology using an ABI PRISM 7700 sequence detector as previously described (19).

Fluorocytometry. Fluorocytometric analysis was used to study the expression of selected cell surface markers. For each antibody, 1×10^5 to 5×10^5 cells were used in 5-ml polystyrene round-bottomed tubes (Becton Dickinson Labware, Franklin Lanes, N.J.). The cells were washed with cold PBS supplemented with 2% human serum at $250 \times g$ for 5 min and then incubated for 30 min on ice with primary antibodies. The primary antibodies used were anti-CD40, -CD80, and -CD86 (Serotec, Oxford, England) and anti-MHC class I (clone W6/32) and -HLA-DR (clone L243) (a kind gift of Lorenzo Piemonti). After two additional washes with PBS plus 2% human serum, the cells were incubated for an additional 30 min with the appropriate secondary antibody conjugated to either fluorescein isothiocyanate or phycoerythrin (Sigma). The cells were then further washed with PBS plus 2% human serum by centrifugation at $250 \times g$ for 5 min. Both infected and uninfected cells were fixed with PBS containing 3% formaldehyde before analysis. Fluorocytometric analysis was performed by use of a FACScan analyzer (Becton Dickinson, San Jose, Calif.). Appropriate gating was used to exclude dead cells, and at least 10,000 events were acquired for each sample.

Measurement of cytokine and chemokine release. The levels of cytokines and chemokines (IL-12 p70, TNF-α, IL-10, RANTES, and macrophage inflammatory protein 1β [MIP-1β]) released into the culture supernatants were measured by ELISAs (R&D Systems, Minneapolis, Minn.) performed according to the manufacturer's instructions. The IL-12 ELISA was reported to have no cross-reactivity or interference with IL-12 p40 or IL-12 p35 monomers or homodimers. ELISA results were normalized for cell numbers and then used to calculate means with appropriate standard deviations (SD) for multiple donors. *P* values were calculated by the two-tailed paired *t* test. For data derived from two donors, ELISA results were used to calculate mean values with appropriate standard errors (SE).

Allogeneic T-lymphocyte proliferation assay. Enriched T-cell preparations were obtained as follows. Peripheral blood mononuclear cells from healthy blood donors were obtained from buffy coats by standard gradient separation procedures. The cells (5×10^6) were washed in RPMI and incubated in complete medium containing 0.5% carbonyl iron (Sigma) for 60 min at 37°C on a rotating wheel; phagocytic cells were then removed with a magnet as previously described (5). After this procedure, residual monocytes accounted for <1% of the cell population, as confirmed by fluorocytometry with an anti-CD14 monoclonal antibody. For mixed leukocyte reactions, DC were pretreated with UV-inactivated HHV-6 A at an approximate MOI of 1 or with uninfected culture medium

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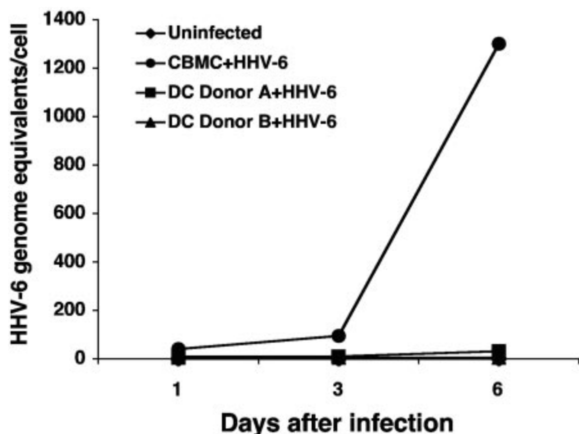

FIG. 1. Lack of productive HHV-6 infection in DC. DC or PHA-activated CBMC were infected with purified HHV-6 A virions at an approximate MOI of 1. After 1, 3, and 6 days, the cells were harvested and analyzed for viral DNA content by use of a quantitative calibrated real-time PCR assay. Human β-actin DNA was measured in parallel to normalize the cell numbers. The data shown are from two representative DC donors and one CBMC donor and are expressed as HHV-6 genome equivalents per cell.

overnight and then either left immature or matured with IFN-γ and LPS as described above. Different numbers of DC were then cocultured for 5 days at different ratios in 96-well flat-bottomed plates (Nunc) together with 10^5 heterologous lymphocytes/well; DC-to-lymphocyte ratios ranged from 1:5 to 1:100. Allogeneic T-lymphocyte proliferation was evaluated by measuring the incorporation of [methyl-^{3}H]thymidine (0.8 μCi/well; Amersham Biosciences, Freiburg, Germany), which was added to the cultures during the last 6 h before harvesting. The cells were then harvested, and radioactivity was measured with a Wallac LKB counter (Perkin-Elmer, Shelton, Colo.).

RESULTS

Lack of HHV-6 replication in DC. To investigate the effects of HHV-6 on DC, we initially evaluated the ability of viral strains representing the two major viral variants, A and B, to replicate in human peripheral blood-derived DC. PHA-activated human CBMC were tested in parallel as a control. Figure 1 shows that the exposure of immature DC to HHV-6 A (strain GS) did not result in the accumulation of viral genome equivalents over time, as evaluated by a quantitative calibrated real-time PCR assay; likewise, no other signs of productive infection, such as cytomorphological changes, loss of cellular viability, or expression of the nuclear phosphoprotein pp41, an early-late viral antigen, were detected throughout the observation period (not shown). In contrast, the same HHV-6 stock initiated vigorous viral replication in CBMC, as revealed by the progressive accumulation of HHV-6 genomes over time (Fig. 1). Similar results (not shown) were obtained for HHV-6 B (strain PL-1).

Effect of HHV-6 on the expression of DC membrane differentiation markers. Next, we investigated the effects of HHV-6 on the maturation of human DC, as assessed by changes in the expression of selected surface markers that are modulated during DC maturation. As shown in Fig. 2A, pretreatment with HHV-6 A (strain GS) had little effect on immature DC expression of CD40 and CD80, while there were slight changes in the expression of MHC class I (upregulated) and HLA-DR (downregulated). In contrast, in DC differentiated by IFN-γ and LPS, pretreatment with HHV-6 markedly reduced the upregulation of all of the differentiation markers examined (Fig. 2B). Similar data (not shown) were obtained with DC treated with HHV-6 B.

HHV-6 inhibits the production of IL-12 by human DC. As illustrated in Fig. 3, unstimulated DC failed to produce significant levels of IL-12 p70, regardless of the pretreatment with HHV-6; in contrast, stimulation with IFN-γ and LPS induced a vigorous production of IL-12 p70, but such production was dramatically suppressed by pretreatment with either HHV-6 A (panel A) or HHV-6 B (panel B). A dose-dependent IL-12 inhibition effect was seen with serial dilutions of the viral stocks (not shown). Notably, UV light inactivation of the viral stocks did not eliminate the suppressive activity (>90% reduction of

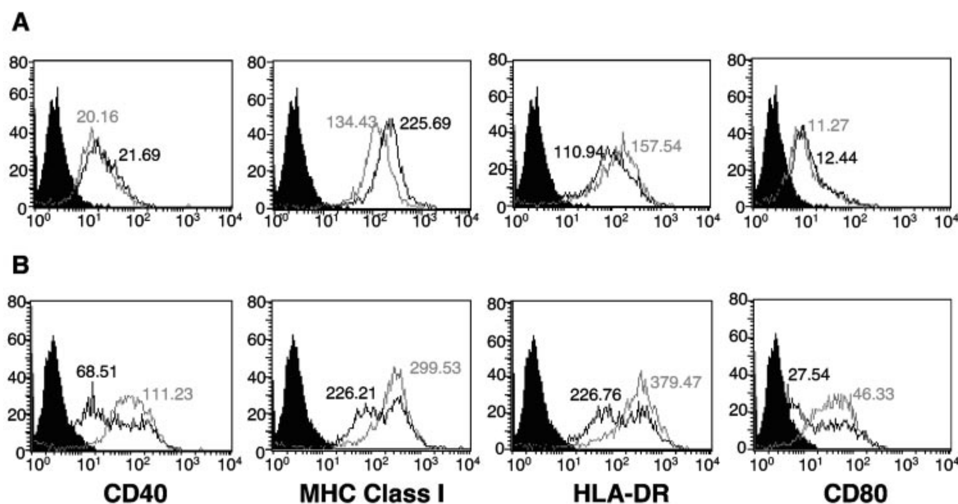

FIG. 2. Inhibition of IFN-γ- and LPS-induced DC maturation by HHV-6. DC were preexposed to purified HHV-6 A virions and then cultured for 40 h in the absence (A) or presence (B) of IFN-γ and LPS. The cells were then harvested and analyzed for surface expression of CD40, MHC classes I and II, and CD80 by fluorocytometry. Profiles of HHV-6-treated DC are shown as black lines, while those of uninfected controls are shown as gray lines. The number on each line denotes the mean fluorescence intensity value. Representative data from one of three donors tested are shown.

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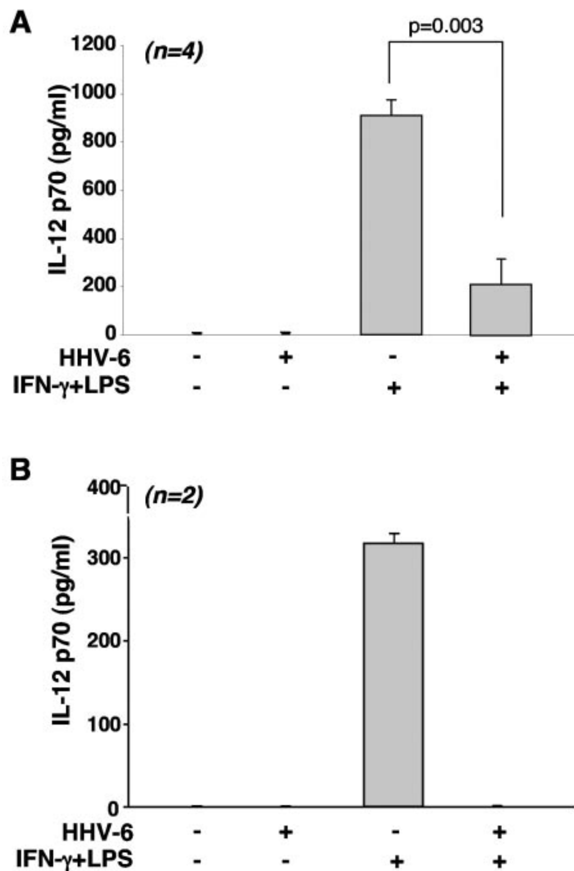

FIG. 3. Inhibition of IL-12 p70 production from primary human DC by HHV-6. Human peripheral blood-derived DC were preexposed to purified HHV-6 virions and then stimulated with IFN-γ plus LPS or left unstimulated. Control DC were not preexposed to HHV-6. Culture supernatants were analyzed for IL-12 p70 levels by ELISA 24 h after the addition of LPS. (A) Mean results (± SD) for DC from four donors that were pretreated with HHV-6 subgroup A (strain GS). (B) Mean results (± SE) for DC from two donors that were pretreated with HHV-6 subgroup B (strain PL-1).

IL-12 release with UV-treated virus), thus formally proving that the HHV-6-induced inhibition of IL-12 p70 in DC is independent of virus replication. These results demonstrated that preexposure to HHV-6 A or B profoundly affects the capacity of human DC to produce IL-12 p70.

HHV-6 does not significantly influence the production of other cytokines and chemokines by DC. HHV-6 infection has been reported to cause an increased production of IL-10 and TNF-α (2), two cytokines that can modulate DC maturation (4, 7). Thus, we tested the possibility that the HHV-6-mediated inhibition of DC maturation was related to the autocrine production of these cytokines. Only a limited, statistically nonsignificant, increase in the production of IL-10 was detected in supernatants of HHV-6-treated DC stimulated with IFN-γ and LPS (Fig. 4A), while the release of TNF-α was slightly decreased, but again the difference did not reach statistical significance (Fig. 4B). These results suggest that the dramatic effects induced by HHV-6 on DC maturation and IL-12 production cannot be primarily ascribed to changes in the levels of IL-10 and TNF-α, even though in the case of IL-10 it cannot be excluded that the levels of cytokine production had already reached plateau levels upon IFN-γ and LPS stimulation.

We also evaluated whether the levels of other soluble factors produced by DC could be influenced by pretreatment with HHV-6. Figure 4C and D show that the production of the CC chemokines MIP-1β and RANTES upon IFN-γ and LPS stimulation was not affected by HHV-6 A; likewise, there was no change in the spontaneous release of such chemokines by immature DC. These data indicated that the suppression of IL-12 p70 by HHV-6 is selective and does not result from a generalized functional impairment of DC.

Lack of IL-12 p70 inhibition by HHV-7. Since a down-modulation of IL-12 production has also been reported for *Human cytomegalovirus*, another member of the *Betaherpetovirinae* subfamily (22), we evaluated whether this observation could be extended to the third known human β-herpesvirus, HHV-7. As seen with HHV-6, no productive HHV-7 infection of DC was documented by morphological observations and fluorocytometry (data not shown). Figure 5 shows that pretreatment of IFN-γ- and LPS-stimulated DC with concentrated HHV-7 virions at the same MOI as that used for HHV-6 failed to inhibit the production of IL-12 p70. Also, the pretreatment of DC with HHV-7 did not induce the production of IL-12 p70 by unstimulated DC. These data suggest that the inhibition of IL-12 p70 is not a general characteristic of all betaherpesviruses.

Inhibition of DC-induced allogeneic T-lymphocyte stimulation by HHV-6. To investigate the functional relevance of the DC maturation and cytokine secretion defects observed after HHV-6 treatment, we assessed the effect of HHV-6 on the ability of DC to stimulate the proliferation of allogeneic T cells. Considering the remarkable susceptibility of T cells to a productive HHV-6 infection, at variance with a previous study (13), we used a UV light-inactivated virus in order to exclude a loss of function related to virus-induced cytopathic effects and cell death. As shown in Fig. 6, uninfected immature DC weakly stimulated the proliferation of allogeneic T cells (panel A), whereas, as expected, T-lymphocyte proliferation was strongly enhanced when DC were previously differentiated with IFN-γ and LPS (panel B). However, pretreatment with HHV-6 caused a marked reduction in the proliferation of T cells stimulated by either immature or mature DC (Fig. 6). In contrast, in agreement with the IL-12 production results (Fig. 5), no reduction in allogeneic T-cell proliferation was seen upon the exposure of DC to HHV-7 (data not shown).

DISCUSSION

The observation that several viruses have evolved strategies to suppress DC functions provides strong in vivo support for the concept that DC play a central role in the development of effective antiviral immune responses (1). In this report, we evaluated the effects of HHV-6, a potentially immunosuppressive betaherpesvirus, in a well-established in vitro model that mimics the functional maturation of DC. We observed a complex pattern of interference with DC functions by both HHV-6 A and B. Pretreatment with HHV-6 impaired the ability of DC to fully differentiate in response to IFN-γ and LPS treatment, as demonstrated by a reduced expression of maturation-associated surface markers. Moreover, the production of IL-12

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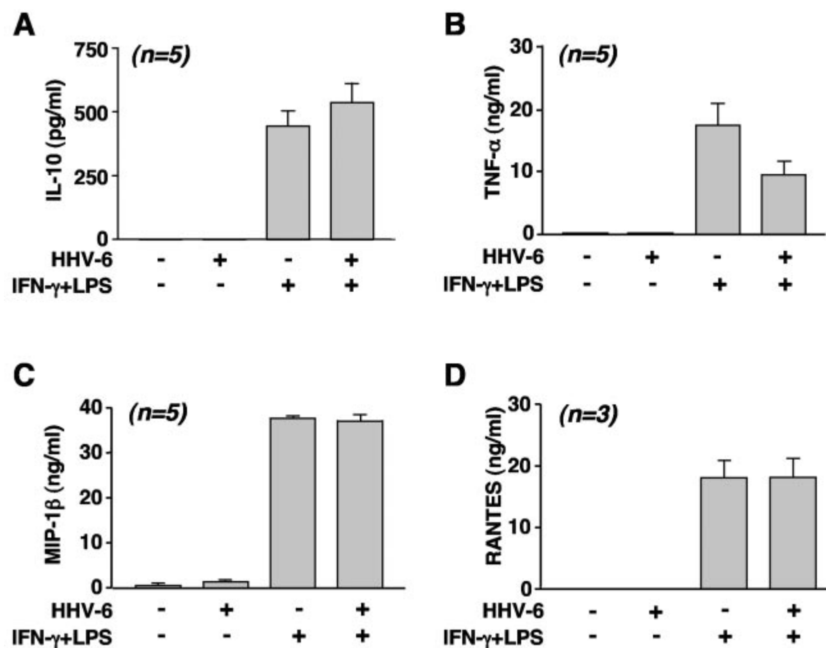

FIG. 4. Failure of HHV-6 to modulate IL-10, TNF-α, MIP-1β, and RANTES production from activated DC. Culture supernatants of DC that had been preexposed to purified HHV-6 A virions and then either left unstimulated or stimulated with IFN-γ and LPS were tested for IL-10 (A), TNF-α (B), MIP-1β (C), and RANTES (D) levels by ELISAs. Mean results (± SD) for five donors are shown in panels A to C, and mean results for three donors are presented in panel D.

p70, a critical cytokine for Th1 polarization, was dramatically and selectively inhibited. It is important to emphasize that we were unable to document productive HHV-6 infections in our DC cultures, in evident contrast with the results of a previous study in which DC cultures were shown to effectively sustain HHV-6 replication, as revealed by the expression of late viral antigens (13). The reasons for these discrepancies are unclear at present. The observation of late viral antigen expression in the latter study may have been related to the different culture conditions used or, alternatively, to residual T-cell contamination of DC cultures. Notably, such prominent expression of late viral antigens was not accompanied by any signs of cytopathic effects or loss of DC viability (13), reinforcing the concept that a large proportion of the DC population in these experiments was indeed spared from the full expression of the viral lytic cycle.

As suggested by our previous results with differentiated human macrophages (27) as well as by studies of measles virus (14), the dramatic effects of HHV-6 on DC maturation and IL-12 secretion in the absence of detectable viral replication most likely resulted from the engagement of the primary HHV-6 receptor molecule, CD46 (25). Our results with a UV light-inactivated virus, which is unable to replicate but maintains the ability to engage the cell surface receptor, corroborate this hypothesis. CD46 serves as a regulator of complement activation, protecting cells from autologous complement-mediated damage; due to this critical physiological function, it is expressed at high levels on the surfaces of virtually all human cells, including DC. Moreover, CD46 provides a key link between innate and adaptive immune responses (18, 21). Indeed, CD46-mediated signaling was shown to induce downstream effects that significantly modulate various immune functions, including T-cell costimulation (3) and polarization toward a T-regulatory cell phenotype (15) as well as the recruitment of SHP-1 and increased nitric oxide production in macrophages (17). These observations illustrate the complexity of CD46-mediated signaling, which is further complicated by the diversity in CD46 isoform-specific tyrosine phosphorylation (21). While the precise CD46 signaling pathway induced by HHV-6 binding to human DC remains to be defined, our results are compatible with a role of viral envelope-mediated CD46 en-

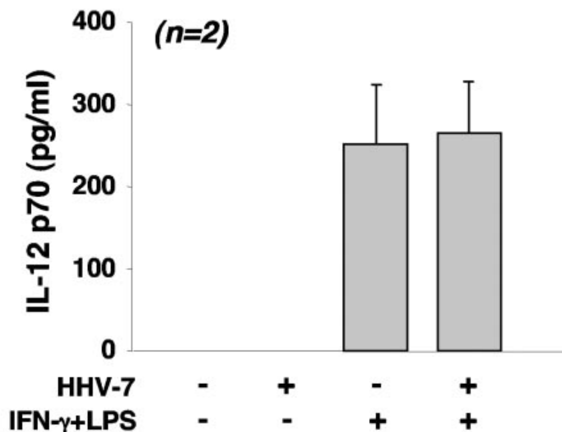

FIG. 5. Failure of HHV-7 to inhibit IL-12 p70 production from primary human DC. Human peripheral blood-derived DC were preexposed to purified HHV-7 virions at an approximate MOI of 1 and then stimulated with IFN-γ plus LPS or left unstimulated. Control DC were not preexposed to HHV-7. Culture supernatants were analyzed for IL-12 p70 levels by ELISA 24 h after the addition of LPS. Mean results (± SE) for DC from two donors are shown.

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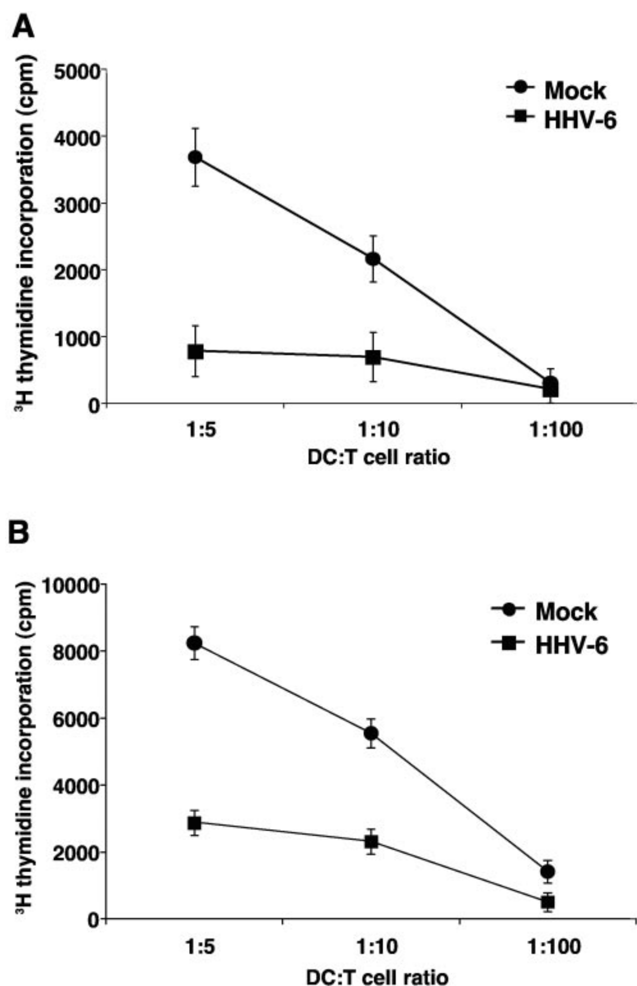

FIG. 6. Reduced proliferation of T cells by allogeneic DC pretreated with HHV-6. Peripheral blood-derived DC were treated with purified UV-inactivated HHV-6 virions overnight and then matured with IFN-γ and LPS or left immature; mock-treated DC were exposed to uninfected culture medium under the same conditions. A total of 10^5 allogeneic T cells were incubated with various numbers of either immature (A) or mature (B) DC. Five days later, the cells were incubated with radiolabeled thymidine and then were harvested and analyzed. Representative data (means ± SD for triplicate wells) for one of three donors tested are shown.

gagement in the selective functional impairment of HHV-6-exposed DC, which occurs in the absence of detectable viral replication.

A critical question that we tried to answer is the functional relevance of the DC defects induced by exposure to HHV-6. For this purpose, we measured the antigen-presenting capability of HHV-6-treated DC in allogeneic T-cell proliferation assays, demonstrating a markedly reduced ability to stimulate T-lymphocyte responses. Note that we employed UV-inactivated HHV-6 stocks in order to rule out potential cytopathic effects related to viral replication in T cells. Diverse mechanisms may underlie such an effect. Allogeneic T-cell responses are affected by a complex array of membrane-bound and soluble factors related to DC differentiation. Among the former, the level of expression of major histocompatibility antigens and costimulatory molecules may have a significant impact on the efficiency of T-cell activation. Among the soluble factors secreted by DC upon stimulation, IL-12 plays a crucial role (23). We have documented an effect of HHV-6 on the expression of both membrane molecules (MHC classes I and II, CD40, and CD80) and soluble factors (IL-12), which may cooperatively account for the reduced T-cell stimulatory activity. Nevertheless, a role of other, still undefined mechanisms cannot be excluded at present.

Even though the molecular mechanisms underlying the impaired DC maturation after contact with HHV-6 are still incompletely understood, the present study raises specific issues that pave the way for future investigations. First, HHV-6 treatment induced a trend towards a reduction of TNF-α and an enhancement of IL-10 production, which in combination may contribute, at least in part, to diminishing IL-12 production. Second, the production of other soluble factors released upon DC activation, such as RANTES and MIP-1β, was not negatively affected by HHV-6. Third, the immunomodulatory effects of HHV-6 are not common to all members of the *Betaherpetovirinae* subfamily, since they were not reproduced by the closely related betaherpesvirus HHV-7. It is noteworthy that HHV-7 does not use CD46 as a cellular receptor. Thus, our results suggest that IL-12 p70 suppression is a specific effect that is not derived from a generalized abatement of DC functions; on the other hand, the overall strategy of HHV-6 is emerging as a coordinated activity of multiple mechanisms focused on the impairment of DC maturation and function.

Several lines of evidence suggest that HHV-6 may act as an immunosuppressive agent (8, 20). However, the clinical observations are still limited, and the potential mechanisms of this immunosuppressive activity are still largely unknown. One implication of the dramatic IL-12 suppression observed herein is the concept that HHV-6 may cause immunosuppression by blocking Th1-polarized immune responses. To provide additional support for these in vitro observations, we will need to verify them in vivo by testing the maturation and IL-12-producing ability of DC isolated from patients with active HHV-6 infections. The observed functional impairment of DC is also consistent with the notion that HHV-6 may act as a cofactor during the course of human immunodeficiency virus type 1 (HIV-1) infection, accelerating the progression toward full-blown AIDS (20). In this respect, it is noteworthy that peripheral blood mononuclear cells from patients infected with HIV-1 were shown to produce lower levels of IL-12 upon in vitro stimulation with *Staphylococcus* proteins than did uninfected controls (6). Studies on the status of HHV-6 infection will be important to elucidate the role played by this herpesvirus in the IL-12 downmodulation documented for HIV-infected patients.

In conclusion, we have shown that HHV-6 prevents the full maturation of human DC in the absence of viral replication and cytopathic effects, illustrating a novel mechanism for HHV-6-mediated immunosuppression. Interestingly, another virus that uses CD46 as a cellular receptor, i.e., measles virus, is also associated with immunosuppression (14). Unfortunately, formal proof of the role of CD46 binding in the observed DC effects is difficult to achieve due to the fact that the anti-CD46 antibodies that may be used to block the HHV-6–CD46 interaction are themselves functionally active and sufficient to affect APC maturation and function (14, 27). In

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addition, antibody-mediated blocking experiments with APC are complicated by the documented effects of Fcγ-receptor ligation, which is per se sufficient to cause IL-12 downmodulation (28). Regardless of these technical hurdles, the shared use of CD46 by measles virus and HHV-6 is compatible with the concept that binding to this receptor molecule, which is emerging as a key link between innate and cognate immune responses, does not simply represent a cell surface anchoring maneuver, but rather is a key mechanism of microbial pathogenesis. Understanding the fine molecular mechanisms of HHV-6-mediated DC dysregulation may be useful for devising novel therapeutic approaches to specifically counteract the immunosuppressive effects of microorganisms that engage the CD46 receptor.

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