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The $\gamma_1$34.5 Protein of Herpes Simplex Virus 1 Is Required To Interfere with Dendritic Cell Maturation during Productive Infection$^{\nabla}$

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The $\gamma_1$34.5 protein of herpes simplex virus 1 is an essential factor for viral virulence. In infected cells, this viral protein prevents the translation arrest mediated by double-stranded RNA-dependent protein kinase R. Additionally, it associates with and inhibits TANK-binding kinase 1, an essential component of Toll-like receptor-dependent and -independent pathways that activate interferon regulatory factor 3 and cytokine expression. Here, we show that $\gamma_1$34.5 is required to block the maturation of conventional dendritic cells (DCs) that initiate adaptive immune responses. Unlike wild-type virus, the $\gamma_1$34.5 null mutant stimulates the expression of CD86, major histocompatibility complex class II (MHC-II), and cytokines such as alpha/beta interferon in immature DCs. Viral replication in DCs inversely correlates with interferon production. These phenotypes are also mirrored in a mouse ocular infection model. Further, DCs infected with the $\gamma_1$34.5 null mutant effectively activate naïve T cells whereas DCs infected with wild-type virus fail to do so. Type I interferon-neutralizing antibodies partially reverse virus-induced upregulation of CD86 and MHC-II, suggesting that $\gamma_1$34.5 acts through interferon-dependent and -independent mechanisms. These data indicate that $\gamma_1$34.5 is involved in the impairment of innate immunity by inhibiting both type I interferon production and DC maturation, leading to defective T-cell activation.

Herpes simplex virus 1 (HSV-1) is a human pathogen responsible for localized mucocutaneous lesions and encephalitis (51). Following primary infection, HSV-1 establishes a latent or lytic infection in which the virus interacts with host cells, which include dendritic cells (DCs), required to initiate adaptive immune responses (36). Immature DCs, which reside in almost all peripheral tissues, are able to capture and process viral antigens (15). In this process, DCs migrate to lymph nodes, where they mature and present antigens to T cells. Mature DCs display high levels of major histocompatibility complex class II (MHC-II) and costimulatory molecules such as CD40, CD80, and CD86. Additionally, DCs release proinflammatory cytokines such as interleukin-12 (IL-12), tumor necrosis factor alpha, alpha interferon (IFN-α), and IFN-β, which promote DC maturation and activation. An important feature of functional DCs is to activate naïve T cells, and myeloid submucosal and lymph node resident DCs are responsible for HSV-specific T-cell activation (2, 45, 52). Moreover, DCs play a direct role in innate antiviral immunity by secreting type I IFN.

HSV-1 is capable of infecting both immature and mature DCs (20, 24, 34, 38, 42). A previous study suggested that HSV entry into DCs requires viral receptors HVEM and nectin-2 (42). Upon HSV infection, plasmacytoid DCs detect viral genome through Toll-like receptor 9 (TLR9) and produce high levels of IFN-α (16, 23, 30, 40). In contrast, myeloid DCs, which are major antigen-presenting cells, recognize viral components through distinct pathways, independently of TLR9 (16, 36, 40). It has been suggested previously that HSV proteins or RNA intermediates produced during viral replication trigger myeloid DCs (16, 40). Indeed, a protein complex that consists of HSV glycoproteins B, D, H, and L stimulates the expression of CD40, CD83, CD86, and cytokines in myeloid DCs (41). Hence, DCs sense HSV through TLR-dependent and -independent mechanisms (16, 40, 41). Nevertheless, HSV replication compromises DC functions and subsequent T-cell activation (3, 20, 24, 42). HSV-1 interaction with immature DCs results in the downregulation of costimulatory molecules and cytokines (20, 34, 38, 42). While HSV-2 induces rapid apoptosis, HSV-1 does so with a delayed kinetics in human DCs (4, 20, 38). HSV-1 is also reported to interfere with functions of mature DCs (24, 39). Upon infection, HSV-1 induces the degradation of CD83 but not CD80 or CD86 in mature DCs (24, 25). Additionally, HSV-1 reduces levels of the chemokine receptors CCR7 and CXCR4 on mature DCs and subsequently impairs DC migration to the respective chemokine ligands CCL19 and CXCL12 (39).

Although HSV infection impairs DC functions, viral components responsible for this impairment have not been thoroughly investigated. It has been suggested previously that the virion host shut-off protein (vhs) of HSV-1 contributes par-

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tially to the viral block of DC activation (43). This activity is thought to stem from the ability of vhs to destabilize host mRNA. Emerging evidence suggests that ICP0 perturbs the function of mature DCs, where it mediates CD83 degradation via cellular proteasomes (25). Findings from related studies show that ICP0 inhibits the induction of IFN-stimulated genes mediated by IFN regulatory factor 3 (IRF3) or IRF7 in other cell types (11, 27, 32, 33). However, the link of ICP0 activities to DC maturation remains to be established. Recently, we found that $\gamma_1$34.5, an HSV virulence factor, associates with and inhibits TANK-binding kinase 1 (TBK1), an essential component of TLR-dependent and -independent pathways that activates IRF3 and cytokine expression (49). Interestingly, an HSV mutant lacking $\gamma_1$34.5 stimulates MHC-II surface expression in glioblastoma cells (47). These observations raise the hypothesis that $\gamma_1$34.5 may modulate DC maturation during HSV infection.

In this study, we report that $\gamma_1$34.5 is required to perturb DC maturation during HSV infection, leading to impaired T-cell activation. Wild-type virus, but not the $\gamma_1$34.5 null mutant, suppresses the expression of costimulatory molecules as well as cytokines in DCs. We provide evidence that the viral block of DC maturation is associated with reduced IFN-α/β secretion. Furthermore, the expression of $\gamma_1$34.5 inhibits DC-mediated allogeneic T-cell activation and IFN-γ production. IFN-neutralizing antibodies partially reverse DC maturation induced by the $\gamma_1$34.5 null mutant. These results shed light on the role of $\gamma_1$34.5 relevant to DC maturation and T-cell responses in HSV infection.

MATERIALS AND METHODS

Mice. BALB/c and C57BL/6 mice were purchased from Harlan Sprague Dawley Inc. and housed under specific-pathogen-free conditions in biosafety level 2 containment. Groups of 5-week-old mice were selected for this study. Experiments were performed in accordance with the guidelines of the University of Illinois at Chicago.

Cells and viruses. Vero cells were obtained from the American Type Culture Collection and propagated in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS). Myeloid DCs were generated as described previously (18). Briefly, bone marrow cells were removed from the tibias and femurs of BALB/c mice. Following red blood cell lysis and washing, progenitor cells were plated in RPMI 1640 medium (Invitrogen, Auckland, NZ) supplemented with 10% FBS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, and 20 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF; Biosource, Camarillo, CA) in six-well plates at 4×10^6 cells/well. Cells were supplemented with 2 ml of fresh medium every other day. On day 8, DCs were positively selected for surface CD11c expression by using magnetic beads (Miltenyi Biotech, Auburn, CA) to give a >97% pure population of $CD11c^+$ MHC-II-positive cells. DCs displayed low levels of CD40, CD80, CD86, and MHC-II molecules, characteristic of immature DCs. Purified $CD11c^+$ DCs were cultured in fresh medium with FBS and GM-CSF and used in subsequent experiments.

HSV-1(F) is a prototype HSV-1 strain used in these studies (12). In recombinant virus R3616, a 1-kb fragment from the coding region of the $\gamma_1$34.5 gene was deleted (8).

Viral infection. Purified $CD11c^+$ DCs were plated into 12-well plates (5×10^5 cells/well) and infected with HSV-1(F) or R3616 at the multiplicities of infection indicated below. After 2 h of incubation, cells were washed with phosphate-buffered saline (PBS) and resuspended in RPMI 1640 medium supplemented with 10% FBS and 20 ng/ml of GM-CSF. At different time points after infection, cells were harvested for analyses. For in vivo analysis, groups of five mice were anesthetized by intraperitoneal injection with ketamine (100 mg/kg of body weight) and xylazine (5 mg/kg). HSV-1(F) or R3616 (2×10^5 PFU) was inoculated bilaterally onto the surfaces of scarified corneas of BALB/c mice. On days 1, 3, and 5, whole eyes were collected from sacrificed mice. The corneas were digested with collagenase type I (Invitrogen, Carlsbad, CA) at 3 mg/ml for 2 h at 37°C. The digested tissues were passed through a 70-μm nylon cell strainer and spun down at 2,000 rpm for 5 min at 4°C. The final pellet was resuspended in complete RPMI 1640 medium, and the single-cell suspension was used for further analysis.

Reverse transcription-PCR (RT-PCR) analysis. Total RNAs from mock-infected or virus-infected DCs were extracted using the RNeasy kit (Qiagen Inc., Valencia, CA). Equal amounts of RNA from each sample were employed to synthesize cDNA by using random primers as suggested by the manufacturer (Invitrogen, Carlsbad, CA). cDNAs were then subjected to PCR amplification for the analysis of ICP27, UL30, and UL44 RNA and 18S rRNA by using specific primers (primers for 18S rRNA were CGCAGCTAGGAATAATGGAA and TTATGACCCGCACTTACTGG; primers for ICP27 RNA were CTGGAATC GGACAGCAGCCGG and GAGGCGCGACCACACACTGT; primers for UL30 RNA were ACTAACTTCGACTGGCCCTTC and CCGTACATGTCG ATGTTCAAC; and primers for UL44 RNA were GCCGCCGCCTACTACCC and GCTGCCGCGATCGTGATG). PCR products were separated on a 1.5% agarose gel and visualized with ethidium bromide under UV light.

Mixed lymphocyte reaction. Spleens were harvested from C57BL/6 mice after cervical dislocation. Single-splenocyte suspensions were prepared by forcing tissue through a fine wire mesh with a syringe plunger and then repeatedly pipetting it into culture medium. After red blood cell depletion, $CD4^+$ T cells were purified with microbeads according to the instructions of the manufacturer (Miltenyi Biotech, Auburn, CA) and used as the responder cells. Stimulator cells were bone marrow-derived DCs from BALB/c mice and were further treated with UV light before use. The responder cells (10^6) were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE; Invitrogen, Carlsbad, CA) and cocultured with the DC stimulator cells (2×10^5) in 2 ml of medium. After 48 h, the proliferation of the responder $CD4^+$ T cells was evaluated using a FACSCalibur and the data were analyzed by the gating of CFSE-positive cells with CellQuest Pro software (BD).

Flow cytometry. Cells were stained with fluorescein isothiocyanate (FITC)- or phycoerythrin-linked monoclonal antibodies (MAbs) according to the instructions of the manufacturer (eBioscience, San Diego, CA). Briefly, cells were blocked with Fcγ MAb (0.5 μg/ml) for 30 min at 4°C. After being washed with PBS, cells were stained with isotype-matched antibodies and anti-CD11c-phycoerythrin, anti-MHC-II-FITC, and anti-CD86-FITC antibodies for 30 min on ice with gentle shaking. Samples were processed and screened using FACSCalibur, and data were analyzed with CellQuest Pro software (BD).

Flow cytometry analyses of the production of cytokines IL-6, IL-12, IFN-α, and IFN-β in cells were performed as follows. Single-cell suspensions were stimulated in 96-well plates with anti-CD3 (5-μg/ml) and anti-CD28 (5-μg/ml) MAbs for 12 h at 37°C in 5% CO_2, after which monensin (2 μg/ml) was added and the plates were incubated for 4 h. After being washed twice with PBS, cells were blocked with 1 μl of Fcγ MAb (0.5 μg/ml) for 30 min at 4°C and fixed with 4% paraformaldehyde at 4°C for 15 min before being permeabilized with buffer (eBioscience, San Diego, CA) at 4°C for 10 min. After being washed once with PBS, cells were stained with appropriate isotype controls and anti-IL-6-FITC, anti-IL-12-FITC, anti-IFN-α-FITC, and IFN-β-FITC antibodies (PBL Laboratories, Piscataway, NJ). Samples were processed and screened using FACSCalibur, and data were analyzed with CellQuest Pro software (BD).

To determine viral infectivity, DCs mock infected or infected with viruses were fixed in 4% paraformaldehyde (Sigma) and permeabilized in permeabilizing buffer (eBioscience, San Diego, CA). Cells were blocked with 5% normal mouse serum (Sigma), incubated with a MAb against HSV-1 ICP27 (Virusys, Sykesville, MD), and allowed to react with a goat anti-mouse FITC-conjugated antibody (Santa Cruz Biotech, CA). ICP27 expression was evaluated by flow cytometry.

IFN bioassay. Culture media from mock-infected or virus-infected DCs were collected and treated with UV light to inactivate virus. Where indicated, 30 μg/ml of neutralizing antibodies specific to mouse IFN-α/β (PBL Laboratories, Piscataway, NJ) was added to the media. Samples were incubated with Vero cells overnight and washed with PBS. Vero cells were subjected to infection with vesicular stomatitis virus expressing green fluorescent protein (VSV-GFP; 10 PFU/cell). At 10 h after infection, cells were harvested and analyzed by flow cytometry using FACSCalibur and data were analyzed with CellQuest Pro software (BD).

Plaque assay. To determine the titer of infectious virus, virus-infected DCs were harvested and freeze-thawed three times. Eye tissues were collected from mice and mechanically homogenized. Samples were serially diluted in 199v medium, and viral yields were titrated on Vero cells at 37°C.

Immunohistochemistry analysis. Tissue sections for immunohistochemistry analysis were deparaffinized with xylene and rehydrated through a series of graded ethanols. Endogenous peroxidase activity was quenched using a 0.3% H_2O_2-methanol bath followed by several washes with PBS. HSV-1 antigens were detected using a 1:1,000 dilution of an HSV-1-specific antiserum raised in a

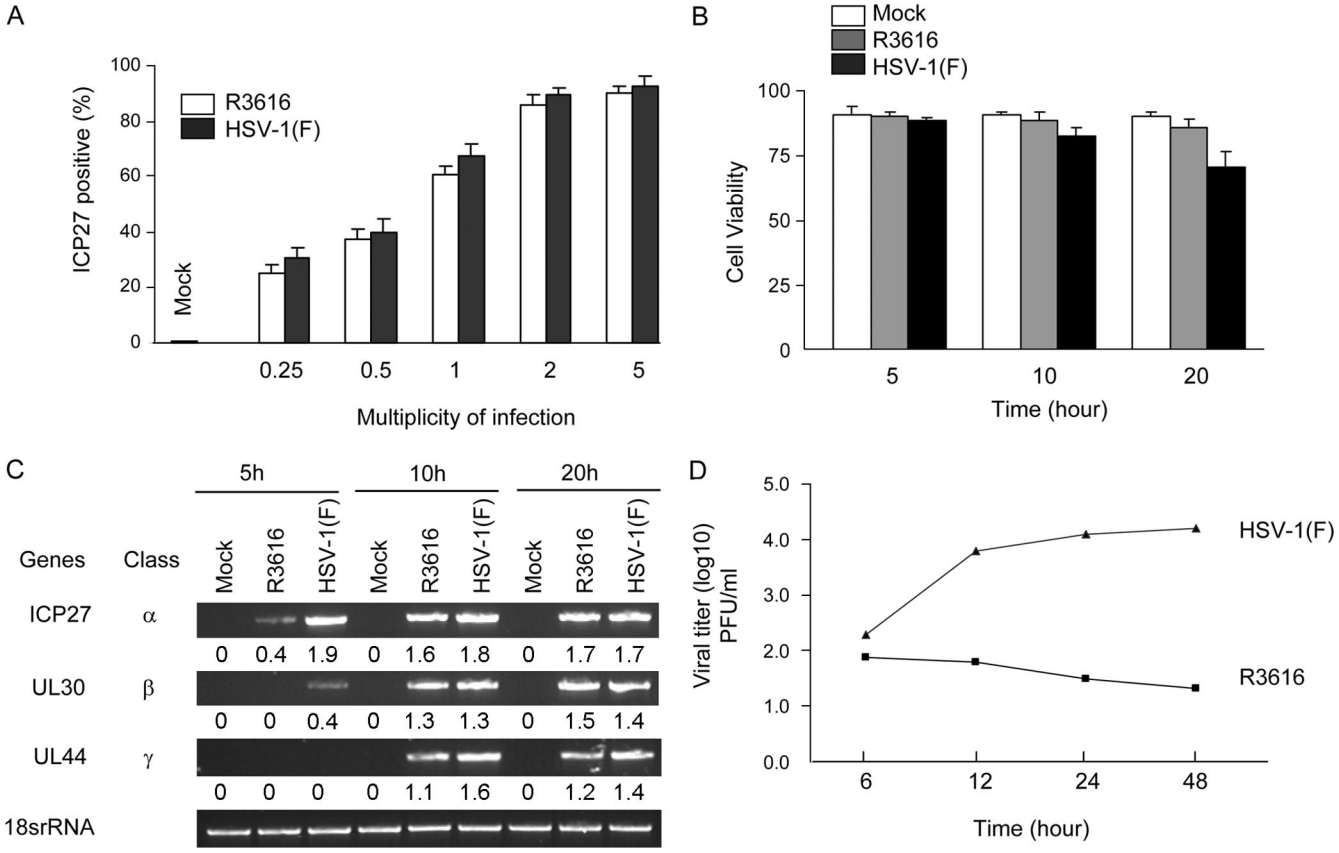

FIG. 1. (A) Viral infection of immature CD11c$^+$ DCs. Bone marrow-derived DCs grown in the presence of GM-CSF were mock infected or infected with wild-type virus HSV-1(F) or R3616, lacking the $\gamma_1$34.5 gene, at different multiplicities of infection. At 9 h after infection, viral infectivity was determined by examining ICP27 expression as described in Materials and Methods. (B) Effects of viral infection on cell viability. Immature DCs were mock infected or infected with viruses (2 PFU/cell). Cell viability was measured by the trypan blue exclusion method at the indicated time points. (C) Viral gene expression in immature DCs. Cells were mock infected or infected with viruses as described in the legend to panel B. Total RNAs were extracted and subjected to RT-PCR amplification for the analysis of ICP27 (α gene), UL30 (β gene), and UL44 (γ gene) RNA and 18S rRNA. The DNA bands were quantified using NIH ImageJ software. Numbers denote the ratios of the amounts of ICP27, UL30, and UL44 RNA to the amount of 18S RNA. (D) Viral growth in immature DCs. Cells were infected with HSV-1(F) or R3616 (2 PFU/cell). At different time points, cells were harvested and freeze-thawed three times and virus titers on Vero cells were determined.

rabbit (Dako) as described previously (48). Tissue sections were incubated with primary antibody at 43°C prior to the addition of biotinylated anti-rabbit immunoglobulin secondary antibody, avidin-horseradish peroxidase, and 3,3′-diaminobenzidine tetrahydrochloride (0.04%) in 0.05 M Tris-HCl (pH 7.4) and 0.025% H_2O_2 as a chromogen (Ventana Medical Systems, Tucson, AZ).

RESULTS

HSV-1 lacking the $\gamma_1$34.5 gene is capable of infecting immature DCs. As an initial step, we sought to compare the susceptibilities of DCs to infection with the $\gamma_1$34.5 null mutant and wild-type virus. Purified CD11c$^+$ DCs were generated from bone marrow in the presence of GM-CSF. These cells, constituting 95% of CD11c$^+$ CD11b$^+$ conventional DCs, were exposed to wild-type HSV-1(F) and R3616 which lacks the $\gamma_1$34.5 gene. ICP27 expression, as a measure of infectivity, was examined by fluorescence-activated cell sorter analysis. As shown in Fig. 1A, the number of ICP27-positive cells increased in a multiplicity-of-infection-dependent manner. At a multiplicity of infection of 2, more than 85% of cells were positive for ICP27 expression. A higher dose did not increase infectivity. HSV-1(F) and R3616 infected DCs comparably. A cell viability assay showed that at a multiplicity of infection of 2, 90% of DCs infected with R3616 were viable throughout infection (Fig. 1B). A similar result for HSV-1(F)-infected DCs at 5 or 10 h postinfection was seen. There was a slight reduction in the viability of HSV-1(F)-infected cells at 20 h, when 75% of cells were viable.

To assess viral gene expression, total RNA extracted from infected DCs was subjected to RT-PCR amplification (Fig. 1C). At the early time point (5 h), ICP27 expression was detectable in both HSV-1(F)- and R3616-infected cells, but its level was 3.75-fold higher in HSV-1(F)-infected cells. UL30 was expressed only weakly in HSV-1(F)-infected cells, and its level was 3.75-fold lower than that of ICP27. No UL44 was detectable. As virus infection progressed to late time points (10 and 20 h), basically the same levels of ICP27 and UL30 were observed. Levels of UL44 were 14 to 30% higher in HSV-1(F)-infected cells than in R3616-infected cells. We further measured the production of infectious virus in immature DCs infected at 2 PFU per cell. The results presented in Fig. 1D show that HSV-1(F) replicated to a titer of 6.2×10^3 PFU/ml at 12 h postinfection and increased to 1.9×10^4 PFU/ml at 48 h

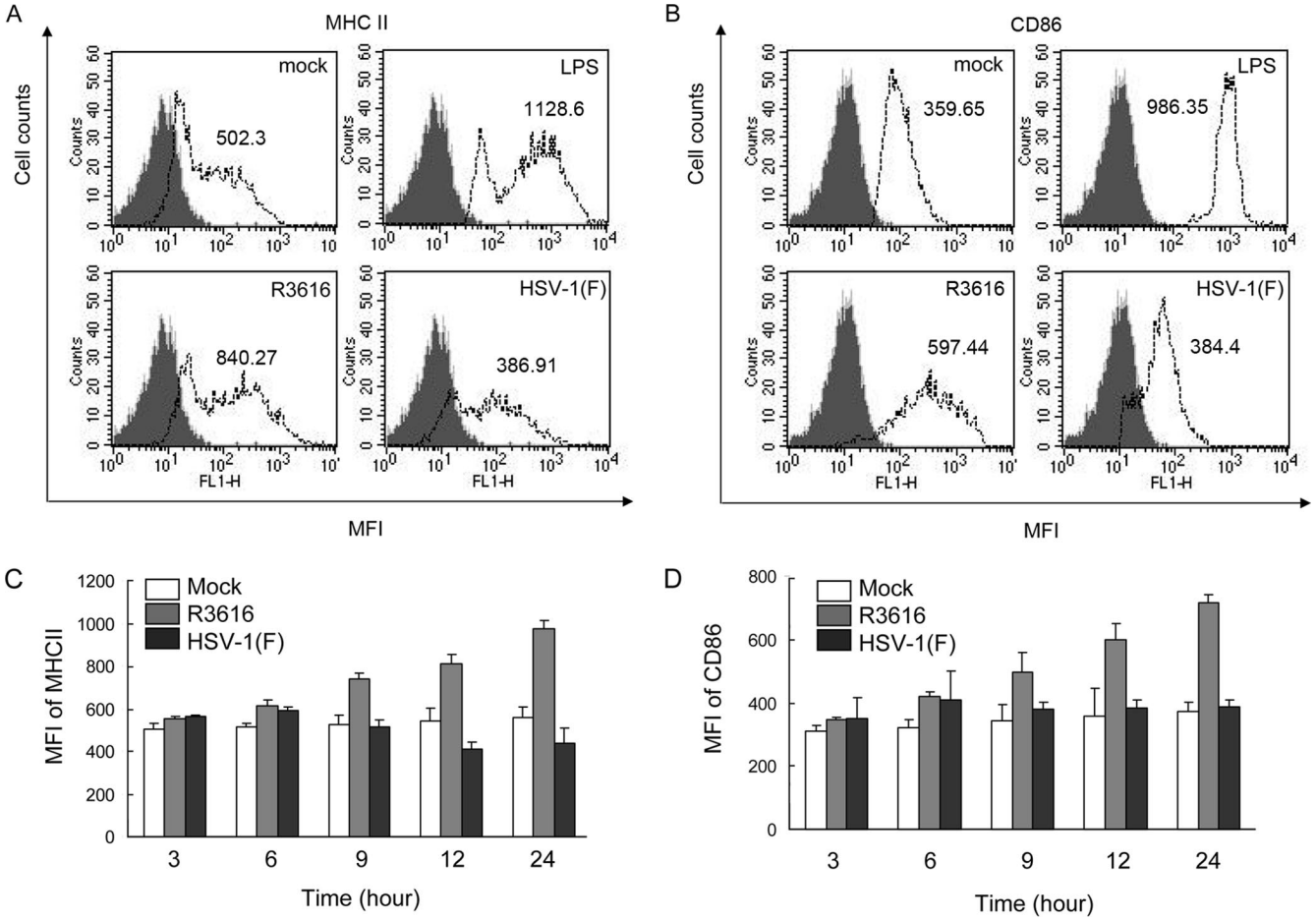

FIG. 2. Effects of $\gamma_1$34.5 on the expression of cell surface molecules. (A) MHC-II expression. Immature DCs were mock infected or infected with HSV-1(F) or R3616 (2 PFU/cell). As a positive control, cells were treated with LPS (0.5 μg/ml). At 12 h postinfection, cells were assessed for MHC-II expression by flow cytometry. Shaded areas, control staining by isotope antibody; open areas, staining by a specific antibody. Numbers denote levels of mean fluorescence intensity (MFI). (B) CD86 expression. Immature DCs, mock infected or infected with viruses as described in the legend to panel A, were stained for CD86 expression. (C) Time course of MHC-II expression. Immature DCs were mock infected or infected with viruses (2 PFU/cell). At 3, 6, 9, 12, and 24 h postinfection, cells were stained for MHC-II expression. (D) Time course of CD86 expression. Cells were treated as described in the legend to panel C and stained for CD86 expression.

postinfection. In contrast, R3616 barely grew, with virus yields remaining at approximately 1.2×10^2 PFU/ml over the course of infection. Wild-type virus and the $\gamma_1$34.5 null mutant were able to infect immature DCs and express viral RNA, but the $\gamma_1$34.5 null mutant was severely impaired in viral production.

The $\gamma_1$34.5 protein is required to suppress HSV-induced maturation of DCs. Based on the above-described analyses, we assessed the impact of $\gamma_1$34.5 on DC maturation. Immature CD11c$^+$ DCs, mock infected or infected with viruses (2 PFU/cell), were subjected to fluorescence-activated cell sorter analysis at 12 h after infection (Fig. 2A and B). We chose to use a multiplicity of infection of 2 because the majority of cells (>85%) are infected and viable after virus infection at this level. As expected, lipopolysaccharide (LPS) induced the upregulation of MHC-II and CD86 expression compared to that in control mock-infected cells. R3616 also stimulated the expression of MHC-II and CD86, although the magnitude was lower than that for LPS. In contrast, HSV-1(F) reduced the expression of MHC-II slightly and had no stimulatory effect on CD86. To monitor the kinetics of costimulatory-molecule upregulation, virus-infected DCs were analyzed over a 24-h period. As illustrated in Fig. 2C and D, in HSV-1(F)-infected cells, the expression of MHC-II and CD86 was maintained at basal levels, which were comparable to or slightly lower than those in mock-infected cells at the time points examined. Similar phenotypes in cells infected with R3616 were seen at 3 and 6 h after infection. As infection progressed to 9 h, R3616 stimulated MHC-II and CD86 expression significantly, and expression further increased at 12 and 24 h after infection. These results suggest that $\gamma_1$34.5 is required to suppress HSV-induced upregulation of costimulatory molecules in immature DCs.

We next analyzed cytokine production by intracellular staining with antibodies against IL-6, IL-12, IFN-α, and IFN-β. As shown in Fig. 3A and B, both HSV-1(F) and R3616 stimulated the expression of IL-6 and IL-12 throughout infection compared to that in mock-infected cells. At 24 h after infection, R3616 induced more cells to produce IL-6 and IL-12 than HSV-1(F). However, the expression patterns for IFN-α and IFN-β were different (Fig. 3C and D). Compared to the pro-

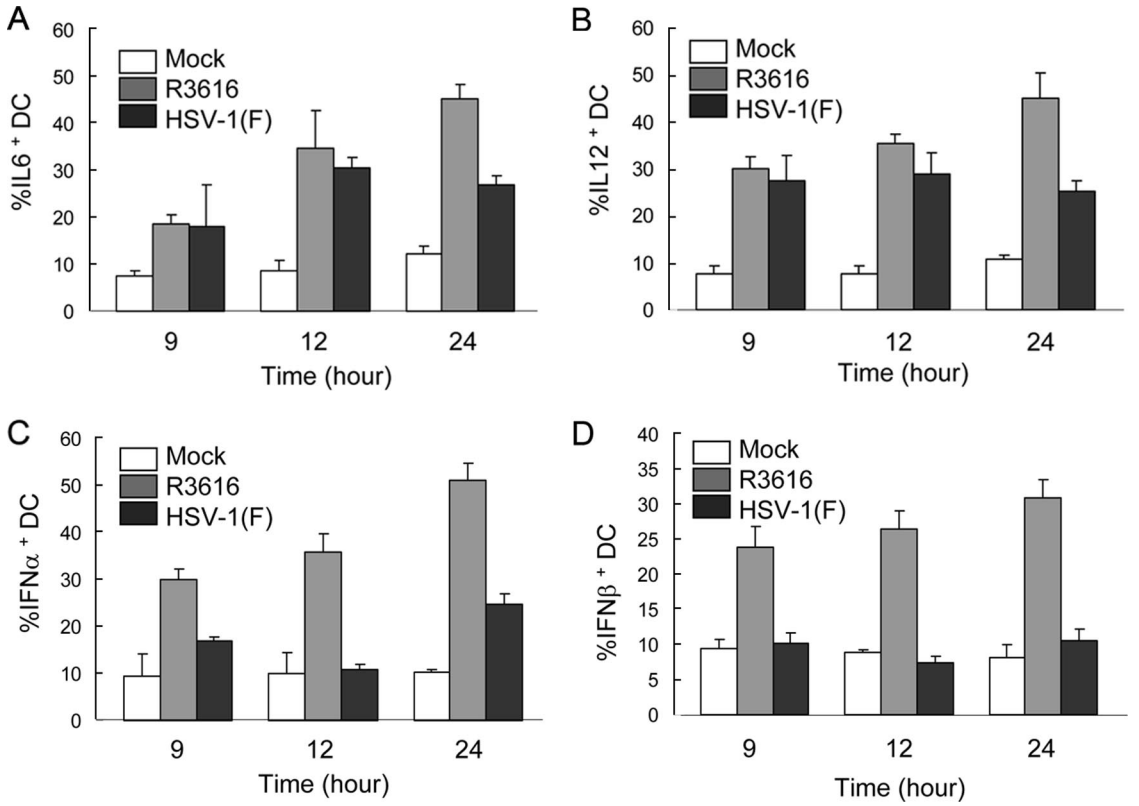

FIG. 3. Effects of $\gamma_1$34.5 on cytokine expression. Immature DCs were mock infected or infected with HSV-1(F) or R3616 (2 PFU/cell). At 9, 12, and 24 h postinfection, cells were processed and analyzed by flow cytometry, as described in Materials and Methods. (A) IL-6 expression. Cells were stained with anti-IL-6 antibody. (B) IL-12 expression. Cells were stained with anti-IL-12 antibody. (C) IFN-α expression. Cells were stained with anti-IFN-α antibody. (D) IFN-β expression. Cells were stained with anti-IFN-β antibody. The data are representative of results from two or three independent experiments with triplicate samples.

portion among mock-infected cells, HSV-1(F) infection modestly increased the proportion of IFN-α-positive DCs. This effect was not seen for IFN-β-positive cells at any of the time points examined. In striking contrast, R3616 infection increased IFN-α- and IFN-β-positive cells drastically. Thus, unlike wild-type virus, the $\gamma_1$34.5 null mutant stimulated IFN-α/β expression in DCs. These results suggest that $\gamma_1$34.5 is involved in blocking the maturation of DCs during HSV infection.

The interference of $\gamma_1$34.5 with DC maturation is linked to reduced IFN secretion. We further evaluated the IFN-α/β secretion among DCs by a bioassay. $CD11c^+$ DCs were mock infected or infected with HSV-1(F) or R3616, and the media were collected and irradiated with UV. The conditioned media, irradiated with UV, were incubated with Vero cells in the presence or absence of neutralizing antibodies against IFN-α/β. Vero cells were then subjected to infection with VSV-GFP, a virus sensitive to IFN. In this assay, the GFP signal inversely correlates with the IFN level. As revealed by flow cytometry analysis (Fig. 4A), in the absence of neutralizing antibodies against IFN-α/β, only 8.4% of mock-infected or 4.4% of HSV-1(F)-infected cells remained GFP negative. In contrast, 46.3% of R3616-infected cells were GFP negative, indicative of increased IFN secretion. Remarkably, the addition of antibodies against IFN-α/β to R3616-infected cells reduced GFP-negative cells to 6.27%. Neutralizing antibodies had modest effects on cells mock infected or infected with HSV-1(F), which is due likely to a low level of IFN production. We conclude that the $\gamma_1$34.5 null mutant indeed stimulated IFN-α/β production in DCs and that wild-type virus suppressed IFN-α/β expression.

To determine whether IFN secretion was required for DC maturation, DCs, mock infected or infected with viruses, were treated or left untreated with anti-IFN-α/β antibodies. At 12 h after treatment, cells were analyzed for MHC-II and CD86 expression. As shown in Fig. 4B and C, R3616 but not HSV-1(F) stimulated the expression of MHC-II and CD86 in DCs untreated with anti-IFN-α/β antibodies. Notably, treatment with anti-IFN-α/β antibodies led to a decrease in MHC-II and CD86 expression when DCs were infected with R3616. This reduction was partial but statistically significant (33%). Treatment with anti-IFN-α/β antibodies had a minor effect on HSV-1(F)-infected or mock-infected cells. Hence, these experimental results suggest that the inhibition of IFN production by $\gamma_1$34.5 contributes to impaired DC maturation. In addition, $\gamma_1$34.5 appears to exert its activity independently of IFN production.

Viral modulation of DC maturation is independent of viral DNA replication. To test whether viral DNA replication is linked to DC maturation, DCs were mock infected or infected with viruses in the presence or absence of phosphonoacetic acid (PAA; 400 μg/ml), a viral DNA polymerase inhibitor. At 12 h postinfection, cells were stained for the expression of

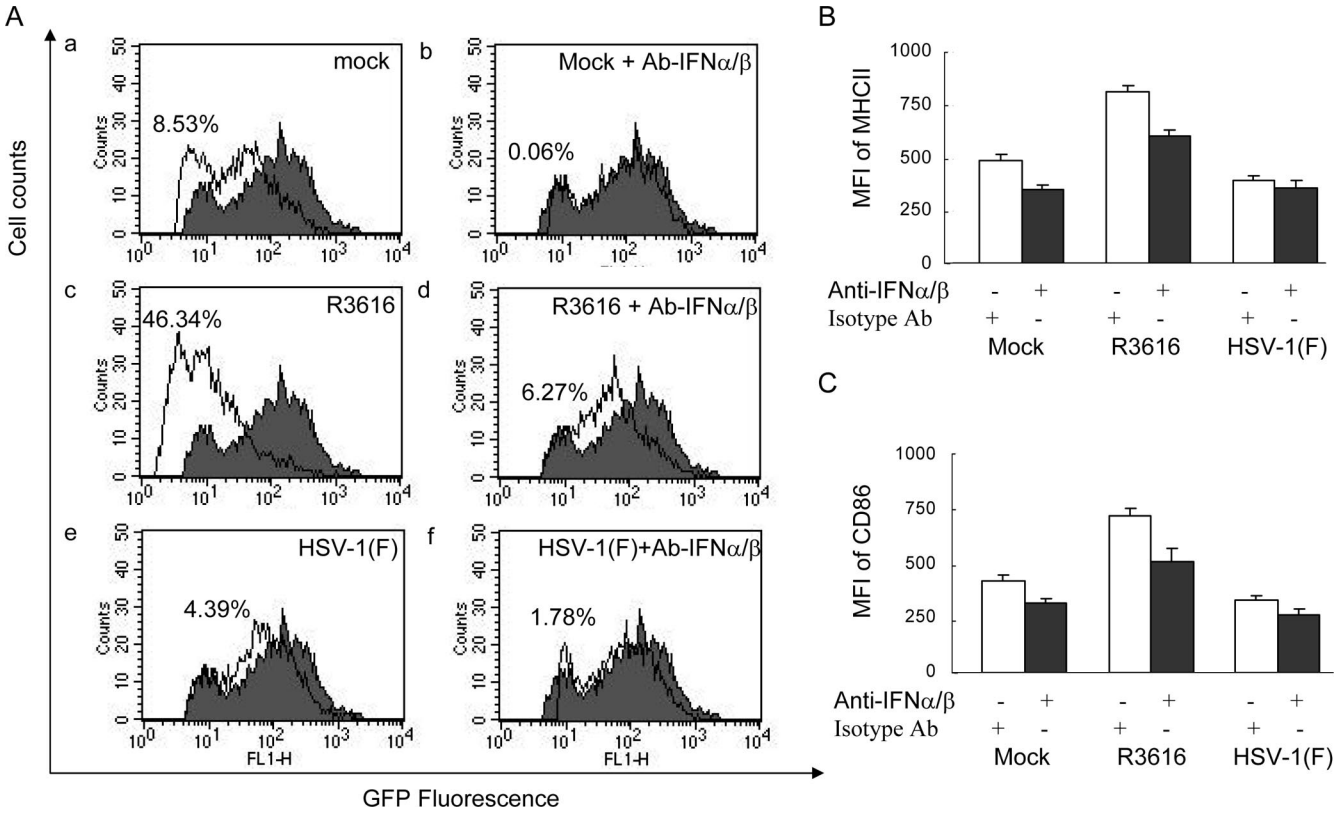

FIG. 4. (A) IFN-α/β secretion in immature DCs. Cells were mock infected or infected with HSV-1(F) or R3616 (2 PFU/cell) in the presence or absence of anti-IFN-α/β antibodies (Ab-IFN-α/β; 10 μg/ml). At 12 h postinfection, the media were collected and irradiated with UV light (0.25 J/cm^2). Vero cells, incubated with the media overnight, were then subjected to infection with VSV-GFP (10 PFU/cell). Cells were then assayed for a GFP signal by flow cytometry analysis. Filled areas represent VSV-GFP-positive cells. Open areas denote VSV-GFP-negative cells. Numbers are percentages of GFP-negative cells. (B) Effect of anti-IFN-α/β antibodies on MHC-II expression. Immature DCs were mock infected or infected with viruses (2 PFU/cell) in the presence (+) or absence (−) of anti-IFN-α/β antibodies (10 μg/ml). At 12 h postinfection, cells were stained for MHC-II expression and analyzed by flow cytometry. MFI, mean fluorescence intensity; Ab, antibody. (C) Immature DCs treated as described in the legend to panel B were stained for CD86 expression. The data represent results from one of two or three independent experiments with triplicate samples.

MHC-II, CD86, IFN-α, and IFN-β. As shown in Fig. 5A, unlike mock infection or HSV-1(F) infection, R3616 infection stimulated MHC-II expression. Similarly, R3616 but not HSV-1(F) stimulated the expression of CD86 (Fig. 5B), IFN-α (Fig. 5C), and IFN-β (Fig. 5D). Notably, these phenotypes were not affected by PAA, an inhibitor of viral DNA replication (data not shown), suggesting that HSV modulation of DC maturation is independent of viral DNA replication.

The $\gamma_1$34.5 protein attenuates the capacity of DCs to stimulate T-cell activation. Because functional DCs stimulate T-cell responses, we determined the effect of $\gamma_1$34.5 on T-cell activation. Immature DCs were mock infected or infected with viruses at 2 PFU per cell. At 12 h after infection, cells were treated with UV light to inactivate viruses. In parallel, a set of uninfected cells were stimulated with LPS as a control. The cells were cocultured with allogeneic CD4$^+$ T cells for 48 h, and cell proliferation was analyzed by flow cytometry. As shown in Fig. 6A, CD4$^+$ T cells alone exhibited 2.2% spontaneous proliferation whereas LPS stimulated a strong level of proliferation, with 89.72% of CD4$^+$ T cells being activated. Mock-infected DCs activated T-cell proliferation by 47.38%, which represents a background level. R3616-infected DCs induced a significantly higher level of T-cell proliferation (67.7%) than mock-infected cells. However, wild-type HSV-1(F)-infected DCs stimulated T-cell proliferation weakly (29.25%).

We next measured IFN-γ expression by CD4$^+$ T cells after coculture with DCs (Fig. 6B). As expected, T cells alone showed a background level of IFN-γ production (0.98% of cells were positive for isotype antibody staining) and LPS induced the expression of IFN-γ (resulting in 53.26% IFN-γ-positive cells). Similarly, R3616-infected DCs induced a higher percentage of IFN-γ-positive T cells (39.35%) than mock-infected DCs (19.88%). However, HSV-1(F)-infected DCs induced a background level of IFN-γ (13.85% IFN-γ-positive cells). Together, these results suggest that the $\gamma_1$34.5 protein is required to inhibit naïve T cells from expressing IFN-γ and differentiating into Th1 cells by modulating DC maturation.

The $\gamma_1$34.5 protein is required to suppress the maturation of DCs in vivo. To further examine $\gamma_1$34.5, we assessed DC maturation in an ocular infection model. Mice were mock infected or infected with viruses (2×10^5 PFU/eye). Single-cell suspensions were prepared from the eye tissues after infection, and phenotypes of DC11c$^+$ DCs were analyzed by flow cytometry.

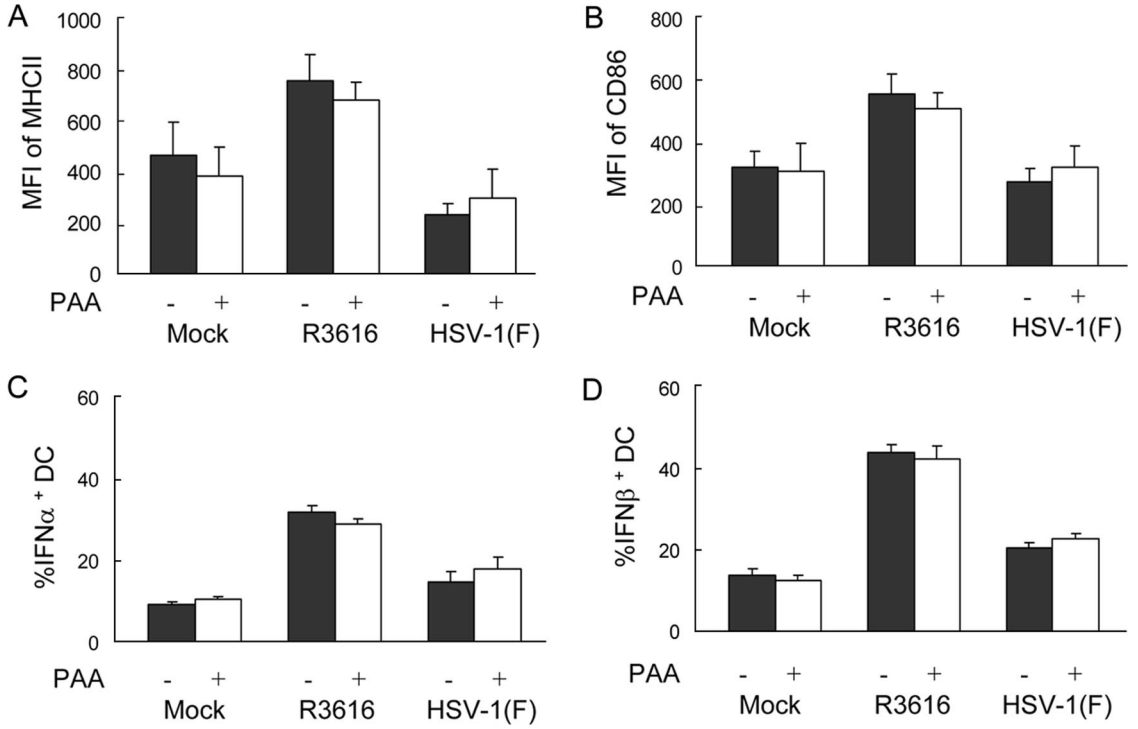

FIG. 5. Effect of viral DNA replication inhibitor on DC maturation. Immature DCs were mock infected or infected with HSV-1(F) or R3616 in the absence (−) or presence (+) of PAA (400 μg/ml). At 12 h postinfection, cells were stained for the expression of MHC-II (A), CD86 (B), IFN-α (C), and IFN-β (D). The data are representative of results from three independent experiments. MFI, mean fluorescence intensity.

As depicted in Fig. 7A and B, on days 1, 3, and 5, R3616 infection consistently stimulated higher levels of MHC-II and CD86 on DCs than mock infection. HSV-1(F)-infected cells expressed lower levels of MHC-II and CD86 than R3616-infected cells. Thus, the $\gamma_1$34.5 protein blocked the expression of costimulatory molecules in DCs of infected mice.

Cytokine assays revealed that fewer than 10% of DCs from mock-infected mice expressed IL-6 and IL-12 on days 1, 3, and 5 (Fig. 7C and D). However, infection with both R3616 and HSV-1(F) resulted in an increased number of DCs producing IL-6 and IL-12. Notably, HSV-1(F) induced slightly less IL-12 than R3616 over the course of infection. In addition, there was a low level of IFN-producing cells (less than 10%) among DCs from mock-infected mice (Fig. 7E and F). A similar pattern among DCs from mice infected with HSV-1(F) was seen throughout infection. In contrast, there was a prominent increase in IFN-positive DCs from R3616-infected mice, which was evident on days 1 and 3. These results indicate that $\gamma_1$34.5 is involved in blocking viral induction of IFN in DCs in vivo.

As a parallel approach, we examined viral replication in the eye. As illustrated in Fig. 8A, HSV-1(F) replicated efficiently in the eye, with titers reaching 5.5×10^5 PFU/ml on day 1, 3.9×10^4 PFU/ml on day 3, and 4.4×10^4 PFU/ml on day 5. In contrast, R3616 barely replicated over the course of infection. There was a 1,000-fold reduction in viral yield compared to that for HSV-1(F). These phenotypes were also mirrored by the results of immunohistochemistry analysis (Fig. 8B). Viral antigens were detectable only in the eyes infected with wild-type virus. Thus, viral replication correlated with the inhibition of DC maturation by $\gamma_1$34.5 in vivo.

DISCUSSION

Previous studies established that $\gamma_1$34.5 is essential to promote HSV virulence (8, 50). In HSV-infected cells, viral DNA replication triggers the double-stranded RNA-dependent protein kinase R (PKR), which phosphorylates the α subunit of eukaryotic initiation factor 2 (eIF-2α), leading to translation arrest (7, 9). As a countermeasure, $\gamma_1$34.5 forms a complex with protein phosphatase 1 that dephosphorylates the α subunit of eukaryotic initiation factor 2 (14). Consistently, the $\gamma_1$34.5 null mutant is virulent in PKR knockout mice but not in wild-type mice (8, 26). However, $\gamma_1$34.5 null mutants with secondary mutations inhibit the PKR activity but remain attenuated in vivo (6, 35). A working model to reconcile these phenotypes is that $\gamma_1$34.5 has additional activities involving, for example, viral egress and autophagy (5, 46). Recently, $\gamma_1$34.5 was found to inhibit TBK1 (49), which regulates TLR-dependent and -independent pathways (22). The data presented in this study indicate that $\gamma_1$34.5 is required to suppress DC functions, which are key mediators of intrinsic resistance to HSV (21). The deletion of the $\gamma_1$34.5 gene stimulated the expression of CD86, MHC-II, and proinflammatory cytokines such as IFN-α/β in DCs. These phenotypes were also seen in an ocular infection model. Remarkably, the expression of $\gamma_1$34.5 inhibited the ability of DCs to induce T-cell activation. Hence, $\gamma_1$34.5 perturbs innate and adaptive immunity.

Our work underscores the importance of IFN and HSV inhibition in DC maturation. Unlike wild-type virus, the $\gamma_1$34.5 null mutant caused drastic induction of IFN production, which paralleled an upregulation of costimulatory molecules. The

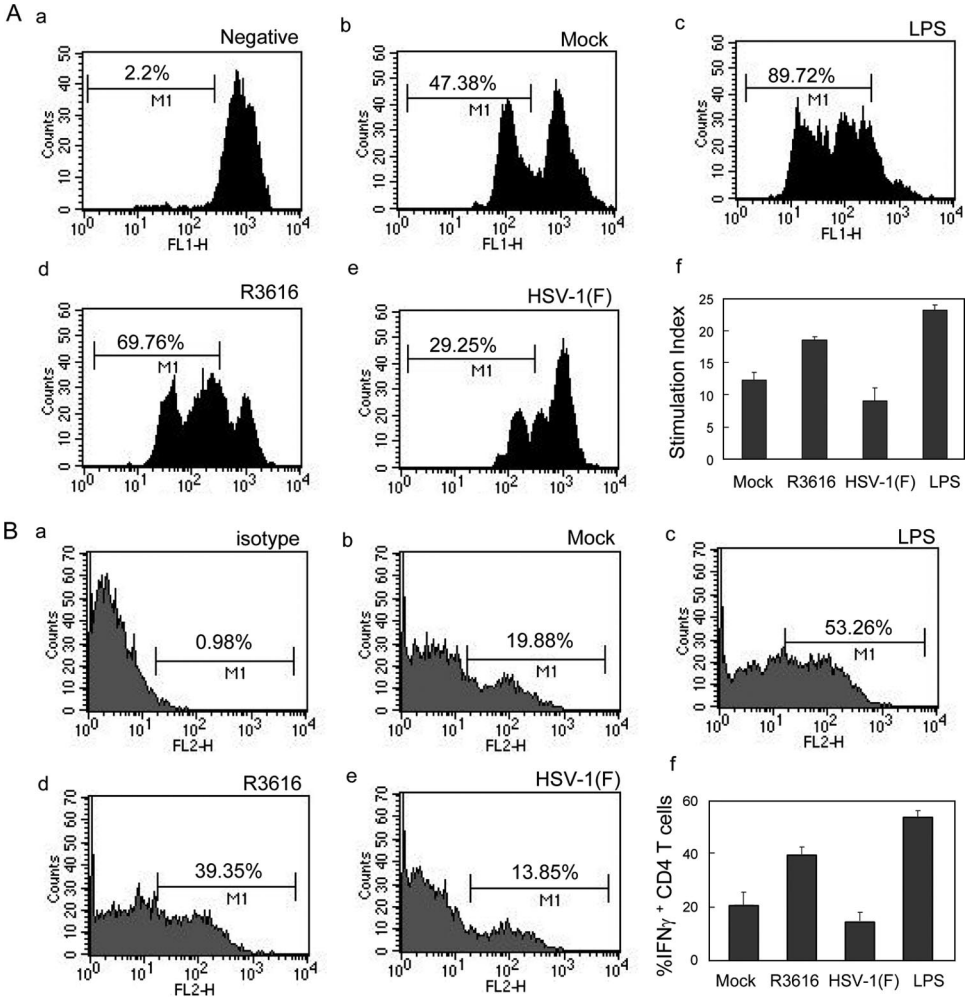

FIG. 6. Activation of naïve CD4 T cells by DCs. Immature DCs were mock infected or infected with HSV-1(F) or R3616 (2 PFU/cell). In parallel, cells were treated with LPS (0.5 μg/ml) as a positive control. At 12 h postinfection, cells were irradiated with UV light (at 0.25 J/cm^2) and used to stimulate allogeneic CD4$^+$ T cells in vitro for 48 h. (A) T-cell proliferation was measured by using CFSE activity and expressed as the percentage of proliferation relative to the baseline population. The plots show spontaneous T-cell proliferation (a) and proliferation resulting from stimulation with mock-infected DCs (b), LPS-treated immature DCs (c), R3616-infected DCs (d), and HSV-1(F)-infected DCs (e). (f) The stimulation index was calculated as the ratio of stimulated proliferation to spontaneous proliferation. M1 denotes gating of CFSE-positive cells. (B) IFN-γ production in CD4$^+$ T cells was assessed by flow cytometry analysis. The plots depict the percentages of T cells showing isotype antibody staining (a) and anti-IFN-γ staining after stimulation with mock-infected DCs (b), LPS-treated DCs (c), R3616-infected DCs (d), and HSV-1(F)-infected DCs (e). (f) Percentages of IFN-γ-positive T cells after incubation with DCs infected with the indicated viruses. The data are representative of results from three independent experiments.

addition of IFN-neutralizing antibodies partially reversed the phenotype in DCs exposed to the $\gamma_1$34.5 null mutant. These results suggest that the inhibition of IFN production by $\gamma_1$34.5 contributes to the suppression of DC maturation. In this context, it is notable that IFN is able to induce the upregulation of MHC and costimulatory molecules, such as CD80 and CD86 (13, 29, 44). In fact, IFN stimulates DC maturation during viral infection (1, 17, 37). In HSV-infected cells, IFN production from myeloid DCs is believed to mediate bystander activation of uninfected DCs (37). However, analyses with influenza virus and Sendai virus suggest that IFN is not essential for DC maturation (28). These observations support the view that besides IFN, additional cellular components regulate DC maturation. Interpreting within the framework of this model, we also observed that the inhibition of IFN-neutralizing antibodies had only partial effects on DC maturation during HSV infection. A working hypothesis to explain these results is that $\gamma_1$34.5 may function through IFN-dependent and -independent mechanisms.

The $\gamma_1$34.5 protein is a leaky late protein which is expressed both early (2 to 4 h) and late in infection (10, 31, 49). This pattern raises the question of how $\gamma_1$34.5 works. Several models can be envisioned. First, $\gamma_1$34.5 acts to modulate innate immune signaling at the early stage of infection. Recent studies suggest that HSV-1 infection triggers DC maturation through multiple pathways (37). In addition to inducing IFN, viral glycoproteins activate NF-κB and p38 mitogen-activated protein kinase signaling pathways in DCs. Therefore, $\gamma_1$34.5 may inhibit one or more of these pathways leading to DC maturation. Another possibility is that $\gamma_1$34.5 may interfere with TLR-

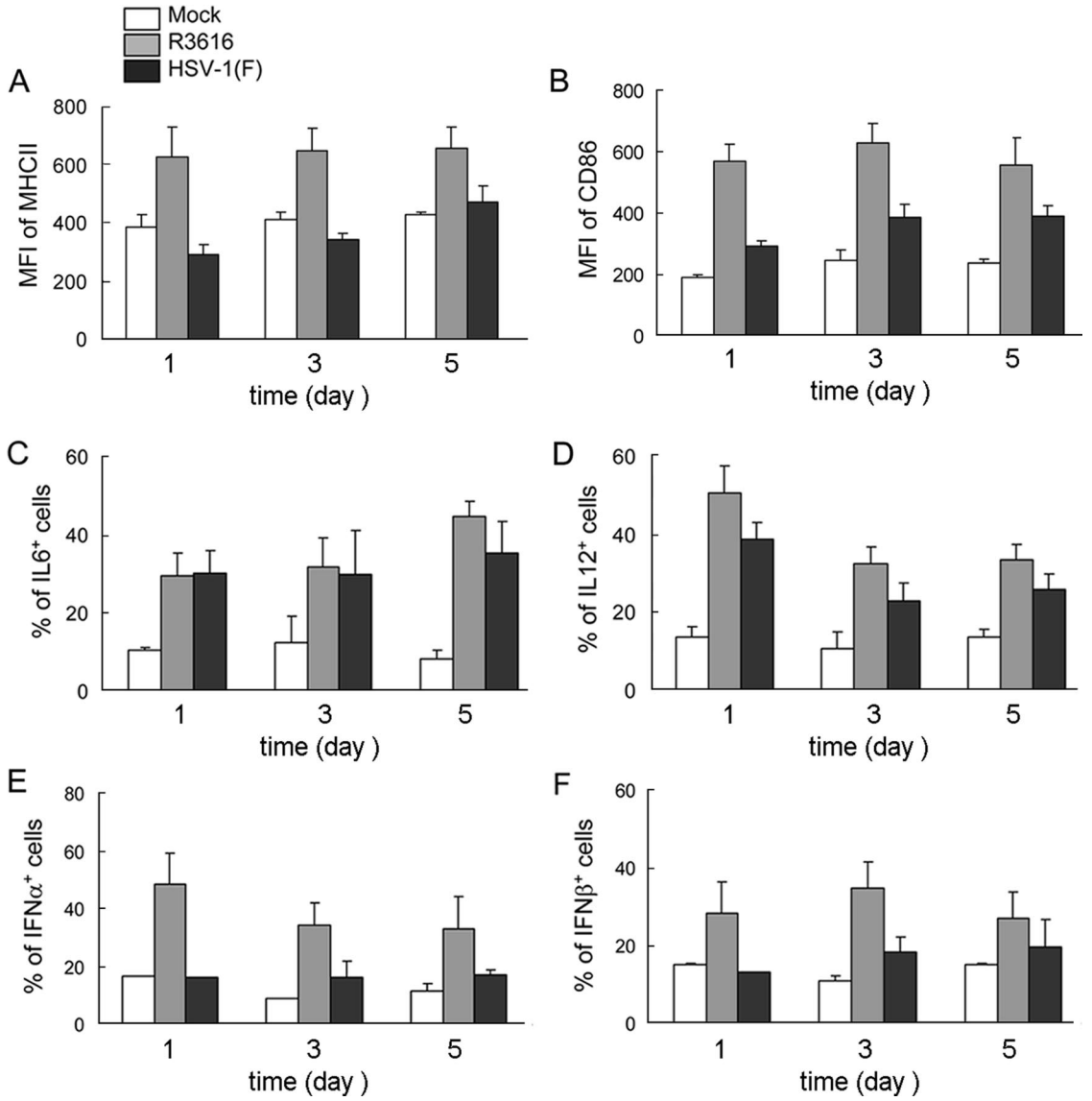

FIG. 7. Modulation of DC maturation by $\gamma_1$34.5 in vivo. Groups of five mice were mock infected or infected with HSV-1(F) or R3616 via corneal scarification (2×10^5 PFU/per eye). On days 1, 3, and 5 after infection, the eye tissues were isolated and single-cell suspensions were prepared. Cells were stained for the expression of MHC-II (A), CD86 (B), IL-6 (C), IL-12 (D), IFN-α (E), and IFN-β (F) by gating on CD11c-positive cells. The data are representative of results from two or three independent experiments. MFI, mean fluorescence intensity.

dependent and -independent pathways because signals from these pathways promote DC maturation (17, 28). In this respect, it is interesting that $\gamma_1$34.5 associates with TBK1 (49), which is essential for cytokine expression. Second, $\gamma_1$34.5 functions to regulate a host or viral protein at the late stage of infection. For example, the inhibition of the PKR response by $\gamma_1$34.5 may contribute to a block of DC maturation. Since DCs with PKR deficiency mature normally in response to viral infection (17, 28), we believe that this hypothesis is less likely. Another possibility is that $\gamma_1$34.5 may regulate a viral protein required to inhibit DC maturation. We observed that PAA did not reverse the inhibitory effect of wild-type virus on DCs, suggesting that a late viral protein or function is dispensable. Third, $\gamma_1$34.5 may target a yet unknown cellular factor that inhibits viral gene expression and indirectly block DC maturation. Additional experiments are needed to test these hypotheses.

DCs have been postulated previously to play a crucial role in mediating resistance to HSV-1 (21). We observed that in infected DCs the $\gamma_1$34.5 null mutant barely replicated but that wild-type virus replicated efficiently, with an approximately 100-fold increase in virus yield compared to that of the null mutant. Similarly, in the mouse ocular infection model, wild-type virus, but not the $\gamma_1$34.5 null mutant, replicated efficiently in the eye. Notably, viral replication in DCs inversely correlated with the production of IFN both in vitro and in vivo. As IFN plays a critical role in antiviral responses, it is likely that the ability of $\gamma_1$34.5 to inhibit IFN production directly facilitates productive infection in DCs.

Results from several studies show that conventional submu-

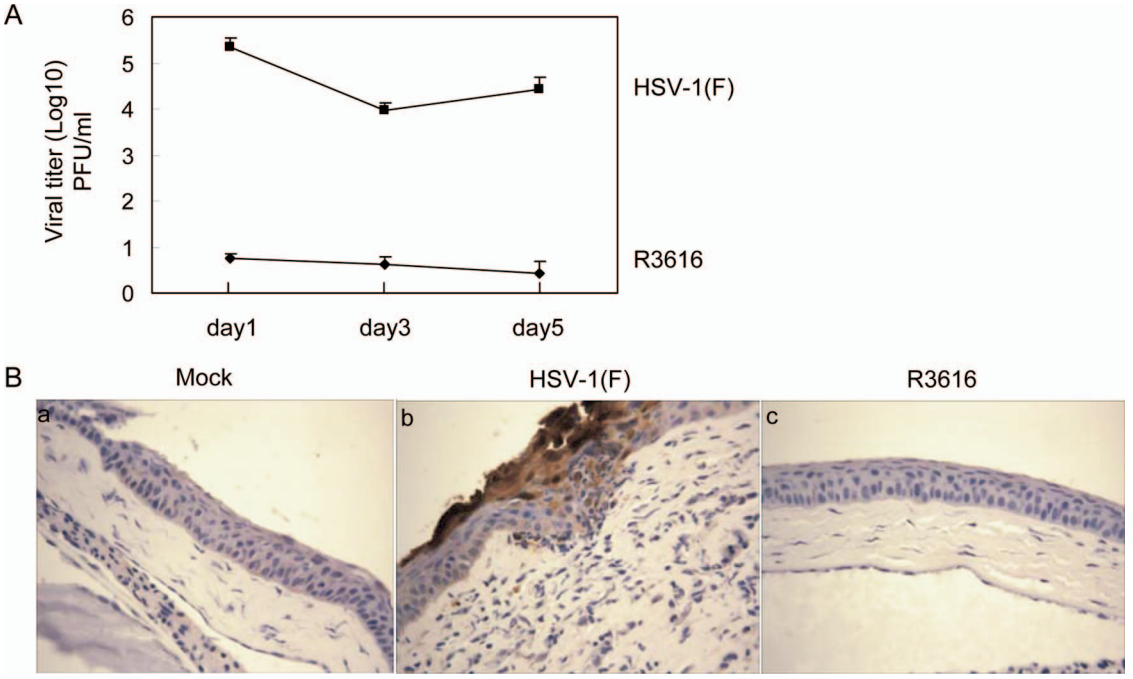

FIG. 8. (A) Viral replication in the eye. Mice were mock infected or infected with viruses as described in the legend to Fig. 7. Viral yields from the eyes of mice were determined on days 1, 3, and 5 after infection. The data are representative of results from three independent experiments. (B) Viral antigens. Thin sections, prepared from the eye tissues obtained on day 5 as mentioned in the legend to panel A, were processed for immunohistochemistry analysis with anti-HSV-1 antibody as described in Materials and Methods. Representative images from each group are shown. HSV-specific staining is shown in brown.

cosal and lymph node resident DCs are responsible for HSV-specific T-cell activation (2, 45, 52). The ablation of DCs impairs the activation of NK cells and $CD4^+$ and $CD8^+$ T cells in response to HSV-1 (21). We noted that the suppression of DC maturation by the $\gamma_1$34.5 protein led to the inhibition of T-cell activation in vitro. Two lines of evidence support this argument. DCs infected with wild-type virus suppressed $CD4^+$ T-cell proliferation, whereas DCs infected with the $\gamma_1$34.5 null mutant stimulated proliferation. Similarly, DCs infected with wild-type virus inhibited IFN-γ secretion from $CD4^+$ T cells, whereas DCs infected with the $\gamma_1$34.5 null mutant stimulated IFN-γ secretion. The precise mechanism by which $\gamma_1$34.5 inhibits T-cell activation is unknown. Emerging evidence suggests that TBK1 is required for optimal T-cell responses (19). As $\gamma_1$34.5 inhibits the kinase activity of TBK1 (49), it is possible that such an interaction negatively regulates DC functions, which leads to impaired T-cell activation. This model may partly explain why $\gamma_1$34.5 is required for viral virulence in vivo. Work is in progress to address this issue.

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REFERENCES

1. **Ahmed, M., K. L. Brzoza, and E. M. Hiltbold.** 2006. Matrix protein mutant of vesicular stomatitis virus stimulates maturation of myeloid dendritic cells. J. Virol. **80:**2194–2205.
2. **Allan, R. S., C. M. Smith, G. T. Belz, A. L. van Lint, L. M. Wakim, W. R. Heath, and F. R. Carbone.** 2003. Epidermal viral immunity induced by CD8α+ dendritic cells but not by Langerhans cells. Science **301:**1925–1928.
3. **Bjorck, P.** 2004. Dendritic cells exposed to herpes simplex virus in vivo do not produce IFN-alpha after rechallenge with virus in vitro and exhibit decreased T cell alloreactivity. J. Immunol. **172:**5396–5404.
4. **Bosnjak, L., M. Miranda-Saksena, D. M. Koelle, R. A. Boadle, C. A. Jones, and A. L. Cunningham.** 2005. Herpes simplex virus infection of human dendritic cells induces apoptosis and allows cross-presentation via uninfected dendritic cells. J. Immunol. **174:**2220–2227.
5. **Brown, S. M., A. R. MacLean, J. D. Aitken, and J. Harland.** 1994. ICP34.5 influences herpes simplex virus type 1 maturation and egress from infected cells in vitro. J. Gen. Virol. **75:**3679–3686.
6. **Cassady, K. A., M. Gross, G. Y. Gillespie, and B. Roizman.** 2002. Second-site mutation outside of the U_S10-12 domain of $\Delta\gamma_1$34.5 herpes simplex virus 1 recombinant blocks the shutoff of protein synthesis induced by activated protein kinase R and partially restores neurovirulence. J. Virol. **76:**942–949.
7. **Chou, J., J. J. Chen, M. Gross, and B. Roizman.** 1995. Association of a M(r) 90,000 phosphoprotein with protein kinase PKR in cells exhibiting enhanced phosphorylation of translation initiation factor eIF-2α and premature shutoff of protein synthesis after infection with $\gamma_1$34.5− mutants of herpes simplex virus 1. Proc. Natl. Acad. Sci. USA **92:**10516–10520.
8. **Chou, J., E. R. Kern, R. J. Whitley, and B. Roizman.** 1990. Mapping of herpes simplex virus-1 neurovirulence to $\gamma_1$34.5, a gene nonessential for growth in culture. Science **250:**1262–1266.
9. **Chou, J., and B. Roizman.** 1992. The $\gamma_1$34.5 gene of herpes simplex virus 1 precludes neuroblastoma cells from triggering total shutoff of protein synthesis characteristic of programmed cell death in neuronal cells. Proc. Natl. Acad. Sci. USA **89:**3266–3270.
10. **Chou, J., and B. Roizman.** 1986. The terminal *a* sequence of the herpes simplex virus genome contains the promoter of a gene located in the repeat sequences of the L component. J. Virol. **57:**629–637.
11. **Eidson, K. M., W. E. Hobbs, B. J. Manning, P. Carlson, and N. A. DeLuca.** 2002. Expression of herpes simplex virus ICP0 inhibits the induction of interferon-stimulated genes by viral infection. J. Virol. **76:**2180–2191.
12. **Ejercito, P. M., E. D. Kieff, and B. Roizman.** 1968. Characterization of herpes simplex virus strains differing in their effects on social behaviour of infected cells. J. Gen. Virol. **2:**357–364.
13. **Gallucci, S., M. Lolkema, and P. Matzinger.** 1999. Natural adjuvants: endogenous activators of dendritic cells. Nat. Med. **5:**1249–1255.
14. **He, B., M. Gross, and B. Roizman.** 1997. The $\gamma_1$34.5 protein of herpes

simplex virus 1 complexes with protein phosphatase 1α to dephosphorylate the α subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase. Proc. Natl. Acad. Sci. USA **94:**843–848.

15. **Heath, W. R., G. T. Belz, G. M. Behrens, C. M. Smith, S. P. Forehan, I. A. Parish, G. M. Davey, N. S. Wilson, F. R. Carbone, and J. A. Villadangos.** 2004. Cross-presentation, dendritic cell subsets, and the generation of immunity to cellular antigens. Immunol. Rev. **199:**9–26.
16. **Hochrein, H., B. Schlatter, M. O'Keeffe, C. Wagner, F. Schmitz, M. Schiemann, S. Bauer, M. Suter, and H. Wagner.** 2004. Herpes simplex virus type-1 induces IFN-alpha production via Toll-like receptor 9-dependent and -independent pathways. Proc. Natl. Acad. Sci. USA **101:**11416–11421.
17. **Honda, K., S. Sakaguchi, C. Nakajima, A. Watanabe, H. Yanai, M. Matsumoto, T. Ohteki, T. Kaisho, A. Takaoka, S. Akira, T. Seya, and T. Taniguchi.** 2003. Selective contribution of IFN-alpha/beta signaling to the maturation of dendritic cells induced by double-stranded RNA or viral infection. Proc. Natl. Acad. Sci. USA **100:**10872–10877.
18. **Inaba, K., M. Inaba, N. Romani, H. Aya, M. Deguchi, S. Ikehara, S. Muramatsu, and R. M. Steinman.** 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J. Exp. Med. **176:**1693–1702.
19. **Ishii, K. J., T. Kawagoe, S. Koyama, K. Matsui, H. Kumar, T. Kawai, S. Uematsu, O. Takeuchi, F. Takeshita, C. Coban, and S. Akira.** 2008. TANK-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines. Nature **451:**725–729.
20. **Jones, C. A., M. Fernandez, K. Herc, L. Bosnjak, M. Miranda-Saksena, R. A. Boadle, and A. Cunningham.** 2003. Herpes simplex virus type 2 induces rapid cell death and functional impairment of murine dendritic cells in vitro. J. Virol. **77:**11139–11149.
21. **Kassim, S. H., N. K. Rajasagi, X. Zhao, R. Chervenak, and S. R. Jennings.** 2006. In vivo ablation of CD11c-positive dendritic cells increases susceptibility to herpes simplex virus type 1 infection and diminishes NK and T-cell responses. J. Virol. **80:**3985–3993.
22. **Kawai, T., and S. Akira.** 2006. Innate immune recognition of viral infection. Nat. Immunol. **7:**131–137.
23. **Krug, A., G. D. Luker, W. Barchet, D. A. Leib, S. Akira, and M. Colonna.** 2004. Herpes simplex virus type 1 activates murine natural interferon-producing cells through Toll-like receptor 9. Blood **103:**1433–1437.
24. **Kruse, M., O. Rosorius, F. Kratzer, G. Stelz, C. Kuhnt, G. Schuler, J. Hauber, and A. Steinkasserer.** 2000. Mature dendritic cells infected with herpes simplex virus type 1 exhibit inhibited T-cell stimulatory capacity. J. Virol. **74:**7127–7136.
25. **Kummer, M., N. M. Turza, P. Muhl-Zurbes, M. Lechmann, C. Boutell, R. S. Coffin, R. D. Everett, A. Steinkasserer, and A. T. Prechtel.** 2007. Herpes simplex virus type 1 induces CD83 degradation in mature dendritic cells with immediate-early kinetics via the cellular proteasome. J. Virol. **81:**6326–6338.
26. **Leib, D. A., M. A. Machalek, B. R. Williams, R. H. Silverman, and H. W. Virgin.** 2000. Specific phenotypic restoration of an attenuated virus by knockout of a host resistance gene. Proc. Natl. Acad. Sci. USA **97:**6097–6101.
27. **Lin, R., R. S. Noyce, S. E. Collins, R. D. Everett, and K. L. Mossman.** 2004. The herpes simplex virus ICP0 RING finger domain inhibits IRF3- and IRF7-mediated activation of interferon-stimulated genes. J. Virol. **78:**1675–1684.
28. **Lopez, C. B., A. Garcia-Sastre, B. R. Williams, and T. M. Moran.** 2003. Type I interferon induction pathway, but not released interferon, participates in the maturation of dendritic cells induced by negative-strand RNA viruses. J. Infect. Dis. **187:**1126–1136.
29. **Luft, T., K. C. Pang, E. Thomas, P. Hertzog, D. N. Hart, J. Trapani, and J. Cebon.** 1998. Type I IFNs enhance the terminal differentiation of dendritic cells. J. Immunol. **161:**1947–1953.
30. **Lund, J., A. Sato, S. Akira, R. Medzhitov, and A. Iwasaki.** 2003. Toll-like receptor 9-mediated recognition of herpes simplex virus-2 by plasmacytoid dendritic cells. J. Exp. Med. **198:**513–520.
31. **McKay, E. M., B. McVey, H. S. Marsden, S. M. Brown, and A. R. MacLean.** 1993. The herpes simplex virus type 1 strain 17 open reading frame RL1 encodes a polypeptide of apparent M_r 37K equivalent to ICP34.5 of herpes simplex virus type 1 strain F. J. Gen. Virol. **74:**2493–2497.
32. **Melroe, G. T., N. A. DeLuca, and D. M. Knipe.** 2004. Herpes simplex virus 1 has multiple mechanisms for blocking virus-induced interferon production. J. Virol. **78:**8411–8420.
33. **Melroe, G. T., L. Silva, P. A. Schaffer, and D. M. Knipe.** 2007. Recruitment of activated IRF-3 and CBP/p300 to herpes simplex virus ICP0 nuclear foci: potential role in blocking IFN-beta induction. Virology **360:**305–321.
34. **Mikloska, Z., L. Bosnjak, and A. L. Cunningham.** 2001. Immature monocyte-derived dendritic cells are productively infected with herpes simplex virus type 1. J. Virol. **75:**5958–5964.
35. **Mohr, I., D. Sternberg, S. Ward, D. Leib, M. Mulvey, and Y. Gluzman.** 2001. A herpes simplex virus type 1 γ34.5 second-site suppressor mutant that exhibits enhanced growth in cultured glioblastoma cells is severely attenuated in animals. J. Virol. **75:**5189–5196.
36. **Novak, N., and W. M. Peng.** 2005. Dancing with the enemy: the interplay of herpes simplex virus with dendritic cells. Clin. Exp. Immunol. **142:**405–410.
37. **Pollara, G., M. Jones, M. E. Handley, M. Rajpopat, A. Kwan, R. S. Coffin, G. Foster, B. Chain, and D. R. Katz.** 2004. Herpes simplex virus type-1-induced activation of myeloid dendritic cells: the roles of virus cell interaction and paracrine type I IFN secretion. J. Immunol. **173:**4108–4119.
38. **Pollara, G., K. Speidel, L. Samady, M. Rajpopat, Y. McGrath, J. Ledermann, R. S. Coffin, D. R. Katz, and B. Chain.** 2003. Herpes simplex virus infection of dendritic cells: balance among activation, inhibition, and immunity. J. Infect. Dis. **187:**165–178.
39. **Prechtel, A. T., N. M. Turza, D. J. Kobelt, J. I. Eisemann, R. S. Coffin, Y. McGrath, C. Hacker, X. Ju, M. Zenke, and A. Steinkasserer.** 2005. Infection of mature dendritic cells with herpes simplex virus type 1 dramatically reduces lymphoid chemokine-mediated migration. J. Gen. Virol. **86:**1645–1657.
40. **Rasmussen, S. B., L. N. Sorensen, L. Malmgaard, N. Ank, J. D. Baines, Z. J. Chen, and S. R. Paludan.** 2007. Type I interferon production during herpes simplex virus infection is controlled by cell-type-specific viral recognition through Toll-like receptor 9, the mitochondrial antiviral signaling protein pathway, and novel recognition systems. J. Virol. **81:**13315–13324.
41. **Reske, A., G. Pollara, C. Krummenacher, D. R. Katz, and B. M. Chain.** 2008. Glycoprotein-dependent and TLR2-independent innate immune recognition of herpes simplex virus-1 by dendritic cells. J. Immunol. **180:**7525–7536.
42. **Salio, M., M. Cella, M. Suter, and A. Lanzavecchia.** 1999. Inhibition of dendritic cell maturation by herpes simplex virus. Eur. J. Immunol. **29:**3245–3253.
43. **Samady, L., E. Costigliola, L. MacCormac, Y. McGrath, S. Cleverley, C. E. Lilley, J. Smith, D. S. Latchman, B. Chain, and R. S. Coffin.** 2003. Deletion of the virion host shutoff protein (vhs) from herpes simplex virus (HSV) relieves the viral block to dendritic cell activation: potential of vhs^- HSV vectors for dendritic cell-mediated immunotherapy. J. Virol. **77:**3768–3776.
44. **Santini, S. M., C. Lapenta, M. Logozzi, S. Parlato, M. Spada, T. Di Pucchio, and F. Belardelli.** 2000. Type I interferon as a powerful adjuvant for monocyte-derived dendritic cell development and activity in vitro and in Hu-PBL-SCID mice. J. Exp. Med. **191:**1777–1788.
45. **Smith, C. M., G. T. Belz, N. S. Wilson, J. A. Villadangos, K. Shortman, F. R. Carbone, and W. R. Heath.** 2003. Cutting edge: conventional CD8 alpha+ dendritic cells are preferentially involved in CTL priming after footpad infection with herpes simplex virus-1. J. Immunol. **170:**4437–4440.
46. **Tallaozy, Z., W. Jiang, H. W. Virgin IV, D. A. Leib, D. Scheuner, R. J. Kaufman, E. L. Eskelinen, and B. Levine.** 2002. Regulation of starvation- and virus-induced autophagy by the eIF2α kinase signaling pathway. Proc. Natl. Acad. Sci. USA **99:**190–195.
47. **Trgovcich, J., D. Johnson, and B. Roizman.** 2002. Cell surface major histocompatibility complex class II proteins are regulated by the products of the $\gamma_1$34.5 and U_L41 genes of herpes simplex virus 1. J. Virol. **76:**6974–6986.
48. **Valyi-Nagy, T., V. Sheth, C. Clement, V. Tiwari, P. Scanlan, J. H. Kavouras, L. Leach, G. Guzman-Hartman, T. S. Dermody, and D. Shukla.** 2004. Herpes simplex virus entry receptor nectin-1 is widely expressed in the murine eye. Curr. Eye Res. **29:**303–309.
49. **Verpooten, D., Y. J. Ma, S. W. Hou, Z. P. Yan, and B. He.** 2009. Control of TANK binding kinase mediated signaling by the $\gamma_1$34.5 protein of herpes simplex virus 1. J. Biol. Chem. **284:**1097–1105.
50. **Whitley, R. J., E. R. Kern, S. Chatterjee, J. Chou, and B. Roizman.** 1993. Replication, establishment of latency, and induced reactivation of herpes simplex virus $\gamma_1$34.5 deletion mutants in rodent models. J. Clin. Investig. **91:**2837–2843.
51. **Whitley, R. J., and B. Roizman.** 2001. Herpes simplex virus infections. Lancet **357:**1513–1518.
52. **Zhao, X., E. Deak, K. Soderberg, M. Linehan, D. Spezzano, J. Zhu, D. M. Knipe, and A. Iwasaki.** 2003. Vaginal submucosal dendritic cells, but not Langerhans cells, induce protective Th1 responses to herpes simplex virus-2. J. Exp. Med. **197:**153–162.