CD1 Antigen Presentation by Human Dendritic Cells as a Target for Herpes Simplex Virus Immune Evasion

Martin J. Raftery, Florian Winau, Stefan H. E. Kaufmann, Ulrich E. Schaible and Günther Schönbichler

J Immunol 2006; 177:6207-6214; doi: 10.4049/jimmunol.177.9.6207
http://www.jimmunol.org/content/177/9/6207

References
This article cites 79 articles, 41 of which you can access for free at:
http://www.jimmunol.org/content/177/9/6207.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
CD1 Antigen Presentation by Human Dendritic Cells as a Target for Herpes Simplex Virus Immune Evasion

Martin J. Raftery, Florian Winau, Stefan H. E. Kaufmann, Ulrich E. Schaible, and Günther Schönrich

In contrast to MHC molecules, which present peptides, the CD1 molecules have been discovered to present lipid Ags to T cells. CD1-restricted T cells recognize lipids, glycolipids, or lipopeptides of either foreign or self origin as Ags (1). The distantly related CD1 proteins constitute an evolutionarily distinct family of Ag-presenting molecules (2). Humans express five different CD1 molecules (CD1a-e). According to their sequence homology, CD1 proteins can be further divided into group I (CD1 a-c), group II (CD1d), and CD1e representing an intermediate form. Group I members are expressed by thymocytes, activated monocytes, B cells, and dendritic cells (DC) whereas CD1d has a broader distribution (3). Unlike MHC class I, the CD1 molecules function independently of the TAP molecules (4, 5). By trafficking through endosomal compartments, CD1 proteins pick up Ags in a similar fashion to MHC class II molecules (6).

CD1-restricted T cells recognize lipids, glycolipids, or lipopeptides of either foreign or self origin as Ags (7). Activation of T cells by CD1-mediated lipid Ag recognition was first demonstrated in the context of infection with Mycobacterium tuberculosis (8). The CD1b molecule presents various lipid Ags, including lipoarabinomannan (LAM), derived from mycobacteria (9). Besides natural endogenous Ags (10, 11) and bacterial Ags (11–14), CD1d molecules can also present α-galactosylceramide (αGalCer), a foreign lipid derived from a marine sponge (15, 16). NKT cells express a CD1d-restricted invariant T cell AgR and strongly respond to αGalCer (7, 17).

Recent studies provide evidence that the CD1 Ag presentation system has an antiviral role. Patients with NKT cell deficiencies associated with disseminated viral infection have been described (18, 19). In HIV-infected patients, a selective loss of NKT cells occurs (20–23). Moreover, insights gained from CD1d-deficient mice suggest that CD1d-restricted T lymphocytes are required for protection against some viral infections (24–27) but not others (28–32), possibly depending on the virulence of the virus strain used (32). In addition, CD1d-restricted T cells have been reported to play an essential role in virus-induced immunopathogenesis (33, 34). In a number of infection models, therapeutic activation of NKT cells by αGalCer helps to clear the virus (30, 35) or improves disease outcome (24, 33). Stimulation of CD1-restricted T cells has been demonstrated in a transgenic mouse model of hepatitis B virus infection (36) and in patients with hepatitis C (37–39). Finally, the discovery that viruses can interfere with CD1 Ag presentation (40–44) is indirect evidence for a role of CD1-restricted T lymphocytes in antiviral immune defense. So far, the mechanisms leading to activation of CD1-restricted T cells in the course of viral infection remain unclear.

DC, which link innate and adaptive immunity (45), can be infected by a panoply of viruses (46) including HSV (47–54). This ubiquitous pathogen is a member of the herpesvirus family, persists for the lifetime of its host, and causes life-threatening illness in immunocompromised individuals (55). HSV-infected DC lose their capacity to efficiently stimulate MHC-restricted T cells (47–54). On the molecular level, inhibition of MHC class I Ag presentation is mediated by HSV-encoded infected cell protein (ICP) 47, which blocks TAP function (56, 57). Numerous other findings document the ingenious viral strategies to block the MHC I pathway (58). In sharp contrast, knowledge on the interplay between viruses and the CD1 Ag presentation system is virtually lacking. In this study, we analyzed presentation of CD1-restricted Ags by HSV-infected human DC. We show that DC respond to the HSV-encoded TAP blocker ICP47 by enhancing CD1 presentation and that this cellular response, in turn, is counterregulated by the virus.

*Institute of Virology, Charité Medical School, Berlin, Germany; and ‡Max-Planck-Institute for Infection Biology, Berlin, Germany

Received for publication March 14, 2006. Accepted for publication July 31, 2006.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† This work was supported by the Deutsche Forschungsgemeinschaft (SFB421).

‡ Address correspondence and reprint requests to Dr. Günther Schönrich, Institute of Virology, Charité Medical School, Humboldt University Berlin, Schumanstrasse 20/21, D-10117 Berlin, Germany. E-mail address: guenther.schoenrich@charite.de

§ Abbreviations used in this paper: DC, dendritic cell; LAM, lipoarabinomannan; αGalCer, galactosylceramide; ICP47, infected cell protein 47; MOI, multiplicity of infection.
Materials and Methods

Cells and virus

U373 cells were maintained in Eagle’s MEM supplemented with 10% heat-inactivated FCS, 100 IU penicillin, 100 µg/ml streptomycin, and 4.5 mM glutamine. Recombinant virus ICP47Δ, which lacks the MHC class I blocker ICP47, and ICP47Δ rescue virus were gifts from R. L. Hendricks (University of Illinois, Chicago, IL) (59). HSV type I strain F, from which the recombinant viruses were derived, was obtained from American Type Culture Collection (ATCC). Viruses were propagated in Vero E6 cells, the supernatant from lytic cells being cleared of debris by centrifugation and subsequently frozen in liquid nitrogen. After 1 h incubation, cells were washed three times with PBS before resuspension in medium.

CD1 transfectants

For generation of CD1b- and CD1d-transfected cell lines, cDNA derived from immature DC was amplified by PCR using appropriate primer sets and cloned into pEco2 (Invitrogen Life Technologies). Similarly, the ICP47Δ gene was amplified from HSV type I strain F genomic DNA and cloned into pIREs (BD Biosciences). The constructs were transfected into cell lines by electroporation.

Generation of DC

Human DC were generated from monocytes isolated from buffy coat preparations supplied by the German Red Cross (Berlin, Germany). Monocytes were isolated from PBMC by negative selection with Ab-coupled magnetic beads (Miltenyi Biotec), and cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS, 100 IU penicillin, 100 µg/ml streptomycin, 4.5 mM glutamine, 500 U/ml GM-CSF, and 200 U/ml IL-4. After 2 days of culture, DC were used for experiments. Lipoteichoic acid from Staphylococcus aureus was provided by Cayla Sas/InvivoGen and used at concentration of 1 µg/ml.

Antibodies

For phenotypic analyses of cell markers by flow cytometry, the following reagents were used: human CD1a mAb clone HI149 was obtained from ImmunoTools; human CD1b mAb clone 4A7.6.6 (60) and human CD1c clone L161 were purchased from Immunotech; human CD1d mAb clone CD1d42 was obtained from BD Biosciences; clone Nor3.2 was obtained from Chemicon International; human CD1d mAb clone CD1d55 (61) was a gift from S. A. Porcelli (Albert Einstein College of Medicine, New York, NY); and human MHCI mAb clone W6/32 was a gift from G. Moldenhauer (German Cancer Research Center, Heidelberg, Germany). Human β-actin mAb clone AC-15 was supplied by Acris. Isotype-matched control Abs were purchased from BD Biosciences/BD Pharmingen. Rabbit anti-HSV was obtained from DAKOPATTS.

Flow cytometry

Cells in suspension were washed once with ice-cold FACSwash solution (PBS with 1% FCS and 0.02% sodium azide) before being resuspended with the first Ab in ice-cold blocking solution (PBS with 10% heat-inactivated FCS and 0.2% sodium azide) for 1 h. The cells were then washed in ice-cold FACSwash solution and the staining was repeated with PE-coupled anti-mouse secondary Ab. Subsequently, cells were washed in ice-cold FACSwash and then resuspended in 100 µl of PBS with 0.3% formaldehyde. Flow cytometry was performed on a FACS caliber (BD Biosciences). For double-staining of CD1b and HSV Ags, the same procedure was followed except with both primary Abs (CD1b-specific mAb and polyclonal antisera against HSV Ags) or appropriate control Ab (isotype-matched irrelevant Ab) given in the first phase, followed by Cy5-conjugated anti-mouse IgG and FITC-coupled anti-rabbit IgG as secondary Abs.

Endocytosis assay

Cells were stained with the relevant mAb in normal culture medium on ice for 1 h. For analysis of endocytosis, cells were stained with a FITC-coupled secondary Ab and were subsequently incubated for 2 h at 37°C. Cells were then stripped of cell surface-bound Ab by incubation with a 300 mM glycine/1% FCS solution at pH 2 for 3 min before neutralization and fixation. To analyze endocytosis with unlabeled mAb, cells were stained as above but incubated for 4 h at either 4°C or 37°C. Cells were then stained for remaining cell surface-bound mAb with a PE-labeled secondary Ab. Confocal microscopy

U373 transfectants grown on coverslips or immature DC (1 × 10⁵) centrifuged onto slides were fixed/permeabilized by acetone/methanol (1:1) at −20°C for 5 min. Thereafter, slides were washed three times before being stained with mAbs against CD1a (clone HI149), CD1b (clone 4A7.6.5), CD1d (CD1d42), MHC class II (clone L243), or rabbit anti-HSV followed by isotype-specific Alexa 488- or Cy5-coupled Abs, or FITC-coupled goat anti-rabbit Abs. Slides were analyzed on a Leica TCS SP confocal microscope.

Western blot

Cells infected for 18 h were lysed for 1 h and cleared of debris by centrifugation for 1 h. A total of 50 µg (U373) or 150 µg (DC) was treated with peptide-N-glycosidase F (Roche) for 18 h before being loaded onto a 15% polyacrylamide gel. After electrophoresis, transfer onto a polyvinylidene difluoride membrane and blocking membranes were stained with anti-CD1d mAb (clone Nor3.2), stripped, and reconstained with anti-β-actin mAb (clone AC-15). Secondary Abs coupled to HRP were used at a concentration of 1/5000.

T cell assays

A total of 5 × 10⁴ APC (immature DC or U373) was irradiated with 50 Gy (immature DC) or 100 Gy (U373) and pulsed with 10 ng/ml pp65-peptide, 10 µg/ml LAM, or 1–5 µg/ml αGalCer for 30 min before 5 × 10⁴ Ag-specific T cells/well were added. Cells were cultured in triplicate in complete medium in 96-well round-bottom plates for 3 days at 37°C and 5% CO₂ before 10 Ci/well (1 µCi/well; Amersham). Thereafter, cells were harvested and liquid scintillation measurements were performed using a Top Count scintillation counter (Packard Instrument). The specific NKT cell response shown in Figs. 3 and 4 was calculated by subtracting baseline proliferation (APC plus NKT cells) from proliferation in the presence of Ag (APC plus NKT cells plus Ag). The baseline proliferation was in every case below 2000 cpm. LCDA4.7, a LAM-reactive T cell line, was derived from lesions of a leprosy patient (62). The CD1d-restricted NKT cells have been described elsewhere (12). αGalCer was supplied by the Kirin Brewery. In separate assays, supernatant was collected from the reactions and frozen at −20°C before being tested by ELISA for IFN-γ, IL-4, and IL-10 (ImmunoTools).

Results

Altered expression of the CD1 Ag presentation system as a response of human DC to HSV infection

HSV modulates both DC phenotype and function of MHC molecules. It is not known, however, if HSV alters the expression pattern of the MHC-related CD1 proteins, molecules which are largely restricted to DC. We assessed surface expression of CD1 molecules after infection of cell lines transfected with CD1 or endogenously CD1-expressing immature DC (Fig. 1A). At a multiplicity of infection (MOI) of 1, the surface levels of CD1a and CD1c molecules remained unchanged. In contrast, CD1d and CD1b expression both on transfected cells and on DC was enhanced after infection with live HSV. Morphological changes of infected DC were observed by measuring the forward scatter/side scatter profile (Fig. 1B, left panel). Moreover, double staining for CD1b and HSV Ags confirmed up-regulation of CD1b on the surface of HSV-infected DC at low MOI (Fig. 1B, right panel). Interestingly, the small percentage of cells expressing very high levels of viral Ags showed strongly down-regulated CD1b expression. No correlation between this population and apoptosis-induced DNA degradation could be seen (data not given). To analyze the cellular CD1 expression in more detail, we used confocal microscopy. In DC, redistribution of CD1b was associated with the presence and colocalization of HSV-encoded Ags (Fig. 2A). In DC infected with live (MOI = 1) but not UV-inactivated HSV, CD1b lost its characteristic localization in the MHC class II compartment and partially relocated to the cell surface (Fig. 2B). Control stainings (Fig. 2, C and D) show the specificity of the reagents used. Taken together, these data demonstrate that some HSV proteins colocalize with CD1b molecules. Furthermore, at low MOIs, DC also increase surface expression of CD1d and CD1b in response to HSV infection.
Activation of CD1-restricted T cells by HSV-infected human DC

Next, we analyzed the functional consequences of CD1 up-regulation on low titer HSV-infected DC. To this end, HSV-infected immature DC pulsed with lipid Ag presented by CD1b (LAM) or CD1d (αGalCer) were used to stimulate corresponding CD1-restricted human T cell lines (Fig. 3). As expected from previous studies, a T cell line recognizing peptide revealed reduced Ag presentation through MHC class I molecules (data not shown). In marked contrast, HSV-infected DC stimulated CD1b-restricted T lymphocytes more efficiently than control DC (Fig. 3A). A similar increase was seen for CD1d-restricted T lymphocytes (Fig. 3B). Thus, immature DC respond to HSV infection by decreasing their capacity to induce proliferation of MHC class I-restricted T cell lines while simultaneously increasing the proliferation of CD1b- and CD1d-restricted T cell lines.

Impaired response of the human CD1 Ag presentation system to ICP47-negative HSV

As viral proteins can have multiple functions, we hypothesized that ICP47, the only known HSV-encoded MHC class I blocker (56, 57), also interferes with Ag presentation through the MHC class I-like CD1 molecules. We anticipated that in the absence of ICP47, the HSV-induced activation of CD1-restricted T cells would be further enhanced. Therefore, the stimulatory capacity of DC after infection with ICP47Δ, a rHSV lacking ICP47, was determined (Fig. 4). Surprisingly, DC infected with ICP47Δ stimulated CD1b- and CD1d-restricted T cells less efficiently than the rescue virus (the ICP47 gene has been reintroduced into ICP47Δ) or wild-type virus. However, the capacity of ICP47Δ-infected DC to activate CD1-restricted T cells was still higher than that of uninfected DC. These findings suggested that besides other unknown mechanisms, ICP47 is contributing to increased CD1 Ag presentation by HSV-infected DC.

Decreased CD1 surface expression on cells infected with ICP47-negative HSV

In additional experiments, we determined whether the absence of ICP47 influences CD1 surface expression. Indeed, immature DC infected with ICP47Δ showed reduced levels of CD1d and CD1b on the surface as compared with cells infected with rescue virus (Fig. 5A). The ICP47-associated changes in CD1d expression on infected DC was difficult to assess due to the low CD1d baseline expression on uninfected DC. For this reason, we used CD1d-expressing U373 transfectants (U373-CD1d cells). Fig. 5B shows reduced CD1d levels on U373-CD1d cells infected with ICP47Δ in comparison to cells infected with the rescue virus confirming the results obtained with DC. These data imply that ICP47 enhances the density of CD1 molecules on the surface of HSV-infected DC.
Enhanced CD1 Ag presentation by ICP47-expressing cells

As a next step, we examined whether ICP47 up-regulates CD1 expression independently of viral infection. For this purpose, we transiently supertransfected U373 astrogliaoma cells expressing CD1b (U373-CD1b) or CD1d (U373-CD1d) with an ICP47 expression construct (Fig. 6A). ICP47 induced up-regulation of CD1b or CD1d on U373-CD1b or U373-CD1d cells, respectively.

CD1b (U373-CD1b) or CD1d (U373-CD1d) with an ICP47 expression construct (Fig. 6A). ICP47 induced up-regulation of CD1b or CD1d on U373-CD1b or U373-CD1d cells, respectively.
Thus, ICP47 alone is sufficient for increasing CD1 surface expression. Finally, we analyzed the effect of ICP47-induced up-regulation of CD1 surface molecules on T cell activation. CD1-restricted T lymphocytes were stimulated with CD1-expressing U373 cells after ICP47 transfection. In the presence of ICP47, the CD1b-expressing U373 cells stimulated CD1-specific T cells with higher efficiency than control cells transfected with the empty vector (Fig. 6B). Similarly, CD1d-expressing U373 cells presented αGalCer more efficiently to CD1d-restricted T cells when transfected with the ICP47 gene (Fig. 6B). Thus, host cells respond to the HSV-encoded MHC-I blocker ICP47 by enhancing CD1 Ag presentation.

**Viral interference with CD1 Ag presentation in human DC**

So far, we analyzed the reaction of the cellular CD1 Ag presentation system to infection with HSV. However, members of the herpesvirus family are able to interfere with DC function. To visualize potential viral evasion mechanisms cell lines transfected with CD1 or endogenously CD1-expressing immature DC were infected with a high MOI and surface expression of CD1 molecules was determined. Indeed, CD1 molecules were down-regulated on both transfected U373 cells and immature DC at a MOI of 100 (Fig. 7A). In transfected cell lines, the total amount of CD1 protein remained unchanged (MOI = 1) or was slightly decreased (MOI = 100) postinfection (Fig. 7B, left panel). In contrast, at both low and high MOIs the total amount of CD1d protein was found to be diminished in DC after infection with live HSV as compared with UV-inactivated HSV (Fig. 7B, right panel). At low MOI, this block was not reflected by flow cytometry, where an increased density of CD1d was detected on DC (see Fig. 1A) due to the effect of HSV-encoded ICP47 and other unknown mechanisms. Thus, a high viral load reduces both surface expression and the total amount of CD1 protein in DC indicating viral interference with the human CD1 Ag presentation system.

Additional experiments revealed that HSV inhibits internalization of CD1 molecules. After infection with HSV at a high MOI (100), DC were less able to internalize CD1a as measured by uptake (Fig. 8A) or reduction in cell surface Ab staining (Fig. 8B). In contrast, maturation induced by lipoteichoic acid from *Staphylococcus aureus*, a ligand of TLR2, which HSV has been shown to stimulate (63), did not interfere with internalization. However, this viral effect on cell function was not observed with transfected cells as both CD1a and CD1d internalization were not reduced by infection. Collectively, this observation emphasizes the unique nature of DC endogenously expressing CD1 molecules and reveal viral interference with the trafficking of CD1 molecules in DC.

Finally, the capacity of HSV-infected DC to stimulate the release of important cytokines characteristic for NKT cells (IL-4, IL-10, and IFN-γ) was assessed. DC infected with UV-inactivated virus induced more IFN-γ or IL-4 than uninfected DC whereas DC infected with live HSV at a low (1) or high (100) MOI strongly inhibited IFN-γ or IL-10 release by NKT cells (Fig. 9A). IL-4 secretion was not affected by DC that had been infected with a low MOI, most likely because IL-4 production by NKT cells is more rapid than other cytokines (64). To investigate the immediate effect of HSV, DC were mixed with NKT cells directly after infection (Fig. 9B). In this case, the inhibition of cytokine release by HSV was less pronounced. Thus, at low and high titers, HSV interferes with the capacity of DC to stimulate cytokine release, particularly IFN-γ, by NKT cells.

**Discussion**

CD1-restricted T cells have been shown to contribute to the antiviral immune response against HSV in mice (25). The underlying mechanisms and potential viral countermeasures, however, are not defined. Here, we have demonstrated two novel findings. First, human DC respond to HSV infection by enhancing CD1 Ag presentation. Second, this evolutionary pressure has forced the virus...
to develop immune evasion mechanisms that target the CD1 Ag presentation machinery and modulate the function of NKT cells.

Many studies have shown that HSV infection impairs the MHC presentation pathway of DC (47–52). We confirmed that HSV-infected DC have a reduced capacity for presentation of conventional peptides to MHC class I-restricted T cells (data not shown). In sharp contrast, DC infected with the same MOI (1) had a higher density of CD1b and CD1d molecules on the cell surface and could more efficiently induce proliferation of CD1-restricted T cells than control DC. Confocal microscopy analysis suggests that the elevated density of CD1b on low MOI HSV-infected DC is due to redistribution of CD1b from the MHC class II compartment to the cell membrane. Although the increase in CD1d surface expression was only moderate HSV-infected DC induced strong proliferation of NKT cells. This result is in accordance with the observation that even low levels of CD1d molecules on monocytes are associated with potent Ag-presenting capacity (61). The observed up-regulation of CD1b and CD1d molecules at least in part must be due to a posttranslational mechanism as it occurred in transfected cells expressing CD1 molecules under the control of a cellular promoter (ef1α). Such a mechanism could allow presentation of CD1-bound viral Ags or self Ags even during viral shutoff of host protein synthesis.

Indeed, we could identify a posttranslational pathway that enables DC to enhance Ag presentation through CD1b and CD1d molecules in response to HSV. DC infected with mutant HSV lacking ICP47, the only known HSV-encoded MHC class I blocker (56, 57), were surprisingly less efficient stimulators of CD1-restricted T cells than control DC. Confocal microscopy analysis suggests that the elevation of CD1b on low MOI HSV-infected DC is due to redistribution of CD1b from the MHC class II compartment to the cell membrane. Although the increase in CD1d surface expression was only moderate HSV-infected DC induced strong proliferation of NKT cells. This result is in accordance with the observation that even low levels of CD1d molecules on monocytes are associated with potent Ag-presenting capacity (61). The observed up-regulation of CD1b and CD1d molecules at least in part must be due to a posttranslational mechanism as it occurred in transfected cells expressing CD1 molecules under the control of a cellular promoter (ef1α). Such a mechanism could allow presentation of CD1-bound viral Ags or self Ags even during viral shutoff of host protein synthesis.

We found that the density of CD1b molecules on DC infected with live ICP47Δ virus was still lower than on cells infected with UV-inactivated ICP47Δ. This emphasizes that in the absence of ICP47 low titers of HSV can effectively interfere with HSV-infected DC. Similarly, regulation of murine CD1d transcripts by microbial signals and proinflammatory cytokines has recently been demonstrated (65). We conclude that DC try to counteract virus-induced paralysis of Ag presentation through MHC molecules by activating CD1-restricted T cells.

Besides blocking the biosynthesis of MHC class I complexes, numerous other ingenious strategies are used by viruses to interfere with the MHC I Ag presentation (58). The Kaposi’s sarcoma-associated herpesvirus encodes 2 proteins, K3 and K5, which enhance endocytosis of MHC class I complexes. As a consequence, the density of MHC class I molecules on the cell surface is downregulated. These viral proteins also trigger internalization of CD1 molecules (our own unpublished data and Ref. 43). Moreover, the Nef protein encoded by HIV down-regulates surface expression of MHC class I molecules and also CD1 molecules (our own unpublished data and Refs. 40, 42, 44). Thus, viral immunoevasins, which target the endocytic pathway, can interfere with Ag presentation through CD1 molecules. The quantity of CD1 molecules on cells infected with live ICP47Δ was still lower than on cells infected with UV-inactivated ICP47Δ. This emphasizes that in the absence of ICP47 low titers of HSV can effectively interfere with CD1 expression. Given the demonstrated importance of CD1 molecules in viral infections, it is likely that HSV has also developed mechanisms that target the associated Ag presentation pathways thereby preventing maximal activation of CD1-restricted T cells.

In our study, several findings show the existence of viral evasion of the CD1-Ag presentation system. At a low MOI, double staining for HSV Ag and CD1b revealed a small population of DC that carried a high HSV Ag load and, simultaneously, showed a strongly diminished surface expression of CD1b. Accordingly, after infection with a high MOI on both DC and transfected U373 cells the expression of all CD1 molecules was reduced. The observed intracellular colocalization of viral proteins and CD1 molecules together with the reduction in CD1 endocytosis in DC implies the immobilization or intracellular redistribution of CD1 by viral proteins. Moreover, the total amount of CD1d protein in DC
infected with a high MOI of live virus was diminished in comparison to DC infected with UV-inactivated HSV. In addition, internalization of CD1a was found to be impaired in DC infected with a high MOI but remained unaffected in DC that were stimulated through a ligand of TLR2. Neither the reduction of CD1d nor the block in CD1a endocytosis was observed in transfected U373 cells infected with a high MOI of HSV although CD1 surface expression was strongly decreased. Therefore, further viral mechanisms exist that can interfere with the CD1 Ag presentation machinery. Most importantly, even at low MOI the capacity of HSV-infected DC to stimulate cytokine release from NKT cells was impaired despite enhanced CD1d surface expression and increased NKT cell proliferation. DC infected with live HSV strongly inhibited IFN-γ or IL-10 release by NKT cells whereas IL-4 secretion was only slightly affected. The latter finding is in line with a recent report showing that IL-4 production is less dependent on prolonged TCR stimulation than IFN-γ production (64). DC infected at a high MOI were additionally unable to stimulate release of IL-4. Thus, HSV can modulate the quality of CD1-restricted T cell responses induced by DC by interfering with CD1 Ag presentation at multiple levels.

We propose that the outcome of interaction between HSV and the CD1 Ag presentations system in infected DC is determined by two conflicting forces. At one hand, DC switch the balance of Ag presentation from the MHC class I to the CD1 pathway in response to HSV infection. In contrast, HSV uses multiple evasion mechanisms that interfere with the CD1 Ag presentation machinery. Which force prevails may depend on the viral load of the infected DC. Moreover, it is possible that analogous to the MHC I presentation pathway uninfected DC take up apoptotic material derived from dying infected DC and cross-present lipid Ags through their CD1 molecules. In this way, CD1-restricted T lymphocytes could be activated despite viral evasion mechanisms targeting CD1 Ag presentation. Subsequently, these T cells can contribute to virus elimination by performing diverse functions (66): instructing uninfected DC to develop into TH1-inducing mature DC (67–71), lysing virus-infected cells (72, 73), helping B cells to secrete IgS (74–76), and amplifying the antiviral NK response (77–79).

Acknowledgments

We thank T. Kaiser from the joint FACS facility of the Max-Planck Institute for Infection Biology and the Deutsche Rheumaforforschungszentrum (Berlin, Germany) for assistance in flow cytometry; U. Noak for excellent technical assistance; Drs. A. A. Porcelli, R. L. Hendricks, and G. Moldenhauer for helpful reagents; and Kirin Brewery Pharmaceutical (Japan) for αGalCer.

Disclosures

The authors have no financial conflict of interest.

References
