Human cytomegalovirus infection interferes with major histocompatibility complex type II maturation and endocytic proteases in dendritic cells at multiple levels

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INTRODUCTION

Infections with human cytomegalovirus (HCMV) establish a life-long viral latency in immunocompetent individuals, which may lead to active viral replication under conditions of immunosuppression (Söderberg-Nauclér & Nelson, 1999; Söderberg-Nauclér et al., 1997). Active HCMV infection, in turn, is associated with an immunocompromised state, with impaired cellular immune functions and an increased rate of opportunistic bacterial and fungal infections (George et al., 1997; Nichols et al., 2002). These features illustrate that HCMV not only prevents viral clearance, but also efficiently subverts crucial elements of the specific cellular immunity in the infected host, resulting in a compromised immune function in a more general sense (Loenen et al., 2001; Ploegh, 1998). In vitro, HCMV not only inhibits natural killer cell function and myelopoesis (Odeberg et al., 2003; Schrier et al., 1986; Simmons et al., 1990), but also contains several homologues of cellular genes involved in immune regulation, such as major histocompatibility complex (MHC) molecules and Fc and chemokine receptors, as well as an interleukin (IL)-10 homologue (Loenen et al., 2001; Spencer et al., 2002). Increasing evidence suggests that HCMV may also induce immunosuppression by infecting dendritic cells (DCs), the functionally most important type of professional antigen-presenting cell (APC), compromising their function.
HCMV infection of DCs interferes with DC maturation, DC-induced T-cell stimulation and DC migration in response to soluble mediators, as well as expression and function of MHC I and II molecules on DCs (Beck et al., 2003; Grigoleit et al., 2002; Moutaftsi et al., 2002; Sénéchal et al., 2004).

DCs guide the immune function of CD4 T cells via the MHC II-mediated presentation of antigenic peptides. For this task, DCs have developed a complex endocytic machinery that processes complex proteins from exogenous but also endogenous sources to antigenic peptides by a set of endocytic proteases, mostly cathepsins. These proteases not only mediate antigen breakdown, but also, at the same time, degrade the MHC II-associated invariant chain (ii) in a stepwise fashion, so that ultimately fully mature MHC II is loaded with antigenic peptide to be displayed at the cell surface for recognition by CD4 T cells (Trombetta & Mellman, 2005). The functional properties of this MHC II antigen-presentation machinery rely on the coordinated action of endocytic transport and protease activity. HCMV-derived gene products (gpUS2, gpUS3 and pp65) have been implicated directly in interfering with individual components of this system in genetically engineered cellular models (Hegde et al., 2002; Odeberg et al., 2003; Tomazin et al., 1999). A recent report with Langerhans-type DCs generated from CD34 progenitors suggests that HCMV infection alters surface expression and the intracellular distribution of MHC II, independent of the expression of US genes. Changes in MHC II synthesis and maturation, as well as in the function of the MHC II-associated processing machinery, were not observed in this model (Lee et al., 2006).

Human monocyte-derived DCs (Mo-DCs) serve as a model for understanding the cell biology of human DCs, as well as a vehicle for immunotherapy approaches. We have recently demonstrated that Mo-DCs can be infected with HCMV in vitro with an infection efficiency of approximately 80% (Jahn et al., 1999). Here, we directly analysed the impact of HCMV infection on the MHC II antigen-presentation machinery in Mo-DCs and demonstrated that HCMV infection interferes with MHC II biosynthesis and maturation, as well as with endocytic proteolysis.

**METHODS**

**Generation of human DCs.** Monocytes were enriched from peripheral blood mononuclear cells (PBMCs) obtained from buffy coats of HCMV-seronegative blood donors, using anti-CD14 antibodies (Milenyi Biotec). Cells were cultured in RPMI 1640 (Gibco) with 10% FCS and 100 μg gentamicin ml⁻¹, supplemented with 100 ng human recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) ml⁻¹ and 500 IU IL-4 ml⁻¹ (both from R&D Systems). Medium and cytokines were replaced every other day. Cells were harvested on day 7 and characterized by flow cytometry.

**Virus preparation and DC infection.** Human foreskin fibroblasts (HFFs) cultured in MEM5 [minimum essential medium (Gibco) containing gentamicin (100 μg ml⁻¹) and 5% FCS] were used for experiments between passages 10 and 25. For infections, the endothelial-cell-propagated HCMV strain TB40/E was used (Siniger et al., 1999). For high-titre virus preparations, HFFs were infected at a low m.o.i. after six days. Infection, cell-free supernant of infected HFF cultures was concentrated by ultracentrifugation at 80,000 g for 70 min. Pelleted virus particles were resuspended in MEM5 and used for infection of DCs as described previously (Grigoleit et al., 2002). For mock-infection, the TB40/E concentration was replaced by MEM5.

**Flow-cytometry analysis.** Cytometry was performed using fluorescein isothiocyanate- or phycoerythrin (PE)-conjugated mouse monoclonal antibodies (mAbs) against the following surface markers: CD40 (Immunotech), CD80, CD83, CD16, human leukocyte antigen (HLA)-DR, CD14, HLA-A, -B and C (W6/32), CD1a and IgG1 isotype control (all from Pharmingen).

**Western blotting.** Western blotting was performed from whole-cell lysates as described previously (Greiner et al., 2003). Anti-MHC class II polyclonal rabbit antisera were obtained from H. Ploegh (Massachusetts Institute of Technology, Cambridge, MA, USA); cathepsin polyclonal antisera against cathepsins B, H and S and mouse antibody against cathepsin L were raised as described previously (Greiner et al., 2003). Goat antisera against cathepsin Z was purchased from Calbiochem, asparagine-specific endopeptidase (AEP) polyclonal antiserum was provided by C. Watts (University of Dundee, Dundee, UK) and polyclonal rabbit antisera against GAPDH was obtained from Acris.

**Pulse–chase experiments, immunoprecipitation and autoradiography.** Metabolic labelling and immunoprecipitation were performed as described by Driessen et al. (1999). For immunoprecipitation, the TU36 antibody was used, which specifically detects MHC II dimers associated with either invariant chain or antigenic peptide (Ziegler et al., 1986). Precipitates were boiled or left at room temperature and subsequently resolved by 12.5% SDS-PAGE under reducing conditions. Radioactive polypeptides retrieved were visualized using a phosphorimager.

**Real-time quantitative PCR.** RNA was extracted, transcribed into cDNA and amplified as described previously (Tolosa et al., 2003). Relative gene expression was calculated using the 2⁻ΔΔCt method (according to User Bulletin 2, Applied Biosystems). Samples were normalized to 18S rRNA to account for the variability in starting RNA concentrations and conversion efficiency of the reverse transcription reaction. cDNA from immature DCs was used as a calibrator sample and set arbitrarily to 100. The oligonucleotides used in this study were: 18S rRNA-for, 5'-CGGCAGCCCATCCAAAGGAA-3' and 18S rRNA-rev, 5'-GCTGAATTACCGCGGCT-3'; cathepsin B-for, 5'-CTGTGATTCGCCTGCAGTC-3' and cathepsin B-rev, 5'-CCAGGATGTTGCCAACCAG-3'; cathepsin H-for, 5'-ACTGGCCTGTTGGGATGAGC-3' and cathepsin H-rev, 5'-AGGCACACATGCTTCCCTC-3'; cathepsin L-for, 5'-ACCAAGTGGAAGGCGATG-3' and cathepsin L-rev, 5'-TTCCCTTCCCTGTATTCCTG-3'; cathepsin S-for, 5'-ACTCAGAATGTGAATCATGGTG-3' and cathepsin S-rev, 5'-TCTCTGGCATCCGAATATATCC-3'; AEP-for, 5'-GAAGCCACATTCTGTT-3' and AEP-rev, 5'-CATGCTCCCAGGTAGC-3'.

**In vitro processing.** A fragment of myelin basic protein (MBP83-99) was generated and digested with endocytic fractions in vitro as described previously (Beck et al., 2001; Dengel et al., 2003), and proteolytic fragments were identified after separation by HPLC on an Esquire3000Plus ion trap mass spectrometer (Bruker Daltonik), essentially as described previously (Burster et al., 2005).
Expression of results and statistical analysis. Unless stated otherwise, one representative result of three independent experiments is shown. Quantitative results were expressed as means ± SD. Statistical analysis of mRNA expression was performed using a two-tailed Student’s t-test for unpaired samples. Levels of statistical significance are expressed as: *, P<0.05 and **, P<0.01.

RESULTS

Surface expression of MHC II is unaffected in DCs during the early phase of HCMV infection

Immature human mo-DCs were generated ex vivo from PBMCs enriched for CD14-positive cells by immunomagnetic sorting, followed by culture in the presence of IL-4 and GM-CSF, essentially as described previously (Riegler et al., 2000). This consistently yielded >80% CD14-negative, MHC II-positive, CD1a-positive DCs of the immunophenotype published in Riegler et al. (2000). DCs were infected with the endothelial cell-propagated HCMV strain TB40/E as published previously by us with infection efficiencies ranging between 70 and 90% (Riegler et al., 2000; Sinzger et al., 1999). Of note, every individual HCMV-infected DC preparation was controlled for infection efficiency by assessing the expression of HCMV immediate-early (IE) antigen (Fig. 1a). Neither isotype controls nor mock-infected cells showed staining with the anti-HCMV IE antibody.

We have shown previously that 24 and 72 h post-infection (p.i.) corresponded to the expression of IE/early and late genes in this model (Riegler et al., 2000), thus defining the early and late phase of infection. To assess the impact of HCMV on the expression of MHC and co-stimulatory molecules during both phases of infection, we analysed the surface levels of MHC and associated co-stimulatory molecules at both time points by flow cytometry (Fig. 1b and c). After 24 h, the expression of MHC I, but not MHC II, was decreased in HCMV-infected DCs, together with CD40 and CD80 but not CD86, consistent with earlier
studies (Grigoleit et al., 2002). After 72 h, we observed not only further downregulation of MHC I expression and decreased surface levels of CD40, CD80 and CD86, but also a significantly decreased mean expression of MHC II. For CD80, CD86 and CD40, a small fraction of the HCMV-infected cells showed increased expression at 72 h p.i. compared with mock-infected cells. This ‘hump’ is most likely to be a result of uninfected bystander cells that alter their cell-surface expression differently from the infected cells in the population. Two distinct populations of DCs were present in the late phase of infection: one population, accounting for roughly 50% of the cells, had only a moderate decrease in MHC II expression compared with the mock-infected control, whilst in the second population surface MHC II was virtually absent. Given the consistently high efficiency of HCMV infection of 70–90% in our model (Fig. 1a), the presence of these two DC populations with MHC II downmodulation or loss, respectively, could not be explained simply by a significant fraction of uninfected DCs. In addition, MHC I, CD80 and CD86 were consistently reduced in the entire DC population, also strongly arguing against inconsistent DC infection. Thus, HCMV infection of DCs led to a downregulation of MHC I, CD80 and CD40 in the early phase of infection. This was followed by an additional decrease or loss of surface expression for MHC class II and CD86 in the late phase of viral replication.

**HCMV infection blocks the formation of SDS-stable MHC II dimers**

We next wanted to analyse the biochemical basis for altered MHC II surface expression in HCMV-infected DCs. First, we analysed the steady-state expression of MHC class II in immature DCs after 24, 48 (data not shown) and 72 h of infection by Western blotting, covering all phases of viral replication. We did not observe a decrease in the steady-state amounts of total cellular class II α-chain (Fig. 2a) or β-chain (data not shown), even after 72 h of HCMV infection.

In order to assess directly the de novo biosynthesis, maturation and stability of MHC class II in HCMV-infected DCs, cells were metabolically labelled with [35S]methionine/cysteine 24 h after HCMV infection, i.e. during the IE phase of the HCMV replicative cycle. After pulse labelling for 30 min, followed by chase periods of 1, 3 and 5 h in the absence of radiolabelled amino acids, cells were lysed. After normalizing cellular lysates for incorporated radioactivity, immunoprecipitation of MHC class II complexes was performed from cell lysates using the conformational-specific TU36 antibody, which selectively detects αβ complexes associated with either αi or antigenic peptide (Ziegler et al., 1986); thus, only complexes that were immunologically relevant were detected. A fraction of the MHC II complexes that has reached a fully mature state including the acquisition of peptide cargo forms SDS-stable complexes at room temperature (so-called SDS-stable dimers), which dissociate at 95 °C. The presence of these SDS-stable MHC II dimers specifically indicates the fully mature, peptide-loaded state of MHC II complexes.

Polypeptides retrieved with TU36 were resolved by SDS-PAGE, with or without prior boiling, and visualized by autoradiography (Fig. 2b). In mock-infected DCs, newly synthesized MHC II complexes were detectable directly after the pulse and reached a maximum after 1 h of chase.
Their quantities decreased during the following chase periods. Fully mature SDS-stable MHC II dimers were detected after 3 h of chase in mock-infected DCs and remained quantitatively stable at the 5 h chase point. In HCMV-infected DCs, by contrast, lower amounts of radiolabelled MHC II complexes were retrieved after the pulse, suggesting a lower rate of MHC II transcription. During the chase, the amounts of MHC II retrieved in HCMV-infected DCs consistently increased and reached maximum levels at the 3 h chase point. This indicated that radiolabelled polypeptides were incorporated into MHC II complexes over a 3 h period in HCMV-infected DCs, in contrast to mock-infected controls where this process was completed after 1 h. Thus, the assembly of MHC II complexes from the respective individual polypeptides was less efficient in HCMV-infected DCs compared with controls. Strikingly, HCMV-infected DCs failed to form fully mature SDS-stable MHC II–peptide complexes, even at the 5 h chase point. Note that the quantities of MHC II complexes retrieved in mock-infected controls at the 3 or 5 h chase point (where SDS-stable dimers are present) were of the same order of magnitude as in infected samples at the 5 h chase point (compare the boiled samples), yet SDS-stable MHC II dimers were absent from these samples.

At 72 h p.i. (Fig. 2c), we observed a substantial down-regulation of MHC class II biosynthesis in late-infected DCs compared with mock-infected cells. Newly synthesized SDS-stable MHC II dimers were present in mock-infected DCs during this late phase of infection, but again were absent in HCMV-infected DCs. We did not observe accumulation of proteolytic intermediates of Ii during the chase in HCMV-infected DCs, so incomplete Ii processing is unlikely to account for the defect in MHC II maturation identified.

In summary, our results demonstrate for the first time incomplete intracellular maturation of MHC II in HCMV-infected DCs.

**Expression levels of mRNA for MHC II-associated proteins and proteases are reduced following HCMV infection**

In order to analyse the influence of HCMV infection on the transcriptional control of MHC II and related genes in DCs, the respective mRNA levels were quantified by PCR at 24 and 72 h p.i. (Fig. 3). Unmanipulated immature DCs after 7 days in culture were included to detect possible influences due to the handling of mock-infected cells. The transcription rates of CIITA, HLA-DRz, Ii p31 and Ii p41 were significantly downmodulated in HCMV-infected DCs in contrast to mock-infected controls during the early phase of HCMV infection. The expression and activity of endocytic proteases, in particular cathepsins such as cathepsin S, control the activity and function of the MHC II antigen-presentation machinery in DCs. Of note, the transcription levels of all major cathepsins (S, Z, B, H and L) were also significantly reduced already after 24 h of HCMV infection. This was not due to a general reduction in the rate of transcription as a result of HCMV infection, as the levels of CD80 mRNA increased following HCMV infection, whilst that of CD86 remained largely stable. The reduced transcription rate for cathepsins was even more obvious at 72 h p.i. A similar reduction in mRNA expression was observed for cystatin C, a major endogenous inhibitor of cathepsin activity, in contrast to cystatin F. Taken together, these results indicated that, by the early phase of HCMV infection, the transcription levels not only of MHC II-associated proteins but also of endocytic proteases were selectively downmodulated in DCs.

**HCMV infection reduces the expression of endocytic proteases and impairs proteolytic processing**

When HCMV-infected DCs were probed for cathepsin polypeptides by Western blotting (Fig. 4a and b), expression of the cathepsins S and H was slightly reduced, whilst cathepsins Z, B and L and AEP essentially remained stable after 24 h of infection. At 72 h p.i., significant reductions in the levels of cathepsins Z, H, S and L were observed when compared with control cells at the same time point, as quantified by densitometry from three independent experiments. By contrast, the total amount of GAPDH remained unaffected by HMCV infection. Monocytes were included as a control for the distribution of cathepsins (Reich et al., 2007).

To assess whether the reduced expression of major endocytic proteases would also functionally impair proteolytic degradation mediated by the MHC II-associated antigen-processing machinery, we analysed the proteolytic processing of a peptide by endocytic proteases derived from HCMV-infected or mock-infected DCs in vitro. As HCMV-infected DCs could not be generated in quantities that would allow us to isolate endocytic compartments for this type of experiment, we used total cell lysates generated and maintained at pH 5, at which the cytosolic proteolytic machinery is largely inactive, as a source of endocytic proteases. Because residual, non-endocytic protease activity cannot be ruled out a priori under these conditions, we chose the peptide ENPVHFFKNIVTPRTP (MBP83–99) as a model substrate, as the proteolytic patterns generated from this peptide by endocytic proteases are well established (Burster et al., 2005; Dengjel et al., 2005). Cell lysates were generated at pH 5.0 from HCMV-infected DCs at 24 h p.i. (i.e. during the early phase of infection where the changes observed in the cathepsin protein levels were still modest) and compared these with the respective lysates from mock-infected cells with regard to degradation of the peptide substrate (Fig. 4c). We observed a considerably lower rate of MBP 83–99 degradation when the peptide was incubated with cell extracts from HCMV-infected DCs compared with mock-infected cells (in three individual experiments). Whilst almost complete degradation of the intact MBP peptide (retention time 28.7–31.4 min)
occurred with lysates from control cells after 3 h of digestion, a significant fraction of the MBP peptide remained intact when lysates from HCMV-infected DCs were used at the same time point. Importantly, the major proteolytic fragments generated were identical to those observed with purified endocytic fractions from B lymphoblastoid cells and yielded the expected proteolytic products indicative of endocytic proteases such as cathepsins D and S and AEP (Table 1) (Dengjel et al., 2005), as deduced from mass spectrometry-based analysis. This confirmed that the bulk of proteolytic activity assayed here represented endocytic proteases. Whilst degradation of the MBP peptide was significantly less efficient with the proteolytic activity present in infected DCs, the proteolytic pathway and the pattern of proteolytic products remained unchanged. The relatively high amounts of N-terminal fragments indicative of AEP activity in HCMV-infected DCs (ENPVVHFFKN) were attributed to a decreased efficiency of endocytic proteases such as cathepsin S and possibly cathepsin D to degrade this fragment further, rather than to increased AEP activity. Consistent with this interpretation, we detected comparable amounts of the
corresponding C-terminal fragment (IVTPR) in infected versus control DCs. In summary, these data demonstrated that HCMV-infection of DCs functionally impairs the proteolytic activity of endocytic proteases by the early phase of infection.

DISCUSSION

HCMV infection in vivo leads to a defect in cellular immunity. DCs, which are central for initiating and regulating cellular immunity, are permissive for HCMV. Several gene products encoded by HCMV have been implicated in interfering with MHC II function. Therefore, we directly analysed the effect of HCMV infection on the MHC II-associated antigen-presentation machinery in human mo-DCs.

Our results demonstrated that, by the early phase of HCMV infection, DCs failed to mature newly synthesized MHC II complexes to the final stage of SDS-stable dimers. In addition, the transcription rate of MHC II genes was reduced and the synthesis of MHC II polypeptides was delayed during the early phase of infection, whilst MHC II surface expression remained unaffected. These changes were accompanied by a decreased transcription rate of major endocytic proteases such as cathepsins S, Z, B and L and a reduced proteolytic capacity of the total endosomal protease pool. At the late stage of infection, this translated into decreased expression of cathepsin polypeptides and low MHC II surface expression. Thus, HCMV interferes with the MHC II antigen-presentation machinery in professional APCs at multiple levels. As HCMV targets both major arms of the intracellular MHC II-associated machinery (MHC II synthesis and maturation, as well as proteases processing antigen and Ii), several viral proteins may contribute to the interference of HCMV with MHC II function in DCs.

The gpUS2 protein, which is expressed in the late phase of HCMV replication, binds to MHC II in the endoplasmic reticulum and induces rapid retrograde cytosolic transport and degradation of the HLA-DR and HLA-DM α-chains by...
T. Kessler and others

Table 1. Identification and relative abundance of intact MBP83–99 and its proteolytic fragments after incubation with cellular extracts from HCMV-infected versus mock-infected DCs

Proteolytic fragments were resolved by HPLC and identified by mass spectrometry. 'Peak' refers to protein peaks 1–8 displayed in Fig. 4(c). The peptide sequence identified is displayed along with the protease most likely responsible for its generation. 'Mock : HCMV' shows the ratio between the amount of a given fragment detected after incubation of the MBP peptide with lysates from mock-infected and HCMV-infected cells, respectively, as assessed by mass spectrometry.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Peptide sequence</th>
<th>Protease*</th>
<th>Mock : HCMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ENPVVHFFKNIVTPRTP</td>
<td>Undigested</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>ENPVVHFFKNIVTPRTP</td>
<td>–</td>
<td>0.16</td>
</tr>
<tr>
<td>2</td>
<td>ENPVVHFFKNIVTPRTP</td>
<td>Undigested</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>–</td>
<td>0.25</td>
</tr>
<tr>
<td>3</td>
<td>ENPVVHFFK</td>
<td>Cat S/L</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>ENPVVHFFKN</td>
<td>AEP</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>ENPVVH</td>
<td>Cat D</td>
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</tr>
<tr>
<td></td>
<td>FF</td>
<td>Cat S, Cat G</td>
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</tr>
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<td>4</td>
<td>ENPVVH</td>
<td>Cat D</td>
<td>1.13</td>
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<td>5</td>
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<tr>
<td>7</td>
<td>ENPVV</td>
<td>Cat S</td>
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<tr>
<td></td>
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<td>–</td>
<td>0.91</td>
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*Digestion by unspecified protease.

the proteasome (Tomazin et al., 1999), as demonstrated in CIITA-transfected glial cells infected with an adenoviral vector encoding US2. However, similar experiments in DCs have failed to confirm this result (Rehm et al., 2002). US3, an early-phase expressed viral protein, diminishes MHC II-mediated T-cell activation in CIITA-transfected glial cells infected with an adenoviral vector carrying US3. In this model, US3 replaced Ii in newly synthesized MHC II complexes in the endoplasmic reticulum, preventing a coordinated transport of MHC II to the endocytic tract and subsequent MHC II peptide loading (Hegde et al., 2002). The HCMV protein pp65 is an HCMV tegument protein that becomes effective as the virion enters the cell. In gamma interferon-stimulated fibroblasts, pp65 delivered via the laboratory HCMV strain AD169 causes accumulation of HLA-DR in lysosomes, followed by degradation of the α-chain, resulting in a reduced MHC class II surface expression at day 1 p.i. (Odeberg et al., 2003). Our results do not support a significant effect of HCMV-derived proteins on the rate of degradation of newly synthesized MHC II polypeptides. Total levels of newly synthesized MHC II complexes remained unchanged between 2 and 5 h of chase in infected versus uninfected DCs in the early phase of infection. In addition, newly synthesized MHC II was stable for at least 5 h at 3 days after infection and did not increase after proteasome inhibition (data not shown). Finally, the total cellular amounts of the MHC II α-chain were not affected over 3 days of HCMV infection.

The effect postulated for US3 is only partly consistent with the results obtained here: although competition of Ii from the MHC II complex by US3 may result in failure to form SDS-stable MHC II dimers in infected DCs, MHC II complexes in this model are expected to contain lower amounts of Ii, because Ii is displaced by US3. However, we did not detect such a reduction in the amount of full-length Ii incorporated in MHC II complexes compared with amounts of the α polypeptide. In fact, the p41 isoform of Ii appeared to be slightly over-represented in MHC II complexes retrieved from HCMV-infected DCs.

When the effect of HCMV infection was analysed in Langerhans-type DCs generated from CD34 progenitors, Lee et al. (2006) also observed reduced MHC II surface expression and in addition alterations in the intracellular distribution of MHC II, independent of the expression of US genes. However, in contrast to our study, an effect of HCMV infection on the maturation of MHC II SDS-stable dimers was not detected. Furthermore, Lee and co-workers found no influence on the activity and expression of cathepsin S in infected cells. This discrepancy may in part be due to the obvious difference in the cell type examined, as well as the use of UV-inactivated virus. In addition, the simple analysis of total SDS-stable dimers by Western blotting, as performed by Lee and colleagues, mirrors the total pool of fully mature MHC II complexes, rather than just the newly synthesized fraction. A sizeable portion of the MHC dimers detected by Lee and co-workers by Western blot may therefore have been synthesized and matured well before the viral challenge, if one takes into account the long half-life time of MHC II complexes. Interestingly, defective maturation of MHC II dimers, as observed in our study, probably results in aberrant subcellular distribution of MHC II complexes (Driessens et al., 1999) in murine DCs, entirely consistent with the observations of Lee et al. (2006).

Whilst MHC II surface expression was absent in about 50 % of DCs at day 3 p.i., it was only slightly decreased in the remaining cells. Recent publications have shown that the endotheliotropic HCMV strain TB40/E is both genotypically and phenotypically homogeneous with respect to myeloid tropism, as it is not based on a plaque-purified clone (Dolan et al., 2004; Sinzger et al., 2008). Additionally, the antibody used only recognized HLA-DR, possibly skewing the picture due to selective effects on this molecule. This lack of homogeneity in combination with the possible preference of viral proteins for HLA-DR may serve as a basis to explain the distinct DC populations observed with regard to MHC II expression in the late phase of infection. Nevertheless, sorting and independent analysis of the two subpopulations will be necessary to understand fully how HCMV targets MHC class II during infection.
Endocytic proteases, namely cathepsin S, are required in APCs not only for antigen processing but also for MHC II maturation, trafficking, surface delivery and function. Here, for the first time, we have implicated HCMV in manipulation of the endocytic proteolytic system. As our results suggest, this may mostly be the result of the effect of HCMV on the transcriptional control of endocytic proteases during the early infection phase. In murine DCs, a modest modulation in cathepsin S activity is sufficient to control the transit of DCs from an immature phenotype (low surface MHC II, high intracellular MHC II and MHC II still associated with the N-terminal retention signal of Ii) to the mature form (fully processed Ii and high MHC II surface expression) (Pierre & Mellman, 1998). HCMV similarly impairs both the phenotypic and functional maturation of human mo-DCs, interfering with MHC II surface expression and DC function. However, the failure to synthesize fully mature SDS-stable MHC II complexes in HCMV-infected cells, as observed by us, is unlikely to have resulted directly from inefficient Ii processing due to decreased cathepsin S. In this scenario, one would predict the accumulation of Ii processing intermediates upstream of CLIP (p41, p33 and p22), which was not observed in our results. AEP is presently considered the most important endocytic protease for antigen degradation, as demonstrated by its dominant effect on T-cell stimulation induced by either the tetanus toxoid C fragment or MBP (Manoury et al., 1998; Watts, 2004). As cathepsin S and AEP are the dominant proteases for destruction of the MBP peptide analysed here (Beck et al., 2001), the increased stability of the peptide when digested with lysates from HCMV-infected DCs is consistent with the reduced expression of these proteases.

From the viral point of view, it is mandatory to prevent the processing of viral protein in the endocytic compartment by the early phase of infection, because this processing would result in the display of viral antigen complexed with MHC II. The presence of a major portion of HCMV protein in the trans-Golgi network predicts the transport of a relatively large amount of viral protein to the late endosome/MHC II loading compartment, so that viral protein is probably loaded on newly synthesized mature MHC II complexes travelling the same route. The block in the formation of SDS-stable complexes from newly synthesized MHC II may therefore, in particular, prevent the presentation of viral proteins by newly synthesized MHC II in the early phase of infection. In addition, downregulation of the key proteases of MHC II-associated antigen processing may interfere with the processing of viral protein in the MHC II compartment and therefore also impede peptide loading on pre-existing mature MHC II molecules. Thus, the direct effect of HCMV infection on MHC II maturation and protease activity may represent a first line of immediate viral interference with the antigen-presentation machinery before more indirect mechanisms, such as changes in transcriptional control, can be implemented. The MHC II downregulation observed in the late phase of infection may also functionally explain the decreased mixed lymphocyte reaction stimulatory capacity of DCs infected with HCMV for 3 days (Grigoleit et al., 2002).

In summary, HCMV interferes with the machinery of MHC II-mediated antigen presentation in human DCs in a time-dependent fashion at multiple levels, including MHC II maturation, protease activity and expression of MHC II and co-stimulatory molecules, as well as gene transcription control. The current major molecular models for the interaction between HCMV and the MHC II system do not sufficiently explain the biochemical data directly obtained here from HCMV-infected DCs, implicating additional unknown viral proteins and molecular pathways in the interaction between HCMV and MHC II-mediated immunity.

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