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Murine Cytomegalovirus Infection Down-Regulates MHC Class II Expression on Macrophages by Induction of IL-10

Stella Redpath,* Ana Angulo,* Nicholas R. J. Gascoigne,2* and Peter Ghazal2†*

Herpesviruses utilize many strategies for weakening the host immune response. For CMV, this includes avoidance of NK clearance and inhibition of MHC class I and class II presentation pathways. In this study, we report that mouse CMV (MCMV) specifically causes a premature and transient activation of host IL-10 very early in the course of infection, resulting in a dramatic and selective reduction in MHC class II surface expression. The expression of IL-10 is normally late in the immune response to a pathogen, serving to dampen the response by suppression of the production of inflammatory cytokines. In infection of macrophages, we show that MCMV induces the production of IL-10, leading to an early and selective reduction in the expression of MHC class II on the surface of the cells. Inhibition of MHC class II expression was not observed in the presence of neutralizing Abs to IL-10 or in macrophages from IL-10-deficient mice. Moreover, MCMV-infected IL-10-deficient mice developed an early and significantly more robust macrophage MHC class II induction than normal mice. Altogether, our results demonstrate that viral induction of an IL-10 autocrine pathway plays an essential early role in selectively reducing MHC class II expression on the surface of APC prior to stimulation by IFN-γ.


Human CMV (HCMV) is a ubiquitous pathogen, normally benign in the healthy individual, but a serious cause of morbidity and mortality in immunocompromised hosts. Murine CMV (MCMV) serves as a useful animal model for HCMV infection (1). It can cause an acute, disseminated infection, followed by persistent production of infectious virus within the salivary gland for months following infection, finally establishing a lifelong latent state (1, 2).

It is well known that CMV infection results in immunodepression (reviewed in Refs. 1 and 2). Both HCMV and MCMV employ many diverse strategies to avoid detection by the host immune system. Among these strategies is the ability to interfere with expression of MHC class I proteins. A number of different molecular mechanisms, involving specific viral gene products, have been characterized for both HCMV and MCMV (3–6). More recently, several groups have also shown that CMV can cause decreased expression of MHC class II proteins, by more indirect mechanisms (7–9). It has been reported that HCMV inhibits IFN-γ-induced MHC class II expression in endothelial cells via induction of IFN-β (7). HCMV can also interfere with class II on alveolar epithelial cells (8). High doses of MCMV can inhibit class II expression on macrophages from SCID mice, due partly to the induction of IFN-β (9). MCMV and HCMV can impair IFN-γ-induced MHC class II up-regulation, by blocking the action of the IFN-γ by both IFN-α/β-independent mechanisms (10) and disruption of the Jak/Stat signaling pathway (11).

While other studies have investigated the effect of MCMV infection on the up-regulation of IFN-γ-inducible MHC class II, we sought to consider the effect of MCMV infection on non-IFN-γ-induced MHC class II molecules in macrophages. Macrophages are believed to be among the earliest cells infected by MCMV. We report a novel mechanism by which MCMV causes a decrease in the expression of MHC class II in macrophages, by inducing IL-10 production in the infected cells.

Materials and Methods

Animals

Five- to six-week-old female C57BL/6J mice were obtained from The Scripps Research Institute Animal Resources Facility (La Jolla, CA). IL-10-deficient animals (12) were obtained from Drs. B. Balasa and N. Sarvetnick, Department of Immunology, The Scripps Research Institute.

Cells

The mouse macrophage cell line IC-21 (13), the mouse mammary epithelial tumor cell line C127I (14), and the murine fibroblast cell line NIH 3T3 (ATCC CRL1658) were obtained from the American Type Culture Collection (Manassas, VA). C127I cells were maintained in DMEM supplemented with 10% FCS, 10 U/ml penicillin G, 10 μg/ml streptomycin, and 2 mM glutamine. NIH 3T3 fibroblasts were cultured in MEM as above, except with 10% FBS. IC-21 cells were grown in RPMI medium supplemented in the same way, with the addition of 5 × 10−8 M β-ME.

Primary macrophages were elicited from peritoneal exudate cells (PECs) following i.p. injection of 1 ml thioglycollate (Becton Dickinson, Cockeysville, MD) into either C57BL/6 or IL-10-deficient mice. PECs were removed by peritoneal lavage. Cells were plated out at 1–5 × 10⁶/ml, and incubated for 24–48 h at 37°C, 5% CO₂, after which nonadherent cells were washed away with PBS. Before staining with appropriate Abs, macrophages were removed from the plates by gentle scraping, and washed in PBS.

Virus stocks and infections

Viral stocks are routinely tested for mycoplasma, and all stocks used in this study were mycoplasma free. Salivary gland-passaged stocks of MCMV (Smith ATCC VR-1399) were used to infect C57BL/6 or IL-10-deficient mice by i.p. injection. A total of 5 × 10⁶ PFU of MCMV was injected in

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Abbreviations used in this paper: HCMV, human CMV; MCMV, murine CMV; MFI, mean fluorescence intensity; moi, multiplicity of infection; PEC, peritoneal exudate cell.
200 μl of DMEM. Control animals were age and sex matched, and were mock infected with 200 μl of DMEM only. Animals were sacrificed 24 h postinfection, and the spleens were removed. Mononuclear cells were harvested by preparation of a single cell suspension, and removal of erythrocytes by hypotonic lysis.

Stocks of tissue-propagated MCMV viruses were prepared in NIH 3T3 cells, and titers were determined by standard plaque assay on NIH 3T3 cells. For in vitro assays, both the Smith strain of MCMV and the rMCMV RM461 (strain K181) were used. The RM461 rMCMV carries the LacZ gene, and detection of infected cells can be made by staining for β-galactosidase activity. RM461 MCMV was originally obtained from E. S. Mocarski (Stanford University, Stanford, CA). Cells were infected with MCMV at a multiplicity of infection (moi) of 10. Adsorption was for 1 h at 37°C, 5% CO2, with gentle rocking of cells every 15 min. Cells were then washed in PBS before fresh medium was added.

Cells infected with MCMV RM461 were detected by staining for expression of β-galactosidase. Cells were fixed for 5 min at room temperature in 0.5% gluteraldehyde, and then stained for 24 h at 37°C in staining solution (1 M MgCl2, 5 M NaCl, 0.5 M HEPES, pH 7.4, 30 mM potassium ferricyanide, 30 mM potassium ferricyanide, 2% X-Gal (5-bromo-4-chloro-3-indolyl β-D-galactoside) in N,N-diethylformamide). For viral growth kinetic studies (Fig. 1), cells were infected at moi = 0.1. Infectious MCMV from cellular extracts or present in the culture supernatant was measured by standard plaque assay at day 1–8 postinfection.

UV inactivation of virus and tissue culture supernatant was performed using a G15T8 lamp (General Electric, Fairfield, CT) at 9 × 104 Joules.

Abs and flow cytometry

In all flow cytometry experiments, FcγR binding of Abs was blocked by incubation of cells with blocking buffer (PBS, 5% v/v heat-inactivated rabbit serum, 1% w/v sodium azide, 30 min at 4°C), before staining. Expression of MHC class II on IC-21 cells and primary macrophages was detected with B720.2 (ATCC TIB154), followed by goat anti-mouse IgG and FITC-conjugated anti-goat IgG (Sigma, St. Louis, MO). Detection of MHC class II on splenic macrophages and on C1271 cells was made with FITC-conjugated 14-4-4S (for C1271 cells) or 25-9-17 (for splenic macrophages) (both Abs from Pharmingen, San Diego, CA). The macrophage marker F4/80 was detected with anti-F4/80 (ATCC HB198) and amplified with biotinylated anti-rat IgG and streptavidin-R613 (Pharmingen). CD11c (N418) was stained using PE-conjugated Ab (Pharmingen). Analysis of cells was made by flow cytometry with a FACS Calibur instrument (Becton Dickinson, Mountain View, CA). A total of 50,000 events were collected per sample.

Analysis of MHC class II expression on ex vivo splenic cells was made in the following way: C37BL/6 and IL-10-deficient mice were injected i.p. with MCMV (5 × 106 PFU). Twenty-four hours postinoculation, animals were euthanized and spleens were removed. Mononuclear cells were stained for F4/80 or CD11c expression, and sorted into single populations by flow cytometry (FACStar; Becton Dickinson, Mountain View, CA), before staining for MHC class II.

For detection of intracellular cytokine expression, primary macrophages (PECS) were mock infected (with DMEM only) or infected with MCMV at moi of 10 for 24 h. Subsequently, cells were incubated with 10 μg/ml Brefeldin A (Sigma) for 4 h at 37°C, as described (15). Cells were then washed with PBS, permeabilized using a Fix & Perm kit (Caltag Laboratories, Burlingame, CA), and stained using anti-cytokine reagents. The Abs used were PE-conjugated anti-IL-10 (JES5-16E3), anti-IL-4 (11B11), anti-IFN-γ (XM16.2), and a PE-conjugated isotype control (rat IgG1). All Abs were purchased from Pharmingen. Control samples that were not permeabilized were also included.

Neutralization of MHC class II down-regulation with Abs

Macrophages (IC-21) were infected with MCMV, in vitro, as described. Conditioned supernatant from infected or noninfected controls was incubated with an anti-IL-10 Ab (JES5.2A5), or an isotype-matched control (YCATE55) for 30 min at 37°C. Treated supernatants were added to noninfected cells, and the cells were then incubated for 24 h, before staining for MHC class II expression. Control samples of noninfected, nontreated cells and directly infected cells were included.

Results

MHC class II down-regulation in MCMV-infected macrophages

During the acute phase of MCMV infection, macrophages readily aid the spread of the virus to secondary sites of infection (16), and are one of the cellular sites of latency for MCMV (17). Using a macrophage cell line, IC-21, which is susceptible to MCMV infection (Ref. 18 and Fig. 1A), we examined how MCMV infection affected the surface expression of constitutively expressed MHC class II proteins. IC-21 cells are derived from C57BL/6 and express I-Ak MHC class II molecules on their surface. Following infection of IC-21 cells with MCMV in vitro, we observed that the expression of constitutive MHC class II was dramatically decreased (Fig. 2A). The mean fluorescence intensity (MFI) of cells expressing MHC class II decreased from 446 to 173, a reduction of 61%, 24 h after infection (Fig. 2A). To investigate whether MCMV infection of primary macrophages could also cause decreased expression of MHC class II proteins, macrophages were isolated from C57BL/6 mice and infected with MCMV. These cells are also susceptible to MCMV infection (Fig. 1B). Analysis of the MHC class II expression in these macrophages 24 h after infection revealed that class II expression is also significantly reduced in primary macrophages in comparison with the noninfected controls (Fig. 2B). The MFI of MHC class II on macrophages decreased from 1985 to 834, a reduction by 58%, 24 h following MCMV infection (Fig. 2B). A decrease in MHC class II expression was not observed in cells that were treated with UV-inactivated virus (Fig. 2C). Thus, live, infectious virus is necessary for this process, suggesting a requirement for viral gene expression.

We also investigated whether MCMV infection could cause decreased surface expression of another activation marker on the surface of macrophages. In the immune response, activation of T cells requires two signals. The first is provided by ligation of the TCR with its MHC/peptide ligand, and the second is provided by T cell ligands binding to costimulatory molecules on the surface of the APC. We considered whether the costimulatory molecule B7-2 (CD86) on the macrophages was also affected by MCMV infection. In primary macrophages from C57BL/6 mice, we found that...
Reduction of MHC class II expression in uninfected cells by culture supernatant from MCMV-infected macrophages

In the experiments described above, infections were conducted under conditions that limited the proportion of cells infected in the culture to approximately 20%. Since the reduction in expression of MHC class II on the surface of MCMV-infected macrophages affected almost all of the cells, we reasoned that secretion of a soluble factor such as a cytokine from the infected cells could be responsible for the effect. To test this hypothesis, culture supernatants from IC-21 cells infected with MCMV (for 24 h) were transferred to fresh, noninfected IC-21 cells and to control, epithelial-derived tumor cells (C127I). As expected, direct infection of IC-21 cells with MCMV caused a reduction in the level of MHC class II expression, with a decrease in staining from MFI 145 to MFI 78 (Fig. 3B). Significantly, macrophages incubated with the conditioned supernatant for 24 h also showed a decrease in the level of MHC class II detection (MFI 145 to 87) (Fig. 3, A and C). By contrast, reduction in class II expression was not observed in the epithelial tumor cell line, C127I (Fig. 3D). It was important to exclude the possibility that this effect was due to the presence of live virus in the culture supernatant. Supernatants from MCMV-infected IC-21 cells were treated with UV light, to inactivate any live virus present, and then incubated with noninfected IC-21 cells. Live virus was not detected in the UV-treated supernatant (see Materials and Methods for details). MHC class II expression was still decreased in noninfected IC-21 cells incubated with these test supernatants (data not shown), indicating that transfer of any contaminating live virus to noninfected cells is unlikely to be responsible for the effect. These data strongly support the notion that a factor such as a cytokine from the infected cells could be responsible for the effect.

FIGURE 2. Reduction of MHC class II expression in a macrophage cell line (IC-21) and in primary macrophages infected with MCMV. A, Noninfected macrophage IC-21 cells (bold line) and MCMV-infected IC-21 cells, moi = 10 (+MCMV, dashed line), were stained for MHC class II expression, after 24 h in culture. B, Primary peritoneal macrophages, taken from C57BL/6 mice, were either noninfected (bold line) or infected with MCMV (dashed line, +MCMV) in vitro, and then stained for expression of MHC class II after 24-h culture. C, Noninfected macrophage IC-21 cells (bold line) and UV-inactivated MCMV-infected IC-21 cells (+UV-MCMV, dashed line) were stained for MHC class II expression, after 24 h in culture. D, Primary peritoneal macrophages, taken from C57BL/6 mice, were either noninfected (bold line) or infected with MCMV (dashed line, +MCMV) in vitro, and then stained for expression CD86 (B7-2) after 24 h of culture. E, Noninfected epithelial-derived C127I cells and MCMV-infected C127I cells (+MCMV) were stained for expression of MHC class II, after 24 h in culture. Control samples stained with FITC-conjugated secondary reagent alone are shown in each panel as a thin line. A total of 20,000 events were collected for each sample.

FIGURE 3. Culture supernatant from MCMV-infected IC-21 cells is sufficient to cause decreased expression of MHC class II when incubated with noninfected cells. MHC class II expression on the surface of noninfected macrophages (IC-21 cells) (A), on the surface of MCMV-infected (+MCMV) IC-21 cells (B), and on the surface of noninfected IC-21 cells incubated with supernatant for 24 h from MCMV-infected cells (+MCMV sup.) (C) was detected by Ab staining. MHC class II staining is shown as a bold line in each histogram, and staining with FITC-conjugated secondary reagent alone is shown as a thin line. D, Epithelial-derived cells, C127I, both noninfected (dashed line) and treated with MCMV supernatant from IC-21 cells (bold line), were also stained with Ab to detect expression of MHC class II. A total of 20,000 events were collected for each sample. Data are representative of two separate experiments.
A–C are from noninfected primary macrophages, and Ab staining and flow cytometry, as described in Materials and Methods. 

Production of IL-10 by primary macrophages infected with MCMV

Several cytokines reportedly cause down-regulation of MHC class II proteins on APC, including IL-4, IL-10, and IFN-γ by MCMV-infected primary macrophages from C57BL/6 mice. For these experiments, detection of intracellular cytokine expression was measured by flow cytometry using specific Abs. We found that IL-10 was expressed in MCMV-infected macrophages 24 h following infection, but not in the noninfected controls (Fig. 4, A and D). Approximately 20–30% of the cells examined stained positive for IL-10. IL-4, which is not normally produced by macrophages, was included as a negative control. Staining of IL-4 was not detected in macrophages (Fig. 4, B and E). While it is accepted that the majority of IFN-γ is made by NK and T cells, IFN-γ may also be produced by macrophages. It has been reported that low levels of IFN-γ are constitutively expressed by resting macrophages and that it is up-regulated in an autocrine manner, following activation of the cells (21). IFN-γ causes the up-regulation of MHC class II expression at later stages in MCMV infection. IFN-γ was not detected at this early stage (24 h) in the infection (Fig. 4F). These data implicate IL-10 as a candidate cytokine involved in causing down-regulation of MHC class II in MCMV-infected macrophages.

Neutralizing Abs to IL-10 block the effect of MCMV-mediated down-regulation of MHC class II in infected macrophages

To test that IL-10 production by MCMV-infected macrophages is, at least in part, responsible for the down-regulation of MHC class II on the surface of infected cells, we used neutralizing Abs against IL-10. Conditioned (MCMV-infected) culture supernatant removed 24 h postinfection from primary macrophages was incubated with either anti-IL-10 Ab (0.1–20 μg/ml) or an isotype-matched control, and then used to treat noninfected cells (Fig. 5A). The inhibition of MHC class II expression by MCMV-conditioned supernatants was reduced in a dose-dependent manner by the anti-IL-10 neutralizing Ab (Fig. 5A). An isotype control Ab did not neutralize the effect of IL-10 (Fig. 5B). The results of these experiments identify IL-10 as an essential mediator of MCMV down-regulation of MHC class II in macrophages.

Macrophages from IL-10-deficient mice are refractory to MHC class II down-regulation during MCMV infection

To establish a direct role of IL-10 in the reduction of constitutively expressed MHC class II in MCMV-infected macrophages, we next studied cells taken from IL-10-deficient (knockout) mice (12). As expected, when primary macrophages from C57BL/6 mice were infected in vitro, a reduction in MHC class II expression of 30% soluble factor, most likely a cytokine, present in the supernatant from MCMV-infected macrophages, is responsible for the reduction of MHC class II expression on the surface of the cells.

Production of IL-10 by MCMV-infected primary macrophages

FIGURE 4. Production of IL-10 by primary macrophages infected with MCMV. Primary macrophages taken from C57BL/6 mice were infected with MCMV (moi 10) for 24 h. Cells were then cultured with 10 μg/ml Brefeldin A, before harvesting and detection of intracellular cytokines by Ab staining and flow cytometry, as described in Materials and Methods. A–C are from noninfected primary macrophages, and D–F are from MCMV-infected primary macrophages. In each panel, staining with an isotype control Ab is shown as a thin line, and staining with each anticytokine Ab is shown as a bold line. A total of 20,000 events were collected for each sample, and data are representative of three separate experiments.

FIGURE 5. Neutralizing Abs to IL-10 can block the effect of down-regulation of MHC class II in MCMV-infected macrophages. A, Macrophages (IC-21 cells) were infected with MCMV (moi 10), as described. At 24 h postinfection, culture supernatant from infected cells was incubated with an irrelevant, isotype-matched control Ab (YCATE55) at 20 μg/ml, and then stained for expression of MHC class II (bold line). Untreated, noninfected cells were shown as a dotted line (untreated). B, Noninfected macrophages were treated with conditioned culture supernatant, which was incubated with an irrelevant, isotype-matched control Ab (YCATES5) at 20 μg/ml, and then stained for expression of MHC class II (bold line). Untreated, noninfected cells are shown as a dotted line (untreated). C, Macrophages directly infected with MCMV were also stained for expression of MHC class II (+ MCMV, bold line), with noninfected, untreated cells as a control (untreated, dotted line).
FIGURE 6. Decreased expression of MHC class II in primary macrophages 24 h following MCMV infection requires IL-10. Primary peritoneal macrophages from C57BL/6 (A) or IL-10-deficient (IL-10T) mice (B) were infected with MCMV (+MCMV, dotted line) in vitro, cultured for 24 h, and then stained with anti-MHC class II Abs to detect MHC class II expression. Peritoneal macrophages taken ex vivo from acutely MCMV-infected C57BL/6 (C) or from IL-10-deficient mice (D) were stained for detection of MHC class II expression 24 h after infection of the animals. Control samples of macrophages taken from noninfected animals are shown in each panel as a bold line (noninfected), and isotype-matched control Ab-stained samples are shown as a thin line (control). A total of 20,000 events were collected for each sample.

(expression on both F4/80<sup>+</sup> and CD11c<sup>+</sup> cells from C57BL/6. The resting macrophages express very low levels of class II (MFI 8), but this goes down to MFI 5 in the infected cells (Fig. 7A). The resting level of class II on the dendritic cells (CD11c<sup>+</sup>) was much higher (MFI >10<sup>3</sup>), and was dramatically decreased in cells from infected mice (Fig. 7B). Cells from the IL-10-deficient mice showed significant increases in the level of expression of MHC class II following MCMV infection, in both macrophage (F4/80<sup>+</sup>) and dendritic cell (CD11c<sup>+</sup>) populations (Figs. 7C and 5D). The macrophages showed an increase in staining of MHC class II from MFI 14 to more than 10<sup>3</sup> (Fig. 7C). The MCMV-infected dendritic cells also increased class II expression (Fig. 7D). These data suggest that IL-10 plays a pivotal role in the reduction of MHC class II expression in splenic macrophages and dendritic cell populations, in the early stages of infection with MCMV. In contrast, in the absence of IL-10, expression of class II is strongly increased in these cellular populations.

Discussion

Infection of macrophages by MCMV plays an important part in the pathogenesis of CMV disease. During the acute phase of MCMV infection, macrophages are the site of viral replication in a number of organs, including the spleen and peripheral blood (22). Macrophages aid in the dissemination of MCMV to secondary sites of infection, and in addition, monocytes and macrophages are one of the cellular sites of latency for HCMV and MCMV (17, 22–27). In the immune response, activation of macrophages results in increased expression of MHC class II on the surface of the cells.

FIGURE 7. Decreased expression of MHC class II in splenic macrophages following acute infection with MCMV. Pure populations of F4/80<sup>+</sup> and CD11c<sup>+</sup> macrophage cells derived from acutely infected spleen were obtained by flow cytometry, and then examined for MHC class II expression in splenic macrophages and dendritic cell populations.

Reduction of MHC class II in splenic macrophages and dendritic cells following acute MCMV infection

We next evaluated the expression of MHC class II on splenic cells following acute MCMV infection. For this purpose, we compared populations of macrophages (F4/80<sup>+</sup>) and dendritic cells (CD11c<sup>+</sup>) taken from C57BL/6 and IL-10-deficient mice, 24 h following infection. There was a clear decrease in MHC class II expression. After infection, macrophages are the site of viral replication in a number of organs, including the spleen and peripheral blood (22). We next sought to test the in vivo relevance of viral-induced IL-10 on MHC class II expression, by examining the level of MHC class II expression on primary macrophages during infection of IL-10-deficient mice. For these experiments, peritoneal macrophages taken directly from acutely infected C57BL/6 or IL-10-deficient mice were examined ex vivo, cultured for 24 h after i.p. infection. A reduction in the level of MHC class II expression was found on cells from C57BL/6 mice (12.5 ± 3.1% cells infected) and cells from IL-10-deficient mice (19.8 ± 3.9% cells infected) at 24 h (data not shown).

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(from MFI 2850 to 2000) was observed (Fig. 6A). In marked contrast, surface expression of MHC class II in MCMV-infected primary macrophages taken from IL-10-deficient mice at 24 h postinfection was unchanged (Fig. 6B). To determine whether there was a difference in susceptibility of the cells to MCMV infection, we took advantage of a mutant version of MCMV strain RM461, which carries a LacZ gene. Cells were stained for expression of β-galactosidase activity as a measure of cellular infection. Similar levels of infection were observed between cells taken from C57BL/6 mice (12.5 ± 3.1% cells infected) and cells from IL-10-deficient mice (19.8 ± 3.9% cells infected) at 24 h (data not shown).

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Activated macrophages present Ag to CD4 T cells and initiate the coordination of a specific immune response. Importantly, activation of macrophages is an early event in the immune response, and macrophages are a link between the innate and adaptive arms of the immune system. Macrophages are activated by chemokines produced in the acute inflammatory response, and also by cytokines produced by activated lymphocytes, which result in an increased capacity to present Ag to CD4 T cells. This latter event requires sufficient surface expression of MHC class II molecules.

In the work presented in this study, we have shown that MCMV infection of macrophages results in a decrease in MHC class II on the surface of the cells, very early in the inflammatory response. This effect is apparent (in vivo and in vitro) within 24 h of infection prior to stimulation by IFN-γ and is mediated, at least in part, by induction of IL-10 production. IL-10 causes a reduction in the level of expression of MHC class II proteins by interfering with exocytosis and recycling of the proteins (19, 20). This delineates a mechanism by which MCMV infection immediately begins to interfere with the host immune system, and would likely impair the ability of the macrophages to present Ag to CD4 T cells by decreasing MHC class II expression. In addition, IL-10 has other immunosuppressive effects, including inhibition of production of inflammatory cytokines. This early effect illustrates again the remarkable variety of CMV strategies for dampening the immune response. The effect differs from previously reported mechanisms by which MCMV impairs MHC class II expression in macrophages, which involve induction of IFN-β (28), and impairment of IFN-γ induction of MHC class II (10, 11). Since CMV is known to have several strategies to interfere with the host cell expression of MHC class I, it is not surprising that the virus also has several mechanisms to inhibit MHC class II expression. It is also worthwhile to note that IL-10 has been reported to cause down-regulation of MHC class I in studies with tumor cells (29, 30) and B cell lines (29).

The role of cytokines in viral infections is complex, and has not yet been fully characterized. There have been several studies to evaluate cytokine production in MCMV infection. It is known that NK cells contribute to early defense against MCMV. In acute MCMV infection of C57BL/6 mice, it was found that IFN-γ and TNF were produced 48 h postinfection (31), and that NK cells were solely responsible for IFN-γ production in this situation. It has also been shown that the IFN-γ production by NK cells in MCMV infection is dependent on IL-12 induction (32). Additionally, IFN-α/β produced in MCMV infection could mediate IFN-α/β-dependent NK cell cytotoxicity (33). In the same study, it was shown that TNF, IL-12, and IFN-γ were present in the serum of MCMV-infected C57BL/6 mice, 2 to 3 days postinfection (33). It has been demonstrated that in MCMV infection, IFN-α/β can also act as a regulator of IL-12 and IFN-γ (34). Recent reports have also shown that MCMV-mediated hepatitis may be attributable to TNF production (35), independent of NK and T cell contribution, and that peritoneal cells produce IL-1 and TNF-α in MCMV infection (36).

In data presented in this work, we have shown that MCMV-infected macrophages taken from IL-10-deficient mice did not exhibit a reduction in the expression of MHC class II following MCMV infection. Indeed, macrophages from IL-10-deficient mice showed increased expression of class II. This may be a consequence of the elevated serum levels of IFN-γ that are known to be present in IL-10-deficient mice (12). The level of IFN-γ would be expected to rise further following MCMV infection, leading to an increase in the level of MHC class II on affected cells. In contrast, macrophages taken from IL-10-sufficient C57BL/6 mice showed a reduction in the level of constitutively expressed MHC class II following infection. These data strongly suggest a role for IL-10 in causing the reduction in MHC class II levels in MCMV infection of macrophages.

The induction of IL-10 very early in MCMV infection would immediately help the virus to avoid detection by the host adaptive immune system, by reducing the capacity of the infected macrophages to present antigenic peptides to CD4 cells. It has already been reported that at 48 h following MCMV infection, IFN-α/β plays an important role in causing reduction of MHC class II proteins in bone marrow-derived macrophages (28). In MCMV-infected macrophages, the virus can also interfere with MHC class II expression by affecting the IFN-γ receptor via a mechanism that is independent of IFN-α/β involvement (10). Taken together, this suggests that the sequential activation of IL-10 and IFN-α/β-dependent and IFN-α/β-independent response pathways is likely to be important in the ability of MCMV to impair Ag presentation by infected macrophages in the very early stages of infection.

Another Herpesvirus, EBV, encodes a viral homologue of IL-10, which shares many of the cellular cytokine’s biological activities (37). However, there is no indication of any effect on MHC class II expression on B cells infected with EBV (37). IL-10 has also been shown to be important in infections with other intracellular pathogens, including infection of macrophages with Listeria monocytogenes and Mycobacterium bovis (38). It has also been reported that infection of human alveolar macrophages in culture by respiratory syncytial virus induced secretion of IL-10 by the infected cells (39). It thus seems likely that pathogen manipulation of the IL-10-mediated pathways in infections may be a more widespread phenomenon.

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References