Dendritic cell function in cytomegalovirus-infected patients with mononucleosis

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Abstract: Dendritic cells (DCs) are important target cells for human cytomegalovirus (HCMV) infection, and the virus has been shown to hamper the differentiation and maturation pathways of these cells in vitro. In the present study, we examined the function of monocyte-derived DCs obtained from immunocompetent individuals undergoing symptomatic HCMV infection in terms of immunophenotypic characteristics, pinocytosis, lymphocyte stimulation capacity, and cytokine secretion in comparison with DCs obtained from healthy controls. Immature and lipopolysaccharide (LPS)-stimulated DCs obtained from patients actively infected with HCMV expressed significantly lower levels of major histocompatibility complex (MHC) class II molecules. The inhibition of expression of MHC class II molecules by HCMV appeared to be functionally relevant, as mature DCs obtained from patients with HCMV mononucleosis were inefficient in stimulating proliferation of allogenic lymphocytes. Finally, the pattern of cytokine secretion by DCs obtained from patients with HCMV mononucleosis was characterized by a proinflammatory profile with an increased production of interleukin (IL)-1β, tumor necrosis factor α, CC chemokine ligand 2 (CCL2) and CCL3, and reduced secretion of IL-10 upon LPS stimulation. During symptomatic HCMV infection in the immunocompetent host, DCs exhibit an impaired immunophenotype and function. These effects may contribute to the virus-induced immunomodulation, which is often observed in HCMV-infected patients. J. Leukoc. Biol. 79: 932–940; 2006.

Key Words: viral infection · antigen-presenting cells · viral-induced immunomodulation

INTRODUCTION

Human cytomegalovirus (HCMV) infection is usually asymptomatic in immunocompetent adults, but the virus occasionally gives rise to clinical illness, i.e., a self-limited, mononucleosis-like syndrome, characterized by malaise, protracted fever, and peripheral blood lymphocytosis with atypical lymphocytes [1]. The induction of an efficient immune response to the virus is important in preventing progressive disease, as demonstrated by the development of severe syndromes in immunocompromised individuals undergoing HCMV infection. Conversely, HCMV itself can disable host immune defense. In fact, reactivation of previously controlled viral infections has been reported during the acute phase of HCMV infection [2], and active HCMV replication enhances the immunosuppression that already exists in solid organ and stem cell transplant patients, which makes such patients at high risk for invasive bacterial and fungal infections [3, 4].

Immunological impairments, which could be responsible for the depressed host immune function, have been described upon HCMV in vitro and in vivo infection. A loss of delayed-type hypersensitivity reactions to recall antigens [2] and a reduced lymphoproliferative response to mitogens [5] and to specific antigens [6] have been detected in patients with HCMV mononucleosis. In vitro, HCMV has been shown to inhibit natural killer (NK) activity [7] and suppress bone marrow myelopoiesis [8, 9]. Furthermore, the HCMV genome contains homologues of cellular genes, which may interfere with the host immune response, including a major histocompatibility complex (MHC) class I molecule, an Fc receptor, four chemokine receptors, two α chemokines, and an interleukin (IL)-10 (cmvIL-10) homologue [10–12]. Another strategy, which may be effective for the virus in the generation of viral-induced immunosuppression, is attacking and manipulating cells that play a key role in the initiation and control of the immune response, i.e., dendritic cells (DCs). It has been shown that HCMV inhibits DC differentiation and maturation and impairs the ability of mature DCs to stimulate T cell proliferation and cytotoxicity [13–15] and to migrate in response to inflammatory [16] and lymphoid chemokines [17] in vitro. Furthermore, a reduction in the expression of MHC class I and class II molecules has been observed on the surface of immature DCs infected with HCMV, together with an alteration in...
the profile of DC cytokine production [18]. Recently, cmvIL-10 has been shown to alter DC secretion of proinflammatory cytokines upon maturation stimuli [19] and to block lipopolysaccharide (LPS)-induced up-regulation of MHC class I and class II with an impaired capacity of cmvIL-10-exposed DCs to activate T cells [20]. These observations suggest that the secretion of vireokine during HCMV infection could inhibit DC functionality.

As DCs play a pivotal role in the induction of the adaptive immune response, the impairment of their function may be a key mechanism for the generation of sustained immunosuppression and for the induction of HCMV latency in the natural host. However, all the above-mentioned studies have described DC functions in in vitro models of infection, and little is known about the effect caused by the virus on the DC function during the infection in the natural host. We have recently shown that the immunophenotype and functions of monocyte-derived DCs are altered in heart transplant patients undergoing symptomatic HCMV infection [21]. In the present study, we examined the function of monocyte-derived DCs obtained from immunocompetent individuals undergoing symptomatic HCMV infection (i.e., HCMV mononucleosis) in terms of immunophenotypic characteristics, pinocytosis, allostimulatory capacity, and cytokine secretion.

MATERIALS AND METHODS

Patients and study design

The patient population consisted of 14 individuals who attended the Unit of Infectious Disease, G.B. Morgagni General Hospital (Forlì, Italy) and the Unit of Infectious Diseases, Karolinska Institutet (Stockholm, Sweden), during a 16-month period (December 2003–March 2005). Patients manifested a mononucleosis syndrome characterized by the presence of at least two of the following signs and symptoms: 2–4 weeks persistent fever, splenomegaly (n = 14), asthenia (n = 9), arthromyalgia (n = 4), lymphadenopathy (n = 5), and at least two of the following analytical alterations: serum alanine aminotransferase (ALT; > 35 U/l or > 0.58 microkat/l, n = 14), aspartate aminotransferase (AST; > 40 U/l or > 0.58 microkat/l, n = 13), relative lymphocytosis (n = 13). Mononucleosis was spontaneously acquired and not transfusion-related in all cases.

As described previously [22–27], diagnosis of HCMV mononucleosis was defined when a compatible clinical picture was associated with the following laboratory findings: the presence of specific immunoglobulin M (IgM) to HCMV, with or without detectable IgG antibodies against HCMV; a lack of serological evidence of ongoing, primary Epstein-Barr virus (EBV) infection, as indicated by the absence of IgM against the EBV viral capsid antigen (VCA) and the presence of antibodies against EBV nuclear antigens (EBNA); and a lack of serological evidence of acute adenovirus infection, as indicated by the absence of specific IgM. Human immunodeficiency virus (HIV) tests were also performed in three patients of this study group presenting risk factors for HIV transmission and were all negative. Peripheral blood (20–30 ml) was obtained from the patients who fulfilled the inclusion criteria for the study. Buffy coats from 15 normal volunteers (11 HCMV-seropositive and four HCMV-seronegative subjects) were selected randomly and included in the study as healthy, immunocompetent controls. Informed consent was obtained from all patients before their enrollment in the study.

Serological tests for the diagnosis of HCMV, EBV, adenovirus, and HIV infection

HCMV serology was performed using an immunoenzymatic assay [enzyme-linked immunosorbent assay (ELISA)] for the detection of HCMV-specific antibodies. An automated, recombinant, antigen-based HCMV IgM immuno-assay [microparticle enzyme immunoassay (MEIA), Abbott Laboratories, Abbott Park, IL] was used in Morgagni Hospital's diagnostic laboratory, and this test has been reported previously as a sensitive and specific assay for the detection of anti-HCMV IgM [28]. A cytomegalovirus IgM capture assay (Trinity Biotech, Bray, Ireland) was adopted at the Department of Virology in Karolinska Hospital. Anti-HCMV specific IgG were detected by MEIA (Abbott Laboratories) at Morgagni Hospital and by an in-house ELISA at Karolinska Hospital. For EBV serology, an automated, immunoenzymatic test was used for the detection of IgM and IgG against VCA and EBNA (ELISA MicroPlate Assay, Labotech Automated Microplate Analyzer, Adaltis Italia S.p.a., Italy) and for adenovirus, a commercially available kit was used (Adenovirus Serion ELISA classic, Serion Immunodiagnostics GmbH, Würzburg, Germany). Screening for primary HIV infection was performed with the VIDAS HIV DUO (bioMérieux, Marcy l’Etoile, France), a commercially available kit, which combines the detection of p24 antigen and anti-HIV antibodies. Tests were performed according to the manufacturer's instruction as part of the routine diagnostic service.

Cell preparation and culture

Peripheral blood was collected from healthy controls and from patients with HCMV mononucleosis 2–3 weeks after the onset of symptoms. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-separating density gradient centrifugation (Biochrom AG, Berlin, Germany). Monocyte-derived DCs were generated as described previously [29]. Briefly, isolated PBMCs were plated at 5–10 × 10⁶ cells/ml in complete RPMI (BioWhittaker, Walkersville, MD) containing 10% fetal calf serum (FCS). Cells were allowed to adhere for 2 h at 37°C, nonadherent cells were removed, and the adherent cells were washed in phosphate-buffered saline (PBS). Complete RPMI containing 10% FCS, 1000 IU/ml IL-4 (R&D Systems, Minneapolis, MN), and 100 ng/ml granulocyte macrophage-colony stimulating factor (GM-CSF) (R&D Systems) was added, and the cells were cultured for 7 days. During the last 2 days of culture, 100 ng/ml LPS (Sigma-Aldrich Corp., St. Louis, MO) was added to the culture medium as a maturation stimulus. Cell differentiation was monitored by flow cytometry. Immature DCs were collected at Day 5 before exposure to LPS, and mature DCs were harvested at Day 7 after incubation with LPS. Cells were gated on size and scatter parameters to exclude small and condensed residual cells (dead cells and cellular debris), following which 70–90% of the cells obtained from the control group (i.e., healthy blood donors) displayed a typical monocyte-derived DC phenotype [e.g., CD14⁺, human leukocyte antigen (HLA)-DR⁺, CD83⁺].

Immunophenotyping

Antibodies used for cell-surface staining included those recognizing CD1a, CD14, CD80, CD86, HLA-ABC, HLA-DR (BD Biosciences, San Jose, CA), and CD83 (ImmuneTech, Marseilles, France). All antibodies were mouse monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin. Irrelevant mouse isotype controls (BD Biosciences) were included in the analysis. Data acquisition and analysis were performed on a FACScan flow cytometer (Beckton Dickinson, Mountain View, CA) using Lysis II software.

FITC-dextran assay

To evaluate the capacity for uptake of soluble antigens from the culture medium, DCs were incubated with 1 mg/ml FITC-dextran (Sigma-Aldrich Corp.) at 37°C for 1 h. As a negative control, cells were incubated under the same conditions at 4°C. After incubation, cells were washed in cold PBS and analyzed by flow cytometry. Data represent the difference in mean fluorescence intensity (MFI) of DCs after FITC-dextran uptake at 37°C and at 4°C.

Allogenic mixed leukocyte reaction (MLR)

The stimulatory ability of mature monocyte-derived DCs was assessed in an allogenic MLR as described previously [30]. For all assays, PBMCs from one healthy donor served as a source of responder cells. PBMCs were isolated by density gradient centrifugation in a Ficoll-separating solution (Biochrom AG) and resuspended in complete RPMI containing 20% FCS and 10% dimethyl sulfoxide (Sigma-Aldrich Corp.), and aliquots were frozen in liquid nitrogen. Responder PBMCs were thawed immediately before use, washed, resuspended...
Quantification of cytokines and chemokines

Supernatants obtained from immature and mature DC cultures were collected at Days 5 and 7, respectively, and frozen at −80°C. Cytokine [IL-1β, IL-10, IL-12, and tumor necrosis factor α (TNF-α)] secretion was analyzed by using the multiprotein ELISA platform human inflammatory cytokine array, Search Light™ (Perbio Science, Erembodegem, Belgium). Chemokine [CC chemokine ligand 2 (CCL2), CCL3, CCL4, CCL5, I309, and eotaxin] production was determined; CA, cervical adenopathy; pos, positive.

Statistical analysis

Statistical analysis was performed using an unpaired Student’s t-test. Differences were considered significant with P < 0.05.

RESULTS

Patients’ characteristics

We defined a group of 14 patients undergoing HCMV mononucleosis on the basis of clinical data and laboratory findings. As described previously [1, 22–27], the symptoms were systemic. Fever and malaise predominated, and few signs of enlarged lymph nodes or spleen were noted. As shown in Table 1, the hematological (lymphocytosis) and analytical (aminotransferases) hallmarks of infectious mononucleosis were observed in all patients included in the study. Diagnosis of HCMV-induced mononucleosis was established on serological criteria, and all patients showed IgM positivity against HCMV, which suggested the presence of an active HCMV infection (Table 1). Polymerase chain reaction and virus isolation are not performed as routine diagnostic methods in immunocompetent patients undergoing mononucleosis at our hospitals and were therefore not carried out in our patients’ study group. There was no serological evidence of acute EBV, adenovirus, and HIV infection, as revealed by the absence of specific serological markers (data not shown).

DCs obtained from HCMV-infected patients exhibit an altered immunophenotype

The expression of DC surface antigens was investigated by flow cytometry on immature and mature monocyte-derived DCs obtained from patients with HCMV mononucleosis and healthy individuals. The mean expression levels of MHC class II molecules were reduced in immature DCs from HCMV-infected patients as compared with cells obtained from healthy controls (P < 0.05; Table 2 and Fig. 1A). In addition, a reduction in the expression levels of the characteristic DC marker CD1a and of the MHC class I molecules was observed in immature DCs obtained from four patients undergoing active HCMV infection (Fig. 1A). One of these four patients presented cervical lymphadenopathy, and immature DCs of this subject (Patient Number 7, Table 1) also exhibited low expression levels of MHC class II molecules, but no additional correlation was observed between impairment of DC immunophenotype and the type and severity of symptoms among the other patients. We then

### Table 1. Summary of Patients’ Clinical and Laboratory Data

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yr) and sex</th>
<th>Spleen and/or liver enlargement</th>
<th>Additional comments</th>
<th>WBC × 1000/μl</th>
<th>% Lymphocytes</th>
<th>ALT*</th>
<th>AST*</th>
<th>CRP mg/dl</th>
<th>HCMV-IgM (AU)</th>
<th>HCMV-IgG (AU)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>30, M</td>
<td>+</td>
<td>–</td>
<td>9.8</td>
<td>58.0</td>
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<td>1.9</td>
<td>3.5</td>
<td>4.5</td>
<td>190.0</td>
</tr>
<tr>
<td>2</td>
<td>32, M</td>
<td>+</td>
<td>–</td>
<td>12.3</td>
<td>65.0</td>
<td>2.1</td>
<td>1.5</td>
<td>3.2</td>
<td>5.3</td>
<td>41.0</td>
</tr>
<tr>
<td>3</td>
<td>32, M</td>
<td>+</td>
<td>IP</td>
<td>10.8</td>
<td>68.6</td>
<td>1.2</td>
<td>1</td>
<td>1.1</td>
<td>4.6</td>
<td>60.0</td>
</tr>
<tr>
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<td>+</td>
<td>–</td>
<td>7.5</td>
<td>55.8</td>
<td>2.8</td>
<td>2.2</td>
<td>ND</td>
<td>2.9</td>
<td>36.0</td>
</tr>
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<td>1.9</td>
<td>1.5</td>
<td>ND</td>
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<td>6</td>
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<td>+</td>
<td>–</td>
<td>9.4</td>
<td>56.3</td>
<td>2.6</td>
<td>1.3</td>
<td>0.2</td>
<td>3.0</td>
<td>199.0</td>
</tr>
<tr>
<td>7</td>
<td>38, M</td>
<td>+</td>
<td>CA</td>
<td>10.7</td>
<td>65.0</td>
<td>4.6</td>
<td>1.9</td>
<td>6.2</td>
<td>6.2</td>
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</tr>
<tr>
<td>8</td>
<td>31, M</td>
<td>+</td>
<td>–</td>
<td>5.4</td>
<td>ND</td>
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<td>9</td>
<td>46, M</td>
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<td>–</td>
<td>14.2</td>
<td>72.0</td>
<td>8.0</td>
<td>5.0</td>
<td>5.8</td>
<td>5.2</td>
<td>195.0</td>
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<tr>
<td>10</td>
<td>28, F</td>
<td>+</td>
<td>–</td>
<td>8.4</td>
<td>58.0</td>
<td>3.0</td>
<td>1.8</td>
<td>2.9</td>
<td>3.6</td>
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<tr>
<td>11</td>
<td>31, M</td>
<td>+</td>
<td>IP</td>
<td>10.0</td>
<td>51.7</td>
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<td>12</td>
<td>28, M</td>
<td>+</td>
<td>CA</td>
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<td>53.0</td>
<td>1.8</td>
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<tr>
<td>13</td>
<td>34, F</td>
<td>+</td>
<td>–</td>
<td>12.6</td>
<td>63.0</td>
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<td>3.6</td>
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<tr>
<td>14</td>
<td>31, M</td>
<td>+</td>
<td>–</td>
<td>8.9</td>
<td>56.0</td>
<td>3.1</td>
<td>2.0</td>
<td>1.6</td>
<td>16.0</td>
<td>10</td>
</tr>
</tbody>
</table>

* Fold increase compared with normal value. WBC, white blood cell; CRP, C-reactive protein; AU, arbitrary units; IP, interstitial pneumonia; ND, not determined; CA, cervical adenopathy; pos, positive.
investigated if there was any effect caused by HCMV infection on the phenotype of DCs undergoing maturation upon LPS stimulation. No differences were found in the mean expression levels of CD1a, CD14, CD80, CD83, CD86, and MHC class I molecules between DCs from patients with acute HCMV infection and controls (Table 2). However, the mean expression levels of MHC class II molecules were decreased significantly on mature DCs obtained from HCMV-infected patients ($P < 0.05$) as compared with mature DCs obtained from healthy controls (Table 2 and Fig. 1B).

### Table 2. Comparison of the Immunophenotypic Characteristics of DCs Derived from Patients with HCMV Mononucleosis and Healthy Controls at Immature and Mature Stages

<table>
<thead>
<tr>
<th></th>
<th>Pts with HCMV mononucleosis (n = 13)</th>
<th>Controls (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Positive cells MFI</td>
<td>% Positive cells MFI</td>
</tr>
<tr>
<td>CD1a</td>
<td>65 ± 31 293 ± 238</td>
<td>76 ± 14 395 ± 177</td>
</tr>
<tr>
<td>CD14</td>
<td>15 ± 23 8 ± 13</td>
<td>18 ± 12 8 ± 6</td>
</tr>
<tr>
<td>CD80</td>
<td>66 ± 31 23 ± 15</td>
<td>77 ± 23 39 ± 26</td>
</tr>
<tr>
<td>CD83</td>
<td>10 ± 3 3 ± 4</td>
<td>20 ± 19 5 ± 4</td>
</tr>
<tr>
<td>CD86</td>
<td>43 ± 26 20 ± 17</td>
<td>42 ± 20 29 ± 23</td>
</tr>
<tr>
<td>MHC I</td>
<td>96 ± 6 190 ± 141</td>
<td>98 ± 1 241 ± 73</td>
</tr>
<tr>
<td>MHC II</td>
<td>52 ± 28 18 ± 19*</td>
<td>68 ± 17 35 ± 20</td>
</tr>
<tr>
<td>Dextran-FITC</td>
<td>88 ± 11 382 ± 463</td>
<td>72 ± 22 212 ± 277</td>
</tr>
</tbody>
</table>

Cumulative data represent mean ± sd from patients (Pts) with HCMV mononucleosis and healthy controls. *$P < 0.05$ between the two groups (Student’s $t$-test).
DCs from patients with HCMV mononucleosis can uptake antigens efficiently but poorly stimulate allogenic T cells

Immature DCs uptake antigens efficiently and yet lose this capacity upon maturation. Cumulative results of FITC-dextran particle endocytosis by immature DCs obtained from the two groups of subjects did not differ \((P=0.29, \text{ Table 2})\), indicating that immature DCs from patients with active HCMV infection and from healthy individuals internalized comparable amounts of antigen. As expected, DCs decreased their ability to take up antigen after LPS stimulation, and there was no difference between HCMV-infected patients and controls \((P=0.53, \text{ Table 2})\).

Mature DCs are specialized in presenting antigens to specific T cells. Therefore, we further assessed the capacity of mature monocyte-derived DCs from patients undergoing HCMV mononucleosis and controls to induce proliferation of T cells in an allogenic MLR. We observed that the ability of DCs from patients with active HCMV infection to stimulate allogenic PBMC proliferation was reduced significantly when compared with cells obtained from healthy controls \((P<0.05 \text{ between the two groups, Fig. 2})\).

Proinflammatory profile of cytokine secretion in mature DCs obtained from HCMV-infected patients

An important function of DCs during immune response is the production of proinflammatory cytokines. The secretion of the cytokines IL-1β, IL-10, IL-12, and TNF-α was analyzed in DC cultures obtained from patients undergoing active HCMV infection and healthy individuals before and after LPS stimulation. Although IL-10 levels were reduced significantly in supernatants of immature DCs obtained from patients with HCMV mononucleosis as compared with healthy controls \((P<0.05)\), no significant difference was detectable in the production of IL-1β, IL-12, and TNF-α in supernatants of immature DCs obtained from the two groups of subjects (Fig. 3). LPS stimulation resulted in a proinflammatory secretion pattern in DCs obtained from patients undergoing HCMV mononucleosis and a significant increase in IL-1β and TNF-α secretion and a reduction in production of IL-10 (Fig. 3). TNF-α showed the most pronounced boost, with an average fourfold increase in secretion from DC cultures obtained from HCMV-infected patients \((P<0.01)\). No significant difference in IL-12 secretion was observed between the two groups of subjects.

Increased inflammatory chemokine secretion by LPS-stimulated DCs obtained from patients with HCMV mononucleosis

The secretion of the inflammatory chemokines CCL2, CCL3, CCL4, CCL5, I309, and eotaxin was analyzed in DC cultures obtained from patients undergoing HCMV mononucleosis and healthy controls before and after LPS stimulation. No differences were found in chemokine secretion by immature DC obtained from HCMV-infected patients in comparison with healthy individuals. However, maturation by LPS resulted in a proinflammatory secretion pattern of chemokines by DCs ob-

![Fig. 2. Mature DCs from patients with HCMV mononucleosis have an impaired ability to stimulate allogenic T cell proliferation. The proliferative T cell response after coculture with mature DCs obtained from HCMV-infected (P, open symbols with dashed lines) and uninfected (BD, solid symbols) individuals was evaluated in a classical allogenic MLR. Increasing numbers of mature DCs were cocultivated with 10⁵ allogenic PBMCs, and the incorporation of [H]thymidine was evaluated. SI was calculated as follows: SI = cpm (PBMC responders + DC stimulators)/cpm (PBMC responders alone). *, \(P < 0.05\) between the group of patients with HCMV mononucleosis and the group of healthy controls (Student’s t-test).](http://www.jleukbio.org)
tained from patients with active HCMV infection as compared with DCs obtained from healthy subjects, with a significant increase in production of CCL2 and CCL3 ($P<0.05$; Fig. 4).

**DISCUSSION**

DCs are one of the target cells of HCMV infection, and the virus, assisted by the virally encoded cmvIL-10, may hamper the DC differentiation and maturation pathways as well as a wide range of specific functions of these cells, such as the ability to secrete cytokines, to stimulate T cell proliferation, and to migrate in response to chemokines in vitro [13–20]. These inhibitory effects may represent an important viral strategy to induce a generalized immunosuppression, which could lead to a successful virus-host coexistence and to the establishment of virus latency in the host.

We have recently demonstrated that immature monocyte-derived DCs obtained from heart transplant patients undergoing an active HCMV infection exhibit reduced expression of the specific DC marker CD1a and impaired expression of MHC class I and II molecules and that mature DCs from the same group of patients present an altered ability to stimulate T cell proliferation [21]. Transplant patients are ideal subjects to study the effect of HCMV infection on DC function, as they often suffer from severe HCMV infection with high viral load.
and viral systemic dissemination. Conversely, these patients are constantly under immunosuppressive therapy with drugs, such as cyclosporine A and corticosteroids, which have been demonstrated to impair DC function in vitro [31, 32].

Here, we analyzed monocyte-derived DCs obtained from symptomatic HCMV-infected immunocompetent individuals to determine whether DC functions are altered in the absence of interfering factors caused by pharmacological immunosuppression. All patients of our study group were young and previously healthy adults, and they all later fully recovered from the disease without developing complications. However, as almost all acquired HCMV infections in the normal host are clinically silent [33], we cannot exclude that we involuntarily selected a group of individuals with susceptibility to HCMV disease and that these patients may not be representative of completely immunocompetent subjects.

We found that expression of MHC class II molecules was reduced significantly on the surface of immature and mature DCs obtained from patients undergoing HCMV mononucleosis in comparison with healthy controls, and expression of CD1a and MHC class I molecules was reduced strongly on immature DCs in four cases.

Down-regulated expression of MHC class II molecules on the surface of these antigen-presenting cells upon HCMV infection may lead to an impaired ability of DCs to stimulate T cell proliferation. In fact, we found that the stimulatory potential of mature monocyte-derived DCs from patients with HCMV mononucleosis was attenuated significantly against alloantigens as compared with mature DCs obtained from healthy subjects. This finding is in agreement with previously published in vitro and in vivo data [14, 15, 18, 21]. Our results may possibly be explained by the action of a soluble factor. This hypothesis is supported by published in vitro data suggesting that the presence of soluble CD83 released into the culture supernatant could reduce the ability of HCMV-infected DCs to stimulate T cell proliferation [15, 34]. However, further studies are required to characterize the mechanism underlying this viral immunosuppressive effect during the infection in the natural host.

The findings of an altered immunophenotype and impaired immunostimulatory ability in DCs from patients with HCMV mononucleosis are similar to those that we recently observed in heart transplant patients undergoing acute HCMV infection [21], implying that HCMV alters DC immunophenotype and function during infection in immunocompetent and immunocompromised hosts. The expression levels of CD1a and MHC class I molecules, as well as allostimulatory capacity, were overall lower in DCs obtained from transplant patients as compared with those in immunocompetent individuals enrolled in the present study. In transplant patients, we observed a strongly altered immunophenotype in immature DCs obtained from individuals with acute HCMV infection as compared with uninfected patients. No difference was found between LPS-stimulated DCs obtained from HCMV-infected and uninfected transplant recipients, which was probably a result of the general low level of responsiveness to maturation stimuli, which we and others [35] detected in DCs from patients under immunosuppressive treatment. Conversely, we observed an impaired immunophenotype in immature and mature DCs obtained from patients with HCMV mononucleosis as compared with healthy subjects.

A key role among the different functions performed by DCs in controlling immunity and tolerance is the secretion of cytokine and chemokines. This function is fundamental for specific recruitment of immune cells in the infection area and for inducing different T cell responses [36]. Here, we found that acute HCMV infection in immunocompetent individuals induced a proinflammatory pattern of cytokine and chemokine secretion in DCs, with significantly increased production of IL-1β, TNF-α, CCL2, and CCL3 and a reduction in the release of the anti-inflammatory cytokine IL-10. Nevertheless, the infection alone was not enough to activate the secretion of proinflammatory cytokines, as the boost of inflammatory soluble factors was revealed only in DCs obtained from HCMV-infected patients following LPS stimulation. The reduced production of IL-10 by DCs obtained from HCMV-infected patients indicates that this anti-inflammatory cytokine is not involved in impairing expression levels of HMC class II molecules and in subverting the immunostimulatory properties of DCs in our patients’ group. Nevertheless, such viral-induced inhibition of DC function may also be caused by mechanisms other than production of IL-10, such as expression of the glycoproteins US2 and US3 [37] or secretion of a soluble form of CD83 [34] in infected cells. These alternative strategies may account for the impairment of phenotype and immunostimulatory function in DCs, which we observed in patients with HCMV mononucleosis. It is at present unknown whether cmvIL-10 is produced during a natural infection and contributes to the viral-induced impaired immunophenotype and functions of DCs.

Production of inflammatory cytokines, such as TNF-α, in response to HCMV infection would be expected to help in combating the virus infection. However, HCMV has been shown to prevent TNF-α signaling in infected cells by down-regulation of one of the TNF-α receptors at the cell surface [38]. In addition, our group has demonstrated previously that TNF-α, together with interferon-γ, rather than inhibiting replication of HCMV, is responsible for the differentiation of monocytes into HCMV-permissive macrophages, which are refractory to the antiviral effect of these cytokines [39]. TNF-α secretion by HCMV-infected monocytes has also been shown to inhibit T cell proliferation through arachidonic acid and prostaglandin E2 release [40]. Furthermore, an association has been reported between high TNF-α plasma levels and the development of HCMV disease in transplant patients [41, 42], and an inverse correlation has been demonstrated between high IL-10/TNF-α ratio values in plasma and the development of symptomatic HCMV infection [43]. Thus, the amplified proinflammatory cytokine profile, which we detected in LPS-stimulated DCs from patients with HCMV mononucleosis, may play a role in the antiviral response but may also take part in the development of symptoms (i.e., fever, asthenia) and may contribute to immunosuppression.

During acute viral infections, viruses tend to induce production of a restricted subset of inflammatory chemokines, which help to shape a T helper cell type 1-polarized adaptive immune response [44, 45]. In agreement with published data, LPS-
stimulated DCs obtained from HCMV-infected patients secreted significantly higher amounts of the inflammatory chemokines CCL2 and CCL3, which can amplify the inflammatory response leading to viral clearance but with the potential to increase viral dissemination. In fact, it has been shown that a β-chemokine homologue produced by murine cytomegalovirus, MCK-2, through its ability to recruit mononuclear leukocytes, is an important determinant of viral dissemination in the mouse model [46], and a similar function has been suggested for the functionally active α-chemokine homologue UL146 encoded by HCMV [47]. CCL2 and CCL3 are human inflammatory β-chemokines, which are able to recruit monocytes, T cells, and NK cells to the site of infection [48]. Encoding for chemokine homologues and inducing secretion of human inflammatory chemokines may represent two different aspects of the same viral strategy to recruit cells susceptible to infection and spread the progeny in the host tissues, and this strategy may ensure access of the viral particles into the monocye population, where HCMV can establish latency.

In conclusion, during symptomatic HCMV infection in the immunocompetent host, DCs exhibit an impaired immunophenotype and function in terms of decreased lymphocyte stimulation. These cells also show an amplified, proinflammatory cyto-chemokine secretion profile. Attacking cells that play a key role in initiation and control of innate and adaptive immune responses such as DCs may be an effective immune evasion strategy for the virus. As a side effect, HCMV-induced subversion of DC function may blunt the immune response to other pathogens, which would have important clinical implications in the acute phase of infection in immunocompromised patients. For example, HCMV-induced immunosuppression is known to result in solid organ and stem-cell transplant patients being at high risk for severe bacterial and fungal infections. Increased knowledge of the cellular mechanisms underlying the impairment of the host immune system caused by HCMV will not only provide important information about basic virology and immunology but will also be helpful in developing new strategies for the monitoring and treatment of immunosuppression caused by this virus.

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