Persistent Human Cytomegalovirus Infection Induces Drug Resistance and Alteration of Programmed Cell Death in Human Neuroblastoma Cells

Jindrich Cinatl, Jr.,1 Jaroslav Cinatl, Jens-Uwe Vogel, Ruslan Kotchetkov, Pablo Hernández-Driever, Hana Kabickova, Bernhard Kornhuber, Dirk Schwabe, and Hans Wilhelm Doerr


ABSTRACT

Infection with human cytomegalovirus (HCMV) is a common and generally asymptomatic affection in childhood. Its role in neuroblastoma (NB) patients has not yet been elucidated. As evidence grows that HCMV interacts with apoptotic signaling due to the interaction of HCMV gene products with cellular proteins of apoptotic pathways, we used human NB cell line UKF-NB-2 persistently infected with HCMV strain AD169 to study the effects of long-term HCMV infection on programmed cell death of neuroectodermal tumor cells. The cells designated UKF-NB-2AD169 continued to produce infectious virus in successive subcultures over a period of more than 1 year. Up to 20% of cells expressed viral genes or produced infectious virus after initiation of infection. UKF-NB-2AD169 cells were significantly less sensitive to the cytotoxic agents cisplatinum and etoposide than parental (noninfected) UKF-NB-2 cells. These effects were associated with decreased ability of UKF-NB-2AD169 cells to undergo apoptosis and continuous viral replication. UKF-NB-2AD169 showed increased levels of antiapoptosis Bcl-2 protein (up to 12-fold), whereas expression of p53 and c-myc was not changed. Treatment of UKF-NB-2AD169 cells with ganciclovir, abolishing virus production, reestablished sensitivity to chemotherapy, lowered Bcl-2 expression, and facilitated induction of apoptosis to the level of the parental cell line. The results demonstrate that persistent HCMV infection confers resistance to cytotoxic agents on neuroectodermal tumor cells and protects from apoptosis, probably due to increased levels of Bcl-2 protein. Hence, it is conceivable that HCMV infection before or during tumorigenesis may contribute in some NB patients to failure of therapy.

INTRODUCTION

HCMV infection is a ubiquitous pathogen in humans. Infection occurs very often during early childhood. It may produce mononucleosis-like disease but is generally asymptomatic in healthy children (1). Nevertheless, HCMV infection has been associated with the oncogenesis of several carcinomas and embryonal tumors such as Wilms' tumor and NB (2, 3). HCMV DNA, mRNA, and/or antigens have been proven to exist in 30–60% of these tumors. The role of HCMV in the development and clinical course of these malignancies has not been completely elucidated because HCMV-specific gene products and sequences were not detectable after long-term culture of HCMV-infected tumor tissues and cells (2). Due to its tendency to produce persistent organ infections, seroepidemiological linking analysis and interpretation thereof is not conclusive. However, it still remains possible that HCMV modulates malignant behavior of certain tumor cells that are susceptible to HCMV infection acting through molecular pathways involved in signal transduction of cellular activation (4). These effects lead to increased synthesis or activity of several cellular enzymes, such as those associated with the regulation of cell cycle progression (for review, see Ref. 5). The interactions of HCMV gene products with tumor suppressor proteins, such as p53 (6, 7), the retinoblastoma susceptibility protein (8, 9), or retinoblastoma susceptibility protein-related proteins (10), are reminiscent of those mediated by oncoproteins of other DNA tumor viruses. Thus, infection of cells with HCMV may influence cell growth, differentiation, and programmed cell death through various pathways.

Programmed cell death (apoptosis) is a key element of a host organism's defense against viral infections, inhibiting viral spread and persistence. It has been suggested that many viruses have evolved mechanisms blocking the apoptotic process that may enable them to establish latent infections (11, 12). These mechanisms include mimicking or inducing the Bcl-2 protein (13–15), blocking the function of p53 (16, 17), or inhibiting the activity of interleukin-1β-converting enzyme family members (18).

HCMV inhibits apoptosis induced by adenovirus E1A protein or tumor necrosis factor α in human fibroblast cells (19). In cell types expressing p53, such as fibroblasts, smooth muscle cells, or endothelial cells, effects of HCMV on apoptosis could be related to the interaction of HCMV gene products with the p53 tumor suppressor protein (7, 20, 21). However, the influence of HCMV on p53 and apoptosis may be more complex because HCMV morphological transforming region II oncopen proteins, as well, is able to bind to p53 and thereby down-regulates p53-activated transcription (6). In cell types that lack p53 expression, mechanisms of inhibitory effects of HCMV on apoptosis remain less clear. Transfection of p53-negative HeLa cells with HCMV IE1 or IE2 genes resulted in impaired apoptosis induced by tumor necrosis factor α and adenovirus E1A protein but not from UV irradiation (19). These experiments failed to detect a change in the expression of a cellular gene that favors survival, such as bcl-2, or of a cellular gene that favors death, such as bax (19).

In NB, patients can be segregated by risk factors, including age at diagnosis, histology, N-myc amplification, and deletion of the lp36.3 chromosomal region (22, 23). Despite intensive therapy, including ablative therapy with stem cell rescue, survival prognosis remains poor. Even children with favorable risk factors eventually die due to tumor progression (24). Important chemotherapeutic agents applied in NB patients, such as VP-16 and CDDP, act through induction of programmed cell death (25). Inhibition of apoptosis may be an important factor for both tumor progression and resistance to antineoplastic therapy (26). Expression of the apoptosis-suppressing protein Bcl-2 in NB was associated with poor stage disease, unfavorable histology and N-myc amplification (27). About one-third of pretreatment tumor specimens presented enhanced levels of bcl-2 expression, and after treatment, over 80% of tumors showed Bcl-2 protein positivity, suggesting that it may be important for the survival of NB cells (27). Induction of bcl-2 expression and enhanced drug resistance through treatment with retinoic acid was accompanied by induction of neuronal phenotype in NB cells (25). Moreover, augmented Bcl-2...
expression of bcl-2-transfected NB cells paralleled increased drug resistance against apoptosis inducing drugs in a dose-dependent manner (28).

We established productive persistent HCMV infection in several human NB cell lines to study the effects of long-term HCMV infection on virus production and phenotypic properties of tumor cells (5). Previous results show that persistent HCMV infection of NB cell lines is associated with the alteration of phenotypic properties of the cells; i.e., HCMV infection increased N-myc expression, decreased expression of neuronal markers, and stimulated production of growth factors (5, 29). Xenografts of persistently HCMV-infected human NB cells in nude mice showed increased growth and formation of metastasis when compared with noninfected tumor cells (30). These findings suggest that HCMV modulates tumorigenic properties in terms of an increase of malignant potential of NB cells. It is conceivable that an altered response to induction of programmed cell death in NB cells persistently infected with HCMV could contribute to such effects.

In the present study, we observed sensitivity of a NB cell line, UKF-NB-2, persistently infected with HCMV to cytotoxic effects of VP-16 and CDDP, which are known to induce apoptosis of NB cells (27, 31, 32). The sensitivity of infected cells to both drugs was markedly reduced when compared with noninfected cells, and the abolition of HCMV infection by GCV treatment of the infected cells reestablished drug sensitivity to a level of uninfected cells. The induction of drug resistance in NB cells by persistent HCMV infection was accompanied by enhanced bcl-2 expression and unaltered expression of c-myc and p53.

MATERIALS AND METHODS

Cells. To establish persistent HCMV infection, NB cell line UKF-NB-2 (33) was infected with strain AD169 and propagated as described for human NB cell line UKF-NB-4 (29). The infected cell line designated UKF-NB-2AD169 and the parental cell line UKF-NB-2 were grown in Iscove’s Dulbecco’s modified medium (Seromed, Berlin, Germany), supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified 5% CO2 incubator. The cells were subcultured at 7–9-day intervals over a period of more than 1 year (40 subcultures) without addition of uninfected cells. Cells were routinely tested for mycoplasma by the Hoechst 33258 staining method and found to be free of contamination.

Viral Replication in HCMV-infected NB Cells. Virus production was monitored by titration of cell extracts and cell culture supernatants, and virus antigen was examined by immunoperoxidase method using monoclonal antibodies (DuPont, Bad Homburg, Germany) directed against M, 72,000 IE and M, 67,000 late proteins as described previously (29). To assess the number of cells capable of producing infectious virus, infectious center assay was performed by infection of UKF-NB-2AD169 cells with noninfected human foreskin fibroblast monolayers, as described previously (29). The amount of viral gene expression was measured in infected cells by in situ hybridization using HCMV-specific DNA probe prepared from the IE region of AD169 strain as described elsewhere (34).

Growth and Viability of NB Cells in Vivo. Growth characteristics and cell viability of UKF-NB-2AD169 cultures were compared with those of UKF-NB-2 cells at different times after initiation of infection. Cells were seeded at a density of 2 × 105 cells/ml in culture medium and incubated at 37°C with 5% CO2. Viability was determined by dye exclusion method after staining with 0.5% trypan blue solution. Viable cells were counted using a hemocytometer.

Antineoplastic Treatment of NB Cells. To study whether sensitivity to antineoplastic agents differs between UKF-NB-2 and UKF-NB-2AD169 sublines, cells were incubated with different concentrations of CDDP and VP-16. In some experiments, UKF-NB-2AD169 cells were cultivated for 21 days (three subcultures) with anti-HCMV agent GCV (Syntex, Palo Alto, CA) before treatment with antitumor agents. The number of viable cells was determined after 2 days of treatment by the dye exclusion method after staining with 0.5% trypan blue solution.

Measurement of Apoptosis by TUNEL Assay. To detect cells undergoing programmed cell death, the in situ TUNEL method was performed (35) using assay kits from Oncor (Gaithersburg, MD). The cytopsin of UKF-NB-cell lines were incubated with FITC-labeled UTP and terminal deoxynucleotidyl transferase following the manufacturer’s instructions.

Detection of p53, c-myc, and Bcl-2 Proteins by Western Blot. Western blot analysis of cellular proteins was performed at different times after initiation of infection in UKF-NB-2AD169 cells in comparison with UKF-NB-2 cells by methods described previously (29). Monoclonal antibodies to p53, c-myc, and Bcl-2 were obtained from Dako, Oncogene Sciences, and Santa Cruz Biotechnology, respectively. Protein concentration in cell lysates was determined with the Bio-Rad protein assay (Hercules, CA) with BSA as a standard. Samples (20 μg/lane) were fractionated by electrophoresis on a denaturing SDS-7.5% PAGE under reducing conditions. Fractionated proteins were transferred electrophoretically to nitrocellulose membranes. Biotin-labeled goat antimouse IgG was used as a secondary antibody, and visualization was achieved by incubation with streptavidin/horseradish and 3-aminopyrdoyl carbazole as the enzyme substrate.

Electron Microscopy. UKF-NB cells were processed for ultrastructural examination as described previously (29, 34). Briefly, cells were pelleted and fixed with 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Durupan-Epon. Thin sections were contrasted with uranylacetate and lead citrate and viewed with a Jeol JEM, 2000 CX microscope (Akishima, Japan).

Flow Cytometry. To investigate the expression of Bcl-2, 5 × 106 cells were fixed for 30 min in 2% buffered formaldehyde. After washing twice in buffer containing 0.5% Tween 20, cells were incubated for 45 min with FITC-conjugated antibodies against Bcl-2 (Boehringer Diagnostika, Koeln, Germany). Fluorescent intensity was measured by FACScan flow cytometry (Becton Dickinson, Heidelberg, Germany). Data were analyzed using Becton Dickinson Lysis II software. All experiments were repeated three times.

RESULTS

Detection of HCMV-specific Antigens, Viral Production, and HCMV-specific DNA in UKF-NB-2AD169 Cells. Expression of viral antigens, production of infectious virus, and amount of viral DNA in UKF-NB-2AD169 at different times after initiation of infection are shown in Table 1. UKF-NB-2AD169 cells continued to produce infectious virus in successive passages, with a titer ranging from 9 × 105 to 6 × 10^6 and from 2 × 10^3 to 8 × 10^3 pfu per 10^6 cells and 1 ml culture medium, respectively; 11–19, 6–13, and 7–14% of cells produced HCMV-specific antigens, produced infectious virus progeny, and expressed viral gene, respectively.

<table>
<thead>
<tr>
<th>No. of subcultures</th>
<th>Viral antigen expression (% positive cells)</th>
<th>Viral titre</th>
<th>Infectious centre (% infected cells)</th>
<th>Viral genome (% positive cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IE Late</td>
<td>pfu/10^6 cells</td>
<td>pfu/ml</td>
<td>11.3 19.4 10.3</td>
</tr>
<tr>
<td>10</td>
<td>18.8a 16.2</td>
<td>6.1 × 10^4</td>
<td>4.2 × 10^4</td>
<td>12.9 14.1 14.1</td>
</tr>
<tr>
<td>20</td>
<td>11.3 10.8</td>
<td>9.4 × 10^4</td>
<td>1.8 × 10^4</td>
<td>6.2 7.3 7.3</td>
</tr>
<tr>
<td>30</td>
<td>16.2 13.7</td>
<td>5.7 × 10^4</td>
<td>8.2 × 10^4</td>
<td>13.2 12.9 12.9</td>
</tr>
<tr>
<td>40</td>
<td>17.9 14.1</td>
<td>2.1 × 10^4</td>
<td>6.3 × 10^4</td>
<td>12.5 11.6 11.6</td>
</tr>
</tbody>
</table>

* Data represent mean from four measurements.
Cell Growth and Viability in UKF-NB-2 and UKF-NB-2^AD169 Cultures. Growth parameters at various times after initiation of infection were compared with those of uninfected UKF-NB-2 cells at the same passage level. The results showed that population doubling time ranged from 22.5 to 25.6 h and from 28.2 to 31.5 h for UKF-NB-2 and UKF-NB-2^AD169 cells, respectively. Viability of UKF-NB-2 cells, as determined by trypan blue exclusion, was greater than 90%, whereas that of UKF-NB-2^AD169 ranged from 84 to 89% (data not shown).

Persistent HCMV Infection Induced Drug Resistance against CDDP and VP-16 in NB Cells. Parental NB cells UKF-NB-2 and HCMV-infected NB cells UKF-NB-2^AD169 were submitted to different concentrations of CDDP and VP-16. The results of a representative experiment are shown in Fig. 1. After 2 days of treatment, significantly more cells survived in UKF-NB-2^AD169 than in UKF-NB-2 cultures, suggesting that HCMV-infected cells had developed resistance against antineoplastic drugs.

Inhibition of Drug-induced Apoptosis in UKF-NB-2^AD169 Cells. Table 2 shows the effects of CDDP or VP-16 on induction of apoptosis in UKF-NB-2 and UKF-NB-2^AD169 cultures by TUNEL. Similar numbers of cells that underwent spontaneous apoptosis were found in nontreated UKF-NB-2 and UKF-NB-2^AD169 cultures. Treatment with CDDP (2 μg/ml) for 2 days induced programmed cell death in 92–95% of cells in noninfected UKF-NB-2 cultures, whereas only 10–12% of apoptotic cells were found in UKF-NB-2^AD169 cultures. VP-16 at a concentration of 20 μg/ml induced apoptosis in 65–70% of cells in UKF-NB-2 cultures, whereas in UKF-NB-2^AD169 cultures, only 12–14% of apoptotic cells were found.

Ultrastructural Examination of Apoptosis Induced by CDDP in UKF-NB-2^AD169 Cells. In noninfected UKF-NB-2 cultures treated for 2 days with 2 μg/ml CDDP, about 90% of cells showed morphological features typical of apoptosis, mostly characterized by compaction and margination of nuclear chromatin, condensation of cytoplasm, and convolution of nuclear and cell outlines (Fig. 2A). In UKF-NB-2^AD169 cultures treated with CDDP, about 10% of cells with signs of apoptosis were found, i.e., 9-fold less apoptotic cells than in noninfected UKF-NB-2 cultures treated with CDDP. Number of infected cells in UKF-NB-2^AD169 cultures ranged from 4 to 8% as demonstrated by findings of viral nucleocapsids in nucleus and features of virus morphogenesis in the cytoplasm at different times after initiation of infection (Fig. 2, B–D). However, not only infected cells but also adjacent noninfected cells were protected against drug-induced apoptosis (Fig. 2B).

Increased Level of Bcl-2 Expression in UKF-NB-2^AD169 Cells. Analysis of cellular proteins by Western blot showed that p53 remained undetectable in both cell lines (data not shown), and c-myc level was unchanged in UKF-NB-2^AD169 cells (Fig. 3A). Bcl-2 expression was significantly increased in UKF-NB-2^AD169 cells when compared with noninfected UKF-NB-2 cells (Fig. 3B). The enhancement ranged from 5- to 12-fold as measured by scanning densitometry at different times after initiation of infection (not shown). Flow cytometry analysis confirmed elevated amount of cellular Bcl-2 protein in UKF-NB-2^AD169 cells (Fig. 4); the increase in Bcl-2 amount ranged from 8- to 15 fold at different subcultures after initiation of infection (not shown).

Effects of GCV Treatment of UKF-NB-2^AD169 Cells on Drug Sensitivity, Apoptotic Response, and Bcl-2 Expression. The treatment with GCV suppressed production of infectious virus and virus antigens in UKF-NB-2^AD169 cells completely. The sensitivity to cytotoxic effects of CDDP and VP-16 and induction of apoptosis by the drugs in UKF-NB-2^AD169 cells treated with GCV were restored to levels of parental noninfected UKF-NB-2 cells (Fig. 1 and Table 2).

Table 2 CDDP- and VP-16-induced apoptosis in UKF-NB-2, UKF-NB-2^AD169, and UKF-NB-2^AD169 (GCV) cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>UKF-NB-2</th>
<th>UKF-NB-2^AD169</th>
<th>UKF-NB-2^AD169 (GCV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>I</td>
</tr>
<tr>
<td>Untreated</td>
<td>7.1</td>
<td>5.3</td>
<td>7.2</td>
</tr>
<tr>
<td>CDDP (2 μg/ml)</td>
<td>91.7</td>
<td>95.2</td>
<td>11.9</td>
</tr>
<tr>
<td>VP-16 (20 μg/ml)</td>
<td>65.2</td>
<td>69.8</td>
<td>12.2</td>
</tr>
</tbody>
</table>

*Cells were used at 20 (I) and 30 (II) subcultures after initiation of infection and compared with noninfected parental cells at the same passage level. UKF-NB-2^AD169 (GCV) cells were used after three previous subcultures with GCV. Data represent the mean of samples from culture vessels which varied by <10%. A total of eight fields (100–150 cells per field) were counted for each sample.
HCMV INDUCES DRUG RESISTANCE IN NB CELLS

Although we did not show whether an increased level of Bcl-2 alone accounts for increased survival of UKF-NB-2^AD169 cells, in previous studies, transfection with bcl-2 or bcl-xL expression vectors was sufficient to confer resistance to drug-induced apoptosis on different NB cell lines (28, 31). Bcl-2 may play a significant role in HCMV-induced resistance to apoptosis in p53-negative neuronal cells, such as UKF-NB-2^AD169, whereas in other cell types, including fibroblasts, smooth muscle cells, and endothelial cells, HCMV may inhibit apoptosis due to interaction of viral gene products with p53 (6, 7, 20, 21).

Bcl-2 protein protects against neuronal cell death induced by various apoptotic stimuli including viral infection (11, 36), e.g., stimulation of bcl-2 expression in a human neuronal cell line infected with La Crosse virus protected cells from virus-induced apoptosis (37). It has been suggested that blocking of the apoptotic process may be important to enable La Crosse virus to establish latent infection. It is possible that inhibition of apoptosis due to the induction of Bcl-2 is important for establishment of persistent HCMV infection in UKF-NB-2^AD169 cells. The bcl-2 family includes bcl-2, bax, MCL-1, A1, bak, bad, bcl-xL, and bcl-xG and the viral protein BHRF1 of EBV, the E1B M, 19,000 protein of adenovirus, the 5-HL protein of African swine fever virus, and Bcl-2 homologue of herpesvirus saimiri (11, 36, 38, 39). Expression of other cellular genes of the bcl-2 family important for the regulation of cell death could be also changed in UKF-NB-2^AD169 cells. However, our initial experiments failed to detect a change in bax, bcl-xL, or bcl-xG expression in UKF-NB-2^AD169 cells (data not shown).

The reversibility of Bcl-2 induction and inhibition of apoptosis after treatment of UKF-NB-2^AD169 cells with the antiviral agent GCV suggest that the modulation of phenotypic properties of tumor cells depends on a sustaining of HCMV infection. It is of interest to study the regulation of apoptosis in NB cells transfected with HCMV genes, such as those encoding IE proteins, which influence apoptosis in other cell types. On the other hand, persistently infected cells, such as

UKF-NB-2^AD169 cells treated with GCV showed an amount of Bcl-2 protein similar to that found in noninfected UKF-NB-2 cells (Fig. 4).

DISCUSSION

The data of the present study demonstrate for the first time that HCMV infection protects tumor cells against the cytotoxic effects of anticancer drugs. These effects were due to inhibition of apoptosis in association with increased expression of antiapoptotic gene bcl-2.
UKF-NB-2AD169, provide an experimental model for the study of some features of HCMV infection that cannot be observed in cells transfected with viral proteins or in acutely infected cells. Persistent infection with HCMV of two other cell lines, namely osteogenic sarcoma and glioblastoma, led to virus variants with biological properties that differed from those of the initially infecting wild-type virus (40, 41). It is conceivable that interactions between virus and cell metabolism vary in persistently and acutely infected cells.

We did not examine the effects of virus infection on apoptosis at single cell level. However, significantly more cells in UKF-NB-2AD169 cultures were protected against cell death induced by antitumor agents than those being infected with HCMV. These results suggest that noninfected cells, as well, in UKF-NB-2AD169 cultures were protected against apoptosis. There are several possible ways to explain this observation. In a previous study, decreased response to cytotoxic agents in sensitive cells was mediated by intercellular transfer of molecules that rescue cells from the effect of an otherwise toxic environment may be a mechanism common to different cell types (42). In UKF-NB-2AD169 cultures intercellular transfer of molecules from a relatively low number of HCMV-infected cells could influence drug sensitivity of other, noninfected cells. The implementation of drug resistance in noninfected cells could also be due to production of a growth factor(s) by HCMV-infected cells. It has been demonstrated that HCMV-induced activation of host cells may lead to the expression (or overexpression) of growth factors that influence growth, differentiation, and apoptosis of neighboring (noninfected) cells. For example, HCMV infection of different cell types stimulates production of basic fibroblast growth factor (43). This growth factor was shown to upregulate the expression of bcl-2 and to delay apoptosis in various cell types (44, 45).

In this study, we present results dealing with the inhibition of apoptosis in UKF-NB-2AD169 cells induced by antineoplastic drugs. Moreover, UKF-NB-2AD169 cells also showed inhibition of apoptosis induced by serum withdrawal or by oxidative stress induced by buthionine sulfoximine (data not shown). The biological significance of these effects is underlined by findings that xenografts of NB cell lines persistently infected with HCMV increase their growth and metastatic potential in nude mice relative to the noninfected tumor counterparts (30). The inhibition of apoptosis due to Bcl-2 could at least in part account for changes in phenotypic properties of HCMV-infected tumor cells. Statistical analysis demonstrated that bcl-2 gene expression was strongly correlated with features associated with aggressive tumor behavior and poor prognosis in patients with different malignancies, including NB (27). bcl-2-transfected NB cells showed a marked increase in inhibition of apoptosis, together with strong drug resistance (28). This enhanced expression of Bcl-2 protein in some NB tumors may affect the clinical course by providing a mechanism for NB cells to resist cytotoxicity of chemotherapy. When treated for short periods of time, the Bcl-2 expressing NB cells showed enhanced survival and a prolonged proliferative advantage as compared to cells that do not express the protein (28). Because many patients suffering from NB had contact before or after diagnosis of NB with HCMV, we have to postulate that some of the HCMV-seropositive NB patients have established infection of their tumor cells. Because one-third of untreated and approximately 80% of treated patients express elevated Bcl-2 levels (27), we cannot exclude that in some of them this phenomenon is mediated by HCMV infection of NB cells. Considering the survival advantage induced by Bcl-2 expression, HCMV could play a modulatory role in tumorigenesis and response to therapy due to the inhibition of apoptosis in NB cells.

In conclusion, the present results encourage clinical studies to show the frequency of HCMV infection of NB tumor tissues and its correlation with clinical outcome and response to therapy. The finding would be relevant for treatment of HCMV-infected NB patients because the use of antiviral agents, such as GCV, could positively influence both tumor progression and efficacy of antitumoral agents.

ACKNOWLEDGMENTS

We are grateful to Gesa Meincke and Heidi Schiffer for excellent technical assistance.

REFERENCES


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