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Effective treatment of experimental cytomegalovirus-induced encephalo-meningitis in immunocompromised rats with HPMPC

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Abstract

Cytomegalovirus (CMV)-induced encephalomeningitis is a dramatic complication in patients with the acquired immunodeficiency syndrome (AIDS) and treatment of this infection remains a major clinical problem. In order to study the pathogenesis and treatment of CMV-induced encephalomeningitis, we experimentally induced intracranial rat CMV (RCMV) infection in rats that were immunosuppressed by total body X-irradiation. CMV infection was monitored by viral plaque assay for estimation of the viral load. CMV-induced pathology, the presence of CMV-infected cells, as well as the presence of T-lymphocytes and monocytes/macrophages were studied by histopathologic and immunohistochemical staining techniques. The meninges showed CMV infection in mononuclear infiltrative cells and in endothelium of small blood vessels 8 days after intracerebral inoculation. This was accompagnied by multiple haemorraghes and inflammatory cell infiltration. The infection and inflammatory response persisted for at least 21 days p.i. Animals were treated with (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC), 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG), hyperimmune serum (HIS) and both DHPG and HIS combined. Treatment with one dosage of HPMPC at 20 mg/kg effectively reduced virus titers. However, all other treatment modalities were not effective. In conclusion, the pathology of RCMV-induced encephalomeningitis in immunocompromised rats closely resembles that of AIDS patients. The infection is effectively treated by HPMPC.

Keywords: Cytomegalovirus; Encephalomeningitis; HPMPC; Acquired immunodeficiency syndrome

1. Introduction

Cytomegalovirus (CMV) infections are a major problem in immunocompromised patients especially in transplant recipients (Linneman et al., 1978) and in patients with the acquired im-

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munodeficiency syndrome (AIDS). The prevalence of CMV infections in individuals infected with the human immunodeficiency virus (HIV) is high. (Drew et al., 1981; Collier et al., 1987; Quinn et al., 1987). In AIDS patients CMV infections are the most frequent opportunistic infections during the late phase of disease (McKenzie et al., 1991; Wilkes et al., 1988; Salazar et al., 1995). Those infections are associated with symptoms of fever, pneumonitis, hepatitis and retinitis (Meyers et al., 1982). A significant percentage of these patients (Snider et al., 1983; Kalayjian et al., 1993) experience neurological complications, such as encephalitis, meningo-encephalitis and radiculomyelitis (Snider et al., 1983; Schooley, 1990; McCutchan, 1995; Arribas et al., 1996). Although it has been shown that neural tissue obtained from these patients contains CMV antigens or nucleic acids, the role of the virus in the pathogenesis of meningo-encephalitis is not fully understood (Nelson et al., 1988; Fiala et al., 1991).

For treatment of CMV infections in these patients commonly used antiviral drugs are 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) and phosphonoformate (PFA) (Collaborative DHPG Treatment Study Group, 1986; Emanuel, 1990). The use of both compounds is limited by serious side effects such as neutropenia and kidney failure. The effect of alternative treatment modalities like hyperimmune serum (HIS) in AIDS patients is not known. More recently, it has been described that (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) (De Clercq et al., 1986, 1987) is a very potent inhibitor of herpes viruses (Yang and Datema, 1991). The advantage of HPMPC over DHPG is the very high selectivety and antiviral activity against human CMV.

Other advantages are that the drug is highly specific and active against rat (RCMV) and mouse CMV (Neyts et al., 1990; Stals et al., 1991, 1996). The intracellular halflife of the active metabolite is long and in animal studies the effect of HPMPC is long lasting (Neyts et al., 1990; Stals et al., 1991). In immunocompromised rats HPMPC completely inhibited viral replication in all internal organs and effectively reduced the mortality from RCMV infection.

In this report we describe CMV infection in rats at day 8 and 21 after intracerebal infection of rat CMV (RCMV). We compared the therapeutic effect of HPMPC on this infection to the effects of DHPG, HIS and both HIS and DHPG combined.

2. Material and methods

2.1. Animals

Inbred specified pathogen free (SPF) male Brown Norway (BN) rats 8 weeks old, were bred at the department of experimental animal services at the University of Maastricht, The Netherlands. Animals were used for the experiment at a total body weight between 140–180 g.

The rats were immunosuppressed 1 day before infection by 5 Gray total body irradation (TBI) as described previously (Stals et al., 1991).

2.2. RCMV infection

The rat cytomegalovirus (RCMV) Maastricht strain, was obtained from a pool of salivary glands of infected laboratory rats (Bruggeman et al., 1982, 1983). The virus pool used underwent 7 passages in laboratory rats. The salivary glands were homogenized and the supernatant was collected after centrifugation and stored at $-70°C$. Each rat received 10^5 plaque forming units (PFU) of RCMV in a volume of 20 μl by intracerebral injection. Controls received 20 μl of a noninfected salivary gland supernatant.

2.3. Antiviral treatment

The antiviral agents ganciclovir, 9-(1,3-dihydroxy-2-propoxymethyl)guanine was purchased from Syntex (Palo Altho, CA). (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) (Gilead Sciences, Foster City, CA) was a gift from Dr E. De Clercq, Leuven, Belgium. Hyper immune serum (HIS) was prepared in rats as described by Stals et al. (1990). In this study HIS was used at a neutralization titer NT of 640 and was given intravenously (i.v.). The antiviral

Table 1
Experimental groups and treatment regimens used

Treatment regimens of rats harvested at day 8 p.i.	n[c]	Treatment regimens of rats harvested at day 21 p.i.	n[c]
HPMPC (10 mg/kg)[a]	5	—	
HPMPC (20 mg/kg)[a]	4	—	
HPMPC (40 mg/kg)[a]	5	HPMPC (40 mg/kg)[a]	5
DHPG (40 mg/kg)[b]	5	DHPG (40 mg/kg)[b]	3
HIS (NT 640)[a]	5	HIS (NT 640)[a]	5
DHPG (40 mg/kg)[b] + HIS (NT 640)[a]	5	DHPG (40 mg/kg)[b] + HIS (NT 640)[a]	5
Infected, but not treated	5	Infected, but not treated	4
Not infected	5	—	

[a] Treatment adminsitred at day 0
[b] Treatment administred daily from day 0 to day 5
[c] n = number of rats per group

compounds (HPMPC and DHPG) were administered by intraperitoneal (i.p.) injection. All treatments were given 1 day after RCMV infection. The treatment regimens used for each group are outlined in Table 1.

2.4. Survival, specimen collection and detection

All rats were examined daily for the presence of disease, as described by Stals et al. (1991) and survival was recorded. To study the effect of treatment on the course CMV infection, brain tissue was obtained at two measure points after infection (day 8 and 21 p.i.). To study the effect of treatment on the spread of virus within the body, the spleen was sampled at these two measure points. At that time the presence of infectious virus and viral antigens was measured. In addition, brain tissue was analysed for histopathological changes. The number of infectious virus particles determined in organs was counted by plaque assay using rat embryonic fibroblasts, as described previously (Bruggeman et al., 1982). The presence of viral antigens was evaluated by immunohistochemistry. For this purpose organs were fixed in paraformaldehyde-lysine-periodate and embedded in parafin. For detection of RCMV antigens mouse monoclonals (McAb) were used, directed against cytoplasmic (McAb 35) and nuclear (McAb 8) antigens (Bruning et al., 1987). The infection load was graded by two independent observers based on the number of RCMV immunoreactive cells in the sections. This number was expressed as point score units (PSU), as described previously (Li et al., 1996). The quantity of CMV reactive cells scored from 0 to 5 (0: no staining; 1: sporadic cells; 2: few cells; 3: moderately dense; 4: dense; 5: very dense). Sections of brain tissue, 4 μm thick and embedded in parafin were analysed for the presence of inflammatory cells by the immunoperoxidase technique. Monoclonal antibody W3/13 (Sera-lab, Crawly Brown, UK) were used for the detection of T-lymphocytes and monoclonal antibody ED-1 (kindly supplied by Dr C. Dijkstra, University of Amsterdam, The Netherlands) for detection of monocytes and macrophages were used. In addition, routine haematoxylin and eosine staining was carried out.

2.5. Statistical analysis

The score of positive cells in different groups was compared with the aid of the two tailed non-parametric Mann-Whitney U test. For comparison of the number of infectious virus particles measured by plaque assay the non parametric Mann-Whitney U test was also used. P values < 0.05 were regarded as statistically significant.

3. Results

3.1. Effect of treatment on virus titers at day 8 p.i.

A total of 8 days after intrathecal inoculation of 10^5 PFU RCMV the brains harbored high numbers of infectious virus. In these animals generalized infection was observed by the presence of high numbers of infectious virus in the spleen (Table 2). Treatment with HPMPC at a dosage of 20 and 40 mg/kg significantly reduced the virus titers in both brain tissue and spleen. HPMPC treatment at 10 mg/kg reduced the virus titer in both organs, but not completely. The other treatment regimes (DHPG, HIS, and both, DHPG and HIS combined) all failed to reduce the number of infectious CMV in the brains significantly. In contrast, these compound had a moderate effect on the number of infectious virus particles in the spleen.

3.2. Effect of treatment at day 21 p.i.

Intrathecal injection of RCMV resulted in persistence of infectious virus for at least a period of 3 weeks (Table 3). At that time CMV was also present in the spleen, indicating general infection during the chronic phase of disease. No significant difference was found in the infectious virus load between the acute and the chronic phase of infection (days 8 and 21, respectively), which indicates continuous viral replication in these immunosuppressed animals for a period of at least 21 days.

As described for the day 8 p.i., HPMPC treatment at 40 mg/kg significantly decreased the number of RCMV in the brain. In addition, at that dosage HPMPC completely inhibited RCMV infection in the spleen, indicating that the drug prevented further spread of the infection through the body. The other treatment modalities (DHPG, HIS and both combined) did not significantly reduce virus titers in the brain and the spleen.

Table 2
Effect of antiviral treatment on the RCMV infection in brain and spleen at day 8 post infection

Treatment of rats	n[a]	Virus titer	
		Brain[b]	Spleen[b]
HPMPC (10 mg/kg)	5	1.19 (2.67)	1.31 (1.80)[c]
HPMPC (20 mg/kg)	4	0.72 (1.44)[c]	0.00 (0.00)[c]
HPMPC (40 mg/kg)	5	1.36 (1.87)[c]	0.00 (0.00)[c]
DHPG (40 mg/kg)	5	6.58 (0.22)	4.64 (0.50)
HIS (NT 640)	5	6.79 (0.18)	4.73 (0.71)
DHPG (40 mg/kg)+ HIS (NT640)	5	6.22 (0.18)	2.10 (1.95)
None (control)	5	6.54 (0.47)	6.05 (0.41)

[a] Number of animals per group.
[b] Virus titer in brain and spleen at day 8 p.i. was measured and is expressed as $\log_{10}$ PFU/g organ tissue (mean ± S.D.)
[c] Significant difference ($P<0.05$) compared to the nontreated (control) group.

Table 3
Effect of antiviral treatment on the RCMV infection in brain and spleen during the chronic phase of infection

Treatment of rats	n[a]	Virus titer	
		Brain[b]	Spleen[b]
HPMPC (40 mg/kg)	5	1.26 ± 1.76[c]	0.00 ± 0.00[c]
DHPG (40 mg/kg)	6	6.31 ± 0.43	2.79 ± 2.41
HIS (NT 640)	5	6.37 ± 0.52	3.80 ± 0.74
DHPG+HIS	5	5.84 ± 0.61	3.72 ± 0.30
None (control)	5	5.61 ± 1.45	4.79 ± 1.25

[a] Number rats per group.
[b] Virus titer in brain and spleen harvested at day 21 p.i. is expressed as $\log_{10}$ PFU/g organ tissue (mean ± S.D.).
[c] Significant difference ($P<0.05$) compared to the nontreated (control) group.

3.3. Pathology of the brain and meninges and the effect of antiviral therapy

CMV in meninges and brain tissue was localized immunohistochemically using RCMV-specific monoclonal antibodies. As presented in Table 4 the meninges of untreated rats harbored high numbers of virus-infected cells at day 8 p.i. (cell score = 3.7 (0.3) median (range)). At 21 days p.i. the number of virus-infected cell declined to a score of 2 (1) median (range). In the brain tissue only few virus-infected cells were observed (median PSU (range) 1 (0) at day 8 and 1 (1) at day 21).

Table 4
Effect of antivirals on the presence of RCMV-infected cells within the meninges and in blood vessels within the meninges

Treatment	Virus infected cells[b] in:			
	Meninges		Blood vessels within the meninges	
	Day 8 p.i.	Day 21 p.i.	Day 8 p.i.	Day 21 p.i.
HPMPC (40 mg/kg)[a]	0.2 ± 0.2[a]	0 ± 0.0[a]	0.0 ± 0.0[a]	0 ± 0.0[a]
DHPG (40 mg/kg)[b]	3 ± 0.3	1.6 ± 0.2	1.4 ± 0.2	0.6 ± 0.1
HIS (NT 640)[a]	1.2 ± 0.3[a]	1.4 ± 0.2	0.8 ± 0.1[a]	0.8 ± 0.0
HIS (NT 640)[a] + DHPG (40 mg/kg)[b]	2.8 ± 0.2[a]	2.4 ± 0.3	1.6 ± 0.3	2.8 ± 0.4
None (control)	3.7 ± 0.3	1.7 ± 0.4	2.2 ± 0.3	1.2 ± 0.1

[a] Significant difference $P<0.05$ compared to nontreated RCMV infected (control) rats.
[b] Virus infected cells expressed as median (range).

In the meninges and to a significantly lesser extent in the brain, scattered hemorrhages with foci of necrosis were present. CMV antigen was often detectable within these necrotic regions, as shown immunohistochemically. In the blood vessels numerous giant endothelial cells were noticed (data not shown). The enlarged cells contained basophylic intranuclear and intracytoplasmic inclusions which are typical for an active CMV infection. Immunohistochemical staining proved the presence of RCMV antigens in these cells.

While in non-infected rats nearly no inflammatory cells were noticed, in the CMV-infected animals numerous inflammatory cells infiltrated the meninges and brain. These infiltrates predominantly consisted of monocytes/macrophages, and to a lesser extent T-lymphocytes. Nearly half of the macrophages present in the meninges and brain were reactive with monoclonals 8 and 35, indicating that these cells contained viral antigens. Infiltrates were extensively present at day 8 p.i. Thereafter, the inflammatory response declined. In addition, endothelial cells, mainly those present in the small vessels, reacted with McAb 8 and 35.

The effect of treatment with HPMPC, DHPG, HIS and both, DHPG and HIS combined, on the quantity of virus-infected cells in brain and meninges was evaluated. As presented in Table 4 HPMPC at a dosage of 40 mg/kg significantly reduced the number of virus-infected cells in the meninges. In the blood vessels of these organs the effect was even more pronounced. In contrast, treatment with DHPG, HIS and both, DHPG and HIS combined, had hardly any effect on the number of CMV-infected cells. In the brain of untreated animals the number of virus-infected cells was low. Therefore, evaluation of the effect of antivirals was not performed. None of the four antiviral regimes had any effect on the inflammatory response in the meninges and in the brain of RCMV-infected rats. This was true for both measure points: day 8 and 21 p.i.

3.4. Survival

All animals submitted to this study survived at least during a period of 21 days. Only the HIS treated group and the control group became ill, but they all survived.

4. Discussion

This study describes the pathologic features of CMV infection in the brains and meninges of immunosuppressed rats after intracerebral inoculation of RCMV. In addition, it describes the effect of administration of antiviral treatment modalities, HPMPC, DHPG, HIS and both, DHPG and HIS combined, on the course of infection.

The rat model described mimics the pathology of CMV-induced meningo-encephalitis in AIDS patients. Histochemical evaluation of brain and

meninges from RCMV-infected rats revealed acute infection accompanied with signs of inflammation. Particulary, in the meninges high amounts of virus-infected cells were present. The virus-infected cells were localized mainly in and around the blood vessels, and consisted predominantly of monocytes/macrophages and endothelial cells. The presence of CMV-infected cells present in blood vessels of infected organs is also described in AIDS-patients (Hawley et al., 1983). CMV infection in and around bloodvessels is accompanied by a local inflammatory response. Earlier, it has been described that after inoculation of RCMV in the foot-pad of rats polymorphonuclear granulocytes infiltrated the perivascular region during the first days post infection, followed by infiltration of CMV-infected monocytes/macrophages. The inflammatory response reached the highest level at about 1 week after infection (Persoons et al., in press).

Another interesting observation is the detection of viral antigens in the endothelium of meninges and brain during the acute phase of infection. The presence of CMV in the endothelium of the brain is also described in postmortem specimen of AIDS patients (Morgello et al., 1987). It suggests that endothelium could act as a portal for entry of the virus into the central nervous system.

An important role of endothelial cells in the pathogenesis of CMV infection is suggested by Grefte et al. (1993), who indicates that infected endothelial cells are detectable in systemically infected immunocompromised patients. Predominantly, endothelial cells of small vessels become infected by CMV, while the endothelium of large vessels is exceptionally infected (Span et al., 1993). Recent data of Vossen et al. (1996) indicate that endothelial cells consist of a very heterogenous population that differently interact with CMV. Infection of these cells seems to be relevant for the pathogenesis. The finding of CMV specific antigens and nuclear inclusions of cells in the brain and meninges indicates local replication and is related with brain damage.

Treatment of RCMV-infected rats with DHPG, HIS or both DHPG and HIS combined had hardly any effect on the virus titers in spleen and brain of the animals during acute and chronic CMV-infection. This finding is in contrast with the synergistic effect of DHPG and HIS during generalized infection (Stals et al., 1996; Rubin et al., 1989). In patients with severe CMV disease combined treatment with DHPG and immuneserum appears to be beneficial (George et al., 1993; Question-and-answer-session, 1993). One explanation for therapeutic failure in this model could be poor penetration of the antivirals into the brain area, which permits initial replication cycles of the virus.

It should also be mentioned that antibody does not cross the blood brain barrier unless there is inflammation. Since there is nearly no inflammatory response in the brain this explains the fact that HIS alone or in combination with DHPG does not lead to a reduction in virus titer.

HPMPC treatment significantly reduced virus titers in brain and spleen. This is in agreement with earlier reports about the highly effective and specific effect of the drug (Stals et al., 1996; Neyts et al., 1991). We found, using immunohistochemical methods, that CMV antigens were localized in cells of the brains and the meninges from the rats. In contrast with the lack of antiviral effect of HIS and both HIS and DHPG combined, the number of virus-infected cells were reduced in the meninges at day 8 p.i. by these treatment regimens. Whether this effect was a result of a decreased number of infected cells, or a decreased sensitivity of the immunohistochemical detection technique by interference of the HIS, remains unknown.

High dosages of HPMPC reduced effectively and long lasting the virus titers as well as the number of virus-infected cells in both brain and spleen, while a lower dosage (10 mg/kg) of the drug showed a less pronounced effect.

It should also be mentioned that intrathecal infection may injure the blood brain barrier and that this may possibly alter the penetration of the compound(s) in the brain. Yang and Datema, 1991 demonstrated however that HPMPC is still able to reduce titers of HVS-2 in brains of mice that were injected intraperitoneally and in which treatment was only started at the time the virus had spread to the brains. Neyts et al., 1992 demonstrated that HPMPC efficiently prevents MCMV-

associated morbidity and mortality upon intracerebral infection. However, following long-term but infrequent treatment (once a week) with HPMPC in SCID mice that had been infected intraperitoneally with MCMV, virus replication was found in the brain. In contrast, little virus replication was found in other organs at that time. Thus brain infections may be more refractory to systemic treatment with HPMPC than visceral infections. This may suggest that perhaps higher and more frequent dosages of HPMPC are needed to treat brain infections with HPMPC than those does used in the present study.

In conclusion, we showed that intracerebral RCMV infection in immunocompromised rats closely resembles severe human CMV infection in AIDS patients. It displays a pathology with pronounced local infection of mononuclear infiltrates and endothelial cells of the small vessels. The treatment model seems suitable to study the effect of candidate antiviral drugs on CMV infection of meninges and brain in the severe immunocompromised host. HPMPC significantly reduces the viral load in brain, meninges and spleen. Therefore, we recommend the study of the effect of HPMPC on CMV-induced encephalitis in AIDS patients.

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References

Arribas, J.R., Storch, G.A., Clifford, D.B., Tselis, A.C., 1996. Cytomegalovirus encephalitis. Ann. Intern. Med. 125, 577–587.

Bruggeman, C.A., Meijer, H., Dormans, P.H., Debie, W.M., Grauls, G.E., van-Boven, C.P., 1982. Isolation of a cytomegalovirus-like agent from wild rats. Arch. Virol. 73 (3–4), 231–241.

Bruggeman, C.A., Meijer, H., Dormans, P.H., Debie, W.H., Grauls, G.E., van-Boven, C.P., 1983. Isolation of a cytomegalo-like agent from wild rats. Arch. Virol. 76 (3–4), 189–199.

Bruning, J.H., Debie, W.H., Dormans, P.H., Meijer, H., Bruggeman, C.A., 1987. The development and characterization of monoclonal antibodies against rat cytomegalovirus induced antigens. Arch. Virol. 94, 55–70.

Collaborative DHPG Treatment Study Group, 1986. Treatment of serious cytomegalovirus infections with 9-(1,3-dihydroxy-2-propoxymethyl)guanine in patients with AIDS and other immunodeficiencies. New Engl. J. Med. 314, 801–805.

Collier, A.C., Meyers, J.D., Corey, L., Murphy, V.L., Roberts, P.L., Handsfield, H.H., 1987. Cytomegalovirus infection in homosexual men. Relationship to sexual practice, antibody to human immunodefiency virus and cell-mediated immunity. Am. J. Med. 82, 593–601.

De Clercq, E., Holy, A., Rosenberg, I., Sakuma, T., Balzarini, J., Maudgal, P.C., 1986. A novel selective broad-spectrum anti-DNA virus agent. Nature (London) 323, 464–467.

De Clercq, E., Sakuma, T., Baba, M., Pauwels, R., Balzarini, J., Rosenberg, I., Holey, A., 1987. Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines. Antiviral Res. 8, 261–272.

Drew, W.L., Mintz, L., Miner, R.C., Sands, M., Ketterer, B., 1981. Prevalence of cytomegalovirus infection in homosexual men. J. Infect. Dis. 143, 188–192.

Emanuel, D., 1990. Treatment of cytomegavirus disease. Sem. Hematol. 27 (2), (Suppl. 1), 22–27.

Fiala, M., Mosca, J.D., Barry, P., Luciw, P.A., Vinters, H.V., 1991. Multi-step pathogenesis of AIDS—role of cytomegalovirus. Res. Immunol. 142, 87–95.

George, M.J., Snydman, D.R., Werner, B.G., 1993. Use of ganciclovir plus cytomegalovirus immune globulin to treat CMV pneumonia in orthotopic liver transplant recipients. Transplant Proc. 4, 22–24.

Grefte, A., Van der Giessen, M., Van Son, W., The, T.H., 1993. Circulating cytomegalovirus (CMV)-infected endothelial cells in patients with an active CMV infection. J. Infect. Dis. 167, 270–277.

Hawley, D.A., Schaefer, J.F., Schultz, D.M., Muller, J., 1983. Cytomegalovirus encephalitis in acquired immunodeficiency syndrome. Am. J. Clin. Pathol. 80 (6), 874–877.

Kalayjian, R.C., Cohen, M.L., Bonomo, R.A., Flarigan, T.P., 1993. Cytomegalovirus ventriculoencephalitis in AIDS. A syndrome with distinct clinical and pathologic features. Medicine 72, 67–77.

Li, F.L., Grauls, G., Yin, M., Bruggeman, C.A., 1996. Correlation between the intensity of cytomegalovirus infection and the amount of perivasculila in aorta allografts. Transplant Int. S1, 340–344.

Linneman, C.C., Dunn, C.R., First, M.R., Alvira, M., Schiff, G.M., 1978. Late-onset fatal cytomegalovirus infection after renal transplantation. Arch. Intern. Med. 138 (8), 1247–1250.

McCutchan, J.A., 1995. Cytomegalovirus infections of the nervous system in patients with AIDS. Clin. Infect. Dis. 20, 747–754.

McKenzie, R., Travis, W.D., Dolan, S.A., Pittaluga, S., Feuerstein, I.M., Shelhamer, J., Yarchoan, R., Masur, H., 1991.

The causes of death in patients with human immunodeficiency virus infection: A clinical and pathologic study with emphasis on the role of pulmonary disease. Medicine 70, 326–343.

Meyers, J.D., Flurnoy, N., Thomas, E.D., 1982. Non bacterial pneumonia after allegenic marrow transplantation a review of 10 years experience. Rev. Inf. Dis. 4, 119–1132.

Morgello, S., Cho, E-S., Nielsen, S., Devinsky, O., Petito, C.K., 1987. Cytomegalovirus encephalitis in patients with Acquired Immuno Deficiency Syndrome. Human Pathol. 18, 289–297.

Nelson, J.A., Reynolds-Kohler, C., Oldstone, M.B.A., Wiley, C.A., 1988. HIV and HCMV coinfect brain cells in patients with AIDS. Virology 165, 286–290.

Neyts, J., Balzarini, J., Naesens, L., De Clercq, E., 1992. Efficacy of (*s*)-1-(3-hydroxy-2-phoshonylmethoxypropyl) cytosine and 9-(1,3-hidroxy-2-propoxy methyl)guanine for the treatment of murine cytomegalovirus infection in severe combined immunodeficiency mice. J. Med. Virol. 37, 67–71.

Neyts, J., Snoeck, R., Balzarini, J., De Clercq, E., 1991. Particular characteristics of the anti-human cytomegalovirus activity of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine (Cidofovir) in vitro. Antiviral Res. 16, 41–52.

Neyts, J., Snoeck, R., Schols, D., Balzarini, J., De Clercq, E., 1990. Selective inhibition of human cytomegalvirus DNA-synthesis by (*S*)-1-(3)-hydroxy-2-phosphonylmethoxypropy) cytosine [(*S*)-HPMPC] and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG). Virology 179, 41–50.

Persoons, M.C.J., Stals, F.S., van Dam Mieras, M.C.E., Bruggeman, C.A., 1997. Multiple organ involvement during experimental cytomegalovirus infection is associated with disseminated vascular pathology. J. Pathol., in press.

Question-and-answer-session, 1993. Chaired by Snydman, D.R., Update on CMV infection in solid-organ transplantation. Transplant Proc. 25 (4), 27–28.

Quinn, T.C., Piot, P., McCormick, J.B., Feinsod, F.M., Taelman, H., Kapita, B., Stevens, W., Fauci, A.S., 1987. Serologic and immunologic studies in patient with AIDS in North America and Africa. The potential role of infectious agents as cofactors in human immunodeficiency virus infection. JAMA 257, 2617–2621.

Rubin, R.H., Lynch, P., Pasternack, M.S., Schoenfeld, D., Medearis, D.N. Jr., 1989. Combined antibody and ganciclovir treatment of murine cytomegalovirus-infected normal and immunosuppressed BALB/c mice. Antimicrob. Agents Chemother. 33, 1975–1979.

Salazar, A., Podzamczer, D., Reñe, R., Santin, M., Perez, J.L., Ferrer, I., Fernandez-Viladrich, P., Gudiol, F., 1995. Cytomegalovirus ventriculoencephalitis in AIDS patients. Scan. J. Infect. Dis. 27, 165–169.

Schooley, R.T., 1990. Cytomegalovirus in the setting of infection with human immunodefiency virus. Rev. Inf. Dis. 12 (7), S811–S819.

Snider, W.D., Simpson, D.M., Nielsen, S., Gold, J.W.M., Metroka, G.E., Rosner, J.B., 1983. Neurological complications of Acquired Immune Defiency Syndrome: Analysis of 50 patients. Ann. Neurol. 14, 403–418.

Span, A.H., Frederik, P.M., Grauls, G., van-Boven, G.P., Bruggeman, C.A., 1993. CMV induced vascular injury: an electron-microscope study in the rat. In vivo. 7 (6A), 567–573.

Stals, F.S., Bosman, F., van-Boven, C.P., Bruggeman, C.A., 1990. An animal model for therapeutic intervention studies of CMV infection in the immunocompromised host. Arch. Virol. 114, 91–107.

Stals, F.S., De Clercq, E., Bruggeman, C.A., 1991. Comparitive activity of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl and 9-(1,3-dihydroxy-2-propoxymethyl)guanine against rat cytomegalovirus infection in vitro and in vivo. Antimicrob. Agents Chemother. 35, 2262–2266.

Stals, F.S., Wagenaar, S.S., Kloover, J.S., Vanagt, W.Y.R., Bruggeman, C.A., 1996. Combination of ganciclovir and antibody for experimental CMV infectious. Antiviral Res. 29, 61–64.

Vossen, R.C.R.M., Derhaag, J.G., Slobbe-van Drunen, M.E.P., Duijvestijn, A.M., Van Dam Mieras, M.C.E., Bruggeman, C.A., 1996. A dual role for endothelial cells in cytomegalovirus infection? A study of cytomegalavirus infection in a series of rat endothelial cell lines. Virus Res. 44, 65–74.

Wilkes, M.S., Felix, J.C., Fortin, A.H., Godwin, T.A., Thompson, W.G., 1988. Value of necropsy in acquired immune defiency syndrome. Lancet 2, 85–88.

Yang, H., Datema, R., 1991. Prolonged and potent therapeutic and prophylactic effects of (*S*)-1[(3-hydroxy-2-phosphonylmethoxy)propyl] cytosine against herpes simplex virus type 2 infections in mice. Antimicrob. Agents Chemother. 35, 1596–1600.