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Treatment of murine cytomegalovirus salivary-gland infection by combined therapy with ganciclovir and thymic humoral factor $\gamma 2$

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Abstract

An optimal therapeutic regimen against primary CMV salivary-gland infection has not yet been developed. We used a murine CMV (MCMV) model system to assess the ability of combined thymic humoral factor THF-$\gamma 2$ immunotherapy and ganciclovir (GCV) antiviral chemotherapy to eliminate detectable viral DNA from salivary glands of infected animals. Mice in different experimental groups were inoculated intraperitoneally with MCMV, treated, and then sacrificed either 2 weeks or 3 months later. To amplify and detect MCMV DNA in infected salivary-gland tissue, we developed a sensitive polymerase chain reaction (PCR) using a glycoprotein B gene primer pair that amplifies a 356 bp segment. During the acute phase of the infection, the detection of high titers of infectious virus in the salivary glands correlated with a strong PCR amplification signal. Although active virions could not be recovered from untreated animals 3 months after viral inoculation, the PCR assay detected a latent MCMV genome. Treatment with either GCV alone or THF-$\gamma 2$ alone had little or no effect on the presence of MCMV DNA. By contrast, combined treatment with THF-$\gamma 2$ and GCV significantly reduced the amount of salivary-gland MCMV DNA to below the limit of PCR detection. The results presented here, and experimental data from previous MCMV research in our laboratories, imply that elimination of the virus from the salivary glands could be due in part to

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THF-γ2 restoration of the various MCMV-suppressed, cell mediated immune-responses. Combining THF-γ2 immunotherapy and GCV antiviral chemotherapy may be an important step toward an effective therapeutic regimen that has the potential to prevent the establishment of viral latency ensuing from primary MCMV salivary-gland infection.

Keywords: Chemo-immunotherapy; Cytomegalovirus; gB gene; Ganciclovir; PCR; Thymic humoral factor THF-γ2

1. Introduction

Eliminating primary cytomegalovirus (CMV) infection from the salivary glands is important for patients who are at risk for any form of immune suppression. CMV, an ubiquitous DNA virus from the herpes family, affects 80% of the population.

The pathogenesis of CMV disease is normally characterized by a benign, self-limiting acute phase of viral infection and a persistent latent phase (Ho, 1991; Koszinowski et al., 1990; Yuhasz et al., 1994). Serodiagnosis of CMV antibodies in the general population has revealed that approximately 80% of adults have been exposed to the virus without clinical symptoms. The clinical outcome of CMV infection is determined by the ability of infected individuals to mount a protective humoral and T-cell-mediated, cellular immune response (Ho, 1991; Koszinowski et al., 1990; Kirchner, 1983; Mercer and Spector, 1986). In immunocompetent, seropositive individuals, CMV infection remains latent and asymptomatic. CMV becomes a hazard only in situations where the host immune system becomes impaired, such as patients with human immunodeficiency virus (HIV) infection or recipients of organ-transplants. In such individuals, preventing reactivation of a latent virus is especially important because it can lead to both single- and multi-organ CMV disease, which is associated with a high rate of morbidity and mortality. In immunocompromised patients, secretion of reactivated latent salivary-gland human CMV (HCMV) via the saliva may explain such oral manifestations of CMV infection as ulcerations (Glick et al., 1991), aphthous stomatitis (Glick et al., 1991; Schubert et al., 1993; Heinic et al., 1993), necrotizing gingivitis (Lucht et al., 1993), and acute periodontal infection (Glick et al., 1991; Dodd et al., 1993). Another serious form of the disease is acquired by transplacental transmission of either primary or reactivated maternal CMV infection. Congenital CMV infection is a major cause of severe birth defects and brain damage in the child (Yow, 1989; Lynch et al., 1991; Lamy et al., 1992), even in asymptomatic mothers.

The predominant antiviral agent currently used for treating HCMV disease is ganciclovir (GCV), a nucleoside analog that inhibits the replication of the virus. Immunocompromised patients initially respond to GCV, but HCMV-infected cells persist in the absence of an effective antiviral immune response. When GCV therapy is discontinued, CMV disease recurs; the estimated remission rate ranges from 60 to 80% (Ho et al., 1989; Feinberg and Hoth, 1991; Laughlin et al., 1991). Increasing the dosage of GCV is not feasible because such treatment is limited by a marked dose-related, bone-marrow suppression, often resulting in such severe neutropenia that therapy must be withdrawn (Feinberg and Hoth, 1991). Moreover, antiviral drug resistance has become an area of increasing clinical importance in treatment of the herpesviruses, including CMV (Coen, 1991; Laughlin et al., 1991; Alain et al., 1993). The severe side effects of this drug, coupled with the need to maintain the immunocompetence of the infected individual, emphasize the need to guide new, alternative therapeutic strategies toward amplifying the immune system.

Thymic humoral factor THF-γ2 (Burstein et al., 1988) is an immunomodulatory agent that has a marked therapeutic effect against viral infections without toxic side effects (Trainin et al., 1986; Katorza et al., 1987; Handzel et al., 1985, 1990). THF-γ2 participates in T-cell differentiation and proliferation in all three lymphoid cell

compartments: the bone marrow, the thymus and the peripheral lymphoid system Trainin, 1990; Trainin et al., 1994, 1996). Treatment of murine and of human bone marrow cells with THF-γ2 in vitro was found to increase their capacity to proliferate into granulocyte-macrophage colonies (GM-CFC). In both animals (Trainin et al., 1986; Rager-Zisman et al., 1990) and humans (Handzel et al., 1990), THF-γ2 has been shown to stimulate CD4 and CD8 T-lymphocytes that participate in cellular and humoral immune responses. THF-γ2 augmented multiple T-lymphocyte functions in mice, including the proliferative responses to T-cell mitogens, mixed lymphocyte reaction, helper effect in antibody production and cytotoxic responses. THF-γ2 was found to increase the ConA-induced IL-2 production by spleen cells from normal mice and also raised the levels of IL-2 in experimental models of immune impairment such as neonatal thymectomy or chemotherapy in murine plasmacytoma. In MCMV-infected animals, THF-γ2 immunotherapy not only reversed the immunosuppressive effect of the virus (Katorza et al., 1987; Rager-Zisman et al., 1990) but also enhanced the therapeutic potential of virus-specific immunocytes that were obtained from MCMV-immune donor mice (Rager-Zisman et al., 1990). THF-γ2 also augments natural killer (NK) cell activity in MCMV-infected mice (Rager-Zisman et al., 1994) as well as in humans with certain viral infections (Handzel et al., 1990).

The goal of the present study was to assess the ability of THF-γ2 immunotherapy to augment GCV antiviral chemotherapy against primary MCMV salivary-gland infection in immunocompetent BALB/c mice. To detect the presence of salivary-gland CMV, we amplified and detected MCMV DNA with a sensitive polymerase chain (PCR) reaction, using a pair of synthetic oligonucleotide primers from the DNA sequence of the glycoprotein B (gB) gene (Rapp et al., 1990). Unlike GCV treatment alone, the combination chemo-immunotherapy, initiated 24 h after virus inoculation, significantly reduced the amount of MCMV DNA in the salivary glands to levels that were barely detectable by PCR.

2. Materials and methods

2.1. Animals

BALB/c female mice, aged 8 weeks, were obtained from the Jackson Laboratory (Bar Harbor, Maine, USA).

2.2. Virus and virus titrations

The Smith strain of MCMV was obtained from the American Type Culture Collection (ATCC) (Rockville, MD). The preparation of highly virulent virus pools from MCMV-infected salivary glands has been described in detail elsewhere (Katorza et al., 1987). Virus stocks containing $0.5–1 \times 10^8$ PFU/0.1 g of tissue were stored at -70°C in 10% dimethyl sulfoxide until use. Eight-week-old mice in three independent experimental groups (10–15 mice/group) were inoculated intraperitoneally (i.p.) with 5×10^4 PFU of stock virus, given various treatments, and then sacrificed either 2 weeks (acute infection) or 3 months later (latent infection). A semiquantitative plaque assay (Rager-Zisman and Merigan, 1973) on primary mouse embryo fibroblasts (MEF) was used to determine the titer of MCMV in salivary gland suspension (SGS) pools.

2.3. Drugs and treatments

Thymic humoral factor-γ2 (THF-γ2) is a synthetic octapeptide (Leu–Glu–Asp–Gly–Pro–Lys–Phe–Leu) (Burstein et al., 1988) of calf thymus origin (Trainin and Small, 1970). A stock solution of the peptide was maintained in sterile phosphate-buffered saline (PBS) solution at a concentration of 1 mg/ml and stored at -20°C. For in vivo experiments, the concentration of the stock THF-γ2 solution was adjusted to 400 μg/ml in PBS. Treated animals received 14 daily i.p. injections of 40 μg/0.1 ml THF-γ2, starting immediately after virus inoculation. The dosage was chosen on the basis of previous studies (Katorza et al., 1987; Rager-Zisman et al., 1996)

Ganciclovir (GCV)-Cymevene™ 500 mg/vial, (Syntex Pharmaceuticals, Maidenhead, UK) was

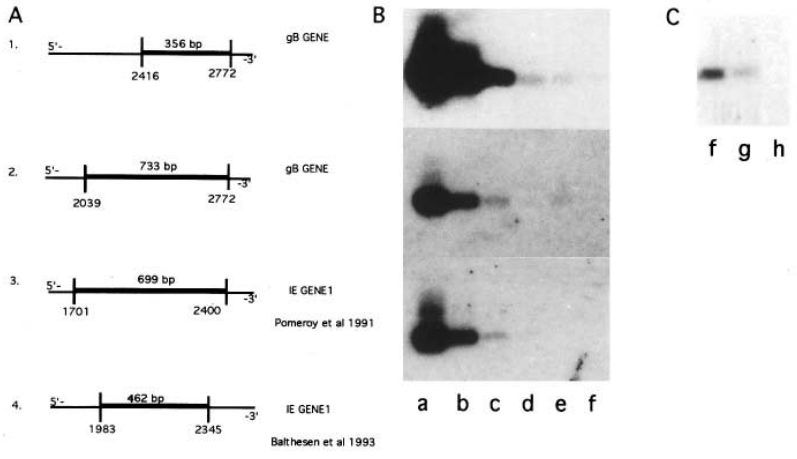

Fig. 1. PCR analysis of MCMV DNA. The sensitivity of the gene amplification of the selected primers was examined by reconstitution experiments in which known amounts of purified MCMV DNA were diluted in a constant amount (1 μg) of cellular DNA extracted from uninfected mice. (A) Representation of the oligonucleotide primer pairs tested for PCR amplification of the MCMV gB gene and IE gene 1. (B and C) Comparison of the sensitivity of detection of MCMV DNA, after PCR amplification and Southern hybridization, using the primers prepared from the gB gene, which amplified a 356 bp segment (1) with two different pairs of primers selected from the IE gene 1 (3 and 4). Primer pair 2 is not shown because it was less sensitive. Lanes a–g represent serial dilutions in 1 μg cellular DNA of 10 ng (a), 1 ng (b), 100 pg (c), 10 pg (d), 1 pg (e), 100 fg (f) and 5 fg (g), cellular DNA only (h). (C) Longer exposure of lanes (f), (g) and (h). Note that the highest degree of sensitivity was obtained by gB gene primer pair (1).

dissolved in sterile PBS immediately before use. Each GCV-treated animal received a daily dose of 100 μg (i.p.) of the drug on days 1, 2, 3, 4 and 8, 9, 10, 11 post infection.

2.4. Purification of MCMV from salivary glands and preparation of MCMV DNA

To obtain MCMV DNA, purified virus was prepared in the following manner: 14 days after inoculating the animals with 5×10^4 PFU MCMV, salivary glands were excised, pooled and 10% (w/v) SGS was prepared. Cell debris were removed from the suspension by centrifuging at 3000 rpm for 5 min. The supernatant containing the virus was layered on a cushion of 5% Ficoll (Sigma Chemical Co., St. Louis, MO, USA) and centrifuged at 25 000 rpm for 2.5 h at 4°C in a Beckman Ti-50 rotor. The virus was resuspended in Tris-EDTA (TE) buffer and then centrifuged at 30 000 rpm for 2 h at 4°C in the same rotor. The final pellet containing the virus was then resuspended in TE buffer and stored at −70°C. MCMV DNA was extracted from SGS of infected mice with a proteinase-K-based lysis buffer (2 mM $MgSO_4$, 10 mM KCl, 10 mM $(NH_4)2SO_4$, 20 mM Tris-HCl (pH 8.8), 0.1% Triton X-100, and 100 μg/ml proteinase K). The DNA was then purified by a series of phenol-chloroform extractions and ethanol precipitation.

2.5. Detection of MCMV DNA by polymerase chain reaction (PCR)

To identify MCMV DNA, we developed a highly sensitive PCR assay to amplify MCMV DNA. We synthesized three DNA oligonucleotide primers that were selected from the published sequence of MCMV gB gene (Rapp et al., 1990; Fig. 1A). The sense primer was based either on the cDNA sequence No. 2416–2443: 5′–AAG–CAG–CAC–ATC–CGC–ACC–CTG–AGC–GCC–3′ or No. 2039–2062:5′-ATC-TTC-CAG-GAC-GGG-AAG-GTC-GTC-3′ and the antisense No. 2745–2772: 5′-CCA-GGC-GCT-CCC-GGC-GGC-CCG-CTC-TCG-3′.

For each gene amplification sample, an aliquot of salivary-gland DNA (1 μg) was added to a

reaction mixture containing 200 μM dNTP, 100 pmol primer, 1.0 mM $MgSO_4$, 10 mM KCl, 10 mM $(NH_4)2SO_4$, 20 mM Tris-HCl (pH 8.8), 0.1% Triton X-100, and 2 U of vent polymerase (Biolabs, Beverly, CA, USA) in a total reaction volume of 50 μl. Samples were amplified for 30 cycles in an automated thermal cycler (Perkin Elmer-Cetus, Emeryville, CA, USA). Each cycle consisted of denaturation for 60 s at 94°C, annealing for 90 s at 68°C, and primer extension for 120 s at 72°C. To evaluate the sensitivity of detection of the gB oligonucleotide primers, we synthesized two sets of oligonucleotide primers from the IE gene 1 sequence. The base sequence of the first set was as follows (Pomeroy et al., 1991): 5′-ATC-AAT-CAG-CCA-TCA-ACT-CTG-CTA-CCA-ACA-3′ and 5′-ATG-GTG-AAG-CTA-TCA-AAG-ATG-TGC-ATC-TCA-3′. The base sequence of the second set was as follows (Balthesen et al., 1993): 5′-ATT-GTT-CAT-TGC-CTG-GGG-AGT-TT-3′ and 5′-ATC-TGG-TGC-TCC-TCA-GAT-CAG-CTA-A-3′.

Gene amplification was accomplished using vent polymerases. The lower limit of detection for this method (gB primers) under the conditions of our experiments was 5 fg of purified viral DNA, corresponding to about 20 copies of the MCMV genome. To verify the presence of DNA in each experiment, parallel actin amplification was performed using the following oligonucleotides: 5′-GAG-ACC-TTC-AAC-ACC-CCA-GCC (sense) and 5′-GGC-CAT-CTC-TTG-CTC-GAA-GTC (antisense).

2.6. Analysis of PCR-amplified DNA

To verify the identity of the amplification products, we hybridized the DNA with a ^{32}P-labeled oligonucleotide probe synthesized according to the glycoprotein B gene sequence no. 2574–2594: 5′-TCG-TCA-GGA-AGC-CCG-TGA-CG-3′, (reverse and complemented probe) or for the IE gene: 5′-AGC-GTA-TCC-ACA-CAT-GTG-GTA-GT-3′ (Balthesen et al., 1993).

Samples (15 μl each) were separated on 1% agarose gel. The gels were then washed twice with depurination solution (0.25 M HCl) for 15 min, twice with denaturation solution (0.5 M NaOH, 1.5 M NaCl) for 15 min, and twice with neutralization solution (1 M ammonium acetate, 0.02 M NaOH) for 30 min. After washing, the gels were southern transferred overnight onto nitrocellulose paper (Schleicher and Schuell, Germany). Filters were washed in 2 × standard saline citrate (SSC) for 5 min and then cross-linked (150 mJ in Gene Linker, BioRad). Pre-hybridization was carried out for 4 h at 58°C in a buffer composed of 6 × SSC, 16.67 mM EDTA, 5 × Denhardt's solution, and 0.2 mg/ml salmon-sperm DNA. Hybridization was carried out overnight at 58°C with the ^{32}P-end-labeled oligonucleotide probe. The filters were washed three times with 3 × SSC for 30 min at 58°C. Autoradiography was carried out at −70°C with one intensifying screen and Fuji Medical X-ray film.

3. Results

3.1. Detection of PCR-amplified MCMV DNA

Fig. 1B shows a comparison of the detection sensitivity of the selected oligonucleotide primers of the gB gene (upper) with that of the IE gene 1 (middle and lower). Because the gB gene primer pair amplifying a 356 bp segment was the most sensitive, we used this pair to detect the MCMV genome in the salivary glands of infected mice. To determine the sensitivity of the selected primers, we carried out reconstruction experiments, where a known amount of purified MCMV DNA was diluted in a constant amount (1 μg) of cellular DNA that was extracted from uninfected mice. Fig. 1B shows the results of a representative experiment, where as little as 5 fg of purified viral DNA, corresponding to about 20 copies of the MCMV genome, were detected (Fig. 1C). Conversely, when a constant amount of uninfected mouse cellular DNA was increased and then diluted with the same quantities of purified viral DNA, the sensitivity of PCR detection decreased. When cellular DNA was not added to the purified viral DNA, the sensitivity of PCR detection increased (data not shown).

3.2. Detection of the MCMV genome in salivary-gland tissue during the acute and latent phases of infection

Balb/c mice were inoculated i.p. with 5×10^4 PFU of MCMV and then sacrificed either 2 weeks (acute phase of infection) or 3 months later (latent stage). Pools of at least three mice of three experiments were examined at each time point, representative autoradiographs of PCR amplification of salivary-gland DNA, summarized in Fig. 2, indicate that by day 14, a large amount of viral DNA, corresponding to a high titer (5×10^6 PFU/g tissue) of infectious virus, was present in the salivary glands (Fig. 2, lane A). After the acute infection was resolved (3 months later) and $< 5 \times 10^1$ PFU of infectious virus was recovered, PCR continued to detect a low copy number of MCMV gB DNA (Fig. 2, lane B). No MCMV DNA could be detected in uninfected control mice (Fig. 2, lane C).

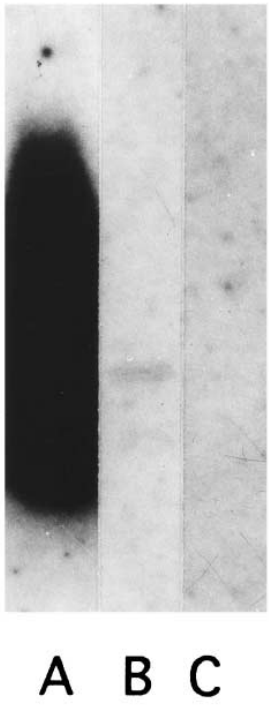

Fig. 2. Detection of MCMV gB DNA in the salivary glands of MCMV-infected mice. Pooled SGS-DNA (from three mice for each timepoint) was subjected to PCR amplification and Southern hybridization, using the primers prepared from the gB gene. Lane (A) amplified gB sequence of MCMV DNA 2 weeks after infection; lane (B) 3 months after infection; lane (C) uninfected mouse DNA.

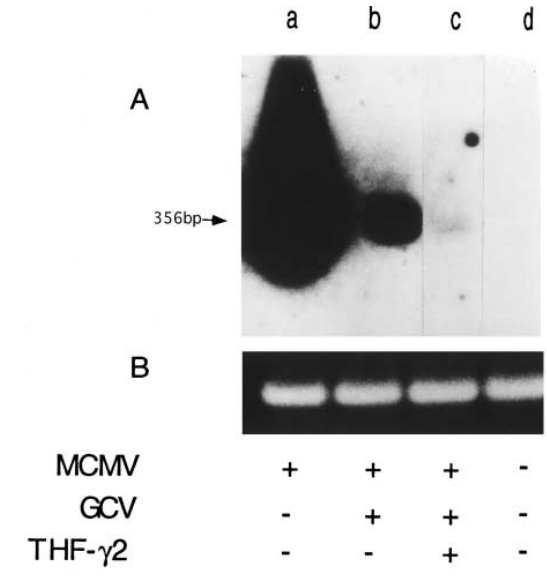

Fig. 3. Detection of MCMV gB DNA in salivary glands of MCMV-infected mice following combined therapy with GCV and THF-γ2. SGS-DNA (prepared from three individual mice) 2 weeks after infection, was subjected to PCR analysis with primer pair 1(A) (see Fig. 1). Lane (a) untreated mice; lane (b) after GCV treatment alone; lane (c) after combined therapy with GCV and THF-γ2; lane (d) uninfected control mouse DNA. (A) gB DNA in salivary glands. (B) Parallel actin DNA amplification.

3.3. Effect of GCV treatment on the presence of MCMV in the salivary glands

The results of GCV treatment alone on the presence of MCMV in the salivary glands of infected mice was assessed by both virus titration and PCR amplification of viral DNA. Three animals, in two experiments, were examined for each treatment. Representative autoradiographs of PCR amplification of salivary-gland DNA are shown in Fig. 3. In animals that were treated with GCV alone, titer of infectious virus showed a 100-fold reduction (4.5×10^4 PFU/g tissue), compared to MCMV titer in infected, untreated mice. MCMV DNA was only partially reduced in the salivary glands (Fig. 3, lanes a and b).

3.4. Effect of combined GCV and THF-γ2 chemo-immunotherapy on the presence of MCMV DNA in the salivary glands

In addition to GCV, MCMV-infected mice received a daily dose of 40 ng THF-γ2 for 14 consecutive days, beginning immediately after virus inoculation. The results showed that treatment with THF-γ2 alone had little effect on the presence of infectious virus or MCMV DNA after virus inoculation (data not shown). The results of the PCR amplification (Fig. 3, lane c) indicated that unlike treatment with either GCV (lane b) or THF-γ2 alone, the combined chemo-immunotherapeutic regimen profoundly reduced both the number of MCMV DNA copies and the titer of infectious virus (4.5×10^2 PFU/g tissu), by day 14 after virus inoculation.

4. Discussion

The results of this study clearly indicate that antiviral chemotherapy affecting only viral DNA replication should be supplemented by agents that are effective at the cellular level, such as active and passive immunotherapeutic modalities. We found that combining GCV antiviral chemotherapy and THF-γ2 immunotherapy downregulated salivary gland MCMV DNA to barely detectable levels by the 14th day after viral inoculation. That GCV treatment alone does not prevent latent MCMV infection has been noted by others (Katzenstein et al., 1986). The unpublished observations in an earlier study (Rager-Zisman et al., 1990) on recipients of THF-γ2-treated spleen cells surviving a fatal MCMV infection and the results of the present study provide evidence that immunotherapy alone had a moderate effect on viral replications in salivary glands of infected mice. Clearly, treatment with a single chemotherapeutic or immunotherapeutic antiviral agent cannot eliminate both CMV virions and CMV DNA from the salivary glands. The results presented here support the findings of Pavic et al. (1993) who found that therapy with individual recombinant cytokines that are known to inhibit MCMV infection could not limit virus replication in vivo. Agah et al. (1991) reported that IL-2-activated murine bone-marrow cells showed potent MCMV-specific cytotoxic potential against infected targets in vitro, but neither the lymphokine nor the bone marrow cells alone were as effective as their combination. Because numerous host and viral factors and multiple protective mechanisms are involved in CMV clearance, combination therapy is obviously desirable. Thus, successfully combining GCV and THF-γ2 in an MCMV model is an important step forward toward preventing the establishment of viral latency after primary HCMV.

The immune system plays a key role in controlling CMV infection. As long as the immune system can provide adequate protection, CMV infection remains latent and asymptomatic in seropositve individuals (Ho, 1991).

In addition, we have previously shown that CMV-neutralizing antibody levels were increased in THF-γ2-treated survivors of a fatal MCMV infection (Rager-Zisman et al., 1990), implying that the octapeptide also stimulates the Th2 CD4 subpopulation of T lymphocytes that facilitate the production of specific antibody. Anti-CMV antibody can attack the mature virions that have escaped the anti-DNA activity of GCV. Because GCV treatment has no effect on the synthesis of CMV polypeptides (Mar et al., 1982), CMV-specific antibody recognizes cultured HCMV- and MCMV-infected cells that continue to produce both cytoplasmic and cell-surface CMV antigens in the presence of GCV (Barnett et al., 1995).

Latency is an integral feature of the pathogenesis of cytomegalovirus infection and disease. The PCR amplifies and detects min amounts of MCMV DNA copies in infected tissue from latently infected animals, where virus is not detectable by standard tissue culture assays and southern hybridization techniques (Klotman et al., 1990). Nested enzymatic amplification of the 700-bp (Pomeroy et al., 1991) or the 200-bp region (Collins et al., 1993) of exon 4 of the major immediate early (IE) gene has been used to detect MCMV DNA in such diverse organs as the heart, kidney, liver, lung, spleen, brain, and salivary glands of latently infected mice. In the present study, we developed the PCR amplification assay

to a very high degree of sensitivity to detect min amounts of MCMV DNA copies in salivary-gland tissue. The PCR assay is often used to evaluate the efficacy of antiviral treatments in humans.

We concluded that the gB gene marker is suitable for monitoring the efficacy of anti-CMV therapy because a pair of synthetic oligonucleotide primers amplifying a 356 bp segment was even more sensitive than two pairs of oligonucleotide primers that were synthesized from MCMV IE gene 1 (Pomeroy et al., 1991; Balthesen et al., 1993). The correlation between a strong PCR amplification signal and the presence of highly titered virus during the acute stage of MCMV infection confirmed that the DNA detected in this assay represents an active virus genome. Moreover, when infectious virus could no longer be isolated from salivary-glands tissue 3 months later, the PCR was able to amplify salivary-glands MCMV DNA, confirming that this pair of oligonucleotides can detect the latent genome persisting in the target tissue.

In immunocompetent animals, CD8 lymphocytes clear MCMV from all organs except salivary gland tissue, where the activity of CD4 cells is required (Koszinowski et al., 1990). CD4 cells can fully compensate for CD8 deficiency, clearing virus from all tissues, including the salivary glands (Jonjic et al., 1990). Other host defense mechanisms against CMV infection include: virus-neutralizing antibody; macrophages; antibody-dependent cytotoxicity; natural killer (NK) cells; interferon (IFN)-α, IFN-β, and IFN-γ; and tumor-necrosis factor (TNF)-α.

THF-γ2 restores defective CD4 T lymphocyte populations (Rager-Zisman et al., 1994). The protective effector functions of CD4 lymphocytes are associated with the release of interleukin-2 (IL-2) (Reddehase et al., 1987), IFN-γ (Lucin et al., 1994), and TNF-α (Pavic et al., 1993). Because THF-γ2 also augments multiple immune functions and stimulates the production of IL-2 (Trainin et al., 1994; Trainin et al., 1996) it is likely to be more effective than a single immunomodulating agent as an adjunct to antiviral chemotherapy. THFγ2-induced augmentation of IL-2 production, for example, would enhance some or all IL-2-mediated immune mechanisms involved in MCMV immunity.

Based on the results presented here and experimental data from prior studies, we suggest that the clearance of MCMV DNA from the salivary glands is due to THF-γ2-induced upregulation of the MCMV-suppressed, Th1 CD4-mediated cellular immune response against CMV-infected cells. We conclude that the successful virustatic effect of GCV chemotherapy combined with THF-γ2 immunotherapy mirrors the complementary activities of the two agents, each exerting its antiviral activities via different and independent pathways. GCV chemotherapy is directed against viral DNA replication and thereby suppresses the productive infection without eliminating all CMV-infected cells. Previous research in our laboratories has shown that the antiviral effect of THF-γ2 is due to its immunomodulatory effect on immune T cells, thus promoting the elimination of residual salivary glands cells containing mature virions and latent viral DNA. Others have shown that GCV can be administered in combination with other antiviral drugs (Freitas et al., 1993a,b) or with CMV-specific immunoglobulins (Dentamaro et al., 1992) without compromising the efficacy of GCV against CMV. We report here that GCV can also be safely combined with active immunomodulatory agents, such as THF-γ2. It is unlikely that a single immunomodulating factor would replace the anti-viral function of Th1 CD4 cells as was indeed demostrated in the case of rIFN-γ in MCMV infection (Lucin et al., 1992). We believe that the ability of the immunomodulator THF-γ2 to potentiate the cellular immune response substantiates the antiviral activity of GCV and offers a new approach toward clearing acute CMV infection, thus preventing the establishment of latent MCMV infection in the salivary glands.

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