Characterization of a strain of murine cytomegalovirus which fails to grow in the salivary glands of mice

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Characterization of a tissue culture-adapted strain of murine cytomegalovirus (MCMV), the Vancouver strain, which demonstrated altered tissue tropism in mice was undertaken to help understand the mechanism of pathogenesis of cytomegaloviruses. The Vancouver strain grew to a limited extent in the spleen but failed to grow in the salivary glands of inoculated mice. This mutation probably arose during multiple in vitro passaging of the parental Smith strain. The Vancouver strain replicated more quickly and produced a greater yield of virus per cycle than the Smith strain in vitro, resulting in a larger plaque size. In addition to these phenotypic differences, the Vancouver strain was found to have a 9.4 kb deletion spanning the XhoI I/L junction of the parental Smith strain (0.960 to 0.995 map units), and a 0.9 kb insertion which mapped to the EcoRI K fragment (0.37 to 0.47 map units). Analysis of virus-induced proteins at various times post-infection identified only one major change in Vancouver strain-infected cells, the absence of a 42K protein found in Smith-infected cells at early and late times.

Introduction

Cytomegaloviruses (CMVs) are ubiquitous agents which infect a number of animals including man (Weller, 1971; Plummer, 1973). As a group they are highly species-specific; thus human CMV (HCMV) cannot be studied in an animal model. Infection of mice with murine CMV (MCMV) has therefore become the most widely used in vivo model system (Griffiths & Grundy, 1987; Hudson, 1979; Plummer, 1973). In general a balanced relationship exists between host and CMV, and infections are normally subclinical. However, HCMV infection of an individual with an immature immune system (congenital and perinatal infection) or infection/reactivation in an immunosuppressed individual (transplant recipients, patients undergoing cancer therapy, and patients with AIDS) can result in severe disease. Organs infected by HCMV or MCMV include salivary glands, spleen, liver, kidney, lymph nodes, lungs, adrenal glands, ovaries and pancreas (McCordock & Smith, 1936; Medearis, 1964; Cheung & Lang, 1977; Mims & Gould, 1979; Ho, 1982; Baskar et al., 1983). A great deal of information has been gained about the epidemiology and pathogenesis of CMV but the molecular mechanisms involved in virus infection, tissue tropism and persistence require elucidation if successful treatment or prevention of disease attributable to CMV is to become a reality.

The salivary glands are a major site of persistence of CMVs, and the site from which chronic shedding of virus can lead to dissemination through the population. A number of interesting features of MCMV replication in salivary glands have been reported. Rapid attenuation of virulent MCMV by passage in vitro and subsequent rapid restoration of virulence by passage of virus in vivo was described over 20 years ago (Osborn & Walker, 1970). Since then, it has been determined that the salivary glands are the only source of virulent virus, and that virus isolated from any other organ is as avirulent as that passaged in tissue culture (Selgrade et al., 1981). It is not likely that this effect of salivary gland passage is due to genetic changes in the virus because the reversion to and from virulence occurs in a single passage. In a recent study, in vivo passage of virus through salivary glands was found to increase binding of the virus to target cells through sialic acid residues, in addition to the N-acetylglucosamine residues recognized by attenuated virus (Ravindranath & Graves, 1990) which could increase the virulence of MCMV by allowing a wider tissue tropism, or by facilitating attachment to permissive cell types.

In this report, we have characterized a strain of MCMV (Vancouver) which fails to grow in the salivary glands of inoculated mice, but grows to higher titre in
tissue culture than the parental Smith strain. Analysis of genetic changes in Vancouver strain DNA and an examination of alterations in protein expression between the Vancouver and Smith strains are reported.

Methods

Cells and virus. The 3T3-L1 cell line was obtained from the ATCC (CCL 92.1) and routinely grown in DMEM (Gibco) containing d-glucose, l-glutamine, 1 mg/ml streptomycin, 1 unit/ml penicillin, 1 ug/ml of the antifungal agent Econazole (Cilag) (complete medium) and 10% foetal bovine serum (FBS), in a humidified incubator at 37 °C and 5% CO₂. Infected cells were maintained in complete medium containing 5% FBS. The Smith strain of MCMV was purchased from the ATCC (VR-194) in 1987 and passed twice in 3T3-L1 cells. The Vancouver strain, obtained from Dr J. B. Hudson (Division of Medical Microbiology, University of British Columbia, Vancouver, Canada), was isolated after multiple passage of the Smith strain in tissue culture (passed a number of times in mouse embryo fibroblasts, followed by 11 passages in 3T3-L1 cells between 1982 and 1990). Stock virus was prepared by infecting 3T3-L1 cells with a low multiplicity of 0.01 p.f.u./cell, and harvesting the cells 24 h after 100% c.p.e, was isolated after multiple passage of the Smith strain in tissue culture (passed a number of times in mouse embryo fibroblasts, followed by 11 passages in 3T3-L1 cells between 1982 and 1990). Stock virus was prepared by infecting 3T3-L1 cells with a low multiplicity of 0.01 p.f.u./cell, and harvesting the cells 24 h after 100% c.p.e. was observed. The cells were sonicated to release infectious virus, and stored at -70 °C in 1 ml aliquots. The infectivity of the Smith virus stock was 6.2 x 10⁶ p.f.u./ml, whereas that of the Vancouver virus stock was 9.6 x 10⁶ p.f.u./ml.

Infection of mice. Adult female CD-1 mice between 4 and 6 weeks of age were infected intraperitoneally with MCMV as noted. Mice were killed at various times post-inoculation by asphyxiation with CO₂. Organs were removed, homogenized and resuspended as a 10% (w/v) homogenate in complete medium containing 5% FBS. Homogenates were stored in aliquots at -70 °C and standard plaque assays were used to determine infectious virus titres.

Growth in tissue culture. Exponentially growing 3T3-L1 cells were infected with MCMV. The inoculum was removed 2 h post-infection and the cells were washed twice with Hanks’ balanced salt solution (HBSS) and then once with medium at 37 °C for 5 min in order to remove reversibly bound infectious virus (Hodgkin et al., 1988). This medium was then removed and replaced with 2 ml of complete medium containing 5% FBS. At various times post-infection, the supernatant was removed and reserved in order to assay for infectious virus. The cells were washed three times in PBS, harvested by scraping into fresh medium and sonicated in order to release intracellular virus. The samples collected at the various time points were subjected to a standard plaque assay to test for the production of infectious virus.

Restriction endonuclease digestion and agarose gel electrophoresis. Conventional electrophoresis was carried out at room temperature in 1 x Tris-acetate–EDTA buffer at 5 V/cm. In some experiments, DNA was end-labelled with [³²P]dATP (ICN) and the Klenow fragment of DNA polymerase I (BRL) after cleavage with restriction endonuclease. Gels containing end-labelled DNA were fixed in 10 x 10⁻³ M formamide, 5 x Denhardt’s solution, 5 x SSC, 25 mM-sodium phosphate buffer pH 7.0, and 0.1 M-glycine/ml of sheared, single-stranded salmon testes DNA. Hybridization was allowed to proceed for 16 to 24 h at 42 °C in prehybridization buffer containing 1 x Denhardt’s solution and heat-denatured, labelled DNA probe. The probes were labelled with [³²P]dCTP (ICN) using the Random Primers DNA Labelling System (BRL), or a QuickPrime kit (Pharmacia). After hybridization, the filters were washed twice in 0.1 x SSC/0.1% SDS for 5 min at room temperature, twice in 0.2 x SSC/0.1% SDS for 5 min at room temperature, twice in 0.1 x SSC/0.1% SDS for 15 min at 65 °C and once in 2 x SSC for 5 min at room temperature. The filters were then air-dried, and subjected to autoradiography for ³²P-labelled probes or developed according to manufacturer’s instructions for the non-radioactively labelled probes.

[³⁵S]Methionine labelling of infected cell proteins. 3T3-L1 cells were infected with MCMV at a high multiplicity (2 to 10 p.f.u./cell), and incubated for various times. The cells were starved in cysteine/methionine-free medium for 30 min prior to the addition of methionine-free medium containing 5% FBS and 50 µCi/ml of [³⁵S]methionine/cysteine, 85:15 (ICN, Tran³⁵S-label). Immediate early (IE) protein production was enhanced by incubation in the presence of 50 µg/ml of cycloheximide (Sigma) from 30 min prior to infection until the addition of labelling medium. At this time the cells were washed three times in HBSS to remove the cycloheximide, and 10 µg/ml of actinomycin D (Sigma) was included in the labelling medium to prevent early (E) protein transcription. To enhance the production of E proteins, cycloheximide was added at 4 h post-infection for 4 to 6 h. The cycloheximide block was then reversed by washing with HBSS prior to the addition of labelling medium. The E proteins were labelled for 2 h, in the presence of 50 µg/ml of phosphonoformic acid (Sigma). Late (L) proteins were labelled from 24 to 28 h post-infection.

Antibody production. All antisera were raised in mice. Adult female CD 1 mice were inoculated intraperitoneally with 1 x 10⁴ p.f.u. of infectious MCMV (both Smith and Vancouver strains). The mice were then boosted at 30 and 60 days post-infection with an intraperitoneal injection of 1 x 10⁴ p.f.u. of infectious MCMV. Two weeks following the third injection, the mice were bled, the blood was allowed to clot overnight, and the immune serum was then removed and stored in aliquots at -20 °C until used.

Immune precipitation. IE, E and L virus-induced proteins were identified by immune precipitation of infected cells with hyperimmune mouse serum. The infected cell lysates used for each experiment were divided into four aliquots and precipitated with anti-Smith or anti-Vancouver serum or with antiserum from mice immunized with sonicated control cells. Labelled infected cells were lysed in immune-precipitation (IP) buffer containing 10 mM-Tris–HCl pH 7.4, 1 mM-EDTA, 150 mM-NaCl, 0.5% (w/v) sodium deoxycholate and 1% (v/v) NP40. Five mm-PMSF (BRL) was added to inhibit serine proteases. The infected cell lysate was then centrifuged in an Eppendorf microcentrifuge at 14000 r.p.m. for 30 min. One to 5 µl of immune mouse serum was added to the supernatant, and allowed to react for 1 h.
on ice. Fifty μl of Staphylococcus aureus cells (Pansorbin Cells; Calbiochem) was then added for 1 h. The antibody/antigen/cell complexes were pelleted by centrifugation in an Eppendorf microcentrifuge at 12000 r.p.m. for 3 min, and washed twice with IP buffer and once with wash buffer (50 mM-Tris-HCl pH 7.4, 100 mM-NaCl). The pellets were then resuspended in 40 to 60 μl of PAGE loading buffer (45 mM-Tris-HCl pH 8.8, 2% SDS, 16% glycerol, bromophenol blue), and boiled for 2 min. The samples were centrifuged for 3 min in an Eppendorf microcentrifuge at 12000 r.p.m. before being loaded onto polyacrylamide gels.

PAGE. Infected cell proteins were separated under reducing conditions on 8 to 20% gradient polyacrylamide gels in a Hoeffer SE600 gel apparatus. Fifteen μl of samples was loaded per well, and the samples were electrophoresed in a discontinuous buffer system (Laemmli, 1970) under a constant current of 30 mA per gel until the bromophenol blue dye front reached the bottom of the gel. Protein Mr standards (BRL High Range) were included on each gel. The gels were stained with 0.1% (w/v) Coomassie blue (Sigma) in a fixative solution of 25% methanol/7% acetic acid, destained in 25% methanol/7% acetic acid/2% glycerol, and then soaked in 25% (v/v) methanol/2% (w/v) sodium salicylate before being dried and subjected to autoradiography.

Results

In vivo growth of the Vancouver strain

To determine the ability of the Vancouver strain to replicate in vivo, mice were inoculated intraperitoneally with MCMV, killed at various times, and the spleen, liver, kidneys and salivary glands were removed and assayed for infectious virus. No virus was ever recovered from mock-infected animals (n = 10). The results of the virus titration from the spleen, liver, kidney and salivary glands of the infected mice following a typical experiment are presented in Fig. 1. Inoculation of 1 × 10^4 p.f.u. Smith virus resulted in detectable replication in the visceral organs of mice, but 1 × 10^6 p.f.u. of the Vancouver strain virus was required before infectious virus could be detected. The peak virus titre in the spleen, liver and kidneys was detected at 3 days post-inoculation with both the Smith and Vancouver strains (Fig. 1a, b, c). After the 100-fold higher inoculum was used, the Vancouver strain gave comparable titres in spleen and higher levels of replication in kidney and liver. The most dramatic difference in the ability of MCMV to replicate in vivo was manifest in the salivary glands of inoculated mice (Fig. 1d). Although infectious virus was not isolated from mice inoculated with the Vancouver strain despite the higher inoculum, the Smith strain replicated to titres of 10^5 p.f.u./ml.

In vitro growth of the Vancouver strain

During routine titration of virus stocks, it was observed that the plaques formed by the Vancouver strain were larger than those of the Smith strain (data not shown). To determine whether the difference in plaque size was a reflection of a faster replication cycle and/or a greater yield of infectious virus per cycle, a one-step growth
Fig. 2. Exponentially growing 3T3-L1 cells were infected with 1 p.f.u./cell of Smith (▲) or Vancouver (●). Duplicate samples were removed at various times and the titre of each sample was determined by standard plaque assay as for Fig. 1. (a) Released or supernatant virus; (b) cell-associated virus.

Fig. 3. Agarose gel electrophoresis (0.8%) of Smith (lanes 1, 3 and 7) and Vancouver (lanes 2, 4 and 8) DNA digested with HindIII (lanes 1 and 2), XbaI (lanes 3 and 4) and EcoRI (lanes 7 and 8), and end-labelled with [32P]dATP. Lanes 5 and 6 contain HindIII/XbaI-digested λ DNA as markers (sizes in kb). Arrowheads (●) highlight differences in the Vancouver strain as compared to the Smith strain.

Viral genome analysis

A comparison of the restriction endonuclease profiles of the two strains of MCMV after cleavage with HindIII, XbaI and EcoRI (Fig. 3) revealed a number of differences in the Vancouver strain relative to the Smith strain. These included one additional HindIII fragment between the HindIII H and I fragments, a larger XbaI D fragment, the loss of the XbaI I fragment, a larger XbaI L fragment, the loss of the EcoRI F fragment, and an additional EcoRI fragment designated K+ between the I and J fragments (see arrows in Fig. 3).

CHEF analysis of the high Mr fragments generated by cleavage of viral DNA with HindIII (Fig. 4) demonstrated that the HindIII D and E fragments detected in the Smith strain were not seen in the Vancouver strain of MCMV. There was one fragment generated by cleavage of the Vancouver strain DNA with HindIII (designated ΔE), which was not seen with the Smith strain, but it was too small to compensate for a deletion of both the HindIII D and E fragments and still correlate with the EcoRI and XbaI restriction endonuclease cleavage results. A deter-

curve for each of the two viruses was generated (Fig. 2). The release of progeny Vancouver strain virus preceded that of the Smith strain by 3 h, and the yield of Vancouver strain virus obtained by 32 h post-infection was 10-fold higher than that for the Smith strain (Fig. 2a). An examination of intracellular virus (Fig. 2b) revealed that the same lag in the production of infectious Smith virus was maintained, suggesting that a process prior to the release of virus was responsible for the differences seen in Fig. 2(a).
mination of the size of the genomes of the Smith and Vancouver strains (total size of the restriction endonuclease fragments generated by cleavage of the genome with EcoRI and XbaI, data not shown) precluded the possibility of a Vancouver strain deletion involving the entire HindIII D and E fragments of the Smith strain. The total size of the Vancouver strain genome was calculated to be approximately 227 kb, as opposed to 235 kb for the Smith strain, rather than a size of 207 kb which would be expected if both the HindIII D and E fragments of the Vancouver strain had been deleted.

MCMV DNA cleaved with restriction endonucleases and subjected to conventional agarose gel electrophoresis followed by Southern blotting and hybridization is depicted in Fig. 5. The identification of fragments was made in comparison to an ethidium bromide-stained gel (Fig. 5c). Hybridization to the HindIII E probe revealed a loss of the EcoRI F and the HindIII E fragments coupled with the appearance of an additional smaller fragment in each profile of the Vancouver strain compared to the Smith strain, and a loss of the Vancouver XbaI I and L fragments to yield an additional fragment of intermediate size (Fig. 5a). Taken together, these results indicated that there was a deletion in the Vancouver strain compared to the Smith strain, which

Fig. 4. CHEF electrophoresis (1-0% agarose) of Vancouver (lane 1) and Smith (lane 2) DNA digested with HindIII. Letter designations A to L are for the Smith strain.

Fig. 5. Southern blot analysis of Smith (lanes 1, 3 and 5) and Vancouver (lanes 2, 4 and 6) strain DNA digested with EcoRI (lanes 1 and 2), HindIII (lanes 3 and 4) or XbaI (lanes 5 and 6) and probed with 32P-labelled (a) HindIII E or (b) HindIII D probes. • denotes fragment(s) specific for the Smith strain, ▲ denotes fragment(s) specific for the Vancouver strain. (c) Ethidium bromide-stained gel; ◀ denote fragments marked in (a) and (b).
included part of the EcoRI F and HindIII E fragments and also the restriction endonuclease site that separated the XbaI I and L fragments (XbaI I/L junction).

Homology of the HindIII AE fragment of the Vancouver strain with the HindIII E fragment of the Smith strain was confirmed by cross-hybridization on Southern blots of HindIII-cleaved MCMV DNA subjected to CHEF electrophoresis. Initially the filter was probed with the cloned [32P]dCTP-labelled HindIII E insert which hybridized to the HindIII E fragment of the Smith strain and to the HindIII AE fragment of the Vancouver strain. The filter was stripped of this probe and rehybridized to [32P]dCTP-labelled AE, which yielded the same results (data not shown).

Hybridization to the HindIII D fragment probe (Fig. 5b) revealed an insertion in the EcoRI K, HindIII D and XbaI D fragments of the Vancouver strain compared to the Smith strain. From this result, we concluded that during the CHEF analysis of the HindIII cleavage fragments (Fig. 4), the HindIII D fragment of the Vancouver strain comigrated with a higher Mr fragment.

The size of each cleavage fragment for each virus strain was determined (data not shown) and was used to calculate the size of the insertion into the HindIII D fragment and the deletion in the HindIII AE fragment of the Vancouver strain. The size of the insertion was calculated as approximately 0.9 kb, by averaging the difference between the Vancouver fragment containing the insertion and the corresponding Smith fragment for each enzyme. Similarly, the size of the deletion was calculated as approximately 9.4 kb.

The order of EcoRI fragments in the region between 0.37 to 0.47 map units has not been previously published (Mercer et al., 1983; Ebeling et al., 1983). As this area contained the EcoRI K fragment insertion in the Vancouver strain, an attempt was made to map this region. Viral DNA was cleaved with EcoRI and subjected to agarose gel electrophoresis, followed by Southern blotting and hybridization. Probes generated by cleavage of the cloned HindIII D fragment insert with BamHI were used to establish linkage between the EcoRI cleavage fragments. The results (Fig. 6) established an EcoRI fragment order of BQeKaP in both the Smith and Vancouver strains of MCMV.

**Genome map comparison of the Smith and Vancouver strains of MCMV**

The results from the restriction endonuclease digestions and cross-hybridization analysis were used to construct HindIII, XbaI and EcoRI maps for the Smith and Vancouver strains of MCMV (Fig. 7) based on previously published MCMV maps (Ebeling et al., 1983; Mercer et al., 1983). Examination of Fig. 7 revealed that the Smith and Vancouver strains of MCMV were identical except for an insertion in the EcoRI K fragment of the Vancouver strain, and a deletion spanning the XbaI I/L junction.

**Analysis of virus-specific proteins**

Experiments were conducted to determine whether the deletion of 9-4 kb of DNA in the Vancouver strain of MCMV included genes expressed in the parental Smith strain. PAGE of proteins immune-precipitated with anti-Smith serum is shown in Fig. 8. Eleven IE virus-specific proteins, ranging in size from 108K to 30K, were detected in both strains including the three abundant IE proteins. A minor 47K Smith IE protein appeared to be replaced by a slightly larger 49K IE protein in Vancouver strain-infected cells (Fig. 8a).

Analysis of immune-precipitated E proteins revealed 16 ranging in size from 208K to 40K in the Smith-infected cells (Fig. 8b). The only difference between the...
Salivary gland growth-negative MCMV strain

Fig. 7. Restriction endonuclease maps of the Smith and Vancouver strains of MCMV.

Fig. 8. Autoradiogram of [3S]methionine-labelled proteins from mock-infected (lanes 1 and 4), Smith-infected (lanes 2 and 5) or Vancouver-infected (lanes 3 and 6) cells which were immune-precipitated with anti-Smith (lanes 1 to 3) or anti-Vancouver (lanes 4 to 6) serum and subjected to gradient SDS-PAGE (8 to 20%). Protein size standards are indicated to the left of the figures. (a) IE proteins. • denotes Vancouver protein with altered mobility. (b) E proteins. ▲ denotes position of protein missing from the Vancouver strain. (c) L proteins. ▼ denotes position of protein missing from the Vancouver strain.

Smith and Vancouver proteins was the absence in the Vancouver strain of a 42K protein present in the case of the Smith strain (Fig. 8b, lanes 2 and 3). The anti-Smith serum precipitated this protein in the Smith strain, but not the Vancouver strain or the mock-infected cells. In addition, as would be expected, the protein was not detected in any of the samples precipitated with the anti-Vancouver serum (Fig. 8b, lanes 4 to 6).

Immune precipitation analysis of the late proteins was conducted with cells pulse-labelled from 24 to 28 h post-infection. Seventeen infected cell proteins ranging from 208K to 32K in size were detected in the Smith-infected cells following immune precipitation with the anti-Smith serum and PAGE (Fig. 8c). The only difference between the Smith- and Vancouver-infected cells was the absence in the Vancouver strain of the 42K protein present at E and L times post-infection in the Smith-infected cells (Fig. 8c, lanes 2 and 3). Again, this protein was not detected in immune precipitation experiments using the anti-Vancouver serum (Fig. 8c, lane 5).
Discussion

MCMV mutants unable to grow in the salivary glands of mice (Sgg− phenotype) have been previously described by a number of investigators. Temperature-sensitive mutants of MCMV described by Tonari & Minamishima (1983) and Sandford & Burns (1988) showed little or no ability to replicate in the salivary glands of inoculated mice. In neither case was the defect responsible for this phenotype characterized further. However rescue of the temperature sensitivity, and not the attenuation for salivary gland growth of the isolate, has since demonstrated that they were caused by independent mutations (Kumura et al., 1990). In a recent study, a deletion of 300 bp in an E gene (Sgg1) which maps to the HindIII J region of the MCMV genome resulted in a virus which showed a greatly reduced ability to grow in the salivary glands and lungs of mice but was indistinguishable from wild-type for growth characteristics in tissue culture or in the liver and spleen of inoculated mice (Manning et al., 1992). In comparison, the Vancouver strain mutant is unable to replicate in the salivary glands following intraperitoneal inoculation and shows decreased replication in the spleen, liver and kidneys of inoculated mice. Thus, the growth characteristics of this Sgg1 mutant (Manning et al., 1992) seem to differ from those of the Vancouver strain and the Sgg1 gene does not map to the large deletion in the Vancouver strain, suggesting that there may be more than one gene affecting the salivary gland growth of MCMV.

Contrasting with its restricted growth in vivo, the Vancouver strain was found to replicate to higher titres in tissue culture, a reflection of both a faster replication cycle and a greater yield of infectious virus per cycle. Analysis of the Vancouver strain genome revealed a 0.9 kb insertion into the EcoRI K fragment and a 9.4 kb deletion spanning the XbaI I/L junction of the Smith strain. The EcoRI K fragment is contained within the HindIII D fragment, which in turn has been shown to harbour the MCMV origin of replication (M. J. Masse, personal communication). Whether or not an insertion into this region would affect the ability of the Vancouver strain to replicate more quickly in tissue culture remains to be investigated. It is more likely, however, that the overall 8.5 kb deletion of the Vancouver strain genome might result in faster viral DNA replication.

The deletion of 9.4 kb of the Vancouver strain genome demonstrates that this region is not essential for replication of the virus in tissue culture. It is probable, however, that this region of the genome does play a role during growth in vivo and is required for transport or productive infection of the salivary glands. In association with the Sgg phenotype, only one major alteration in MCMV proteins was identified in the Vancouver strain relative to the Smith strain, namely the absence of a 42K protein detected at early and late times in Smith-infected cells. When anti-Vancouver serum was used, the 42K protein could not be detected in either Smith- or Vancouver-infected cells suggesting that not only was it not precipitated from cells infected in vitro but it was not made in vivo as shown by a lack of antibody production against this protein in Vancouver-infected mice. Other differences found were manifest as minor alterations in migration of Vancouver strain-specific proteins.

Characterization of the genes responsible for the replication of MCMV in the salivary glands would be useful for designing a live attenuated vaccine strain of HCMV. The deletion of part of the HindIII E fragment of the Smith strain of MCMV resulted in a virus which not only failed to replicate in the salivary glands of infected animals, but also showed decreased replication in all other organs examined. Production of antiserum against the Vancouver strain revealed, however, that a significant immune response is initiated following inoculation. Most importantly, immune precipitation experiments using the anti-Vancouver serum demonstrated that a response to the major IE protein was elicited. This is significant since the immune response to this protein has been demonstrated to be sufficient to protect mice from lethal challenge with wild-type virus (Koszinowski et al., 1987a, b; Reddehase et al., 1986). If the gene collinearity between HCMV and MCMV holds true, the identification and deletion of the corresponding gene involved in the Sgg− phenotype of HCMV could result in reduced dissemination of an HCMV vaccine in the population. Thus, further investigation of such a virus strain is warranted. Experiments to rescue the salivary gland growth defect of the Vancouver strain have been successfully completed and an analysis of the Sgg+ recombinants is underway (J. Boname, unpublished results).

References


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