Archives of Virology 62, 1—29 (1979)

Archives of Virology

The Murine Cytomegalovirus as a Model for the Study of Viral Pathogenesis and Persistent Infections*

Brief Review

By

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Accepted June 12, 1979

Introduction

I have two main reasons for presenting this review on murine cytomegalovirus (MCMV). The first is a response to the increasing popularity of this virus as an experimental system. The second reason is my belief that MCMV can be used as a valid and practical model for the study of several phenomena of current interest, including: pathogenesis; interactions between viruses and the immune system, including viral immunosuppression; control of persistent infections; and congenital infections. Such studies are all the more important now in view of the general realization that these phenomena all apply to human CMV infections.

In the discussion that follows, the term persistent infection refers to an infection which persists beyond the initial acute phase of the infection or period of optimal virus replication. Two types of persistent infection are distinguished for MCMV viz: chronic infection, characterized by the production of a relatively low level of virus; and latent infection, a situation characterized by the absence of infectious virus, but in which the presence of at least one viral attribute can be demonstrated. It is possible that both types of persistent infection can be present in the same animal but in different tissues.

Fundamental Properties of MCMV

The mouse virus, like other CMV's, is a member of the *herpetoviridae*. It shares a number of common properties with other CMV's, and there may be some justification for assigning the CMV's to a sub group within the family. However, this

* Abbreviations: ADCC, antibody-dependent cytotoxic cell; ALS, anti-lymphocyte serum; C3, third component of complement; CMI, cell-mediated immunity; CMV, cytomegalovirus; Con A, concanavalin A; EBV, Epstein-Barr virus; HCMV, human cytomegalovirus; HSV, herpes simplex virus; IF, interferon; MCMV, murine cytomegalovirus; LPS, lipopolysaccharide (bacterial); PHA, phytohemagglutinin; PWM, pokeweed mitogen; SHF, serum hyporeactivity factor.

0304-8608/79/0062/0001/$ 05.80

may be premature, since only the mouse and human CMV's have been studied in detail so far, and it is already apparent that these two differ in certain basic features (63). This is illustrated by the summary of their fundamental properties in Table 1. Nevertheless, differences between herpes viruses *in vitro* may simply reflect adjustments to life in tissue culture, whereas the CMV's do appear to show uniformity in behaviour in their natural hosts.

The mouse CMV grows readily in embryo cultures, giving rise to several thousand viral genomes per infected cell (108), from which approximately 100 PFU can be obtained. The potential infectivity is probably greater than this, since a typical multicapsid virion may contain several infectious genomes, but will only register as a single infectious entity (66).

Table 2 summarizes the available data concerning the susceptibility of different cell cultures to MCMV. The information is incomplete in the sense that few of these cultures have been tested at different phases of the cell cycle, a factor which has been shown to be important at least in fibroblasts (115). The virus also has the capacity to 'adapt' to growth in cells from different species (82, 133a).

Considering the large size of the MCMV genome, it is not surprising that the virus replication cycle incorporates a number of controlling elements in its transcriptional and translational patterns. These features have been studied in our laboratory (109, 20, 21) and by KIM *et al.* (84, 111). It would be interesting to investigate these controls in relation to non-productive infections.

Table 1. *Comparison of some fundamental properties of MCMV, HCMV and HSV*

Property	MCMV	HCMV	HSV
Mol. wt. of genome ($\times 10^6$)	132 (112, 113)	150 (42)	99 (53, 136)
Number of viral polypeptides	>38 (84, 20)	>32 (37, 46, 166)	>47 (59)
Multicapsid virions	Yes (65)	No	No
Centrifugal enhancement of infectivity	Yes (66, 125a)	Yes (66)	No
Effect on host DNA synthesis	No effect prior to viral DNA replication; gradual decrease later (64a)	Stimulation in quiescent cells (146, 31)	Inhibition (7, 136, 140)
Effect on host RNA synthesis	Slight decrease (113)	Stimulation (169)	Inhibition (7, 136, 140)
Effect on host protein synthesis	Gradual decrease late in infection (84, 20)	Little effect (46)	Inhibition (59, 136)
Thymidine kinase	Inhibition; no viral enzyme (114)	Stimulation; no viral enzyme (36)	Viral-coded enzyme (69)
Viral DNA polymerase	Yes (115a)	Yes (62a)	Yes (174a)
Range of permissive cells *in vitro*	Mouse cells; can adapt to other cell types (82; see Table 2)	Restricted to human fibroblasts (131)	No restriction (7, 136)

References are in brackets

Table 2. *Cell susceptibility to MCMV* in vitro

Species of origin	Cell line	Virus yield
Mouse a)	Embryonic- and adult-fibroblasts; 3T3, 3T6; MKSA (SV40 transformed kidney cells); whole brain	++[a]
b)	L/929	+ to ++ (cyclic)
c)	Y-1 (adrenal cells); spleen culture; primary kidney	+
d)	T-lymphocytes; L5178Y (leukemic lymphocytes)	−
Rat	Cerebellum	++
Hamster	BHK	+
Rabbit	Primary kidney; RK13	+
Sheep	Primary fetal brain	++
Monkey	BSC-1	+
Human	HeLa; Hep-2; WI-38; KB; diploid fibroblasts (human fibroblasts	− + with apodemus sylvaticus CMV)

[a] ++ = yields > 10 PFU/cell; + = yields <1 PFU/cell; − = no virus replication
References: 57, 64, 82, 83, 111a, 132, 133a

Pathogenesis—Primary Infection

Introduction

It is over 40 years since McCordock and Smith (94) reported on the distribution of MCMV in tissues of infected mice. Many studies have followed, but apart from yielding a more detailed knowledge of the effect of the virus upon certain tissues and cells, these have not really given us a clear picture of viral pathogenesis. Most of these studies have utilized the intraperitoneal route of infection, sometimes intracerebral, neither of which can be considered a natural route of transmission. It is generally assumed that the results of such modes of infection are relevant to a natural infection, although this assumption remains to be verified. The early studies of Mannini and Medearis (95) showed that the virus can be transmitted between cage mates (probably orally), but not from cage to cage, and in fact it has been shown more recently that intranasal inoculation with a relatively small dose of virus will lead to a successful infection in the lungs (71).

It is clear however from the various studies reported that MCMV has a propensity for many tissues, and for a variety of functionally different cells within these tissues. The general picture that emerges is one of extensive replication of the virus in whatever tissue it can gain access to (without an impressive viremia), which is controlled within a few days and replaced by a chronic infection in many tissues. The latter may be terminated completely or else it changes into a latent infection (without overt signs of the presence of virus). Reactivation of the infection can be brought about by various treatments. Wild mice commonly harbor a persistent infection, although fortunately for investigators many laboratory colonies appear to be completely free of MCMV.

The more general features of pathogenesis will be discussed first. These will be followed by a more detailed description of individual tissues; and a comparison of the cell types involved with the *in vitro* studies.

General Features

McCORDOCK and SMITH (94) were the first workers to study mice experimentally inoculated with salivary gland homogenates of MCMV. Intraperitoneal and intracerebral inoculations were used, and histological sections were prepared from many tissues. Lesions, indicated by inclusion-containing cells and/or inflammatory reactions, were evident in various cells in the liver; spleen; lymph nodes; adrenal cortex; kidney glomeruli; intestinal mucosa; fat; connective tissue; lung; and in certain cells of the ovarian theca interna, and acini of both submaxillary gland and pancreas.

Other interesting findings were: (i) the inability of spleen- and liver-derived virus to establish disseminated infection in other mice (a point to be discussed in more detail later); ii) the strain variation in susceptibility of mice to the virus. The latter point has been reiterated in many of the subsequent studies, and more recently a genetic basis for this feature has become apparent (see section on genetics).

MANNINI and MEDEARIS (95) examined several parameters relevant to MCMV infection. They noted that the infection was inclined to be more severe if the infection was carried out in younger animals, with greater virus dosage, and by intravenous inoculation rather than intraperitoneal, intranasal, oral or orbital routes. These generalisations have since been verified by many other workers, with MCMV and other virus infections. The most likely explanations are that younger immature mice cannot cope with infection as readily as adults, and the severity of a tissue lesion is proportional to the amount of virus reaching a tissue.

Intranasal and oral administration of virus gave rise to infection in the lungs and salivary glands, and occasionally visceral tissues, thus mimicking what is probably the natural route of infection (95, 71).

The influence of the age of the mouse was illustrated most dramatically by the study of SCHWARTZ *et al.* (152). They infected newborns via the retina, although the uvea appeared to be the most sensitive area and probably accounted for transmission to the blood. Subsequently most of these mice, which had stunted growth, died over ensuing days, and at autopsy revealed considerable lesions in spleen, thymus, lymph nodes and bone-marrow. Although other organs were proportional to body weight, the impressive observation was the widespread destruction of lymphoid cells, a feature not seen in infected adults (see below). Evidently the interaction between MCMV and lymphoid cells is complicated by the state of maturity of the tissue, and probably the stage of differentiation of each cell type. The actual cause of mortality was not clear however, since the authors also noted blood-borne bacteria in the infected animals. In retrospect it is possible that the mice may have died from bacterial infection, since it is known that MCMV predisposes mice to other microbial infections (48, 47).

Numerous studies have documented a typical humoral antibody response to the virus, and more recently a cell-mediated response. This will be discussed further in a later section. Titers of virus in the blood seem to be relatively low however, and the virus that is blood-borne is cell-associated (70). This point deserves consideration, since it may mean that virus reaches tissues in a form less accessible to marcophages.

Some strains of mice during chronic infection produce immune complexes in renal glomeruli. These complexes comprise MCMV antigens, IgG and C3. In some instances autoimmune reactions occur as well (123). These features are important and might constitute useful models for the study of viral immune complex disease. Similar findings have also been reported for human CMV (162).

Liver

Most of the early documentation on the effects of MCMV infection on liver tissue was obtained by RUEBNER and colleagues (142, 143), who used the intraperitoneal route of infection. Their conclusions have been confirmed since. Initially (from 2 days p.i.) characteristic nuclear and cytoplasmic inclusions were seen (by electron microscopy) in parenchymal cells. The viral capsids were assembled within the nucleus and subsequently appeared in densely staining cytoplasmic bodies. These bodies were composed of viral capsids embedded in an amorphous matrix which gave a positive staining reaction for acid phosphatase (considered to be a lysozomal enzyme). Kupffer cells were also infected.

Over the next few days focal lesions (presumably due to damaged parenchymal cells) increased in size. Meanwhile the titer of virus increased up to 4 days p.i. after which it rapidly decreased to an undetectable level. These workers did not comment on the presence of an inflammatory reaction, although others have since indicated that one can be seen several days after infection (122, 18).

One of the most intriguing aspects of the liver infection is the significance of the dense cytoplasmic inclusions. RUEBNER *et al.* (143) suggested that these bodies represented associations between virions and lysozomes. Although there is no direct evidence for this, their argument is based on three points: i) relatively little 'infectious virus' is recovered from the liver, despite the large number of particles seen in thin sections, and none is detectable by one month after infection; ii) the presence of a lysozomal enzyme (acid phosphatase) within the cytoplasmic inclusions, but which is absent from nuclear inclusions; iii) the apparent proliferation of lysozomes (especially around the Golgi zone) in infected cells. Although these arguments are in accord with the concept of lysozomal destruction of virus, there are nevertheless alternative interpretations. Thus while the yield of infectious MCMV for liver is low, and it is difficult to establish disseminated infection in mice from liver homogenates, it is still possible to establish infections in salivary glands of the recipients (126). In other words liver-derived virus is 'infectious'. Also similar dense bodies have been seen in MCMV-infected mouse embryo cells, and in HCMV-infected fibroblasts, where they appear to be compatible with efficient production of infectious virus (65, 157, 147, 165). Thus the significance of the dense bodies is unknown, but their presence does not correlate with destruction of virus.

Spleen

RUEBNER *et al.* (143) reported that inclusions were observed soon after intraperitoneal infections, particularly in reticulum cells surrounding the follicles (presumably the initial site of entry into the tissue). More details were added by later workers (61, 154, 78, 106). The development of the virus particles in the nucleus, and their association with dense bodies in the cytoplasm, resembled the process described for the liver.

Numerous workers have reported several reproducible features of the spleen response to MCMV infection (usually intraperitoneal), notably: i) suppression of immunoresponsiveness; ii) enlargement, iii) necrosis; The first feature warrants a separate discussion (see below). The latter two features seem to be mutually exclusive. A recent comprehensive study by MIMS and GOULD (106) has put the situation in a somewhat clearer perspective. It appears that only certain strains of mouse are susceptible to necrosis and this correlates with high titers of virus, ie. $>10^5$ PFU/g. spleen, whereas absence of necrosis correlated with low titers ($<10^4$ PFU/g) and enlargement of the spleen. CHALMER (18, 19) has indicated that necrosis of visceral organs, including spleen, is characteristic of the susceptible H-2^b haplotype. These mice also give a marked inflammatory response.

MIMS and GOULD (106) investigated the necrosis response in more detail. The first cells producing virus (indicated by fluorescent antibody staining) were medium to large mononuclear cells in the perifollicular areas and red pulp. These cells were not phagocytic, although virus was subsequently found in macrophages and small lymphocytes. Foci of necrosis, apparently originating with infected cells, then spread and sometimes persisted for several weeks.

Other workers have noted the involvement of reticulum cells and megakaryocytes during the early stages of infection (127). One of the intriguing questions arising from these observations is why various types of cell resident in the spleen may be susceptible to infection, whereas the replacement cells of the same type are not, at a time when infectious virus prevails. Male and female mice responded identically in the study of MIMS and GOULD (106), but newborns gave high titers of virus without corresponding necrosis. Again this brings into focus the relevance of the state of maturity of the tissue, since the lymphoid organs are not fully developed at birth (152).

It appears from the studies of MIMS and GOULD (105, 106), and of LOH and HUDSON (91), that the cells initially involved in the infection are not lymphocytes, although these may subsequently become infected. Macrophages certainly do take up the virus and may be important in controlling its spread. However there are other ill-defined cells in the spleen, and some of these may well be involved.

It is possible that necrosis may simply reflect the presence of virus-susceptible cells, governed by the particular H-2 haplotype. In the absence of such cells, the spleen may enlarge due to increasing numbers of mononuclear cells. Clearly there is the need for a comprehensive study of various relevant parameters in a given inbred strain of mouse, before any meaningful picture of spleen pathogenesis can be drawn. It is also pertinent to ask whether splenic necrosis or enlargement could possibly result from the anticipated smaller dose of virus that the tissue would receive during a natural infection. All the phenomena discussed in this section could conceivably be artefacts.

Other Lymphoid Tissues

Much less attention has been given to lymphoid tissues other than the spleen. Descriptions have been fragmentary and have implied that changes are similar to the spleen. These involve necrosis of lymphocytes and other lymphoid cells, followed by eventual recovery of the normal architecture in survivors. The thymus is exceptional in that newborns do not recover the loss of cortical cells, and in this

organ the epithelial cells also contain virus inclusions (152). The immune capacity of surviving animals was not examined however. It is interesting that the latter study was performed on mice receiving intraorbital infection, which presumably gave rise to a low level viremia. Since the amount of virus reaching the various lymphoid tissues in this case may well have mimicked a natural infection, then the results are meaningful. Various unidentified bone marrow cells were found to be susceptible to the virus. This could have important consequences, depending upon which kinds of stem cell were affected.

Blood Cells

Although viremia is a normal phase of MCMV infection, very little infectious virus is detected in a free form, in contrast to many other types of virus infection. Most blood borne MCMV is associated with cells in the 'buffy coat' (70), a finding which also applies to human CMV (33). Primary MCMV infection results in significant changes in the peripheral blood population (127). The hematocrit value decreases between 3 and 7 days p.i. followed by recovery to normal by 15 days. Leukocytes show a similar time course, the initial decrease being due to mononuclear cells which recover, while polymorphs do not decrease but increase in number, followed by a return to normal. Platelet counts also drop considerably in the first few days and then recover by 7 days p.i. The time of minimal cell numbers, at least for mononuclear leukocytes and platelets, corresponds to the time of maximal virus production in spleen and lymphoid tissue, and may reflect a decrease in the appropriate stem cells. This could then result in a transient overproduction of certain cells. No long term deficiencies seem to have resulted from the infection.

An alternative explanation for the apparent fluctuation in cell numbers is that the virus brings about alterations in lymphocyte traffic (178) which eventually correct themselves.

Lungs

Very little attention was given to lung involvement in the earlier studies on MCMV pathogenesis. This is an important concern however, since reactivation of human CMV is often accompanied by pneumonitis with serious consequences (23). The classical study of MANNINI and MEDEARIS (95) did at least demonstrate infection in the lungs following oral or intranasal inoculation.

More recently BRODY and colleagues (14, 15) have examined the lungs of ALS-treated mice subjected to a low dose sub-cutaneous inoculation of MCMV, a condition resembling the immunosuppressed patient. Also JORDAN (71) administered a similar low dose of virus intranasally and examined the effects on the lungs.

In BRODY and CRAIGHEAD's study (14) tissue-culture passaged virus was used. This caused little effect unless the mice had been on a prior ALS regimen. In the latter case the given inoculum of 100 PFU corresponded to one LD_{50}, most of the deaths occurring between 21 to 25 days p.i. The virus replicated to relatively high titers in the lungs ($>10^6$ PFU/g. at 27 days p.i.) and persisted for at least two months in the survivors. Virus particles were observed only in capillary monocytes and alveolar macrophages. The alveolar septa enlarged, apparently because

of the accumulation of infected monocytes, and fluid accumulated in the interstitial spaces. The septa apparently retained their integrity despite the edema and the continuing spread of virus. The authors suggested that these changes were irreversible and that the severity of the edema determined whether or not death would occur.

In a subsequent study, BRODY *et al.* (15) attempted to trace the origin and transport of the proteinaceous interstitial fluid by using horseradish peroxidase as a marker. They concluded that the fluid was derived from blood vessels and passed through the alveolar septum by pinocytotic cell-to-cell transfer. Exactly how the virus was able to induce this process to occur was not discussed.

JORDAN (71) inoculated animal-passaged virus intranasally and established infection in the lungs without prior ALS treatment. The success of this endeavour was probably due to the use of non-attenuated virus. The lungs were the primary target of infection and yielded 10^5 PFU/g within 5 days. Thereafter the virus spread via the blood to other organs, while the lung titer diminished. The pneumonitis that occurred was similar to the description given above i.e. thickened alveolar septa due to infiltration by macrophages/monocytes, and the accumulation of proteinaceous fluid in the air spaces. A larger dose of virus gave rise to a similar course of events, but the pneumonitis was correspondingly more extensive and severe and some animals died.

Salivary Glands

These organs have been relegated to consideration after the other tissues only because they appear not to be a primary target for MCMV. No matter what route of infection is used, evidence for replication is not obtained for at least 8 days. Although the virus presumably seeds the salivary glands from the blood, there still seems to be a considerable delay in initiating the infection. Studies of the glands *in vitro* might cast light on the reason for this, but such studies have not been reported.

Most of the work has concentrated on the submaxillary glands, although certain specific cell types in all three salivary glands appear to be susceptible. The infection is characterized by an acute phase, giving high yields of virus (10^7 to 10^9 PFU/g.), followed by a transition to a chronic phase, the duration of which varies with mouse strain.

Detailed descriptions of the infection have been given by several groups (143, 55, 73). Virus replication, which was not accompanied initially by necrosis or other alterations to the tissue, was localized in the acinar cells of submaxillary, parotid and sublingual glands. There was further restriction too, in that mucus-secreting areas of these glands were affected much less frequently. The intranuclear development of the virus was similar to spleen and liver. In contrast the cytoplasmic inclusions were quite different. The latter consisted of vesicles which contained various numbers (sometimes more than 100) of fully enveloped virions. These vesicles fused with the plasma membrane and liberated the virions into the lumen, from which the virus was presumably transported along the ducts into the mouth as part of the salivary secretions. The infected cells did not appear to be damaged, and it is possible that the virus simply utilized the cell's normal secretory mechanism as a means of exit.

HENSON and colleagues extended their observations in an attempt to determine what factors controlled the infection (55, 56). At the time of optimal virus production ie. during the third week p.i., all virus was still restricted to some (not all) acinar cells. An inflammatory response could be observed after 16 days. This consisted of mononuclear cells and macrophages, but no polymorphs. This point heralded the onset of a phase of degeneration of the infected cells, although the basement membrane separating the acinar cells from the connective tissue remained intact. Normal appearing cells adjacent to the infected ones were also destroyed in the process. Eventually the inflammatory response was replaced by a healing process and the integrity of the acinus was restored.

HENSON and STRANO (56) suggested that, since the basement membrane remained intact, the infiltrating lymphocytes produced a non-specific cytotoxic factor which was responsible for cell killing. However, if the cells expressed viral antigen on their basal surface, a standard T-cell response could have been evoked.

Whatever the mechanism of the termination of infection might be, it is clear that only the acinar cells support virus replication. The duration of the infection probably depends upon the speed with which the mouse can mount an effective inflammatory response, the C3H/Anf strain being apparently much more efficient in this than ICR/HA mice, which give a long drawn out chronic infection lasting for several months. In support of this hypothesis, it was found that cortisone administration prolonged considerably the production of virus in C3H submaxillary glands (55), presumably by abrogating the inflammatory response.

Two important questions remain unanswered. How does the virus gain access to the acinar cells in the first place? If the virus originates in the blood, as supposed, then it must be able to cross the basement membrane. This could explain why it takes several days before virus replication initiates. Secondly, assuming that the virus does use this route of transport, then why are none of the other cells in the tissue affected? This question is obviously applicable to many of the other tissues already discussed, since within each tissue there seems to be a restricted population of cells which is susceptible to the virus.

Central Nervous System

Most of the work reported on CNS involvement has utilized intracerebral or intra-orbital inoculation, which has given the virus direct access to tissues it might not normally encounter. Nevertheless at least some of the observations must be relevant to human CMV infections, since the latter are often associated with mental retardation, microcephaly and audiovisual impairment in infected infants, and with retinitis in adults (49, 175, 131, 167, 151).

MARGOLIS and KILHAM (98) and DAVIS and colleagues (28, 29), inoculated suckling mice intracerebrally (with tissue-culture and animal-passaged virus respectively) and observed cytopathic effects within two days. A generalized encephalitis resulted, with considerable necrosis and inflammatory response, which involved many parts of the brain. Numerous cell types, including neurons in different areas, glial cells and macrophages, all showed typical nuclear and cytoplasmic inclusions. Cell fusion was a prominent feature reported by MARGOLIS and KILHAM and these authors indicated that this may have been the mode of trans-

mission of virus ie. by the recruitment of neighbouring uninfected cells into polykaryocytes.

These observations are also reflected by *in vitro* studies, in fetal and adult brain tissue, from which the general impression is that all recognizable cell types of the CNS show CPE with typical CMV inclusions and cell fusion (149, 176, 64). Thus if the virus gains entry to the CNS, it should be able to disseminate widely. In contrast peripheral nerve tissue seems to be more resistant, although even here the infection brought about degeneration of myelin (149).

A different story emerged from the work of SCHWARTZ *et al.* (151) who inoculated salivary gland-passaged virus intraorbitally (near the retina) in weaned mice. An inflammatory response was evident after one day and eventually involved most of the vascular tissues of the eye. Again many cell types produced virus over a period of several days, and the visual receptors degenerated. However, the neuronal layers of the retina (ganglion layer, inner nuclear layer and inner plexiform layer) were spared, and showed no involvement despite their proximity to the site of virus invasion.

One possible reason for the discrepancy, regarding susceptibility of neurons, is that the mature retina contains no dividing neurons, whereas the immature CNS tissues and explanted tissues may contain dividing cells. The situation is not as simple as this however, since human CMV chorioretinitis in adults involves neurons as well as other cells (32, 182, 1, 151).

Fetus

One would anticipate, on the basis of the foregoing considerations, that various tissues of the fetus would be susceptible to MCMV. The limited amount of information available suggests that this is so. In addition, cultures derived from fetuses are normally used for propagating the virus. However, the important question that merits attention is: can the virus gain access to the fetus *in vivo*? In human, bovine, porcine and guinea pig CMV infections this can happen (101, 130, 50, 79, 35, 26, 86); but doubt has been expressed about MCMV, mainly because of the negative transmission results obtained (100, 70, 117); and the supposed barrier to transplacental infection offered by the mouse placenta. For these reasons investigators have given more consideration to nutritional deprivation as a cause of fetal abortion in MCMV-infected mothers (88). The explanation is not so simple, however, and further discussion of this problem will be reserved until other features of congential CMV infection are brought into context.

Types of Cell Affected

In an earlier section the variety of cell types which could support MCMV replication *in vitro* was discussed. Most of the cell cultures studied were composed of cells of fibroblast origin, derived from embryonic or newborn tissues. In some tissues *in vivo* the virus seems to be capable of spreading unrestricted among different types of cell (eg. brain and liver); but in others the virus shows strong specificity (eg. spleen, lung, salivary glands), Table 3. However the virus itself clearly does not determine this specificity. Thus in most tissues cells of the monocyte-macrophage line are susceptible, whereas the susceptibility of neurons is de-

termined by their location. The latter situation is governed by either the state of differentiation of the neuron, or by its tissue environment, and as yet we have no clues as to what kind of factors are involved. Studies of explanted tissues might shed some light on this problem, although such systems are often complicated by the breakdown of tissue architecture and necrosis, and by the proliferation of fibroblasts. Despite these problems some attempts have been made. Nerve tissue derived from CNS seems to retain the ability to disseminate the virus freely, whereas peripheral nerve tissue is much more restrictive (149, 176). The virus had no effect upon the development of fetal otocysts *in vitro*, mimicking the *in vivo* result (29).

More recently tracheal rings were maintained in culture and infected. The virus replicated in the epithelial cells, causing loss of cilia and a hyperplasia in the epithelial layer, and also in connective tissue fibroblasts, but not in chondrocytes (96). This illustrates the point that under appropriate circumstances even epithelial cells can support virus replication, although it remains to be seen if they do so *in vivo*.

Table 3 summarizes these observations on viral susceptibility of different cell types.

Table 3. *Types of cell susceptible to MCMV* in vivo

Tissue	Cell types known to replicate the virus
Liver	Parenchymal cells; Kupffer cells
Spleen	Reticulum cells; macrophages; megakaryocytes; some lymphocytes
Thymus	Lymphocytes, especially in newborns; epithelial cells
Bone-marrow	Various unidentified stem cells
Lungs	Macrophages/monocytes
Salivary glands	Some acinar cells
CNS	Macrophages; glial cells; some neurons
Embryo	Many cell types

Genetic Basis of Variation in Response

It has been known for many years that the overall susceptibility of mice to MCMV is strongly dependent upon the strain of mouse. In fact the nature and duration of specific tissue lesions is also influenced in this way (56, 106). In addition, Drosi and colleagues (34) have isolated, from wild mice, a strain of MCMV to which Swiss mice are resistant.

Recently Chalmer *et al.* (17, 18, 19) have been able to correlate these effects with the H-2 haplotype. Thus not only is mortality dictated by certain H-2 loci, but so is the nature and severity of tissue damage.

In general, infection in susceptible H-2^b and H-2^d strains is characterized by necrosis (with little inflammation) in liver, spleen, lymph-nodes and bone-marrow, with death occurring at higher virus doses. Genes other than H-2 also contribute to the detailed histopathology. In contrast, in the more resistant strains such as H-2^k, similar events occur during the first 24 hours p.i., but the lesions do not increase significantly thereafter. Inflammatory responses are also noticeable in these strains. Macrophages may be important in determining the outcome of the virus-

tissue interaction, depending upon their ability to restrict and clear the virus. In addition each strain of mouse displays characteristic changes in blood cells in response to the infection.

It is clear from these studies that a complete understanding of MCMV pathogenesis must take into account the genetic background of the mouse under study, and future pathogenetic studies should be carried out with defined strains.

Virulent and Attenuated Virus

In this context too, the studies on pathogenesis have lacked consistency. Virus stocks, although ultimately all derived from the original Smith strain, have undergone diverse histories in different laboratories. Some workers have used only virus which has been repeatedly passaged in mice; others have used exclusively tissue-culture passaged virus; while some others have used stocks with mixed or even 'confused' histories. Despite the lack of attention usually paid to this parameter, MCMV workers are fortunate in that the virus adapts rapidly to either of two states viz: virulent or attenuated (126). A single passage of the virus in mouse embryo cultures is enough to 'attenuate' the virus i.e. it no longer replicates significantly in liver and spleen, although it retains its normal potency for submaxillary glands. The change is fully reversible and a single passage in this salivary gland restores maximum infectivity for liver and spleen. The molecular basis for this change in property is unknown but is under current investigation in our laboratory.

At least one minor genetic change has occurred among the current MCMV stocks. The strain designated K181 by Osborn differs significantly from our standard Smith strain in terms of virulence in mice, and also shows slight changes in restriction endonuclease patterns, although DNA-reassociation kinetics indicated the identity of their genomes (107). It is quite likely, therefore, that the various stocks of MCMV have at least minor genetic differences, just as there are differences in the genomes of the various strains of HCMV and HSV (81, 156, 54). Such differences could contribute to pathogenetic differences, just as the mouse strains were seen to do.

Immunosuppression

Introduction

Murine CMV is immunosuppressive, according to various indicators of immune responsiveness *in vivo* and *in vitro*. Thus infected mice have a reduced capacity to respond to foreign antigens or interferon-inducing agents; are able to accept heterologous skin grafts for longer than their uninfected counterparts; and their spleen cells respond less well to mitogenic stimulation *in vitro*. In turn infected mice produce antibody, interferon and at least one type of T cell-mediated response. Although these are probably all ramifications of a sequence of interconnected events in the animal, it is more convenient to discuss each parameter independently, and then attempt correlations. The features of immunosuppression will be considered first, and this will be followed by a discussion of possible mechanisms and various aspects of recovery from the primary infection.

The MCMV-Infected Mouse

Within 2—3 days p.i. mice showed a reduced capacity to respond to sheep red blood cells (125, 60, 9). This effect was noticeable before the peak time of virus production in visceral organs, and was most dramatic around 7 days p.i. It was followed by a gradual recovery to normal in surviving mice. In addition infected mice rapidly lost their capacity to produce interferon (at least serum interferon) in response to NDV (125, 78) and various chemical inducers (168), although they could provide interferon in response to MCMV itself, albeit transiently (see below).

Infected mice also showed prolonged survival of skin grafts, especially when grafted within several days of the infection (60), although at later times grafts were rejected normally. Antibody forming cells were also reduced shortly after infection (27).

These results are indicative of a reversible suppression in B-cell and T-cell function during the acute phase of the infection. Mice which survive the pathological consequences of the infection then recover their normal immune responsiveness.

Spleen Cell Responses to Mitogens

Spleen cultures established from MCMV-infected mice invariably show reduced responses to several mitogens viz: Con-A, PHA, LPS and PWM; and to mixed leukocyte cultivation, as measured by uptake of tritiated thymidine (60, 9, 155, 10, 11, 77, 78). The general features of these effects are: i) the immunosuppression is detectable before the peak time of virus replication; ii) it precedes clinical signs of infection and tissue pathology; iii) it is not due to cytolysis of lymphocytes; iv) it occurs in strains of mice which differ with respect to subsequent spleen pathology, ie. whether they show splenic necrosis or splenomegaly; v) it is always followed by recovery to normal or near-normal response within days; vi) moreover the effects are for the most part consistent among laboratories in the face of technical differences and idiosyncracies.

The kinetics of these responses closely parallel the *in vivo* attributes described above, and probably reflect a generalized but transient suppression in all immune responses.

Immunosuppression in vitro

A model system was set up in our laboratory for the purpose of studying the immunosuppression in more detail. Spleen cultures derived from normal mice were infected with MCMV in *vitro* and several parameters were examined over a period of several days. The rationale for this approach was that, if the suppressive effect of the virus could be mimicked in culture, then this would afford us a means of analyzing the mechanism in some detail without the problem of fluctuating cell populations which occurs *in vivo* (127). Furthermore we could then vary the amount of virus added to the cultures, and also vary any other factor which might be important.

In this system we found that MCMV did indeed suppress the spleen cell responses to con A and to LPS, although cell viability was not affected (67). Other salient features of the system are as follows: i) spleen cells from all mouse strains examined suffered a diminished response to mitogens following infection; ii) the

effect represented a real inhibition in the synthesis of DNA and RNA by the lymphocytes (92). This was an important point to establish since all of the investigators using the *in vivo* infected spleens assumed that changes in exogenous thymidine uptake reflected corresponding changes in DNA synthesis. In fact in MCMV-infected fibroblasts this correspondence does not hold true (114, 64a). In addition macrophages can secrete significant amounts of thymidine into the cultures under some conditions, and this could dilute the exogenous labelled thymidine (124, 158); iii) a very small number of cells, having properties similar to B-lymphocytes, replicated the virus; iv) macrophages took up most of the input virus and some of them could persist as infectious centers; v) virus could be reactivated from these macrophages by cultivating them with syngeneic or allogeneic fibroblasts; vi) UV-inactivated virus did not inhibit cell response to mitogens; vii) reconstitution experiments indicated that the immunosuppression effect was mediated via infected macrophages; viii) animal-passaged and tissue-culture virus were both immunosuppressive. Further details of these studies can be found in a series of recent publications (67, 91, 92).

The system just described is clearly more amenable to detailed analysis than infected spleens *in vivo*; but it does have one important limitation. It cannot take into account the role of the animal responses to the primary infections. For example, we cannot be certain that the persistently infected macrophages would survive *in vivo* in the face of a mounting attack by cytotoxic T-cells (133). Furthermore, some of the spleen cells *in vivo* may be exposed to a greater number of virus particles over a period of days, which might thereby increase the likelihood of lymphocytes becoming infected. Thus it is likely that a full appreciation of the respective roles of the various components may only be realised after further study of both the *in vivo* and the *in vitro* infections.

The Mechanism of Immunosuppression

The subject of immunosuppression has been reviewed from different aspects in recent years (178, 179, 173, 2, 141). Many viruses have been shown to be immunosuppressive, and it is evident from the accumulated data that, although the precise mechanism is not understood for any virus, it is likely that different explanations will be forthcoming. In this connection it should be remembered that the body's immune system is a homeostatic one, and therefore any slight perturbation in one component could profoundly affect the overall system.

Several hypotheses can be advanced to explain the immunosuppressive effects of MCMV. Direct cytolysis of lymphocytes as a mechanism can be rejected because the suppressive effect *in vivo* does not correlate with the nature or severity of the spleen lesions, and the effect is manifest before the peak of virus synthesis (61, 154, 78). Furthermore relatively low virus to cell ratios (MOI <0.01 PFU/cell) suffice to abrogate almost completely the response of T- and B-cells to mitogens. In the *in vitro* system, overall cell viability (ie. of lymphocytes mainly) is unaffected by an MOI of up to 100 PFU/cell. Here most of the virus particles are taken up by macrophages at the expense of lymphocytes, which are only rarely infected (91).

However, it is still possible that certain kinds of lymphocytes are affected directly by MCMV. Thus the immature thymocytes of newborns appear to be especially susceptible to MCMV (152). This indicates that lymphocyte differenti-

ation may be important, and one is reminded here of the array of interactions between lymphocytes and EBV (54, 43).

The degree of immunosuppression, at least *in vitro*, is proportional to MOI but the effect is not competitive with the mitogens. These and other considerations led us to conclude that the effect of the virus is an indirect one, mediated via a minority population of cells which are important to mitogen responses. The three possibilities pertinent to MCMV are: i) alterations in lymphocyte traffic; ii) alteration in macrophage function; iii) augmentation of suppressor cell activity. The latter two are not necessarily distinct, since in some studies suppressor cell activity has been attributed to macrophages (139).

Lymphocyte Traffic

This term refers to the migratory patterns of lymphocytes within the body. The studies of WOODRUFF and WOODRUFF (178—180) showed that a short exposure of thoracic duct lymphocytes to influenza virus or NDV, sufficient to cause slight membrane changes, resulted in profound alterations in the tropic properties of these cells. Instead of migrating to their proper T-cell areas in spleen and lymph nodes, they migrated primarily to the liver. The effect was fully reversible and within 24 hours most of the cells had returned to their proper locations. The significance of these observations lies in the fact that the spleen could be temporarily deficient in B- and T-cells, or at least specific sub-classes of lymphocytes, which could result in decreased immune responses. Although this seems to be an attractive hypothesis, the speed of reversal argues against its applicability to MCMV, since the immunosuppression caused by the latter takes many days to return to normal.

Alterations in Macrophage Function

Many of the immune responses inhibited by MCMV infection utilize macrophage functions. Thus any effect of the virus upon macrophages could conceivably alter the outcome of numerous cellular interactions, either because of impairment of direct macrophage participation in lymphocyte functions, or because of interference in the secretion of regulatory factors (172). Stimulatory properties of macrophages could be depressed, or their inhibitory properties could be augmented.

Several studies have shown a viral suppression of phagocytic and chemotactic responses of macrophages, polymorphs and neutrophils (103, 89, 144) and it has been suggested recently that influenza virus brings about transient immunosuppression in humans by interfering with peripheral blood monocyte function (135).

However, it is equally likely that MCMV would cause immunosuppression by activating macrophages rather than by suppressing their functions. It is clear that while small numbers of macrophages stimulate the responses of spleen cells to various mitogens, activated macrophages are inhibitory (76, 177, 139, 2, 129). SCOTT (153) proposed that the immunosuppression brought about by *C. parvum* was due to activation of the macrophages which ingested the bacteria. According to this concept any stimulus which brings about activation of the macrophage will lead to suppression of immune responses. Such stimuli would include certain microbial infections, T-lymphocyte products, immune complexes (2, 90), and interferons (118, 150).

Once activated, the macrophage can secrete a number of products which are immunosuppressive (2). These include prostaglandins, especially those of the E series (174, 44); thymidine (124, 158); and interferon (45, 118). The prostaglandin effect can be mimicked by dibutyryl c-AMP, which suggests that suppression may be mediated by any substance capable of raising the concentration of cyclic AMP within lymphocytes.

It must not be forgotten, however, that macrophages normally produce other factors, such as mitogenic protein and lymphocyte chemotactic factor (172), which one would expect to counteract the immunosuppressive effects. Possibly the net effect is determined by the precise nature of the macrophage activator, the presence of functionally distinct populations of macrophages, or the type of recipient cell present in the vicinity of the activated macrophage *in vivo*.

The fact that MCMV infection does result in an early burst of IF production which precedes immunosuppression (170), together with the points discussed above, suggest that the macrophage may be the cell responsible. Recovery from immunosuppression would then be explained by either a reversal of activation (due to removal of the virus, or a feedback loop), or by a repopulation of the spleen with normal monocytes and macrophages.

Augmentation of Suppressor Cell Activity

Non macrophage suppressor cells, which can suppress the response of spleen cells to mitogens, have been described (eg. 39), although their reality in some situations has been questioned (139). Assuming that such a class of suppressor lymphocytes does exist, the virus would have to augment their activity considerably to explain the results. WEBB and JAMIESEN (174) described a population of T-cells which responded, within a few hours of exposure to high doses of PHA or Con A, by secreting prostaglandins. The following sequence of events would then be similar to that described for activated macrophages.

Thus the immunosuppressive effect of MCMV could be explained by interaction of the virus with either a macrophage or a suppressor T-cell, leading to the secretion of prostaglandins or IF (presumably type II IF in the case of the T-cells) and the inevitable suppressive effect upon other T-cells and B-cells.

At present the evidence tends to favour the macrophage, since several studies (92, 92a, 105) implicate this cell as the principal target for the virus. But the relevance of tissue architecture, and the regionalisation of functionally different populations of macrophages and lymphocytes within lymphoid tissues (5), are factors which must be considered also. The results of *in vitro* studies, with homogenous suspensions of spleen cells, can only serve to indicate the possibilities.

The Roles of Macrophages

Macrophage-MCMV interactions can be considered from two aspects: i) the role of macrophages in restricting the spread of or 'clearing' the virus; ii) the effect of the virus upon macrophage function, especially as the latter relates to augmentation of lymphocyte functions and 'clearing' of other infecting microbes.

The genetic variation in susceptibility to MCMV has been described in a previous section. SELGRADE and OSBORN (154) attempted to find out if this variation was reflected in the response of macrophages to the virus. The situation is not so

simple however, since animal-passaged and tissue-culture passaged MCMV produces infectious centres, and replicates to some extent, in peritoneal, spleen, liver (Kupffer cells) and alveolar macrophages from various strains of mouse (171, 154, 67, 105, 91). The tissue culture-passaged virus seems to be cleared or removed by macrophages much more effectively than animal-passaged virus (105), which may explain why the former fails to replicate to high titers in some visceral organs (126). Again this emphasizes the fact that one cannot consider viral pathogenesis as an attribute of the virus alone, since the fate of MCMV within a given tissue may be determined by the presence or absence of macrophages at the site of entry. Furthermore, the majority of blood-borne virus appears to be cell-associated rather than free (70), a fact which may influence the localization and fate of the virus within lymphoid tissues.

The effect of the virus upon normal macrophage function has been considered already in its connection with immunosuppression. It is possible that other macrophage functions, such as phagocytosis and intracellular digestion, may be inhibited or even augmented as a result of MCMV infection. These possibilities can be tested. These processes are relevant to concurrent microbial infections, and indeed it has been shown that MCMV-infected mice are particularly susceptible to pseudomonas, staphylococcal and candida infections. A lethal dose of one of the latter organisms is considerably smaller than in normal mice. Mortality appears to be due to the superinfecting microbe rather than MCMV itself, and the effect has been attributed to viral immunosuppression (48, 46). Since the primary defect appears to be a poor clearance of organisms by the tissues (46), then impaired macrophage functions should be considered as an explanation.

This phenomenon has also been noticed in human CMV infection in renal transplant recipients, in which the virus can apparently predispose the patient to fatal fungal and bacterial infection (23).

Immunological Parameters in Human CMV Infections

KANTOR *et al.* (75) reported the association of immunological changes with CMV-mononucleosis. Since then various groups have attempted to correlate humoral and CMI defects with HCMV infections. Generally there is a correlation between impaired CMI responses and corresponding disseminated CMV infection (148, 121, 137, 41, 164, 8, 38). Thus the situation would appear to be similar to MCMV-infected mice, and further study of MCMV may shed light on the mechanisms involved in HCMV infections.

Recovery From Primary Infection

Antibody Response

Neutralizing antibodies against MCMV have not usually been considered to play an important role in the initial stages of recovery from infection. The reason for this is that serum antibody is not detectable for at least a week or so, according to conventional assay techniques. However the incorporation of complement into the assay procedure has enabled workers to detect neutralizing antibodies as early as 3 days p.i. (3). Furthermore, serum taken from mice 3 days p.i. could

immunize other mice against a virus challenge given 24 hours later. This protective effect was not due to interferon, but was apparently due to the presence of IgG (rather than IgM) in the serum (3). Although this indicates a significant role for serum IgG in the recovery phase, it is not clear why the 3 day serum was much more protective than serum taken at 5 or 7 days p.i., which presumably would have contained a higher concentration of anti-MCMV globulin. Perhaps there is a special class of high-avidity IgG synthesized transiently shortly after infection; or there is some other protein responsible which fortuitously fractionates with IgG. Further purification and characterization of the active principle is certainly desirable.

In addition to neutralizing antibody, one would anticipate a role for small amounts of antibody in some kind of ADCC reaction, such as may occur in other herpes infections (138, 141). Thus the participation of humoral responses in controlling MCMV infections cannot be considered insignificant.

More support for this hypothesis may be the demonstration that newborns which suckled immune mothers were protected from a subsequent lethal dose of MCMV (102). Thus the colostrum appears to be protective although it is not clear whether this was due to transfer of IgG or lymphocytes.

Interferon

The relevance of IF to recovery from MCMV infection has often been questioned on the basis of two observations: i) Not all workers have detected serum IF in infected mice (125, 168); ii) MCMV infection *in vitro* is difficult to control by IF (125, 120). Nevertheless, other workers have detected significant amounts of serum IF shortly after infection *in vivo* (78, 170), and the virus does induce IF production in mouse fibroblast cultures within 4 hours (120). There is no obvious correlation of IF production with virus strain, dosage, or route of administration.

When positive results were obtained, they indicated a rapid response to virus infection. Thus KELSEY *et al.* (78) detected serum IF 12 hours p.i. which reached a peak at 36 hours p.i. and then decreased to zero by 4 days p.i. The virus titer in the spleen continued to increase for some days after this. More recently TARR *et al.* (170) reported that serum IF peaked around 2—3 days p.i., decreased over the next few days and was followed by a second burst of IF, which finally diminished to zero by 11 days p.i. These data do support the concept of a role for IF in controlling MCMV infection. On the other hand other workers (163, 3) failed to demonstrate directly a protective role for IF i.e. by inoculation of serum into mice prior to MCMV. Clearly the problem of serum IF is controversial at present, and its resolution requires further analysis.

Other factors need to be considered too. Most of the discussion has focussed on the classical type 1 IF, which may not be relevant to the control of MCMV-induced histo-pathology. In this connection RYTEL and HOOKS (145) reported that spleen cells, taken from mice 7 or 14 days p.i. produced IF (presumed type II) in response to PHA and MCMV-antigens. This parameter should be compared with other parameters of infection. It is interesting that in this study the spleen cell IF response to viral antigens was good, while the IF response to PHA was poor compared to uninfected cultures. This finding correlates with the observations of reduced responses of infected mice to IF-inducers *in vivo* (125, 168), and illustrates

the fact that infected mice can still respond efficiently to the specific virus while being in a general state of 'immunological unresponsiveness'.

But do the mice respond efficiently to MCMV? In spite of the early production of antibody and IF, the visceral organs still continue to replicate the virus and suffer consequent damage. Perhaps these early responses serve only to control dissemination of the virus.

Serum Hyporeactivity Factor (SHF)

This term was coined by STRINGFELLOW *et al.* (168) to explain the state of relative unresponsiveness of MCMV-infected mice to IF-inducers. The effect is temporary *in vivo* and has been attributed to a factor in the serum which interferes with the *in vitro* IF assay. Other viruses apparently give rise to SHF also, although MCMV seems to be the most proficient.

The presence of such a factor could explain the fluctuation in serum IF levels (i.e. as a reflection of a negative feedback loop), and in fact TARR *et al.* (170) have constructed curves which correlate the kinetics of serum IF and SHF production in MCMV-infected mice. These workers also attempted to separate IF and SHF by different kinds of column chromatography, with partial success. This is an intriguing development and further study of the nature of purified SHF, and its relationship to IF, may provide some insight into the relevance of IF to natural infections.

T-Cell Responses

A role for T-cells was indicated by STARR and ALLISON (163), who found that the transfer of syngeneic T-cells from immunized mice conferred protection to recipients from a lethal dose of MCMV, and diminished the severity of liver parenchymal necrosis.

As early as two days p.i. a class of T-cells was detected which transformed in response to MCMV-antigens (77, 62). This population of cells increased to a peak around 15 days, then decreased gradually, although they were still detectable 75 days p.i. In addition QUINNAN *et al.* (133) reported the presence of cytotoxic T-cells which were virus-specific and H-2 restricted (like several other viruses investigated recently). This population appeared only after the peak of virus replication in the spleen, peaked around 10 days p.i. and decreased to undetectable levels by day 30. The relationship of these two cell populations is not yet known, but in any case it is evident that MCMV infection does give rise to T-cell responses similar to those encountered in other virus infections, and their kinetics suggests that they could be important in eliminating antigen-bearing cells in many tissues.

Macrophages

The involment of macrophages has been discussed already. In the present context it is sufficient to reiterate that macrophages may well serve as a kind of filtering device to sequester virus particles entering tissues via the blood, and hence to reduce the chances of contact with other cell types (105, 91). A possible problem is raised by the finding that MCMV may be able to persist in macrophages (67, 91). Such cells *in vivo* could seed virus into the tissue after recovery of the animal from the primary infection, unless they were eliminated by CMI. This remains to be tested.

Recovery in Submaxillary Glands

The previous discussion in this section evidently applies to tissues such as liver and spleen in which the virus infected cells are accessible to blood-borne cells and factors. In many of the tissues, however, the specific cells which replicate the virus are physically separated from the blood and connective tissue by a basement membrane. The question then arises: how are these infected cells destroyed, whilst sparing the overall integrity of the tissue? HENSON and colleagues (55, 56) have attempted to answer this question by electron microscopic analysis of submaxillary glands removed at various times after infection. In this tissue virus replication is restricted to acinar cells and high yields of virus can be maintained for weeks, long after the cessation of replication in visceral organs (see above). These workers concluded from their studies that termination of the acute infection was eventually brought about by an inflammatory response (composed of infiltrating lymphocytes and monocytes/macrophages) which gave rise to a cytotoxic factor that destroyed the infected cells and adjacent 'normal' cells, without perturbing the basement membrane. The reason why some strains of mouse have prolonged submaxillary gland infections may reflect their relatively inefficient inflammatory response. The cells involved in cytolysis may represent one of the T-cell populations mentioned above.

Persistent Infections and Reactivation

In vivo

Cytomegaloviruses commonly persist in their natural hosts as latent infections, from which they can be activated by various manipulations, although the control mechanisms are not understood (175, 131). The murine cytomegalovirus (MCMV) has been the object of most of the experimental studies to date, and these are summarized in Table 4. The relevance of the immune system for maintaining the latent infection *in vivo* has been implicated by the studies of GARDNER *et al.* (40) who activated the virus in healthy wild mice by inoculation of anti-theta serum; by MAYO *et al.* (99) and JORDAN *et al.* (72), both groups demonstrating reactivation of virus in previously infected mice subjected to immunosuppression; and by CHEUNG and LANG (24), who caused reactivation by intraperitoneal inoculation of allogeneic blood. Thus the murine virus is analogous to the human cytomegalovirus, which is frequently activated in patients receiving multiple blood transfusions or immunosuppressive therapy (175).

Table 4. *Reactivation of MCMV*

Animal/tissue	Reactivation stimulus	Reference
Wild mice	Anti-theta serum	40
Laboratory mice	Mock blood transfusion	24
Laboratory mice	Cyclophosphamide	99
Laboratory mice	Anti-lymphocyte serum + corticosteroid	72
Laboratory mice	Graft rejection	181
Spleen, thymus	Cultivation with fibroblasts *in vitro*	58, 67, 122
Salivary gland, prostate	Cultivation with fibroblasts *in vitro*	25

It is not known whether these manipulations work because they activate virus within lymphoid cells, or because they suppress lymphoid cells involved in controlling the virus in other tissues.

The virus can be activated from several nonlymphoid tissues by cultivation *in vitro* with fibroblasts (58, 122, 25, 67). The source of fibroblasts in these instances was either mouse embryonic tissue or the explanted tissue itself. Mouse cytomegalovirus replicates readily in embryonic or adult murine fibroblasts as long as they are dividing (112, 115). Conceivably the virus could be harboured in a latent form in non-dividing fibroblasts or other cells *in vivo*, from which it could be reactivated by the stimulus of explantation.

It was reported by BRODSKY and ROWE in 1958 that the virus could be excreted into the mouth for a year after the initial infection (13). Later studies indicated that this was due to a chronic infection of the salivary glands, which in certain strains of mice was inefficiently terminated (55). The point to emphasize here is that virus can still be produced, in low yields, for many months after the complete disappearance of any sign of infection in visceral organs. Probably other tissues can also give rise to a chronic infection (100). Nevertheless, explantation of spleens and other lymphoid tissues from such mice occasionally resulted in reactivation of the virus (58, 122). This suggests that MCMV can indeed persist in a non-productive state in some kind of lymphoid cell. Since the tissue at the time of explantation was free of infectious virus, it seems reasonable to refer to this situation as a latent infection.

The experimental systems mentioned above have not given any real clues to the identity of the cell types harbouring the persistent MCMV, nor to the mechanisms involved, apart from implicating elements of the immune system. Nevertheless, further study along these lines is desirable because of their relevance to corresponding HCMV reactivations. Thus the mock transfusion system (24) shows similarities to the results of multiple blood transfusion in humans, while immunosuppressive therapy of chronically infected mice (99, 72) obviously relates to the human condition. Graft rejection in mice also led to dissemination of MCMV (181).

It appears that many kinds of perturbation of the immune system can result in reactivation of CMVs in humans and in mice, and at this time it seems premature to speculate on the precise events that must be taking place.

In vitro

Because of the complex nature of the reactivation process *in vivo*, we decided to try and set up model systems for the study of MCMV persistence and reactivation *in vitro*. Spleen cultures, infected *in vitro*, served as one model. The salient features of this model have been summarized above. There are obvious limitations in this kind of study, not the least of which is the fact that a static suspension culture of lymphoid cells in artificial conditions is hardly representative of an intact organ with dynamic cell populations. In spite of this, we have been able to demonstrate the importance of the macrophage in restricting virus spread, and the possible role of this cell type in harbouring persistent viral genomes (91). At the same time the role of lymphocytes has been questioned. In contrast to other virus infections, it is not clear at present that CMV's do rely upon lymphocytes for replication or persistence. But of course the situation could be different *in vivo*.

The second model relates to the ability of MCMV to persist in a non-replicating state in fibroblasts (116).

Cultures of murine 3T3 fibroblasts, maintained in the Go-phase by deprivation of serum growth factors, do not support MCMV replication. The titer of infectious virus decreases over a period of several days. In contrast the number of infectious centers (I.C.), enumerated by plating washed intact cells onto mouse embryo cultures, remains high. Thus a significant fraction of the cell population retains a viable viral genome, which can be induced to replicate upon provision of the appropriate stimulus. The level of infectious centers remains high as long as the cultures remain healthy. If the infected culture is supplied with fresh medium and serum, then the virus replicates and progeny virions appear in the medium (fresh medium alone has no effect).

Further analysis of this system revealed the following features: i) MCMV-DNA can only replicate in S-phase cells; ii) transcription in infected Go-cells occurred only from 16—19 percent of the viral genome, in contrast to about 40 percent in S-phase cells; iii) the transcripts in Go-cells constituted only a single abundance class, compared with two classes in S-phase cells; iv) only five viral polypeptides could be detected in the Go-cells (none of which were virion proteins), whereas productive infection is accompanied by 8 and 38 viral polypeptides early and late during infection respectively.

The relevance of this model system lies in the fact that the majority of cells in the body, including fibroblasts, are in a Go-phase (4). Therefore fibroblasts in various connective tissues might contain persistent viral genomes, which can then be reactivated by contact with dividing cells, or under conditions which stimulate fibroblast proliferation.

If the virus does persist in other cell types *in vivo*, then appropriate *in vitro* models should be examined. Consideration should also be given to the use of 'organ cultures', such as tracheal ring cultures (96). Such cultures may retain elements of control which are lost during dissociation of tissues into single cell suspensions.

Transmission of CMV to Offspring

The Magnitude of the Problem

The human cytomegalovirus is considered to be the most frequent congenital virus infection, and the commonest microbial cause of mental retardation in infants (175, 49, 16, 131, 50).

Studies in several countries have indicated that at least one percent of all live births are accompanied by active HCMV infection (ie. infection as diagnosed by the presence of virus in urine or of IgM antibody). Of these, one in ten to twenty shows clinical symptoms involving CNS and sometimes other sites. Furthermore, it has been reported more recently that newborns with subclinical infections may develop auditory- or learning-disabilities later (51, 160).

Most adults carry the virus as a persistent infection, regardless of the presence of circulating antibody (74, 161). As a consequence most pregnant women are immune but many of these (10—28 percent) secrete the virus into the cervix (119,

110, 159, 150). Thus many newborns are exposed to the virus at the time of delivery and may acquire infection at this time (134.) In addition the virus has been implicated in pregnancy wastage (6, 85).

The possible routes of transmission of HCMV to the offspring are: i) vertical via germ line cells; ii) transplacental to the developing fetus (30); iii) perinatal via cervical secretions; iv) post-natal via breast-milk; v) post-natal by exogenous infection. The presence of the virus in semen (87, 97), sperm (128), cervical secretions (119, 110, 159) and colostrum (52) has been documented.

Needless to say, a suitable animal model would be invaluable to study the relative importance of these transmission routes and the factors which are involved (80).

Among the animal CMV's, evidence has been presented for transplacental transmission of IBRV (79), porcine CMV (35) and guinea-pig CMV (26, 86). The latter system should be well worth extending because of the similarities between human and guinea-pig placental structure, although the virus itself has not been well characterized.

Several workers have attempted to demonstrate transmission of MCMV to offspring, and these studies will now be discussed.

Transmission of MCMV

Infection by MCMV can lead to decreased litter size and concomitant increases in the frequency of abortions (95, 100a, 70, 117, 22). Since infectious virus was never isolated from aborted or viable fetuses, it was generally presumed that the viral genome did not cross the placenta, and therefore fetal death was probably due to nutritional deprivation.

There are compelling reasons for believing this explanation. Thus the mouse placenta is often regarded as a greater obstacle to a virus particle than is the human placenta, due to the larger number of trophoblast layers in the former. The significance of this factor is questionable, however, since LCMV and polyoma virus readily cross the mouse placenta (104, 93). A more convincing argument is the fact that MCMV replicates in proliferating mouse fetal tissues *in vivo* and *in vitro* (70, 117a) and therefore if the virus were transmitted by this route its presence should be manifest as infectious virus particles. This argument is also invalid in view of our recent demonstration of transmission of the viral genome from mother to offspring (22). Viral DNA and antigens could be detected in a few cells from explanted viable embryos, although viral CPE was not evident until the explants had been passaged several times *in vitro*. Thus it is possible that some embryonic cells carry latent MCMV genomes.

These results do not indicate which of the proposed routes of transmission occurs in mice. True vertical transfer via germ line cells is a possibility, since latent viral genomes have been detected, by nucleic acid hybridization analysis, in ovaries and testes of MCMV-infected mice (12). Furthermore, Young *et al.* (183) were able to transmit MCMV to the fetus by inoculating a mixture of sperm and virus. Our studies are compatible with this route, or a transplacental route. Since the virus has a tendency to persist in different tissues, then reactivation in the cervical area, or even in mammary glands, followed by infection of the newborn,

is an additional possibility. It is important to investigate these routes in more detail, and to determine whether the surviving progeny suffer in any way as a consequence of persistent virus. Thus I believe that MCMV does provide a useful model for the study of CMV transmission to offspring.

Concluding Remarks

It should be evident from the foregoing discussion that MCMV can provide us with promising models with which to study various aspects of viral pathogenesis and persistent infections. Some of the more interesting and productive possibilities are the following: i) the molecular basis for adaptation to growth in heterologous host cells; ii) the molecular basis of the phenomenon of rapid attenuation of MCMV and its reversal; iii) the genetic basis of the variation in histo-pathology; iv) the mechanism of immunosuppression; v) the controlling factors involved in the establishment and maintenance of persistent infections in various tissues; vi) the routes of transmission of MCMV to offspring and the consequences.

In most of these areas a combination of *in vivo* and *in vitro* studies is required. These studies should, however, utilize genetically characterized inbred mice, and virus strains with documented histories. Whatever the results of these studies, they are bound to be relevant to human CMV infections, and possibly to other herpes infections as well.

Acknowledgments

The studies performed in this laboratory were supported financially by the Medical Research Council, Ottawa.

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Received June 11, 1979