

Cytomegalovirus Replication and the Host Immune Response¹

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Cytomegalovirus (CMV) is closely associated with host cellular structures, and this has a significant impact upon the immunologic response following infection. CMV may be recovered from a variety of body secretions and fluids during acute infection, and protracted shedding may supervene in some instances. The reasons for a variable host response to CMV infection remain unclear, and the mechanisms responsible for the establishment of persistence have not been worked out. CMV persistence and latency are discussed, and some recently derived relevant data are presented. An animal model has been developed consistent with clinical observations pertaining to CMV transmission with blood. Results obtained in the course of these and other studies support the concept of immunological activation of latent CMV. The timing of CMV infection relative to an unrelated antigenic challenge is probably critical in determining the emergence of immunodepression or enhancement. Some aspects of CMV sero-diagnosis are also reviewed.

INTRODUCTION

It is almost 20 years since cytomegalovirus (CMV) was first isolated independently by Smith, Rowe, and Weller; and yet, rigorous techniques have only recently been widely applied to the study of this virus (or these viruses). The epidemiology of CMV infections is uncertain and at least variable in different populations. The host response to infection is poorly defined; the immunologic response is often variable and confusing. The responsibility of CMV for production of disease, apparently of importance in some special groups, is only partially comprehended. The basic biology of the virus and its impact upon cells seems to be particularly important in light of recent evidence of the oncogenic potential of CMV (1). And yet, while we are still in this state of uncertainty, strains of CMV are being fed and injected into human volunteers in an effort to devise a vaccine (2).

Clearly under these circumstances there is a pressing need to derive data relating to CMV replication, the frequency and effect of the establishment of persistence and latency, the means of virus activation, the timing and nature of the immune response, and the relevance to malformations and disease (including late manifestations) of virus cytopathology and the immunologic response of the host.

The very close association of CMV with host cells, indeed, the derivation of some viral surfaces from nuclear and cytoplasmic membranes, certainly contributes to the ambiguity of the immunologic response. Like many ancient parasites, CMV is, in its natural state, a well adapted commensal; infections probably result in recognizable syndromes and disease only in the presence of host immunologic deficiencies or in situations where the acquisition of infection is delayed and the normal evolutionarily

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derived patterns of host/parasite interaction are interrupted. Ironically, these circumstances most frequently pertain in our own Western society where the processes of civilization have been accompanied by vastly altered patterns of hygiene and social interaction; where prolonged survival is facilitated in the face of debilitating disease and where sophisticated medical techniques have been developed including transfusion, extracorporeal perfusion, deliberate immunosuppression, organ transplantation, lengthy surgical procedures, and anesthesia.

It is our purpose to outline the current understanding of CMV replication, latency, and activation as well as the nature of the immunological response to this virus. Where specific evidence is lacking in man, we rely upon data derived from related systems in experimental hosts. Where appropriate, some testable hypotheses are proposed. Since this represents a status report and not an exhaustive review, the references cited are selective and not inclusive.

CYTOMEGALOVIRUS REPLICATION

The cytomegaloviruses are characterized by a relatively slow cycle of replication, by species specificity (though a few exceptions have been described) by the variable, occasionally sparse production *in vitro* of cell-free virus, and (like other herpes viruses) by the capacity to establish persistent and latent infections. *In vivo* and *in vitro* infected cells enlarge and develop sizable intranuclear inclusions and characteristic cytoplasmic bodies. Viral DNA and antigens are assembled into whole virions within the nucleus. Thereafter, the capsids (with and without dense cores) bud

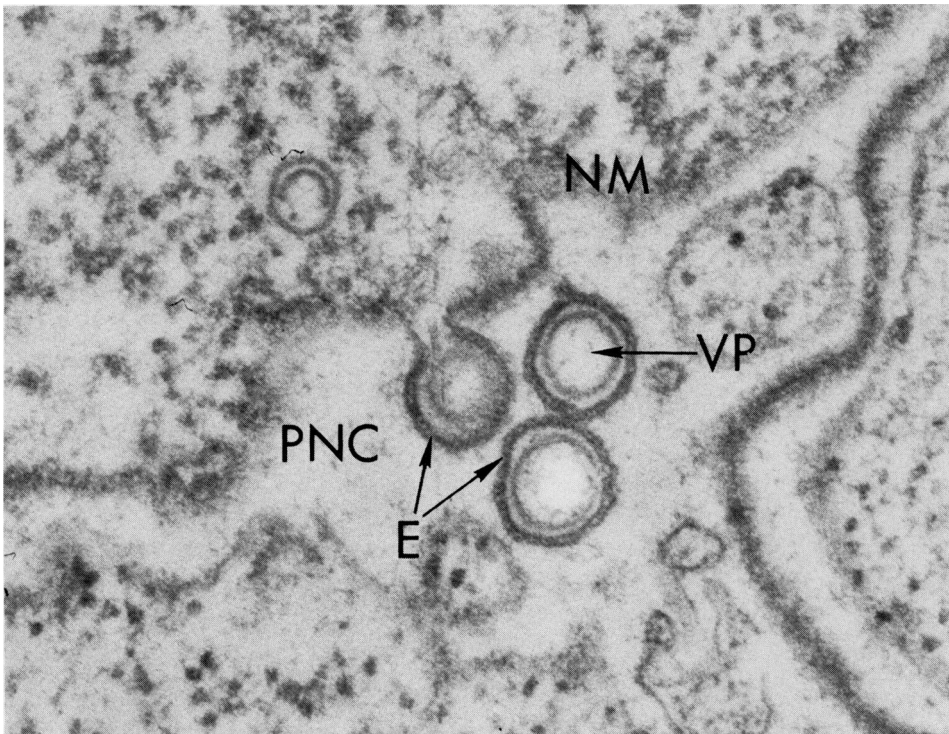


FIG. 1. Murine cytomegalovirus (VP) budding from the nuclear membrane (NM) into the dilated perinuclear cisternae (PNC) and receiving its outer membranous envelope (E) (Uranyl magnesium acetate and lead citrate, X114,000). Reprinted with permission of the *American Journal of Pathology* (from Schwartz *et al.*, 51).

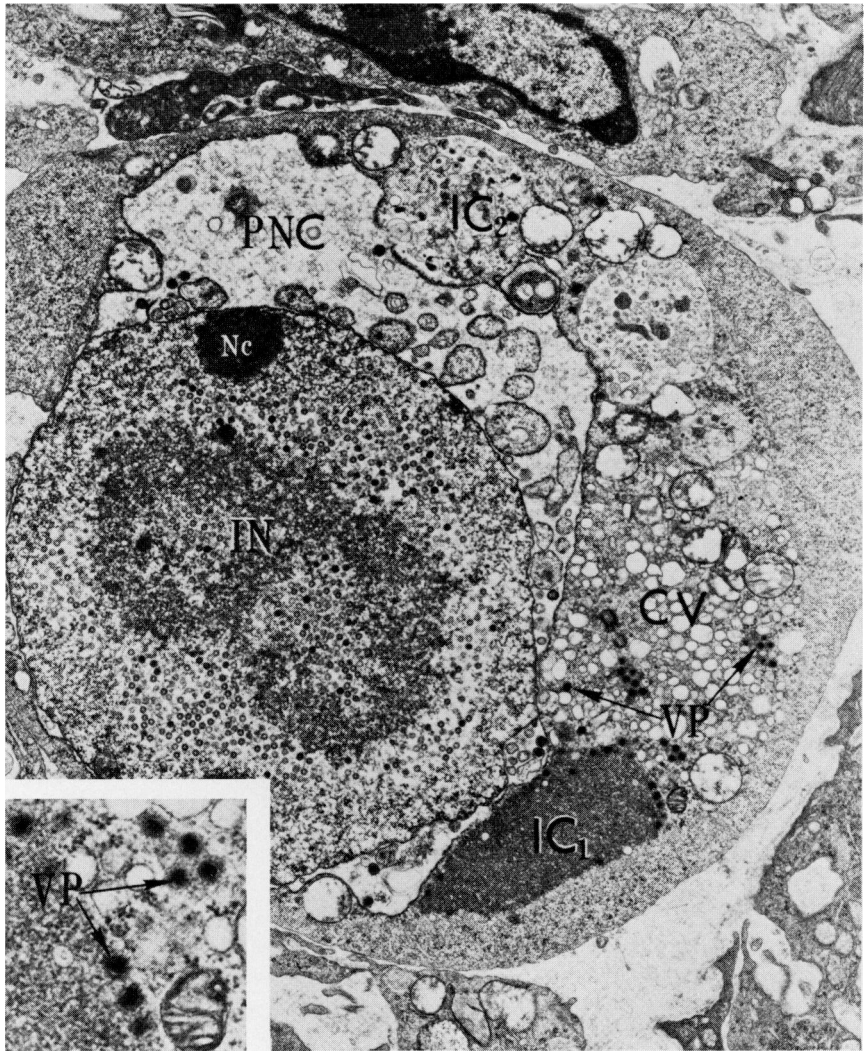


FIG. 2. A cytomegalic cell in the anterior portion of a mouse eye infected intraocularly with murine cytomegalovirus. The nucleus contains peripherally marginated nucleoli (Nc), and a centrally located accumulation of chromatin (intranuclear inclusion, IN) and viral particles (VP). Many of the viral particles do not have dense nuclear cores. Note also the dilated perinuclear cisternae (PNC), cytoplasmic inclusion (IC), viral particles, and vacuoles filled with membranous debris. Inset—The viral particles are shown at a higher magnification (Uranyl magnesium acetate and lead citrate, X10,000; inset, X37,500). Reprinted with permission of the *American Journal of Pathology* (from Schwartz *et al.*, 51).

through and incorporate a bit of the nuclear membrane to acquire an envelope and enter dilated perinuclear cisternae, vacuoles, and tubules in the cytoplasm. The budding and associated acquisition of the viral envelope by a mouse CMV is depicted in Fig. 1. Some membraned virions are then shed directly to the extracellular space. A proportion of the capsids which reach the cytoplasm seem to lose their membranes and acquire new coats from various cytoplasmic surfaces. Additional empty capsids are discharged after acquiring membranes. The various possibilities for acquiring an intact core as well as appropriate or improper membranes may determine the production of a high ratio of particles to infectious virus which has been described for CMV (3). Figure 2 is an electromicrograph of a cell from the anterior portion of a

mouse eye previously infected with murine CMV. Several of the features of CMV replication described above are apparent in this photomicrograph.

Although CMV seems to replicate *in vitro* preferentially in fibroblasts, considerable evidence demonstrates that multiplication *in vivo* occurs in a variety of cells, including many of epithelial morphology. In the face of disseminated disease, CMV has been recovered from or demonstrated in cells of virtually every organ system.

The initial recoveries of CMV were made by Smith from salivary gland (4), by Rowe and co-workers from adenoids (5), and by Weller and his associates from urine (6). A significant proportion of salivary glands derived from unselected pediatric autopsies demonstrate histologic evidence of CMV infection (7). Nevertheless, very few adults shed infectious CMV in saliva (8). The recovery in certain disease states of CMV from washed leukocytes (9–11) as well as biochemical and clinical analogies to Epstein–Barr Virus (EBV) and the epidemiologic evidence suggesting transmission of CMV with blood products (12) has given impetus to the concept that CMV may persist, not only in adenoids, but in a variety of cells of lymphoid origin. And yet, as has been emphasized by extensive studies, virus is only rarely recovered from the circulating cells of healthy individuals (13–15). Viruria is usually prominent and prolonged following congenital and some postnatally acquired CMV infections. Nevertheless, surveys of nonpregnant seropositive adults reveal only rare instances of viruria (8). Recently Lang and Kummer have demonstrated the prolonged presence of CMV in semen in the absence of virus in the blood, urine, or saliva (16). Perhaps the presence of CMV in different organs or cells depends upon the strain of virus, the route of acquisition, the maturity, genetics, and/or immunologic capacity of the host.

THEORIES PERTAINING TO THE MECHANISM OF ESTABLISHMENT OF CMV PERSISTENCE AND LATENCY

1. Persistence

Viral persistence in the presence of humoral antibody is prolonged for years in most instances following congenital acquisition of CMV and often after clinically apparent infection in adults. Nevertheless, indefinite persistence must not be the rule, since active shedding of CMV is demonstrable in very few antibody-positive adults.

Persistent shedding of virus implies the inability of the host to clear virus-infected cells. This suggests the failure of host T cells to recognize and eradicate infected cells which almost certainly manifest new virus-associated surface antigens. It would seem that this failure of cellular immune function must reflect either a defect in T cell function or macrophage processing. The latter is apparently a prerequisite for the direction of T cell-mediated cytotoxicity.

If CMV replicates in or is directly or indirectly injurious to T cells, the virus might interfere with cellular immunity in this fashion. Recently, employing the murine CMV model, Schwartz and his associates have demonstrated that neonatal infection with CMV is associated with damage to T cells and can result in the virtual ablation of the neonatal mouse thymus and the depletion of lymphoid cells in the periarteriolar areas of the spleen (T cell areas) (18). Figures 3 and 4 depict the microscopic appearance of the mouse thymus at 2 weeks of age uninfected and following neonatal CMV infection. Marked depletion of cortical lymphoid cells is apparent as is the overall reduction in organ size. Mice which have recovered after CMV infection during this critical early postnatal period resemble animals which have been neonatally thymectomized, are often small, and may succumb to a variety of intercurrent infec-

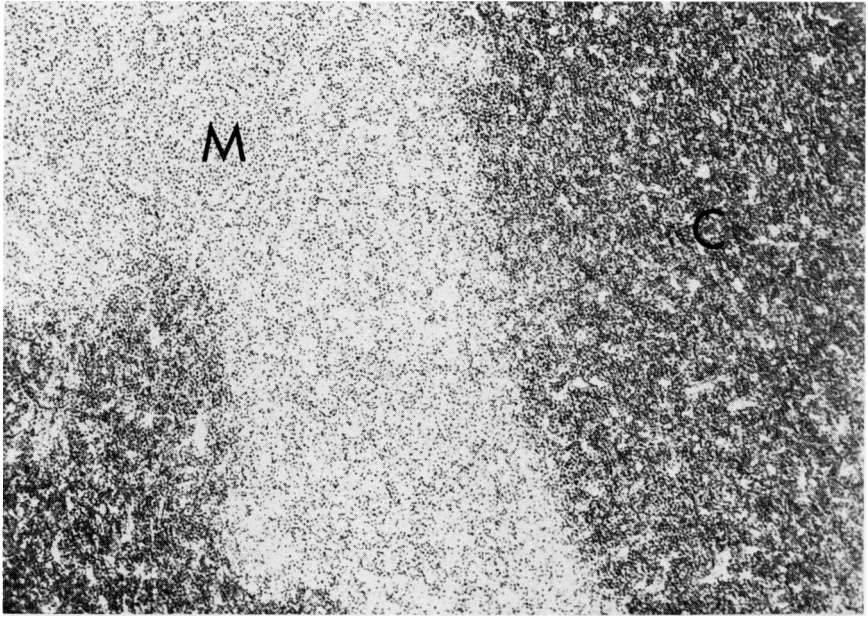


FIG. 3. A section from a normal mouse thymus seen at 2 weeks of age. There is a prominent cortex (C) tightly packed with lymphocytes. The cortex is clearly delineated from the medulla (M) which has a scattering of lymphoid cells within it (H & E, X100). Reprinted with permission of the *American Journal of Pathology* (from Schwartz *et al.*, 18).



FIG. 4. A section from a thymus of a 2-week-old mouse who was infected at birth with murine cytomegalovirus. The corticomedullary junction is indistinct, and there is a marked depletion of lymphoid cells in the cortex. Note that almost an entire thymic lobule can be seen at this magnification (H & E, X100). Reprinted with permission of the *American Journal of Pathology* (from Schwartz *et al.*, 18).

tions. Allowing for the different timing of the development of thymic and T cell maturity in man, similar or related, more selective effects of congenital CMV infection in the human fetus might be responsible for postnatal CMV persistence. This may be analogous to the variable influence upon cellular immunity of Lymphocytic Choriomeningitis (LCM) and Lactic Dehydrogenase (LDH) viruses (19). These infections also have a directly injurious effect upon thymus-dependent lymphoid tissue which, after recovery, results in virus persistence in the face of intact antibody production and unimpaired cellular immunity for other antigens.

If macrophage function is injured by CMV infection of these cells, the processing of viral antigens might be inhibited. Selgrade and Osborne have studied the pathogenesis of murine CMV infection (20) and suggest that macrophages from mouse strains of varying susceptibility to this infection facilitate the inductive phase of cellular immunity to the same extent. They speculate that heritable differences in host susceptibility to CMV infections may be primarily dependent upon lymphocyte rather than macrophage susceptibility. This is the only direct reference we shall make to the very important but scarcely studied area of genetic susceptibility to infection, persistence, and latency of CMV.

The production of antigen-antibody complexes (perhaps related to the presence of nonneutralizing antibody) may interfere with macrophage function. The existence of such complexes in connection with CMV infection has been suspected, and some direct evidence for such complexes is beginning to accumulate. Payne *et al.* (21) make reference to "CMV-immune-complex changes in glomeruli" seen in kidneys removed from one patient at pretransplant nephrectomy and again later from the same individual after graft rejection. Oldstone (22) cites unpublished observations which describe the presence (employing mouse CMV) of granular deposits of host IgG and C₃ in tissues, as well as the recovery of specific viral antibody from injured glomeruli.

Recently we were afforded the opportunity to examine a 14-week-old female infant who died due to a congenital CMV infection. Aside from the customary virologic and morphologic findings of the disseminated virus disease, interesting lesions were found in the kidney, examples of which are depicted in Fig. 5. The glomeruli were hypercellular and showed evidence of endocapillary proliferative changes. In a few of the glomeruli a marked proliferative reaction of the parietal layer of Bowman's capsule was present, reminiscent of the epithelial crescent formation that is frequently found in rapidly progressive (subacute) glomerulonephritis. Fluorescent antibody studies revealed the deposition in a segmental and granular pattern of IgG and C₃ within the glomerular tuft as is often found in immune complex glomerulonephritis. CMV-infected proximal convoluted epithelial cells also exhibited IgG and C₃ on their surfaces. Paradoxically the immune system was apparently harming this patient as well as helping her to cope with the disseminated virus disease.

The persistence of actively replicating virus, possibly suppressed by the presence of antibody, can be demonstrated in some instances by employing explant techniques. Cheung and Lang have recently presented evidence for the prolonged cyclic production of murine CMV in salivary gland explants (23). Extracts of the same glands made at the time of their original dissection were apparently virus negative.

2. Latency

It is proposed that in most instances CMV persistence is not established, either because the quantitative relationships between antibody and antigen are not optimal

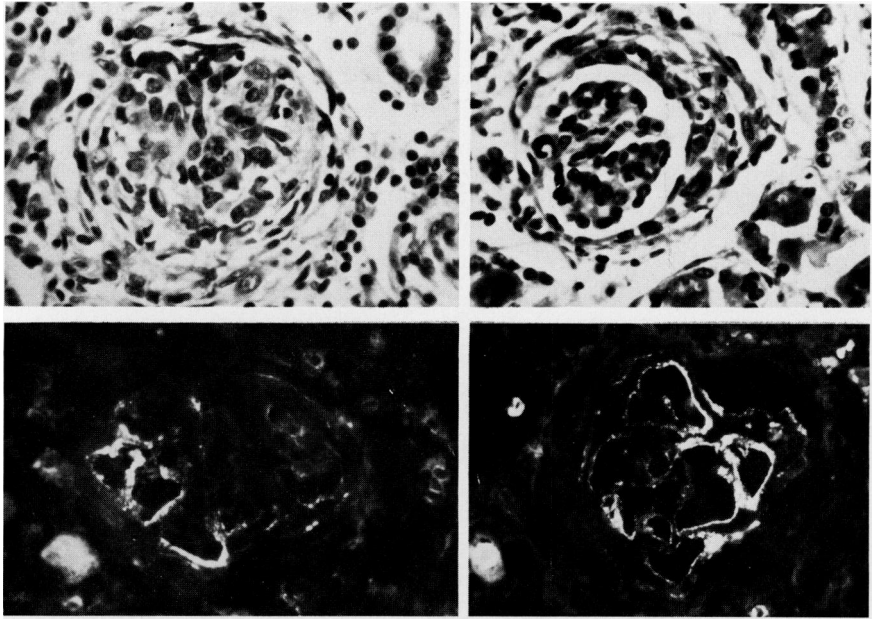


FIG. 5. Sections from the kidney of a patient with a disseminated cytomegalovirus infection. In the upper panels a marked endocapillary proliferation is seen on the left and subcapsular crescent formation is demonstrated on the right. The lower panels show granular deposits of IgG (left) and C_3 (right) within the glomerular tuft. Adjacent infected proximal tubular cells also show a diffuse deposition of these substances (X400). Fluorescent microscopy study performed by Dr. Ralph C. McCoy.

for the production of complexes, or because T cell function is not directly or indirectly impaired by CMV. In some (possibly all) other cases a latent infection is established. In this case it may be that the CMV genome is integrated with the DNA of cellular chromosomes. The analogy to EBV, a closely related virus, may be relevant. Presence of EBV genetic material has been demonstrated in nonproductive lymphoblastoid cells (24, 25), and cloning experiments support the concept that viral genetic material is present in all of the cells of a given culture; even those without demonstrable intact virus or viral antigens (26–28). The need, in order to demonstrate CMV in leukocytes, for prolonged cocultivation of these cells with sensitive feeder layers of fibroblasts is reminiscent of the means required to activate nonproductive viruses such as SV40 (29). The elegant techniques employed by Huang and Pagano (30) are beginning to provide data which will permit the evaluation of these questions as they pertain to CMV infection.

Recent observations of Cheung and Lang suggest that latency of CMV may be more widespread than has been previously suspected (23). Prolonged observations were made of explants of normal prostate and prostate derived from mice previously infected with murine CMV. No cytopathic effects were observed in either case, and the cells (which appeared morphologically identical) were passaged separately several times. When a sufficient quantity of cells from both sources had been accumulated, they were each superinfected with murine CMV in a plaque procedure. It was anticipated that residual virus might be manifest by interference. However, the number of plaques were the same in each case, but a difference in morphology was evident. Small, uniform plaques were seen in the normal prostatic cells, while larger plaques were evident in the prostate cells derived from previously infected mice. This

suggests that all of the prostate cells from the previously infected animal may carry incomplete or defective CMV, and that the superinfection succeeded in rescuing latent virus either by complementation or perhaps by supplying certain critical enzymes.

ACTIVATION OF LATENT VIRUS

Cytomegalovirus is activated in conjunction with pregnancy, transplantation and immunosuppression, perfusion and multiple transfusions, and disseminated (reticuloendothelial) malignancies (31). All of these conditions are accompanied by the presence of a homograft or homograft analogy; most by varying degrees of immunodepression. It is possible that either or both of these factors contribute to the activation of CMV.

It has been suggested that the cellular response to the presence of foreign antigens might activate CMV (15). Precedents exist for immunological activation of latent viruses. The subject has been discussed in a recent review dealing with the activation of mammalian leukemia viruses (32). Initially it appears that the activation of CMV in connection with transfusion and perfusion must relate only to the host response to a homograft (in this case leukocytes) since it seems that immunosuppression is not involved. However, some published data (33) and our own preliminary studies (34) again have clouded the issue since they indicate that perfusion, transfusion, or possibly even extended anesthesia may result in a variable depression of parameters associated with cellular immunity.

In spite of these uncertainties, information is accumulating which supports the concept of immunological activation of CMV. Wu, Dowling, Armstrong, and Ho have been able to enhance the replication of chronic murine CMV in the absence of immunosuppression with allografts of skin and lymphoid cells derived from spleen (35). Olding, Jensen, and Oldstone were also able to initiate synthesis of murine CMV with an allogenic reaction *in vitro* to lymphocytes which they characterize as B cells. Cheung, Smith, and Lang have developed a murine model for transmission and activation of CMV with blood transfusion (37). Blood from previously infected mice, virus-negative by tissue culture assay, was "transfused" intraperitoneally into uninfected allogenic and isogenic hosts. After a latent period of better than 1 month, virus was invariably detectable in the salivary glands of the allogenic recipients and only rarely in the case of isogenic recipients. Transfusion of blood from uninfected animals into previously inoculated but presently virus-negative mice was followed by activation of CMV in both heterologous and homologous recipients. This model appears to mimic the situation in man and may provide a suitable test system for definition of the virus carrier state, the impact of leukocyte depletion, and several additional problems relevant to the risks of CMV infection transmitted by blood products.

Within renal dialysis units Hepatitis B infections have presented a significant problem. The frequency of anicteric Hepatitis B infections and the establishment of the posthepatitis carrier state among dialysis patients in contrast to the unit staff and household contacts has been ascribed to the immunological inadequacies of patients with chronic renal disease (38). This environment seemed an appropriate one in which to assess the risks of CMV activation among patients with compromised immune surveillance systems in the absence of significant allografts, as well as the risk of CMV transmission to their contacts. Drs. Sexton, Gutman, Smith, and Lang have carried out a study of CMV within a hospital and home dialysis program. The data

seem to indicate that neither chronic renal disease nor the dialysis procedure serve to activate CMV.

Immunodepression may be induced by several viruses including CMV (19). However, Simmons and associates at the University of Minnesota have reported the apparent acceleration of allograft rejection in patients with preexisting evidence of CMV infection (39). Initially it appears that these observations are in conflict with the studies which demonstrate CMV-associated immunosuppression as reported in mouse systems by Osborn (40, 41), Schwartz (18), and even by Howard and co-workers (42), some of whom also collaborated with Simmons *et al.* Howard demonstrated prolongation of allograft survival after murine CMV infection and showed that as the interval increased between the initiation of the CMV infection and subsequent grafting, the duration of graft survival was diminished. When CMV infection occurred 13 days prior to skin graft (the longest interval reported), the rejection pattern approached that of the uninfected controls. Employing a similar mouse model, Hamilton, Elliott, Cheung, and Lang have been able to demonstrate that when a skin graft is applied more than 3 weeks after induction of a sublethal CMV infection, the rejection of the allograft is enhanced. At the same time the titers of the persisting CMV infection are increased. The latter effect is notable since it occurs without accompanying immunosuppression. Thus, these apparently conflicting data may be resolved by a careful analysis of the timing of the CMV infection relative to the initiation of the immunologic challenge. Initially, after the virus is introduced, direct injury may be incurred by T cells and/or macrophages which impairs the immune response. When the infection is initiated during a critical phase of T cell maturation as in the studies of Schwartz (18), the damage to cellular immune function can be protracted. CMV infection acquired later in life may be accompanied by transient T cell dysfunction, thus explaining the inverse temporal relationship between infection and immunosuppression reported by Howard (42). The studies of Hamilton (43), which are consistent with the clinical observations of Simmons (39), indicate that when grafting is delayed still longer after CMV infection, the immune surveillance mechanisms may actually be enhanced. The timing of infection relative to antigenic challenge has been shown to alter the degree of immunodepression in other systems; although in the case of Friend leukemia virus, the related immunodepression is said to become ever greater as the interval is increased between infection and administration of the antigen (19).

MEASUREMENT OF THE HUMORAL ANTIBODY RESPONSE OF THE HOST

The importance for the control of CMV infections of cellular immunity is implied by much of the information discussed in the previous sections of this report. The assessment of humoral immunity to CMV has provided most existing pertinent epidemiologic information. Thus, it is sobering to note that all of the existing tests for CMV humoral antibody possess significant drawbacks. The complement fixation (CF) test will identify antibody to most (though not all) strains of CMV. The sensitivity of this test has been repeatedly questioned. In addition the CMV CF procedure may not record the presence of IgM antibody effectively. It is certain that in some instances the CF will yield negative results in congenitally infected newborns possessing IgM antibody. Drs. Waner and Weller, with Dr. Kevy, have demonstrated that longitudinal patterns of CMV CF antibody may vary significantly (44). In 11 individuals followed for 18 months, titers changed at least once from

significant to undetectable levels. We have been able to confirm these findings in the course of the previously mentioned longitudinal study of renal dialysis patients and controls. It may be said that CMV CF titers can wane and well unpredictably!

The CMV neutralization test is less broadly reactive than the CF procedure. Weller and co-workers demonstrated CMV antigenic heterogeneity employing the neutralization test (45), and these observations have been repeatedly confirmed. The plaque technique as well as the addition of complement may sharpen and improve the sensitivity of this test, and the procedure may provide important CMV strain-related epidemiologic information if it becomes feasible to routinely produce high titered type-specific antisera in heterologous hosts.

The indirect hemagglutination (IHA) test yields results which are qualitatively comparable to those of the CF test, though the sensitivity of the IHA procedure is apparently greater than that of the CMV CF test. Some sera, negative by CF, are IHA positive. Less frequently, we have found serums which are CF positive and IHA negative. The accuracy and reproducibility of the IHA procedure depends upon the preparation of serum-free antigens, careful control of buffer pH and composition, the choice of reactive sheep erythrocytes, and effective tannic acid.

The indirect fluorescent antibody technique has also been applied to the study of CMV serology. Although this procedure has also been employed for the overall assessment of CMV antibody, its main application has been to the recognition of specific CMV macroglobulin (46). The measurement of specific IgM antibody has been used most effectively to identify congenitally infected infants as well as some postnatally acquired primary infections. This technique, originally applied to the study of CMV by Hanshaw, has been modified by him to increase the test sensitivity by prolonging the initial incubation of the serum with infected cells. In spite of improvements, the interpretation of the test is frequently subjective. A positive CMV IgM test is depicted in Fig. 6. The reaction of IgM-positive and -negative serum is evaluated with uninfected and infected cells. Although in this instance the reaction of the positive serum with the CMV infected fibroblasts stands out clearly, in other circumstances the residual fluorescence, particularly apparent with positive serum and uninfected cells, can be confusing. The nonspecific reaction of CMV antibodies with normal cellular structures may reflect the incorporation into CMV of elements of cellular membranes.

Dr. Harwood, then a graduate student in microbiology at Duke University, had as her investigative goal to apply the technology of the radioimmunoassay (RIA) to the problem of quantitatively assessing CMV antibody, ultimately hoping to accurately measure the IgM response to CMV. Initially, she worked to devise an RIA procedure for measuring IgG antibody to murine CMV (47). In our experience with the virus neutralization technique, antibodies had been detected in relatively concentrated sera (usually only if diluted less than 1:64). It seemed, therefore, that a radioimmunoassay designed to detect low antibody concentrations, such as the sequential adsorption analysis described by Day (48), would be well suited to the RIA procedure. This technique, after modification for use in the Microtiter system, had the advantage of requiring few reagents and minute quantities of ^{125}I -labeled anti-serum.

It was found that, following sequential adsorption of mouse immune serum on control cellular antigens, species-specific antibodies to mouse CMV were detectable by reacting the test serum with viral antigen prepared from virus-infected cells or virus-infected salivary gland suspension and then adding ^{125}I -labeled goat antiserum

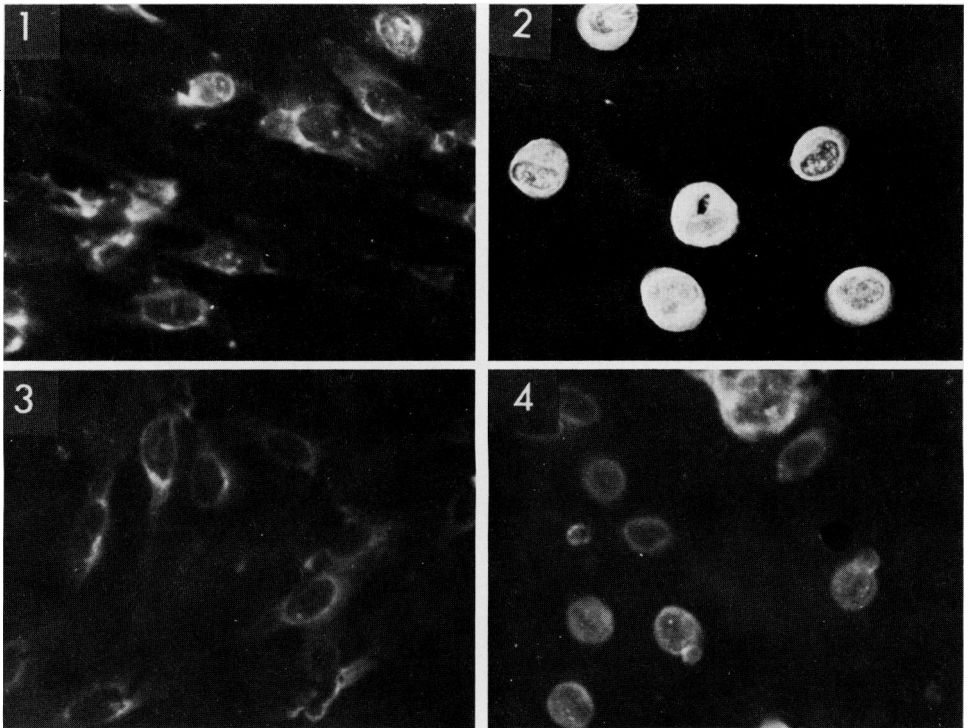


FIG. 6. Indirect fluorescent antibody demonstration of CMV-specific IgM antibody. IgM positive serum on (1) uninfected and (2) infected cells. IgM negative serum on (3) uninfected and (4) infected cells. (Reprinted from thesis of S. E. Harwood, 47).

against mouse immunoglobulin. Although nonspecific binding of both normal and test serum did occur, this nonspecificity was substantially reduced by sequential adsorption. Although it was not possible to completely eliminate the binding of antibodies to control antigen, the relative increase in binding seen when viral antigens were used made antibody activity easily detectable. Further exploitation of this and other RIA procedures may be fruitful. Knez *et al.* have recently reported the development of an RIA technique for the assessment of IgM antibody to CMV (49).

Furukawa, working with Dr. Plotkin, has found that the humoral immune response to CMV may also be detectable in a precipitin test (50). Although two common precipitin lines were detectable using several strains of CMV, some strain differences were recognized and it was felt that this procedure might have applicability to the delineation of CMV variants. In general, the precipitin antibody response following acute infections seemed to parallel the CMV antibody response as measured by CF and IHA. Most precipitin tests have the disadvantage of being relatively insensitive, but it may be that with the application of countercurrent immunoelectrophoretic techniques the sensitivity of this procedure can be enhanced.

There is still a considerable need to sharpen the techniques applicable to assessing the humoral immune response to CMV. Many of the problems experienced in defining the epidemiology of infections caused by this virus can be related to confusing or inadequate procedures. There is a need to further apply techniques such as membrane fluorescence used with success in the study of EBV. In addition it would seem appropriate to extract CMV core antigens in order to test for core antibody, an

approach which has been fruitful in the investigation of Hepatitis B infections. The preparation of core antigens may also facilitate the study of CMV subtypes.

CONCLUSION

This past decade has seen the gradual recognition of the importance of CMV as an opportunistic invader and as the transmissible agent most frequently responsible for congenital infection and injury (at least in the United States and Western Europe). We remain reasonably certain that CMV can be transmitted with and activated by blood transfusion. Employing mouse models, it has been possible to reproduce many of the virus-related findings which have been reported in association with cardiopulmonary bypass perfusion and massive transfusion. These model systems may enable us to more accurately define the carrier state and to determine whether component transfusion will solve this problem. As a matter of fact, it may be possible to solve this and several related clinical problems before we possess an entirely satisfactory understanding of the close and complex virus-host relationships pertaining to CMV. Elucidating these relationships will still be of considerable importance, indeed perhaps a prerequisite, for the development of a vaccine and in order to understand the pathogenesis and significance of CMV persistence, latency, and activation.

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