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Editorials

Human cytomegalovirus infection

Cytomegalovirus comes to the attention of the respiratory physician when it causes pneumonitis in immunosuppressed patients. Before we discuss this and other clinical manifestations of cytomegalovirus infection it is worth reviewing pertinent aspects of the molecular virology, and of the biology of cytomegalovirus in the normal host. As with all microbial agents, this basic information is necessary to the understanding of pathogenesis in molecular terms.

Virology and immunology

Herpesviruses are double stranded DNA viruses and cytomegalovirus is the largest (235 kilobases of DNA). The complete nucleotide sequencing of the virus will soon be achieved (probably the largest contiguous piece of DNA to have been sequenced so far). This will enable the structure of the viral proteins to be predicted from open reading frames in the sequence—some 200 proteins are predicted. As with other herpesviruses, the genes encoding these proteins are expressed in three sequential phases designated immediate early, early, and late. The immediate early and early cytomegalovirus genes code for "non-structural" proteins—that is, they are not components of the virus particle and are detectable only in infected cells. The late gene products are mainly structural proteins of the virus. The mechanisms that regulate cytomegalovirus gene expression are complex, not fully elucidated, and beyond the scope of this review. Control of cytomegalovirus gene transcription depends both on regulatory factors provided by the cell (such as various types of DNA binding proteins, which may be present only in certain cells or at particular stages of the cell cycle) and also on the viral gene products themselves—the products of one class of genes turning on transcription of the next[1] (figure).

The sites where cytomegalovirus persists in normal virus carriers are uncertain, but a small fraction of peripheral blood mononuclear cells has been shown to contain viral DNA by the technique of in situ hybridisation—of the order of one in 1000–10 000 cells—and these are probably monocytes.[2] Cytomegalovirus may well persist at other sites but these await discovery. The state of the viral genome during persistence in the normal individual is also unknown. Herpesviruses in general do not integrate their DNA into the host's chromosomal DNA, but persist as free genomic DNA. Whether expression of only a limited set of viral genes is needed to maintain persistence or whether a continual low level of viral replication occurs in normal carriers is similarly unclear.

The epidemiology of cytomegalovirus has been assessed by standard seroepidemiological techniques, the presence of antibody being taken to indicate carriage of the virus. There is a steadily increasing prevalence of seropositivity with increasing age, so that something over half the adults in developed countries are seropositive. The prevalence rises to virtually 100% in certain groups, such as homosexual men. If anything, the use of antibody as a marker of infection is likely to underestimate the prevalence of virus carriage. Although not yet applied to cytomegalovirus infection, sensitive techniques such as the polymerase chain reaction, which allow the detection of very small amounts of viral DNA, show evidence of persistent virus infection even in antibody negative subjects.[3] Cytomegalovirus may be excreted in saliva, breast milk, urine, and semen and cervical secretions, so that infection may be transmitted via saliva (the likely predominant method of spread in young children) and sexual contact. Cytomegalovirus is, of course, also transmitted by blood (possibly in infected monocytes) and organ allografts.

The immunocompetent host is unlikely to experience any recognisable clinical problems from the carriage of cytomegalovirus. As with other persistent viruses, the host T lymphocyte response appears to be the principal immune effector mechanism maintaining this normal equilibrium between host and virus. The role of cytotoxic T lymphocytes, which are capable of specifically killing virus infected cells, has been studied most. In a mouse model (mouse cytomegalovirus is a different virus from human cytomegalovirus but is biologically similar) cytotoxic T lymphocytes clearly protect against lethal infection.[4] For human cytomegalovirus it has been shown that normal carriers have a rather high frequency of memory cytotoxic T lymphocytes (of the CD8 phenotype) specific for cytomegalovirus in their peripheral blood.[5] Cytotoxic T lymphocytes are known to recognise virus infected cells by virtue of the T cell receptor (and other associated recognition molecules) on the T cell surface binding to a complex between the class I HLA molecules and viral proteins on the infected cell

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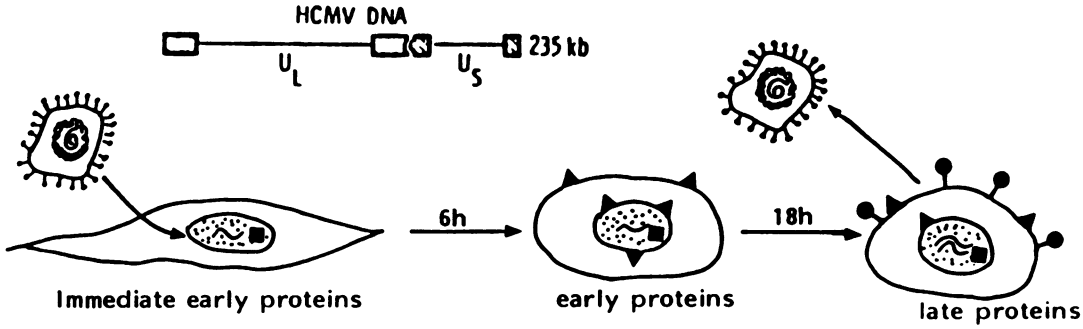

Schematic summary of the transcriptional programme of human cytomegalovirus. The virus genes are expressed in a temporally regulated sequence (see text).

surface. The question of which particular cytomegalovirus proteins are recognised by cytotoxic T lymphocytes has been studied by using recombinant DNA techniques to express isolated cytomegalovirus genes. The immediate early proteins appear to be the predominant proteins recognised,[6] and this is also the case in the mouse cytomegalovirus model. The apparent paradox of how cytotoxic T lymphocytes recognise immediate early proteins on the cell surface even though, as stated earlier, they are located in the nucleus, is readily explained. It is now clear that small peptide fragments derived from viral proteins (rather than the intact proteins) bind to specific regions of the HLA molecules during the intracellular processing and insertion of the HLA molecules into the cell membrane (see ref 7 for further details).

Thus the evidence suggests that the predominant class of viral gene products expressed during persistence of the virus are the same proteins that are recognised by the host's main immunological defence mechanism. This seems likely to provide a mechanism for containing the virus and preventing its dissemination.

Clinical aspects of cytomegalovirus

The clinical problems associated with cytomegalovirus vary according to the host. *Intrauterine infection* (and to a lesser extent perinatal or neonatal infection) may be associated with developmental abnormalities and mental impairment—particularly if the mother is primarily infected during pregnancy.[8] In the *normal immunocompetent person* primary infection may be associated with a syndrome very like infectious mononucleosis (although with a negative result in the monospot test), but it usually goes undetected and clinically significant reactivation is very rare. There have been suggestions that cytomegalovirus may cause several diseases of unknown aetiology, particularly Guillain-Barré syndrome and more recently insulin dependent diabetes.[9] Although of interest, these postulated associations lack convincing proof, and again are not considered further.

In contrast, cytomegalovirus infection in the *immunocompromised host* may cause severe problems. In iatrogenically immunosuppressed patients, particularly those receiving kidney, heart, lung, liver, or marrow transplants, *reactivation* of cytomegalovirus if the patients are already carrying it is common. Frequently this becomes manifest only as excretion of cytomegalovirus in the urine or oropharynx and is not associated with clinical problems, or only with fever, although it may be associated with organ disease as outlined below. *Primary infection* in immunosuppressed allograft recipients is much more likely to result in clinically significant cytomegalovirus induced disease. It has also been suggested, on the basis of restriction enzyme analysis of clinical isolates, that *reinfection* of seropositive recipients of transplants with a different strain of cytomegalovirus may occur and may be associated with more severe disease than reactivation.[10] Cytomegalovirus disease in these patients may take the form of pneumonitis, hepatitis, colitis, oesophagitis, and possibly upper gastrointestinal tract ulceration, and, rarely in this group, retinitis and encephalitis. Cytomegalovirus pneumonitis is clinically indistinguishable from pneumonitis produced by other "opportunist" pathogens in immunosuppressed patients, and is characterised by bilateral interstitial infiltrates on the chest radiograph with associated hypoxaemia. The histopathology is of an interstitial pneumonitis with infiltrating inflammatory cells, together with the distinguishing histological feature of cytomegalovirus infection (from which it was named), the "owl's eye" nuclear inclusions in enlarged (cytomegalic) cells. Cyto-

megalovirus pneumonia is frequently progressive and fatal. For example, in the Seattle bone marrow transplant programme the incidence of cytomegalovirus pneumonia was 17%, with an 85% mortality.[11] Indeed, cytomegalovirus infection produces more problems overall in those with bone marrow transplants than in recipients of other transplants, being the most common infective cause of death and having a rather characteristic peak incidence of around 60 days after transplantation.

Cytomegalovirus is also a major cause of morbidity in patients with the acquired immunodeficiency syndrome (AIDS). Here, however, the cytomegalovirus disease is nearly always associated with reactivation rather than primary infection, as the major populations at risk of AIDS (homosexual men and intravenous drug abusers) are nearly all virus carriers. The frequency of organ infection is rather different from that in recipients of allografts, with gut disease, retinitis, encephalitis, and adrenalitis being more frequent—for instance, clinical retinitis occurs in about 10% of patients with AIDS and nearly always leads to loss of sight if untreated. Pneumonitis clearly related to cytomegalovirus is much less common and although the virus may be isolated from the lung it is usually associated with other opportunist organisms, *Pneumocystis carinii* in particular.[12]

How does cytomegalovirus disease arise in those who are immunosuppressed? Patients are given immunosuppressive regimens designed to suppress the T cell responses concerned in graft rejection, but the same classes of effector T cells also provide surveillance of virus infected cells. The logical assumption is that inability to generate an effective T cell response is responsible for the uncontrolled dissemination of cytomegalovirus infection. This may well be true. It has been shown that there is a diminished frequency of Epstein-Barr virus specific T cells in recipients of renal transplants, and it was reported some time ago that the recovery of recipients of bone marrow transplants from cytomegalovirus disease was associated with the presence of cytomegalovirus specific cytotoxic T cells in their peripheral blood.[13] Of itself, however, this impairment of T cell responses is probably too simplistic as a sole explanation. It has, for instance been suggested that cytomegalovirus pneumonitis may be immunopathologically mediated, the lung injury actually being produced by T cells attacking infected cells. This could conceivably explain the low incidence of pneumonitis in AIDS patients.[14] Although there are well established animal models of tissue injury produced by the immune response against other viruses, there is as yet no firm evidence for this in the case of human cytomegalovirus. A major puzzle is that, although cytomegalovirus productively infects a limited number of cell types in vitro, and its cellular site of persistence is uncertain, the virus is present in many different cell types in different organs in patients dying of cytomegalovirus disease.[15] The reason for this is not clear, but it could imply that certain cells in recipients of transplants may provide whatever factors are required for cytomegalovirus transcription more effectively than cells in normal patients.

The relation of cytomegalovirus infection to graft rejection in recipients of kidney transplants has aroused interest. It has been suggested that cytomegalovirus may increase the risk of graft rejection, though not all studies have shown such an association.[16] One recent study, using in situ hybridisation to analyse the site of cytomegalovirus infection in the transplanted kidney, found that cytomegalovirus positive cells were present almost exclusively in the interstitium and that these were probably infiltrating mononuclear cells.[17] The possibility that graft versus host disease in recipients of bone marrow transplants may be associated with cytomegalovirus infection has also been suggested. In the Seattle series graft versus host disease tended to precede cytomegalovirus disease and to be a risk factor for it but not vice versa.[11] In the context of these suggested relationships between cytomegalovirus and aspects of transplantation immunology, it is of interest that the cytomegalovirus genome encodes a predicted protein with striking homology to class I major histocompatibility molecules, although its function in the virus is as yet unknown.[18]

Diagnosis

VIRUS ISOLATION

Cytomegalovirus may be isolated in tissue culture from urine and throat washings; but asymptomatic excretion may occur in immunosuppressed patients and isolation from blood (buffy coat) is clinically much more significant, for viraemia is usually associated with disease due to cytomegalovirus. The virus is slow to grow in tissue culture and a week to 10 days may be needed for the laboratory to identify cytomegalovirus by its characteristic cytopathic effect. The use of fluoresceinated monoclonal antibodies against immediate early or early proteins, however, allows the virus to be identified in tissue culture within 24 hours. This is a very useful means of obtaining a rapid result (sometimes referred to as detection of early antigen fluorescent foci[19]).

SEROLOGY

Antibody to cytomegalovirus is of more limited use in diagnosis, except in the case of a clear conversion from seronegative to seropositive, indicating primary infection. The presence of IgG indicates that the individual is carrying cytomegalovirus; whereas IgM indicates

recent primary infection, and it may also be present during episodes of reactivation.[20] A rise in titre of IgG antibody may also occur during reactivation. Cytomegalovirus disease often occurs, however, in a clinical setting where antibody responses are suppressed and results are difficult to interpret, making dependence on serological tests unwise.

IMMUNOCYTOCHEMISTRY

Monoclonal antibodies to cytomegalovirus proteins have been used to directly identify cytomegalovirus infected cells in tissue biopsy material and in cells recovered from bronchoalveolar lavage fluid.[21,22] The use of antibodies against immediate early or early proteins may be preferable. Alveolar macrophages appear to be the predominant cells containing cytomegalovirus in lavage fluid.

DNA PROBES

Several recent reports have described the use of DNA probes to detect cytomegalovirus in tissues (lung, liver, gut) or body fluids.[15,23] Such methods are not in routine use, although the use of biotinylated rather than radioactive probes will make it easier to transfer these techniques to the routine diagnostic laboratory. Selection of the right probe is crucial as certain regions of cytomegalovirus DNA show homology to cellular DNA; probes avoiding these regions should be chosen.

A real problem in diagnosis is to determine whether cytomegalovirus when present is the cause of illness or a coincidental bystander. This problem is most acute in determining the cause of pneumonitis in immunosuppressed patients, where other opportunist agents may be isolated in addition to cytomegalovirus. In general, the isolation of the virus from a site in association with clinical evidence consistent with cytomegalovirus disease suggests a causal role for the virus. In the case of cytomegalovirus pneumonitis a combination of a positive culture and cytological or immunocytochemical evidence from cells obtained from lavage fluid has been reported to give a reasonable combination of specificity and sensitivity, although the association of a positive culture with histological evidence from lung biopsy material (if obtainable) is even better.[22,24] In the specific setting of bone marrow transplants, isolation of the virus from lavage fluid by culture with detection of early antigen fluorescent foci has been reported to be sensitive and specific for cytomegalovirus pneumonitis, correlating well with the results of open lung biopsy.[25]

Treatment

ACYCLOVIR

There is currently no absolutely satisfactory treatment for cytomegalovirus. Acyclovir is ineffective in stopping replication of the virus (which does not possess its own thymidine kinase and cannot phosphorylate acyclovir). Despite this the incidence of cytomegalovirus disease is reported to be slightly but significantly lower in recipients of bone marrow transplants given prophylactic intravenous acyclovir.[26]

GANCICLOVIR

The newer nucleoside analogue *ganciclovir* (Syntex; also known as DHPG—dihydroxypropoxymethyl guanine) effectively inhibits replication of the virus in vitro; it is phosphorylated to the monophosphate by a cytomegalovirus induced cellular thymidine kinase. It is finding application in the treatment of cytomegalovirus disease in patients with AIDS[12] and in recipients of allografts. There are no controlled clinical trials clearly showing its efficacy and it would be difficult to justify a trial comparing it with placebo. Published evidence is accumulating, however, to support its ability to suppress cytomegalovirus replication in vivo and produce clinical benefit.[27] There is less evidence of its efficacy for pneumonitis than for colitis or retinitis caused by cytomegalovirus; the reports of relative lack of effect, however, relate mainly to recipients of bone marrow transplants with pneumonitis and it may confer more benefit on patients with heart-lung and renal transplants.[28,29] In patients with AIDS recurrence of cytomegalovirus disease (particularly retinitis) when treatment is stopped is very common, and ganciclovir has now been used for extended periods to prevent relapse of cytomegalovirus retinitis (over a year in some instances). The drug causes neutropenia in about a quarter of patients, an effect that appears to be dose related and is counteracted by concurrent administration of recombinant granulocyte colony stimulating factor (GM-CSF) (this has emerged in trials in which the recombinant GM-CSF was being used primarily to counter the neutropenia induced by chemotherapy with zidovudine). Ganciclovir is available only for intravenous use. Specific details concerning dosage and pharmacology are available from the manufacturers and from reviews,[30] and are still evolving. It has recently been licensed and probably represents the currently most effective drug for serious cytomegalovirus disease.

TRISODIUM PHOSPHONOFORMATE

Trisodium phosphonoformate (Foscarnet) is not a nucleoside analogue but a competitive inhibitor of the cytomegalovirus DNA polymerase (and of the DNA polymerase of other DNA viruses). Although used quite widely (particularly in Scandinavia), it has again not been subjected to controlled trials. It effectively inhibits virus replication in vitro and there is anecdotal evidence of efficacy in vivo. It has to be given

intravenously but is relatively non-toxic, although its similarity to diphosphonates leads to drug deposition in bone and occasional increases in serum calcium concentration. Its use is certainly worth considering in patients to whom ganciclovir cannot be given.[31]

CYTOMEGALOVIRUS IMMUNE GLOBULIN

Cytomegalovirus immune globulin has been used for passive immunisation, and shown in controlled trials to lessen the frequency of clinically significant primary cytomegalovirus infection, when administered prophylactically to seronegative recipients of transplants.[32] There is no convincing evidence of its being of therapeutic benefit when given alone to patients with active cytomegalovirus disease.[33] The concentration of specific cytomegalovirus antibody in these preparations, however, is not very great. Possibly monoclonal antibodies or engineered variants of them might be more effective.

COMBINATION THERAPY

Combination therapy with both ganciclovir and cytomegalovirus immune globulin has recently been reported (from Seattle and from the Sloan-Kettering Institute in New York) to give more encouraging results in the treatment of cytomegalovirus pneumonitis after bone marrow transplants than either alone, with over half the patients surviving in a combined total of 35 patients.[34,35] Combined treatment was given for two to three weeks, with "maintenance" beyond this if patients still had symptoms. Although the comparison is again with historical controls, these reports strongly suggest that further trials of this combination are warranted, although why the two agents combined should be better than either alone is unclear. Such trials would not be easy; they would have to use standardised immunoglobulin and they would probably need to be multicentre and to use a control group treated with ganciclovir alone for comparison.

Vaccines and prevention

There is currently no effective vaccine against cytomegalovirus. A candidate live vaccine derived from the Towne strain of the virus has not been shown to confer significant protection from cytomegalovirus infection on seronegative recipients of renal transplants.[36] There are in addition theoretical objections to the use of live vaccines for any virus capable of establishing persistence. The design of an effective subunit vaccine will depend on elucidation of which viral proteins are capable of eliciting protective immunity.

The most effective way to prevent serious cytomegalovirus disease in transplant programmes is to match donor and recipient for cytomegalovirus, giving seronegative recipients only transplants from seronegative donors and in addition using only cytomegalovirus negative blood products; in this way primary infection may be largely prevented.[37]

Conclusion

Persistent viruses have obviously evolved subtle mechanisms for coexisting with their hosts, as shown by the uncertain pathogenesis of the diseases that result when the normal virus-host relationship is perturbed. Cytomegalovirus disease remains a very considerable problem in immunosuppressed patients; but the promise of more effective chemotherapy, coupled with advances in understanding the basic biology of the virus, suggests that we may soon be able to manage the problem more rationally and effectively. Indeed, the advent of specific chemotherapy may throw light on the extent to which particular clinical syndromes are caused by active cytomegalovirus replication.

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