Opportunities for diagnosing cytomegalovirus in pulmonary infections

Cytomegalovirus (CMV) infection is considered a major cause of morbidity and mortality in organ and bone marrow transplant recipients and patients with the acquired immune deficiency syndrome (AIDS). Untreated CMV pneumonia carries a mortality approaching 100%. Other syndromes may include fever with or without lymphocytosis, retinitis, encephalitis, duodenitis, and colitis, although only the first satisfies strict criteria for a causal association with the virus.¹

Two hypotheses have been proposed to explain the pathogenesis of CMV pneumonia. Either pre-existing lung damage and immunodeficiency reduce the magnitude of virus-induced tissue destruction required to produce pneumonia,² or the pneumonia reflects T cell-mediated immune reactivity directed against CMV antigens.³ The first predicts a preventative role for avoidance of iatrogenic immunosuppression or pulmonary toxins, whereas the second is compatible with a therapeutic role for CMV immunoglobulin. The severity of CMV pneumonia in thoracic allograft recipients which led to the study reported by Egan and colleagues on pages 9-13 of this issue of *Thorax*⁴ is explained either by immunosuppression and pre-existing lung damage² or by T cell immunopathology associated with lung rejection.³

Many strategies may limit the clinical impact of CMV pneumonia. Anti-CMV chemotherapy with ganciclovir or foscarnet are often ineffective in established CMV pneumonia.⁵ Adjunctive CMV immunoglobulin therapy was suggested to block T cell immunoreactivity against CMV antigens, but the pathogenic role of such immunopathology² and the efficacy of immunotherapy remain unproven.⁶ Prophylactic high dose acyclovir is safe and possibly effective.⁷ Pre-emptive ganciclovir treatment when pulmonary CMV infection is detected by bronchoalveolar lavage has been shown to be efficacious.⁸ Nonetheless, the potential benefit of prophylactic ganciclovir for all immunosuppressed patients must be balanced against the possible emergence of pathogenic drug-resistant virus.⁹ Both ganciclovir and foscarnet have major adverse effects – bone marrow suppression and nephrotoxicity, respectively – and the synergistic myelotoxicity of ganciclovir and zidovudine largely preclude their combined administration.¹⁰ Prophylactic CMV immunoglobulin may have a role in high risk CMV seronegative organ transplant recipients whose donors are seropositive.¹¹

Preferential matching of organ transplant recipients and donors for either CMV antibody status or HLA antigens has been proposed, the former to avoid the high risk CMV positive donor, CMV negative recipient combination,¹² and the latter to minimise graft rejection and hence severe CMV disease associated with immunosuppressive antirejection treatment.¹³ Graft survival is improved by HLA matching in renal and cardiac transplant recipients,^{14,15} and graft rejection could be reduced by DR antigen matching in heart transplant recipients.¹⁶ Increased graft rejection consequent on CMV matching at the expense of HLA mismatching could therefore lead to increased iatrogenic immunosuppression. The rapid latex agglutination assay used to determine donor and recipient CMV antibody status immediately before transplantation is prone to produce occasional false negative¹⁷ and false positive results.¹⁸ This might lead to inadvertent matching of seronegative re-

cipients with seropositive donors if the test on the recipient or donor gave a falsely positive or a falsely negative result, respectively. Moreover, iatrogenic immunosuppression is a crucial component of the severity of any CMV disease.¹⁹ The combination of erroneous CMV matching producing a high risk CMV seronegative recipient CMV seropositive donor pairing and marked immunosuppression consequent on HLA mismatching is therefore likely to be a lethal concoction. In addition, CMV matching at the expense of HLA matching will only reduce the clinical impact of CMV disease if severe manifestations are largely confined to seronegative recipients of organs from seropositive donors. However, local experience indicates that fatal CMV pneumonia can occur in previously seropositive renal transplant recipients given intensive antirejection treatment.

HLA matching appears to be associated with a low incidence of graft loss and severe CMV disease in renal allograft recipients, presumably because iatrogenic immunosuppression is thereby minimised.^{13,14,19} At present, heart, heart-lung, and lung allograft recipients and their donors are largely matched for CMV antibody status rather than HLA antigens.¹² Prospective HLA matching in thoracic transplantation should improve its success¹⁶ and also minimise severe CMV disease.¹³

Progress in the rapid diagnosis of CMV disease followed the development of techniques for the early detection of virus replication in cell cultures 24-48 hours after inoculation.²⁰ A viral cytopathic effect visible by light microscopy and indicative of a positive virus isolation result only appears after 1-4 weeks. CMV immediate early or early antigen detection in cell culture by immunofluorescence using specific murine monoclonal antibodies (also known as detection of early antigen fluorescent foci)²¹ is widely available and provides early diagnosis of active and, in particular, pulmonary CMV infection by respective examination of urine or bronchoalveolar lavage fluid.^{8,20,22} Most patients with pulmonary CMV infection either have or will shortly develop CMV pneumonia and should be treated promptly with ganciclovir. Immediate early antigen detection is not as sensitive as conventional cell culture.²⁰ Therefore, if CMV pneumonia is diagnosed, treatment should be continued for a minimum of 14 days. If the rapid test for CMV is negative, treatment started on empirical grounds before the immediate early antigen result is available may be discontinued only if CMV pneumonia is excluded on clinical grounds.

The incomplete effectiveness of treatment of CMV pneumonia, suboptimal performance of available rapid diagnostic tests, and continuing high incidence of CMV disease (particularly pneumonia) in thoracic transplant recipients prompted Egan *et al* to initiate prospective surveillance for CMV infection and disease in their patients.⁴ In this issue of *Thorax* they assess the diagnostic and prognostic significance of quantitation of CMV antigenaemia. Peripheral blood polymorphonuclear neutrophil leucocytes positive for viral lower matrix protein were enumerated in cytospin preparations. High level antigenaemia (>50 antigen positive cells per 2×10^5 neutrophils) was detected 2-44 (median 14) days before onset of CMV disease in five patients (four with pneumonia, one with duodenitis), but six patients who remained disease-free had similar antigenaemia giving a positive predictive value of only 46%. The negative predictive value was 100%.

An alternative approach to the early identification of patients who will subsequently develop CMV disease is the assay of CMV viraemia. Blood anticoagulated with preservative-free heparin is inoculated on human fibroblast cell monolayers, and immediate early antigen detection is performed 24–48 hours later.²¹ In comparison with quantitation of CMV antigenaemia, this approach has a number of disadvantages. It depends on the production and maintenance of fastidious human cell lines which may show toxic effects or succumb to bacterial or fungal overgrowth following specimen inoculation.²⁰ Cell toxicity appears to be a significant problem with blood specimens such that, in one recent study, only one of 15 specimens positive in cell culture gave a positive result in the rapid test.²³ Heparinised blood samples probably only yield positive results when high level viraemia is present, as heparin is a potent inhibitor of CMV replication.²⁴ However, the cut-off for a positive result is likely to be more variable than when CMV antigenaemia is quantitated. With increasing time for specimen transport from patient to laboratory, heparin increasingly inhibits CMV infectivity.²⁴ In contrast, cytolysis of neutrophils is unlikely to selectively affect CMV antigen positive cells and so the relative level of antigenaemia should be unaltered. Nonetheless, CMV viraemia and antigenaemia assays share relatively low positive predictive values.^{4,23} Use of the polymerase chain reaction (PCR) rather than immediate early antigen detection to monitor CMV viraemia may be confounded by the ultrasensitivity of the PCR which can detect viral DNA in peripheral blood mononuclear cells from healthy persons.²⁵

Before introducing regular surveillance for CMV antigenaemia in their immunosuppressed transplant or AIDS patients, clinicians should balance the advantages of early identification of patients likely to progress to severe CMV disease against the logistic problems involved and the lack of cost-benefit analyses. Specimens must be tested in the laboratory within 3–5 hours of collection,⁴ thus requiring a dedicated transport system. The requirement for cyto-centrifugation poses safety problems, particularly if specimens from patients with AIDS are processed, because aerosol generation is inevitable. The work should therefore be confined within a microbiological safety cabinet. All these features will render CMV antigenaemia testing an expensive procedure. However, if specimens are only collected for diagnosis in symptomatic patients, CMV antigenaemia assays are an attractive alternative to immediate early antigen detection.

The considerable cost of regular surveillance for high level CMV antigenaemia might be justifiable if, when all patients with a positive result were treated pre-emptively with ganciclovir or foscarnet, CMV associated mortality or morbidity was reduced. In particular, a consequential reduction in the costs of hospitalisation, drugs, and other care would be persuasive evidence. The low positive predictive value and high incidence of high level CMV antigenaemia in the study reported by Egan *et al.*⁴ might be seen to indicate that a cost-benefit analysis would not favour the introduction of antigenaemia testing. Only 21 of 32 thoracic transplant recipients (66%) remained free of high level antigenaemia and therefore might have been considered not eligible for pre-emptive antiviral chemotherapy. At least half the patients selected to receive pre-emptive treatment on the basis of a possible screening test were not destined to develop CMV disease. As CMV antigenaemia preceded CMV disease by 2–44 days, any pre-emptive antiviral treatment might either be too late or have to be given for at least six weeks. However, the costs of inpatient care on a high dependency unit for transplant

patients will be high, and therefore the outcome of a cost-benefit analysis cannot be predicted.

In conclusion, assay of CMV antigenaemia rather than viraemia is probably the preferred option for prediction of CMV disease if surveillance for pulmonary CMV infection is considered too invasive in immunosuppressed patients at high risk of serious CMV disease. Alternative strategies for the prevention of CMV associated morbidity and mortality, such as improved HLA matching in solid organ transplantation, high dose acyclovir prophylaxis for bone marrow (and perhaps other) transplant recipients,²⁶ and selective CMV immunoglobulin prophylaxis, should nonetheless be pursued. The only option in patients with AIDS appears to be early antiviral therapy as soon as CMV disease is diagnosed. Surveillance for CMV antigenaemia may currently be justified in thoracic transplant recipients, given the high incidence of serious CMV disease. Such surveillance has not, however, been subjected to cost-benefit analysis and may merely be an expensive luxury.

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