Disease resulting from human cytomegalovirus (CMV) infections leads to significant morbidity and mortality in patients with Acquired Immunodeficiency Syndrome (AIDS), transplant recipients undergoing immunosuppressive therapy, and neonates. Antiviral agents are rarely used in CMV-infected neonates, but Valaciclovir (VCV) prophylaxis and laboratory-guided preemptive ganciclovir (GCV) therapy have improved outcomes for AIDS patients and transplant recipients, and second-line antivirals such as foscarin (FOS) and cidofovir (CDV) can be used when treatment fails.

Despite this, emergence of antiviral resistant CMV strains and other potential host or viral factors that contribute to the persistence of CMV continue to complicate the clinical management of immunocompromised patients. Rapid diagnosis of increasing CMV load and antiviral resistant CMV strains in the blood of patients prior to the onset of symptoms ensures appropriate treatment and prevention of potentially life-threatening diseases.

The host circulatory system plays an important role in CMV dissemination, latency and persistence. CMV is initially transmitted via body fluids, fomites, latency and persistence. CMV is initially important role in CMV dissemination, the host circulatory system plays an threatening diseases.

Correlation between cytomegalovirus disease and antiviral resistance

Gillian M Scott
Jenna M Iwasenko
Zubair M Waliuzzaman
David H Miles
William D Rawlinson
Virology Division
Department of Microbiology
South Eastern Area Laboratory Services
Prince of Wales Hospital
Randwick, NSW 2031
Tel: (02) 9382 9188
Fax: (02) 9398 4275
E-mail: scottgi@sesahs.nsw.gov.au

Antiviral resistant CMV strains occur in approximately 30% of patients treated for greater than 2 months, and are associated with increases in circulating viral load and progression of disease. Phenotypic analyses such as plaque reduction assays are used for the diagnosis of antiviral resistant CMV infections, but are time consuming, require a cultured isolate, and are often preceded by detection of CMV antiviral resistance by genotypic analysis.

Restriction enzyme analysis of CMV protein kinase (UL97) PCR products detects the most common mutations that confer GCV-resistance. However, these assays detect 46-70% of CMV antiviral resistant strains and therefore miss a significant percentage of HCMV antiviral resistance. Furthermore, DNA polymerase (UL54) mutations associated with GCV, FOS and CDV resistance continue to emerge with increased usage of these antiviral agents.

Sequencing of DNA polymerase (UL54) and protein kinase (UL97) PCR products is a more sensitive approach for the detection of mutations that confer CMV antiviral resistance. We have developed DNA polymerase (UL54) and protein
kinase (UL97) PCR-sequencing assays for the detection of all known mutations that confer antiviral resistance to CMV. Using these methods, mutations associated with CMV antiviral resistance were previously identified in 17% of blood specimens from high-risk immunocompromised patients.

More recently, antiviral resistant mutations were detected in 35% of specimens sent for testing, indicating the incidence of antiviral resistant CMV strains is increasing in the Australian population (Figure 1). Mixed viral populations of antiviral-resistant and antiviral-sensitive strains can complicate the detection of CMV antiviral resistance using genotypic assays, and isolates circulating in the blood can have different genotypes from those causing end-organ disease. However, it is our experience that genotypic detection of CMV antiviral resistant strains circulating in the blood correlates with symptomatic disease at different sites, and sometimes precedes the development of CMV disease.

Six DNA polymerase (UL54) mutations were detected in blood specimens from patients with clinical evidence of resistant CMV infection that are previously unrecognised in antiviral resistant or sensitive strains. These DNA polymerase mutations are being separately transferred to a sensitive CMV isolate to demonstrate the ability of these mutations to confer antiviral resistance.

Preliminary results indicate a mutation (A834P) in CMV DNA polymerase functional domain III confers resistance to GCV, FOS and CDV, demonstrating the ability of some single DNA polymerase mutations to confer cross-resistance to a number of antiviral agents. These mutations are being investigated further by in vitro DNA polymerase activity assays to determine the mechanism of antiviral resistance and effect on polymerase fidelity.

These studies will enhance our understanding of CMV DNA replication and assist in design of future anti-CMV agents. Furthermore, continued investigations of CMV DNA polymerase will allow the identification of the full complement of mutations that confer resistance to CMV.

Detection of increasing viral load in blood and identification of circulating antiviral resistant strains allow prediction or confirmation of CMV disease in at-risk patients and comprehensive and rapid diagnosis of antiviral resistant CMV infections. Taken together, this information will assist clinicians in decisions regarding appropriate treatment and improve health outcomes for treated patients.

References
12. Li W, Anwar F, Jesurrun J & Erice A. Cytomegalovirus UL97 and glycoprotein B (gB) sequences in tissues from immunocompromised patients with ganciclovir-resistant virus infection. Scand J Infect Dis 1999; 31:549-553.