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Review

A new tool linking human cytomegalovirus drug resistance mutations to resistance phenotypes

Meike Chevillotte[a], Jens von Einem[a], Benjamin M. Meier[c], Feng-Mao Lin[d], Hans A. Kestler[b,c,*], Thomas Mertens[a,**]

[a] Institute of Virology, University Hospital Ulm, D-89081 Ulm, Germany
[b] Internal Medicine I, University Hospital Ulm, D-89081 Ulm, Germany
[c] Institute of Neural Information Processing, University of Ulm, D-89069 Ulm, Germany
[d] Institute of Bioinformatics and Systems Biology, National Chiao Tung University, Hsin-Chu 300, Taiwan, ROC

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ABSTRACT

Drug resistant strains of human cytomegalovirus (HCMV) in patients at risk may increasingly develop into a problem in the clinical setting. Genotypic resistance testing is becoming the method of choice, but requires previous phenotypic characterisation of each newly found mutation. In order to facilitate the interpretation of the patient's CMV sequence data, a web-based search tool was generated that links the sequence to a database containing all published UL97 (protein kinase) and UL54 (DNA polymerase) mutations and corresponding antiviral drug susceptibility phenotypes. It is reasonable to assume that HCMV drug resistance testing will provide relevant data for an adjustment of therapy and on prognosis of clinical outcome. HCMV drug susceptibility testing will become even more important once new drugs will be available for therapy allowing a wider choice of antiviral agents to treat HCMV disease. These topics will also play a pivotal role for optimising antiviral therapy of HCMV- and other viral diseases.

Contents

* Corresponding author at: Institute of Neural Information Processing, University of Ulm, 89069 Ulm, Germany. Tel.: +49 731 5024248; fax: +49 731 5024156.
** Corresponding author at: Institute of Virology, University Hospital Ulm, 89081 Ulm, Germany. Tel.: +49 731 500 65100; fax: +49 731 500 65102.
E-mail addresses: hans.kestler@uni-ulm.de (H.A. Kestler), thomas.mertens@uniklinik-ulm.de (T. Mertens).

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1. Introduction

Human cytomegalovirus (HCMV) is a highly relevant opportunistic pathogen for individuals with a compromised or immature immune system, such as transplant recipients, patients with acquired immunodeficiency disease syndrome (AIDS) or connatal infected children. HCMV is the leading cause of birth defects caused by intrauterine virus infections, but unfortunately, no effective treatment is currently licensed during pregnancy. Without antiviral intervention, HCMV-associated disease represents a main cause of morbidity and mortality in solid organ transplant (SOT) and in bone marrow or hematopoietic stem cell transplant (BMT/HSCT) recipients (Boeckh et al., 2004; Boeckh and Ljungman, 2009; Sia and Patel, 2000; Sun et al., 2008). The incidence of HCMV-related complications and death in AIDS patients has declined due to the introduction of highly active antiretroviral therapy (HAART) (Kedhar and Jabs, 2007; Palella et al., 1998), but still remains a concern in patients with low CD4+ cell counts. Therefore, diagnosis and monitoring of active HCMV infection and, in many cases, long-term antiviral therapy against HCMV are life-saving for patients at risk for severe HCMV disease.

Many factors influence the success of anti-HCMV treatment, such as the underlying disease, the severity of immunosuppression, concentrations of the antiviral drugs, and finally the susceptibility of the patient's viral strain – or viral strains – to the administered antiviral drug. Virologists have to provide clinically validated methods for fast and reproducible drug susceptibility testing in order to (i) determine viral drug resistance as reason for failure of therapy, (ii) optimise antiviral therapy, (iii) provide a new prognostic marker.

This review recapitulates current treatment options for HCMV infection and disease, resistance mechanisms and methods for drug susceptibility testing. In this context, a new tool is presented that links HCMV genotypes to a database of published in vitro drug susceptibility phenotypes, thereby providing information for an optimisation of antiviral therapy. Furthermore, future needs are discussed, such as required clinical validation of antiviral resistance testing using the proposed interpretation system as well as the incorporation of future drugs and drug targets into the database.

2. Currently available systemic drugs and their resistance mechanisms

Three systemic drugs are currently licensed to treat HCMV infections and disease: Ganciclovir (GCV) including its prodrug Valganciclovir (ValGCV), Cidofovir (CDV) and Foscarnet (FOS). All drugs target the viral DNA polymerase, pUL54. GCV acts as nucleoside analogue and has to be initially phosphorylated by the HCMV protein kinase, pUL97 (Cihlar and Chen, 1996; Michel et al., 1998; Sullivan et al., 1992). CDV does not require this initial phosphorylation step and acts as nucleotide analogue. Finally, FOS has a different mode of action by acting as pyrophosphate analogue (Chrisp and Clissold, 1991).

The two viral proteins involved in resistance mechanisms against these three drugs are pUL97 and pUL54. Defined mutations in pUL97 lead to reduced phosphorylation activities resulting in lower levels of monophosphorylated – and thus active – GCV (Baldanti et al., 2002a; Biron et al., 1986). The degree of residual pUL97 phosphorylation activity and reduced drug susceptibility is quite variable, depending on the position of the mutated amino acid (Baldanti et al., 2002a; Chou et al., 1995a, 1995b). Hence, mutations in UL97 exclusively confer resistance to GCV, and about 90% of all GCV resistances detected so far have been attributed to mutations in UL97 (Chou, 1999). Mutations in the polymerase may increase its exonuclease activity, so that the nucleoside analogues GCV and CDV are recognised and excised from the DNA chain more efficiently. Additionally, other polymerase mutations are thought to decrease affinity for antiviral compounds, a mechanism that concerns all three antivirals mentioned and thus may lead to multidrug resistance (Gilbert and Boivin, 2005a).

It has been described that the evolution of antiviral resistance occurs in a stepwise fashion (reviewed in Nijhuis et al., 2009; Müller and Kräusslich, 2009). Prior treatment, the entire virus population naturally contains only a small fraction of less drug susceptible variants. This phenomenon can be explained by a reduction of replicative fitness of those variants. When subsequent antiviral treatment does not suppress viral replication completely, e.g. due to suboptimal levels of antiviral drugs or extreme immunosuppression of the host, a selection process favouring these variants is initiated. Since they can further replicate in the presence of the antiviral drug, they are able to evolve and acquire additional mutations that enhance replicative fitness and possibly increase resistance, finally resulting in a large population of highly resistant and replication competent viruses. Concerning HCMV, this model is supported by the fact that major risk factors for HCMV drug resistance are the residual capacity of the host's immune system to control viral replication and the overall amount and duration of viral replication (Drew, 2000). The clinical virologist has to keep these selection mechanisms in mind when monitoring viral loads in patients responding to antiviral therapy. Furthermore, the moment of antiviral drug susceptibility testing combined with the sensitivity of applied assays has to be critically evaluated.

3. Antiviral drug susceptibility testing of HCMV

3.1. Current methods for phenotypic characterisation of drug susceptibility in HCMV

Before the establishment of genotypic testing, phenotypic testing of clinical isolates was the method of choice for determining HCMV drug susceptibility and it still is in many laboratories. In theory, phenotyping should be advantageous, since drug susceptibility of the clinical isolate including all acquired mutations can directly be assessed. However, major disadvantages of phenotypic testing are the difficulties in obtaining the isolate from a patient's sample – especially after onset of therapy – selection of a sensitive virus population during the isolation process and in general the slow replication of HCMV which delays results up to several weeks. In consequence, phenotypic testing can in most cases only be used for retrospective determination of resistance. Although the method is still used for diagnostic purposes, the main importance of phenotypic testing today relies on the characterisation of newly found mutations after marker transfer experiments in order to provide the necessary basis for genotypic resistance testing.

As recommended by the Food and Drug Administration in 2007, drug susceptibility of a viral strain to a specific antiviral agent should be expressed as the drug concentration that is effective to inhibit viral replication in vitro by 50% (EC_{50}). Today, the gold standard for phenotypic characterisation of HCMV is still the plaque reduction assay (PRA), which measures viral spread in cell culture using different antiviral drug concentrations and counting newly formed viral plaques. However, inter-assay and especially inter-laboratory standardisation of the PRA has been shown to be very difficult (Landry et al., 2000). These difficulties can be attributed mainly to differences in cell cultures used by different laboratories. In consequence, efforts have been made to develop new assays, which allow for a better standardisation. Some are based on reporter cell lines measuring HCMV spread in cell culture reflected by luciferase activity (Gilbert and Boivin, 2005b), green fluorescent protein (GFP) (Ueno et al., 2006; Ueno and Ogawa-Goto, 2009), or enhanced green fluorescent protein (EGFP) (Luganini et al., 2008). Another approach is to use quantitative real-time PCR to detect