A Model for HCMV Infection in Immunosuppressed Patients

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Abstract

We propose a model for HCMV infection in healthy and immunosuppressed patients. First, we present the biological model and formulate a system of ordinary differential equations to describe the pathogenesis of primary HCMV infection in immunocompetent and immunosuppressed individuals. We then investigate how clinical data can be applied to this model. Approximate parameter values for the model are derived from data available in the literature and from mathematical and physiological considerations. Simulations with the approximated parameter values demonstrates that the model is capable of describing primary, latent, and secondary (reactivated) HCMV infection. Reactivation simulations with this model provide a window into the dynamics of HCMV infection in (D-R+) transplant situations, where latently-infected recipients (R+) receive transplant tissue from HCMV-naive donors (D-).

Key words: HCMV, CMV, herpes, transplantation, mathematical model, immunosuppression

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1 Introduction

According to the OPTN/SRTR 2006 Annual Report [34], more than 27,000 organs were transplanted in the United States during 2005. Overall, between 2004 and 2005 there was a 3.7% increase in the number of transplanted organs and a larger 5.0% increase in the number of patients on waiting lists. These numbers reflect trends consistent with previous years, in which the number of patients waiting for transplants, 89,884 in 2005, greatly exceeds the availability of organs. Given these facts and the fact that, as of the end of 2004, there were more than 153,000 persons living with a functioning organ transplant in the United States, optimal care of transplant patients is of utmost importance from patient, supply, and cost perspectives.

Life-long pharmacological immunosuppression is the standard of care for transplant recipients, making them much more susceptible to opportunistic infections. For solid organ transplant (SOT) patients undergoing immunosuppressive therapy, human cytomegalovirus (HCMV) infection is the most significant threat to patient and graft health [11, 25]. Indeed, either directly or indirectly, HCMV infection causes allograft rejection, decreased graft and patient survival, and predisposition to opportunistic infections and malignancies [11, 25]. Approximately 75% of organ transplants without antiviral prophylaxis have some incidence of active HCMV infection in the first year [25], and in a recent retrospective study, Sagedal*, et al.,* showed that HCMV infection is associated with lower survival rates in both high- and low-risk renal transplant patients [30].

HCMV is one of eight known human herpes viruses and the health threat posed by HCMV infection in immunosuppressed patients is due to its life-long latent infection. The prevalence of HCMV in the population varies, but in the developed world approximately 60% of the population is infected [9]. There are four major stages of HCMV infection: primary (or acute), latent (or persistent), active asymptomatic viral replication, and symptomatic viral replication (or disease). The clinical manifestations of primary HCMV infection range from asymptomatic to mononucleosis-like illness in healthy immunocompetent individuals. After primary infection, latent HCMV persists in the nucleus of cells until it is reactivated, beginning a program of lytic replication and lysis, and leading to a new (sometimes asymptomatic) round of active infection and latency. Although there is generally no pathology associated with active HCMV infection in immunocompetent individuals, active HCMV infection can lead to disease in immunocompromised individuals, as in pharmacologically immunosuppressed transplant recipients, individuals undergoing chemotherapy and AIDS patients who are not treated with antiretroviral therapy [7]. HCMV disease is defined as an active infection of HCMV, as detected by antigenemia or virus in the blood, as well as HCMV syndrome, characterized by fever, fatigue, neutropenia, and other symptoms. HCMV disease mostly occurs in severely immunosuppressed patients (such as transplant patients) and rarely occurs in immunocompetent patients [36].

HCMV infection is a significant health threat to immunosuppressed patients. Patient health outcome could be improved with suitable mathematical modeling that could predict the disease course in individuals and one that could suggest optimized treatment strategies.

Mathematical modeling to aid in the understanding of scientific hypotheses is widely used and historically accepted in the physical sciences and engineering communities. As the focus of biological research turns more and more towards understanding complex biological systems across multiple scales, investigators in the biosciences have begun to recognize the value of mathematical modeling coupled with experimental investigations to enhance understanding of mechanisms, pathways, anomalies, etc. The modeling process is

an iterative process (Fig. 1), in which one begins with the real system under investigation and pursues the following sequence of steps: (i) empirical observations, experiments, and data collection; (ii) formalization of properties, relationships and mechanisms which result in a biological or physical model; (iii) abstraction resulting in a mathematical model; (iv) formalization of uncertainty/variablity in model and data resulting in a statistical model; (v) model analysis, interpretation and comparison (with the real system) (vi) changes in understanding of mechanisms, pathways, etc., in the real system; and (vii) design of new experiments.

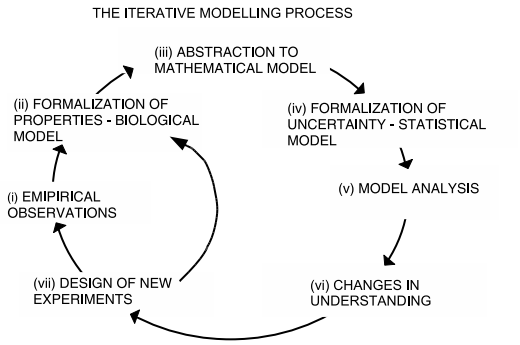

Figure 1: Schematic diagram of the iterative modeling process.

In recent years, development of quantitative techniques, such as real-time PCR measurements of viral load, antigenemia assays for infected cell counts, flow cytometry for T cell subsets, and ELISPOT for virus-specific T cell function, make it feasible to couple experimental investigations with mathematical modeling. In a recent review Funk*, et al.,* summarized investigations in which PCR measurements of viral DNA load are applied to a standard mathematical model for viral load decay kinetics during antiviral treatment to estimate the half-life of HCMV as well as other viruses relevant to transplantation [15]. Various researchers have used this standard on-treatment model to calculate the half-life of HCMV in the blood of kidney transplant [29], SOT [17], and AIDS retinitis [9, 8] patients, for example. In other modeling work, Emery *, et al.,* applied a standard model of infected cell kinetics to measurements of viral load in whole blood to calculate the basic reproductive number R_0, the number of infected cells produced by a single infected cell, and obtain an estimate of antiviral treatment efficacy necessary to prevent HCMV infection.

The use of standard models, such as for viral decay kinetics above, are an important beginning in the abstraction step of the iterative modeling process described in Fig. 1 and can be viewed as special cases of more detailed models. Furthermore, these basic models facilitate our understanding of the complex processes occurring in HCMV infection and can inform more encompassing models of infection dynamics. For example, knowledge of the viral clearance rate tells us about the rate of viral replication in the blood, which must be greater than the clearance rate, during times when the viral load is increasing and estimates of viral growth rate highlight the differences between HCMV-naive and -experienced patients.

The above-mentioned HCMV models have addressed only kinetic behavior. For example, during viral load decay following antiviral treatment, the behavior of the viral load is determined by the antiviral treatment, but does not effect the treatment itself. The next iteration in the modeling process should be dynamic models that account for the "push-and-pull" of processes such as in the interactions between the viral infection and the immune response, in which the viral infection provokes an immune response and is, in turn, attenuated by the immune response. Recently, Wodarz*, et al.,* developed a dynamic model of HCMV infection to

elucidate the potential roles of the innate and adaptive immune responses in the phenomenon of cytotoxic T lymphocyte (CTL) memory inflation [37], the increase of HCMV-specific CTL cells in time relative to the overall T cell population after resolution of the acute infection. Investigation with this model showed that competition between innate and adaptive immune response could be a significant factor in CTL inflation and was consistent with experimental data from mice.

In this work we develop a mathematical model of HCMV infection dynamics at the cellular/viral mechanistic scale for application to individual (whole organism) clinical data and patient health. In Section 2 we present the biological and mathematical models. We formulate the mathematical model as a system of ordinary differential equations that describe the pathogenesis of primary HCMV infection in immunocompetent or immunosuppressed individuals. The mathematical model is mechanistic and physiological with compartments that correspond to cell and virus populations. Although we are ultimately interested in applying this model to HCMV infection in transplant patients, we do not explicitly model donor tissue at this point. In Section 3 we outline how currently available clinical measurements can be applied to this model. In Section 4 we review the data available in the literature and obtain approximate parameter values for the model using available data and mathematical and physiological considerations. In Section 5 we demonstrate that this initial model exhibits appropriate characteristics of primary and latent HCMV infections in immunocompetent individuals. Finally we apply the model to cases of immunosuppression and show that the model exhibits secondary (reactivation) infection following immune suppression.

2 Biological/Mathematical Model

In this initial model we have not tried to formulate a model that reflects all features of the host immune response as well as viral and genetic factors. Instead, our intent is to develop a model that can capture the most salient biological features of HCMV infection in immunosuppressed individuals and, one for which parameters can be plausibly estimated based on longitudinal clinical data. In this model we describe the dynamics of the viral load V and immune response E, as well as actively-infected R_I, susceptible R_S, and latently-infected R_L cells (Table 1). A brief description of the underlying biological model on which we base our mathematical model embodied in (1) below is given first.

Variable	Description	Units
V	viral load (free virus)	virions/μl-blood
E	virus-specific immune effector cells	cells/μl-blood
R_I	actively-infected cells	cells/μl-blood
R_S	susceptible cells	cells/μl-blood
R_L	latently-infected cells	cells/μl-blood

Table 1: State variables for HCMV infection model.

The major reservoir of latent virus is in cells of the myeloid lineage: progenitor cells in the bone marrow and in peripheral blood mononuclear cells derived from these cells [15, 32]. A wider class of susceptible cells, including endothelial and organ-specific cells, are targets during active infection [15, 32]. Since our

focus is on utilizing clinical data, we model relevant cell populations in the blood compartment. In the biological and corresponding mathematical model, the state variables for actively-infected, latently-infected, and susceptible donor-derived cells in the blood compartment are R_I, R_L, and R_S, respectively.

A schematic diagram of the processes contained in our models is presented in Fig. 2. Cellular state compartments are indicated in white circles, while the free virus state compartment is denoted with a gray circle. Processes that are increasing or decreasing the values of the state variables (compartments) are indicated with directed arrows and the rates at which the processes occur are indicated next to the corresponding arrows. For example, a virion V infects a susceptible cell R_S to, collectively, become an actively infected cell R_I with rate kVR_S. Removal from the system is represented by an arrow directed toward the null compartment Ø. Arching arrows that loop back on a compartment represent net death and/or growth process for the corresponding state variable. At the beginning of a primary infection, the R_I, R_L, and E state variables will have value zero.

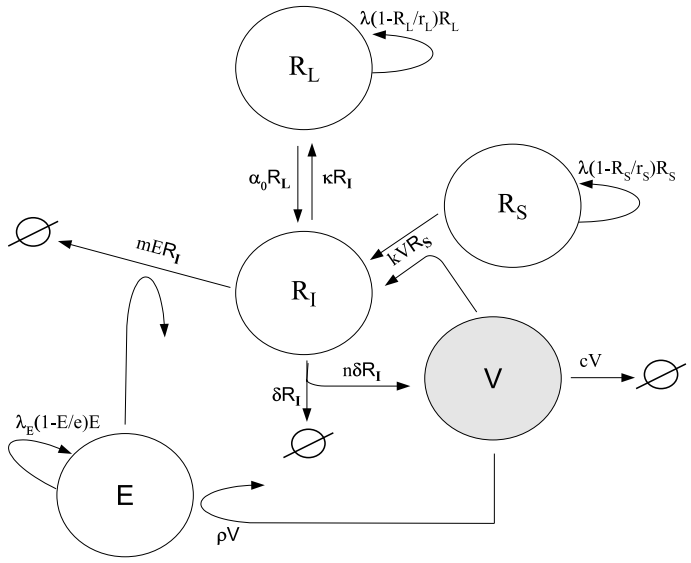

Figure 2: Biological model schematic diagram of key processes in (non-transplant) HCMV infection: infection $V + R_S \rightarrow R_I$, immune-induced cell lysis of infected cells $E + R_I \rightarrow E + \emptyset$, spontaneous reactivation from latency $R_L \rightarrow R_I$, establishment of latency $R_I \rightarrow R_L$, viral induced cell lysis $R_I \rightarrow nV$, viral clearance $V \rightarrow \emptyset$, and maintenance of cell populations (arching arrows). Volume units refer to whole blood.

We model homogeneous populations of latently-infected and susceptible cells, which in the absence of ongoing infection are maintained in an equilibrium state through normal cell division and death through the terms $\lambda(1 - R_S/r_S)R_S$ and $\lambda(1 - R_L/r_L)R_L$. Susceptible cells become infected at rate kR_SV, with f virions infecting each cell (for simplicity in a first model we take $f = 1$ for all computational results presented below). Due to the cytopathic effects of HCMV, actively infected cells die at rate δR_I, and are assumed to produce n virions before death. Free virus is cleared from the blood at rate cV.

There is clinical evidence to suggest that in healthy subjects with latent HCMV there is intermittent low-level viral replication due to spontaneous reactivation of latent virus [7, 22]; we include this conversion of R_L to R_I in our models through the rate term $\alpha_0 R_L$. It is hypothesized that in healthy subjects the immune response prevents these small asymptomatic outbreaks from progressing to the point of producing

detectable symptoms [22, 32]. However, in the absence of a strong immune response, the virions (free virus) released by the reactivated cell can infect nearby susceptible cells and propagate the infection. We model the concentration of virions in the blood through the state variable V. During active infection some cells will progress to a latent state rather than complete the full lytic cycle, a process which is assumed to occur with rate κR_I in the model.

The cellular immune response is the key defense against HCMV infection [25, 26] and is represented by the state variable E in the model, an aggregate compartment of HCMV-specific effector and memory CD8+ T cells (CTLs). We do not model the details of effector cell expansion and contraction during primary infection in this initial model (for examples of this type of modeling see [19, 37]). In the case of primary HCMV infection, Day*, et al.,* showed that an initial diverse polyclonal response to the immunodominant viral epitope (pp65) is followed by a disproportionate contraction of certain clones that were abundant during primary response [5]. The level of detail required to model this complex process is not helpful at this stage. Rather, we seek a qualitative model of the overall response of expansion, clearance, and contraction. In our model, CTLs lyse actively infected cells at a rate mER_I. The concentration of CTLs increases in response to the presence of free virus through the rate term ρV, and this term initiates the immune response during primary infection which begins with $E = 0$. After primary infection, a low level of (memory) CTL cells are maintained through the term $\lambda_E(1 - E/e)E$. Natural killer (NK) cells also participate in control of CMV infection, but we do not model this innate immune response component in this first model.

Immunosuppression therapy throughout the lifetime of a transplant patient is the standard of care to prevent the immune system from attacking donor tissue. We include the parameter ϵ_S, with $0 \leq \epsilon_S \leq 1$, to describe the level of immunosuppression ($\epsilon_S = 1$ corresponds to total immunosuppression). Inclusion of immunosuppression also makes the model relevant to AIDS patients who suffer from disease-induced immunosuppression. The level of immunosuppression can have a significant effect on the risk for HCMV disease, as evidenced by the increased incidence of HCMV retinitis among AIDS patients who are not on antiretroviral therapy. In our model, the factor $(1 - \epsilon_S)$ attenuates the rate of the immune response E and the rate of immune-mediated lysis of infected cells mER_I.

The dynamical ordinary differential equations corresponding to the above biological model are given by

$$\begin{aligned}
\dot{V} &= n\delta R_I - cV - fkR_S V, \\
\dot{E} &= (1-\epsilon_S)\left[\lambda_E\left(1-\frac{E}{e}\right)E + \rho V\right], \\
\dot{R}_I &= kR_S V - \delta R_I - (1-\epsilon_S)mER_I + \alpha_0 R_L - \kappa R_I, \\
\dot{R}_S &= \lambda\left(1-\frac{R_S}{r_S}\right)R_S - kR_S V, \\
\dot{R}_L &= \lambda\left(1-\frac{R_L}{r_L}\right)R_L - \alpha_0 R_L + \kappa R_I.
\end{aligned} \tag{1}$$

Model parameters along with the rationales for the values we assume are discussed in Section 4 and are summarized in Table 2 at the end of that section.

3 Relevant clinical measurements

Because this model is intended to be used with clinical data, a few remarks about the viral load state variable V are required. There is no clear standard for reporting the HCMV load in the literature and measurements are generally taken using either plasma, whole blood, or peripheral blood leukocytes (white blood cells). These three different sources represent measurements of virion DNA, virion plus cell-associated DNA, and cell-associated DNA, respectively. (Note that HCMV is a single-stranded DNA virus so there is only one copy of viral DNA per virion.) For modeling purposes it is preferable to model the concentration of virions in the blood, because cell-associated viral DNA is difficult to quantify; *i.e.,* the number of copies of viral DNA per latently-infected cell is probably characterized by a distribution and the number of copies of viral DNA per actively-infected cell will depend upon the stage of the lytic cycle within each cell.

As stated above, measurements of virions (free virus) are performed using the plasma component of whole blood and are generally reported as copies of HCMV DNA per mL of plasma, but in order to be consistent with the units of the cell compartments should have units of cells per μL of blood. To convert from copies/mL-plasma to copies/μL-blood we need to know the fraction of the whole blood volume that is plasma. The total volume of the blood can be considered to be composed of three subvolumes: plasma, white blood cells (WBCs), and red blood cells (RBCs). The hematocrit (HCT), which is a standard measurement in a Complete Blood Count (CBC), is the percentage of the total blood volume occupied by RBCs. Normal results are in the range 40.7-50.3 for men and 36.1-44.3 for women [24]. Since the volume occupied by WBCs is a small fraction of the total blood volume, we can approximate the fraction of blood volume that is plasma as $1 - HCT/100$. For models that include viral and cellular compartments it would be best to know the value of the fraction of the blood volume that is plasma at each measurement of the plasma viral load, but these numbers are not reported in the literature. Measurements using plasma can be approximately converted to whole blood equivalent by using the median value of the hematocrit $\overline{\text{HCT}}$ in the conversion $V[\text{copies}/\mu\text{L-blood}]=V_p[\text{copies/mL-plasma}]\times(1 - \overline{\text{HCT}}/100)/1000$.

Another practical aspect of utilizing clinical viral load data with mathematical modeling is the proper handling of censored data. That is, measured viral load values often fall below the assay's lower limit of quantification, leading to measurements that are left-censored, i.e., known only to be below the limit. Similarly, right-censored values can occur when measured values lie above the upper limit of quantification for the assay, although this does not appear to occur often with HCMV infection. Left-censored data occur quite frequently, for example during surveillance of transplant patients not undergoing antiviral therapy or following resolution of HCMV infection, either naturally or as a result of antiviral treatment. The Expectation Maximization (EM) Algorithm [6, 23] is a maximum likelihood estimation technique that can be used to obtain reliable parameter estimates with censored data and has been successfully utilized for parameter estimation with models of HIV infection [1, 3].

The HCMV Antigenemia Assay is routinely used to monitor transplant patients for HCMV infection. This assay stains peripheral blood leukocytes (PBLs) (extracted from whole blood) that are positive for pp65, a major protein produced during active HCMV infection. Treated cells are plated and stained cells are counted using a microscope. The results of this test are expressed in the form of HCMV antigen-positive cells per L PBLs, where L might equal 2×10^5, for example. The drawbacks of this assay are that it is time-sensitive, time-consuming processing, and slide interpretation can be subjective [35]. However, this measurement can be used to directly inform the state variable for infected cells R_I if additional information is made available.

The concentration of WBCs (leukocytes) measured in the CBC can be used to convert directly to the state variable R_I in the computational model. For example, if W WBCs per μL-blood are measured in the CBC and A is the result from the antigenemia assay (pp65 positive cells per L leukocytes), then $R_I = AW/L$ cells/μL-blood.

The number of CD8+ T cells per μL of blood can be counted using standard flow cytometry methods. This measurement does not directly correspond to any of the computational model state variables, but can be used along with the PBMC depleted ELISPOT assay to determine values for the HCMV-specific state variable E in the computational model. The PBMC depleted ELISPOT assay measures the ability of the CD8+ T cells to produce specific cytokines (*e.g.*, IFN-γ) in response to target viral epitopes. The test depends upon the peptides used to stimulate the T cells and a panel of overlapping peptides that span the HCMV gene products is desirable, since it best represents the total HCMV-specific CD8+ immune response. The results of this test are expressed in terms of spot-forming cells (SFCs) per N CD8+ T cells, where N equals 1×10^5, for example. The number of HCMV-specific CTL is equivalent to the number of SFCs minus a background count. The result of this assay can be converted to give the concentration of CTL cells E in the computational model. For example, if flow cytometry determines that there are C CD8+ T cells per μL blood and P HCMV-specific CTL per N CD8+ T cells are measured in the ELISPOT assay, then $E = PC/N$.

4 Approximation of model parameters.

Parameter estimation is an important, but often difficult, task in mathematical modeling. With sufficient data all 13 parameters in model (1) can be estimated using inverse problem techniques. To be "sufficient" for parameter estimation, data must generally include longitudinal observations (that capture periods of most rapid dynamic change) and must inform enough state variables to uniquely (at least locally) identify parameters. To begin to estimate parameters we would ideally seek data on primary HCMV infection in immunocompetent individuals where there are fewer parameters to identify, because $\epsilon_S = 0$, $E(0) = 0$, $R_I(0) = 0$, and $R_L(0) = 0$ in this case. Upon reviewing the literature one finds that there are very little data on primary infection in immunocompetent individuals, most likely due to the generally asymptomatic nature of primary HCMV infection in immunocompetent individuals, and very little longitudinal data. Much of the data that are available comes from studies of primary HCMV infection in pregnant women (see, for example, [21, 27, 28]). Significantly more longitudinal data are available in the literature for the more complicated cases of HCMV infection in transplant patients (see, for example, [12, 16, 38]).

One approach, when data are scarce, is to approximate as many model parameters as possible and use a small amount of data to obtain reasonable values for the remaining parameters. In this section we find approximate values for many of the parameters and initial conditions in model (1). This allows us to carry out reasonable exploratory simulations in the absence of sufficient data for parameter estimation. Approximate parameter values also serve as good initial guesses for iterative parameter estimation algorithms used with data. We make the following approximations to obtain parameter values as summarized in Table 2.

(1) The rate of viral induced cell lysis δ can be estimated from measurements of the time to late cytopathology $T_y = 120 - 168$ hours for HCMV [14]. Then $\delta \approx 1/T_y \approx 2 \times 10^{-1}$ day^{-1}.

(2) The total HCMV-specific CD8+ T cell response in seropositive healthy (latently-infected) individuals is 4.6% (median) with a range of $0-36\%$ of the overall CD8+ population in blood [33]. The range of CD8+ cells is $300-900$ cells/μL-blood [18]. Therefore, we expect the equilibrium (latent state) level of HCMV-specific CTL cells $\tilde{E} \approx 0-300$ cells/μL-blood.

(3) Generally, no virions (free virus) are detected in healthy HCMV seropositive individuals [32]. The lower limit of detection for the ultasensitive assays is $L = 20$ virions/mL-plasma. As discussed in Section 3, the ratio of plasma to whole blood volume can be approximated by $1 - \overline{\text{HCT}}$, where $\overline{\text{HCT}}$ is the median hematocrit. Therefore, we expect the equilibrium (latent state) viral load $\tilde{V} \leq L \approx 1.1 \times 10^{-2}$ virions/μL-blood, where we have used $1 - \overline{HCT} \approx 0.57$.

As stated in Section 2, the susceptible, latently-infected, and actively-infected state variables (R_S, R_L, and R_I) represent populations of monocytes in the blood. Some parameters can be approximated based on known properties of circulating monocytes.

(4) The concentration of monocytes in blood is $0.15-0.6\times10^3$ cells/μL-blood [18] and we take the median value to be $r_S \equiv 4\times10^2$ cells/μL. In the case of primary infection, we can then approximate the initial condition $R_S(0) = r_S$. In cases where the susceptible cell population is not significantly depleted during infection (strong immune response), we can approximate $R(t) \approx r_S$.

(5) Reactivation of latently-infected cells R_L occurs when monocytes differentiate into macrophages upon exiting the blood compartment [32]. The half life of monocytes in blood is approximately 71 hours [13]. Therefore $\alpha_0 \approx \ln(2)/71 \text{ hr}^{-1} = 2 \times 10^{-1} \text{ day}^{-1}$.

(6) Approximately 0.01% of peripheral blood monocytes are latently infected [32]. Therefore, we approximate the equilibrium (latent state) concentration of latently-infected monocytes in blood as $r_L \equiv 4 \times 10^{-2}$ cells/μL-blood. In simulations of reactivation, if $R_L(0)$ is unknown, we can approximate $R_L(0) \approx r_L$ and if the latent cell population is not significantly depleted during infection we can approximate $R_L(t) \approx r_L$.

It is helpful to write some of the unknown parameters in terms of quantities we do know (auxiliary parameters).

(7) As mentioned in Section 1, various researchers have measured the exponential decay rate of the HCMV load for patients on effective antiviral treatment. Under the assumption that the antiviral drug completely blocks the release of new virions, the viral load dynamics are given by $V(t) = V(0)e^{-at}$, where a is the absolute value of the (log-linear) slope of the decay. The half-life of the viral load decay is then given by $t_H = \ln(2)/a$. Measurements of t_H in the literature range from 1.5-11.5 days [2, 9, 8, 17, 29]. The viral load decay kinetics of model (1) are approximately given by $dV/dt = -(c+kr_S)V$. Therefore for a specified half-life t_H, we can determine the quantity

$$c + kr_S = \ln(2)/t_H. \tag{2}$$

(8) Another quantity that has been measured is the doubling time t_D of the viral load. Measurements of t_D in the literature range from 0.38-2.1 days [9, 10]. These measurements assume a period of (nearly)

exponential growth of the viral load $V(t) \approx V(0)e^{\ln(2)t/t_D}$. If we consider early times during primary infection before the immune response is significant, model (1) can be approximated by a reduced model

$$\begin{aligned} \dot{V} &= n\delta R_I - cV - fkr_S V, \\ \dot{R}_I &= kr_S V - \delta R_I. \end{aligned} \tag{3}$$

These equations can be solved analytically and, for solutions representing exponential growth and t large enough, $V \propto e^{Ct}$, where $C = -(1/2)(F - A)$, $A = (F^2 + 4\delta(kr_S(n-1) - c))^{1/2}$, and $F = \delta + kr_S + c$. Using $C = \ln(2)/t_D$ and (3) we can find that

$$k \approx \frac{1}{nr_S}\left(\frac{\ln(2)}{t_D\delta}\left(\frac{\ln(2)}{t_H} + \delta + \frac{\ln(2)}{t_D}\right) + \frac{\ln(2)}{t_H}\right). \tag{4}$$

Therefore, for a specified viral load doubling time t_D (and with values for t_H, n, r_S, and δ) the parameter k can be determined. Once k has been determined from (4), c can be calculated from (2).

(9) We can approximate the rate m of cell lysis by immune effector cells by obtaining equilibrium solutions for primary infection with a reduced model

$$\begin{aligned} \dot{V} &= n\delta R_I - cV - fkr_S V, \\ \dot{E} &= \lambda_E\left(1 - \frac{E}{e}\right)E + \rho V, \\ \dot{R}_I &= kr_S V - \delta R_I - mER_I. \end{aligned} \tag{5}$$

and specification of an equilibrium value of the HCMV-specific immune response cells $\tilde{E}$ from above. Then

$$m \approx \left(\frac{\delta}{\tilde{E}}\right)\left(n\left(\frac{c}{kr_S} + 1\right)^{-1} - 1\right). \tag{6}$$

(10) Using the equilibrium solutions from (5) and specifying a value for the equilibrium (latent state) viral load $\tilde{V}$, we can approximate the rate parameter for immune effector cells λ_E as

$$\lambda_E \approx -\frac{\tilde{V}\rho e m^2\left(\frac{\ln(2)}{t_H}\right)^2}{\delta\left(kr_S\, n - \frac{\ln(2)}{t_H}\right)\left(\frac{\ln(2)}{t_H}(em + \delta) - kr_S\, n\delta\right)}. \tag{7}$$

In summary, we can obtain approximate values for model parameters δ and α_0 and for auxiliary parameters t_H, t_D, $\tilde{E}$, $\tilde{V}$, r_S, and r_L. Using these approximate values and values for the unknown parameters ρ, n, and e (model parameters λ and κ are also unknown), we can calculate values for model parameters k, c, m, and λ_E using equations (2), (4), (6), and (7). Table 2 contains an example of parameter values obtained with this approximation methodology. The values chosen for the unknown parameters (ρ, n, e, λ and κ) were guided by the simulations and the requirements of achieving the target values for $\tilde{E}$ and $\tilde{V}$ and obtaining reasonable peak values for the viral load. There is further discussion of these parameter values in Section 5.

	Parameter	Value	Units
Virion induced immune response	ρ	5×10^{0}	cells/(virions $\cdot$ day)
Productivity of infected cell	n	5×10^{1}	(virions/mL)/(cells/μL)
Rate of viral induced cell death	δ	2×10^{-1}	day^{-1}
Rate of viral clearance	c	3×10^{-1}	day^{-1}
Infection rate constant	k	1×10^{-4}	μL/(virions $\cdot$ day)
Immune induced cell lysis	m	1×10^{-1}	μL/(cells $\cdot$ day)
Exit and reactivation rate for monocytes	α_0	2×10^{-1}	μL/(cells $\cdot$ day)
Rate of latency	κ	2×10^{-3}	μL/(cells $\cdot$ day)
Homeostatic replenishment of immune cells	λ_E	4×10^{-2}	day^{-1}
Cell replenishment rate	λ	1×10^{-3}	day^{-1}
HCMV-specific effector cell term	e	9	cells/μL-blood
Level of immune suppression	ϵ_S	0	-
Number of infecting virions per cell	f	1	-
Equilibrium level of susceptible cells	r_S	4×10^{2}	cells/μL-blood
Equilibrium level of latent cells	r_L	4×10^{-2}	cells/μL-blood
Half-life of virions during antiviral treatment	t_H	2	days
Doubling time of the virions	t_D	1.5	days
Equilibrium level of HCMV-specific effector cells	$\tilde{E}$	10	cells/μL-blood
Equilibrium level of virions	$\tilde{V}$	1×10^{-2}	virions/μL-blood

Table 2: Model parameter approximations.

5 Simulations

5.1 Immunocompetent Case

Primary infection. The computational model must describe three types of infection: primary, latent, and secondary (or reactivated) infection. Figure 3 (solid line) illustrates simulation of primary infection in an immunocompetent individual ($\epsilon_S = 0$) using the parameters listed in Table 2 and the initial conditions $(V, E, R_I, R_S, R_L) = (1\times10^{-4}, 0, 0, 4\times10^{2}, 0)$. In Fig. 3 one can see peak values of 420 virions/mL-blood and 19 and 1.8×10^{-2} cells/μL-blood for the viral load, immune response, and actively-infected cells, respectively. The duration to peak for V is dependent upon the particular choice of the initial condition V_0, with smaller values pushing the peak out further in days. The peak in actively-infected cells occurs slightly ahead of the peak viral load (22 *vs.* 24 days), presumably due to the fact that the immune response directly affects R_I and only indirectly V. The peak in the immune response occurs on day 33.

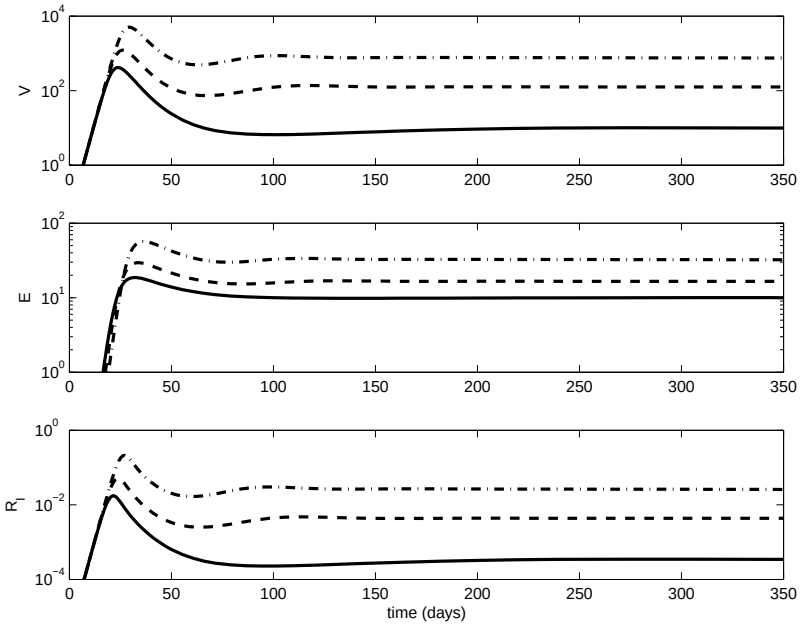

Figure 3: Simulation of primary infection with different degrees of immune suppression: $\epsilon_S = 0$ (solid line), $\epsilon_S = 0.4$ (dashed), and $\epsilon_S = 0.7$ (dash-dot). Plotted, from top to bottom, are the viral load V copies/mL-blood, immune response E cells/μL-blood, and actively-infected cells R_I cells/μL-blood.

Latent infection. In the deterministic model (1) the latent state is characterized by the equilibrium values of the state variables following primary infection. Non-zero equilibrium values of V and R_I represent an ongoing sub-clinical level of infection that is kept in check by the immune system. (A latent state described by intermittent reactivation of latent virus and subclinical infections would require a stochastic model.) For the immunocompetent simulation (Fig. 3, solid line) V, E, and R_I reach equilibrium levels of 9.9 virions/mL-blood and 10 and 3.4×10^{-2} cells/μL-blood, respectively. The equilibrium levels of the viral load and immune response for the immunocompetent simulation are close to the specified auxiliary parameters $\tilde{V}$ and $\tilde{E}$ (Table 2) that were used in the parameter calculations and the equilibrium level of V is just below the ultrasensitive threshold of detection (≈ 10 virions/mL-blood).

The impact of the values chosen for the unknown parameters ρ, n, e, λ and κ (Table 2) on the immunocompetent primary infection results in Fig. 3 are as follows. Decreasing the value of ρ increases the peak values for V, E, and R_I, but has negligible effect on the equilibrium values (Fig. 4). The effect of varying the value of n is most significant on the results for R_I, and has a negligible effect on results for V or E. Decreasing n increases all values for R_I. Decreasing e increases the peak of E, but has a negligible effect on the peaks of V and R_I and does not affect any of the equilibrium values attained. Changing λ or κ by and order of magnitude has negligible effects on any of the results for immunocompetent primary infection shown in Fig. 3 (solid line). However, the results for R_L (not shown) are affected by changes in κ. Finally, it should be noted that decreasing n or ρ may result in damped oscillations in V, E, and R_I after the primary peaks, although the equilibrium values are not affected.

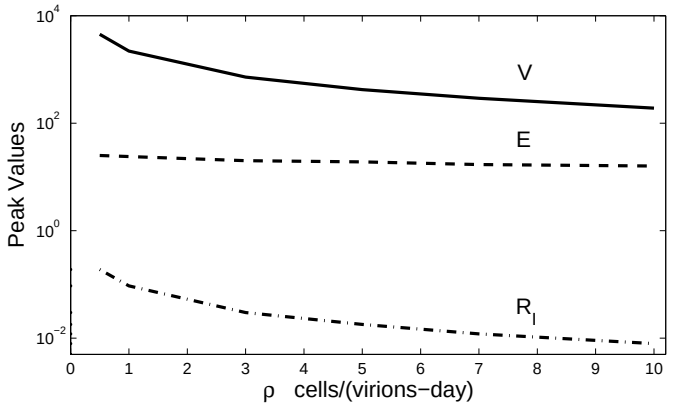

Figure 4: Peak values for V (virions/mL-blood), E (cells/μL-blood), and R_I (cells/μL-blood) as a function of ρ.

5.2 Immunosuppressed Case

Figure 3 also illustrates the effect of immunosuppression on primary HCMV infection, where using the same initial conditions and parameter values as in the immunocompetent case above, the viral load V, immune response E, and actively infected cells R_I are plotted for different levels of immunosuppression: $\epsilon_S = 0.4$ (dashed), and $\epsilon_S = 0.7$ (dash-dot). In Fig. 3, one can see that the peak and equilibrium values of V, E, and R_I increase as the level of immunosuppression increases. The immunosuppressed simulation results can be compared to data from immunosuppressed transplant patients. For example, Sia*, et al.,* measured the plasma viral load just prior to antiviral treatment for 24 transplant patients with HCMV infection and found a range of $0 - 4.61 \times 10^4$ and median of 2.65×10^3 virions/mL-plasma (the lower limit of detection was 288 virions/mL-plasma) [31]. Although these values do not necessarily represent peak values, we can roughly compare them to the range of peak values obtained in these simulations. Using the parameter values in Table 2 and $\epsilon_S = 0 - 0.9$ we obtain simulation results that yield a range of peak viral loads of $\approx 7.40 \times 10^2 - 8.1 \times 10^4$ virions/mL-plasma (note conversion to plasma units); this suggests, at least in this regard, a reasonable first approximation of parameters for model (1).

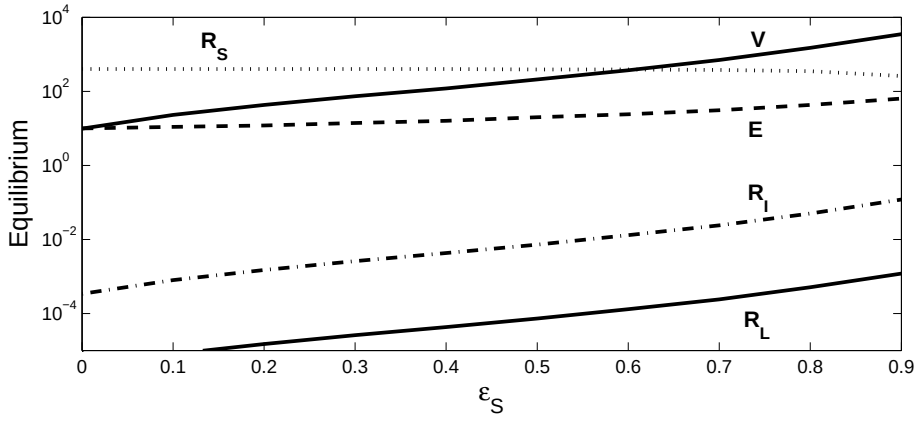

Figure 5: Equilibrium values for state variables following primary infection as a function of the level of immune suppression. Note that V is plotted in units of copies/mL-blood.

Figure 5 illustrates the effects of immunosuppression on the equilibrium (latent state) levels of the state variables that are achieved following primary infection, where it can be seen that the equilibrium values for all state variables, except R_S, increase as the level of immunosuppression ϵ_S increases, consistent with a latent (chronic) infection of increasing severity. For larger values of ϵ_S, the equilibrium viral load is well above normal thresholds of detection, although the infections are still kept in check by the immune response.

Secondary infection - reactivation. The role that immunosuppression plays in HCMV reactivation is important for both transplant and untreated AIDS patients. To investigate secondary infection we simulated immunosuppression of a previously healthy individual with a latent HCMV infection. Specifically, we simulated primary infection in a healthy ($\epsilon_S = 0$) patient and used the equilibrium values of the state variables, which characterize the latent state, as initial conditions for another simulation with immunosuppression. All parameters, other than ϵ_S, are kept fixed in the two simulations. Figure 6 (solid line) displays the simulation results for V, E, and R_I, with an immunosuppression level of $\epsilon_S = 0.7$ following latent infection. The immunocompetent ($\epsilon_S = 0$) latent state levels are indicated with dotted lines. Figure 6 reveals that imposing immunosuppression on a latent infection causes a secondary infection. Following reactivation the equilibrium values of V, E, and R_I remain elevated above the immunocompetent latent values.

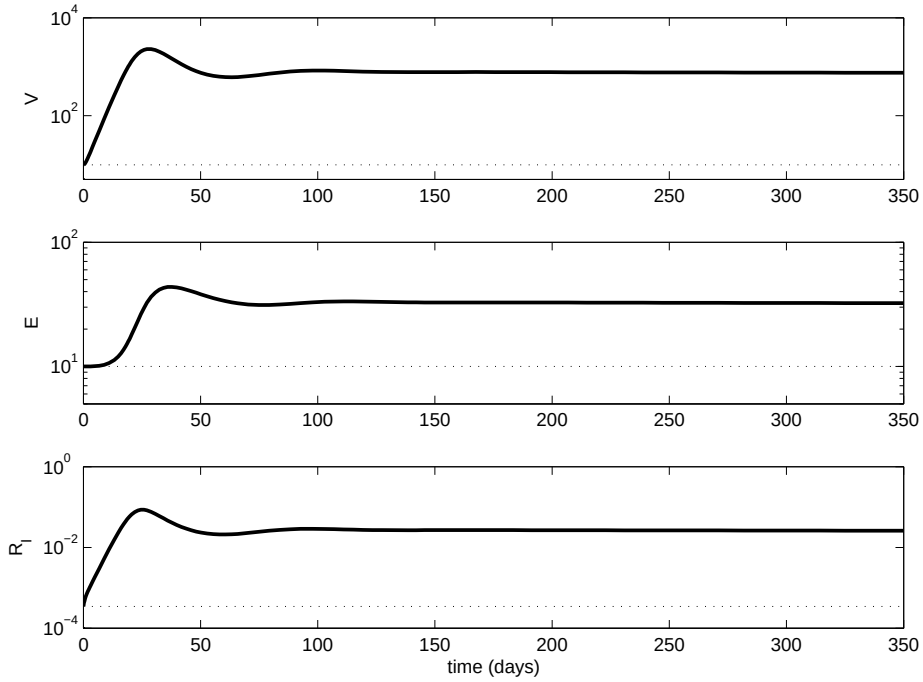

Figure 6: Simulation results for reactivation following immunosuppression ($\epsilon_S = 0.7$). The dotted lines indicate the values of the state variables during the latent state (prior to immunosuppression). Note that in all cases, V is plotted in units of copies/mL-blood.

6 Conclusion

We have derived an initial mathematical model of HCMV infection in both immunocompetent and immunosuppressed individuals. Longitudinal data that inform multiple state variables are necessary for robust parameter estimation. A review of available clinical measurements yields that standard clinical and research measurements such as viral load, antigenemia assay, T cell subsets, and HCMV-specific T cell population, can be applied to the model, but may require additional information that is available, but not generally published/transmitted. In leu of suitable longitudinal data for parameter estimation, we approximated many of the model parameter values using data available in the literature along with mathematical and physiological considerations. Simulations with the approximate parameter values demonstrated that the model is capable of describing primary, latent, and secondary (reactivated) HCMV infection. Within the framework of the deterministic mathematical model, the latent state is characterized by the non-zero equilibrium levels of the viral load and actively-infected cells, representing an ongoing infection that is controlled by the immune system and which is below the level of detection in the immunocompetent case. As the level of immunosuppression is raised in the model, the levels of the viral load and actively-infected cells that characterize the latent state also rise, eventually reaching detectable levels. The model also exhibits secondary infection (reactivation) when immunocompetent latent state (equilibrium) values are used as initial conditions for subsequent simulation with immunosuppression. This type of reactivation scenario serves as a simplified description of (D-R+) transplant scenarios where latently-infected recipients (R+) receive transplant tissue from HCMV-naive donors (D-).

We believe that models such as that derived here can be used in inverse problem simulations to provide information on the types of longitudinal data required to permit better estimation of model parameters for infected individuals and ultimately to allow predictive capabilities (similar to those of the models in [1, 3] for HIV patients) in patients undergoing transplants. Moreover, HCMV infection can serve as a model for the actions of other viruses that cause latent or persistent infections, such as hepatitis C and Epstein-Barr and other herpes viruses [4, 20, 25]. Understanding the dynamics of HCMV infection in immunosuppressed patients may contribute to a better understanding of these other viruses. The model developed above can be applied to other herpes viruses, although parameters will generally be different and some herpes viruses (*e.g.*, HSV) are not cytopathic. Indeed, even within a given infection model, different individuals will have different parameter values, and understanding the extent of heterogeneity in those based on clinical data and how that heterogeneity translates into heterogeneity in longitudinal progression across the transplant patient population will provide increased understanding of disease propagation in populations.

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References

[1] Adams BM, Banks HT, Davidian M, Rosenberg ES, Model fitting and prediction with HIV treatment interruption data, *Bulletin of Mathematical Biology* 69:563–584, 2007.

[2] Asberg A, Humar A, Rollag H, Jardine AG, Mouas H, Pescovitz MD, Sgarabotto D, Tuncer M, Noronha IL, Hartmann A, Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of cytomegalovirus disease in solid organ transplant recipients, *American Journal of Transplantation* 7:2106–2113, 2007.

[3] Banks HT, Davidian M, Hu S, Kepler GM, Rosenberg ES, Modeling HIV immune response and validation with clinical data, *Journal of Biological Dynamics*, 2008,to appear.

[4] Banks HT, Kepler GM, Nguyen HK, Webster-Cyriaque J, Inverse problems and model validation: An example from latent virus reactivation, *J. Inverse and Ill-posed Problems*, 15:19–35, 2007.

[5] Day EK, Carmichael AJ, ten Berge IJM, Waller ECP, Sissons JGP, Will MR, Rapid CD8+ T cell repertoire focusing and selection of high-affinity clones into memory following primary infection with a persistent human virus: human cytomegalovirus, *The Journal of Immunology* 179:3203–3213, 2007.

[6] Dempster AP, Laird NM, Rubin DB, Maximum likelihood from incomplete data via the EM algorithm, *J. Roy. Stat. Soc., Ser. B*, 39:1, 1977.

[7] Dunn HS, Haney DJ, Ghanekar SA, Stepick-Biek P, Lewis DB, Maecker HT, Dynamics of CD4 and CD8 T cell responses to cytomegalovirus in healthy human donors, *Journal of Infectious Diseases* 186:15–22, 2002.

[8] Emery VC, Viral dynamics during active cytomegalovirus infection and pathology, *Intervirology* 42:405–411, 1999.

[9] Emery VC, Cope AV, Bowen EF, Gor D, Griffiths PD, The dynamics of human cytomegalovirus replication in vivo, *Journal of Experimental Medicine* 190:177–182, 1999.

[10] Emery VC, Hassan-Walker AF, Burroughs AK, Griffiths PD, Human cytomegalovirus (HCMV) replication dynamics in HCMV-naive and -experienced immunocompromised hosts, *Journal of Infectious Diseases* 185:1723–1728, 2002.

[11] Fishman JA, Emery V, Freeman R, Pascual M, Rostaing L, Schlitt HJ, Sgarabotto D, Torre-Cisneros J, Uknis ME, Cytomegalovirus in transplantation - challenging the status quo, *Clinical Transplantation* 21:149–158, 2007.

[12] Gamadia LE, Rentenaar RJ, van Lier RAW, ten Berge IJM, Properties of CD4(+) T cells in human cytomegalovirus infection, *Human Immunology* 65:486–492, 2004.

[13] Goto Y, Hogg JC, Suwa T, et al., A novel method to quantify the turnover and release of monocytes from the bone marrow using the thymidine analog 5 '-bromo-2 '-deoxyuridine, *American Journal of Physiology-Cell Physiology* 285:C253–C259, 2003.

[14] Ho M, *Cytomegalovirus: Biology and Infection*, (Plenum Medical Book Company, NY, 1991), pg. 63.

[15] Funk GA, Gosert R, Hirsch HH, Viral dynamics in transplant patients: implications for disease, *Lancet Infectious Diseases* 7:460–472, 2007.

[16] Hadaya K, Wunderli W, Deffernez C, Martin PY, Mentha G, Binet I, Perrin L, Kaiser L, Monitoring of cytomegalovirus infection in solid-organ transplant recipients by an ultrasensitive plasma PCR assay, *Journal of Clinical Microbiology* 41:3757–3764, 2003.

[17] Humar A, Kumar D, Boivin G, Caliendo AM, Cytomegalovirus (CMV) virus load kinetics to predict recurrent disease in solid-organ transplant patients with CMV disease, *Journal of Infectious Diseases* 186:829–833, 2002.

[18] Janeway Jr CA, Travers P, Walport M, Shlomchik MJ, *Immunobiology: The Immune System in Health and Disease*, 6th edition, Garland Science Publishing, New York, pg 471, 2005.

[19] Jones LE, Perelson AS, Opportunistic infection as a cause of transient viremia in chronically infected HIV patients under treatment with HAART, *Bulletin of Mathematical Biology*, 67:1227–1251, 2005.

[20] Kepler GM, Nguyen NK, Webster-Cyriaque J, Banks HT, A dynamic model for induced reactivation of latent virus, *Journal of Theoretical Biology* 244:451–462, 2007.

[21] Lilleri D, Fornara C, Furione M, et al., Development of human cytomegalovirus-specific T cell immunity during primary infection of pregnant women and its correlation with virus transmission to the fetus, *Journal of Infectious Diseases* 195:1062–1070, 2007.

[22] Margolis TP, Elfman FL, Leib D, Pakpour N, Apakupakul K, Imai Y, Voytek C, Spontaneous reactivation of herpes simplex sirus type 1 in latently infected murine sensory ganglia, *Journal of Virology* 81:11069–11074, 2007.

[23] McLachlan GJ, Krishnan T, *The EM Algorithm and Extensions*, John Wiley, New York, 1997.

[24] U.S. National Library of Medicine and National Institutes of Health, MedlinePlus, (March 8, 2007), Medical Encyclopedia, Hematocrit (HCT), Retrieved March 25, 2008, from the World Wide Web: `http://www.nlm.nih.gov/medlineplus/ency/article/003646.htm`.

[25] Pereyra F, Rubin RH, Prevention and tretment of cytomegalovirus infection in solid organ transplant recipients, *Current Opinions in Infectious Diseases* 17:357–361, 2004.

[26] Radha R, Jordan S, Puliyanda D, Bunnapradist S, Petrosyan A, Amet N, Toyoda M, Cellular immune responses to cytomegalovirus in renal transplant recipients, *American Journal of Transplantation* 5:110–117, 2005.

[27] Revello MG, Zavattoni M, Sarasini A, Percivalle E, Simoncini L, Gerna G, Human cytomegalovirus in blood of immunocompetent persons during primary infection: Prognostic implications for pregnancy, *Journal of Infectious Diseases* 177:1170–1175, 1998.

[28] Revello MG, Lilleri D, Zavattoni M, Furione M, Genini E, Comolli G, Gerna G, Lymphoproliferative response in primary human cytomegalovirus (HCMV) infection is delayed in HCMV transmitter mothers, *Journal of Infectious Diseases* 193:269–276, 2006.

[29] Roberts TC, Brennan DC, Buller RS, Gaudreault-Keener M, Schnitzler MA, Sternhell KE, Garlock KA, Singer GG, Storch GA, Quantitative polymerase chain reaction to predict occurrence of symptomatic cytomegalovirus infection and assess response to ganciclovir therapy in renal transplant recipients, *Journal of Infectious Diseases* 178:626–635, 1998.

[30] Sagedal S, Rollag H, Hartmann A, Cytomegalovirus infection in renal transplant recipients is associated with impaired survival irrespective of expected mortality risk, *Clinical Transplantation* 21:309–313, 2007.

[31] Sia IG, Wilson JA, Groettum CM, Espy MJ, Smith TF, Paya CV, Cytomegalovirus (CMV) DNA load predicts relapsing CMV infection after solid organ transplantation, *Journal of Infectious Diseases* 181:717–720, 2000.

[32] Sissons JGP, Bain M, Wills MR, Sinclair JH, Latency and reactivation of human cytomegalovirus, *Journal of Infection* 44:73–77, 2002.

[33] Sylwester AW, Mitchell BL, Edgar JB, Taormina C, Pelte C, Ruchti F, Sleath PR, Grabstein KH, Hosken NA, Kern F, Nelson JA, Picker LJ, Broadly targeted human cytomegalovirus-specific CD4(+) and CD8(+) T cells dominate the memory compartments of exposed subjects, *Journal of Experimental Medicine* 202:673–685, 2005.

[34] United States Department of Health and Human Services, (n.d.) OPTN/SRTR Anual Report, 2006 Annual Report, pgs. I-1,I-2, 2006, Retrieved January 10, 2008, from the World Wide Web: `http://www.optn.org/AR2006/download_instruction.htm`.

[35] Vanpoucke H, Van Vlem B, Vanholder R, Van Renterghem L, Significance of qualitative polymerase chain reaction combined with quantitation of viral load in the diagnosis and follow-up of cytomegalovirus infection after solid-organ transplantation, *Intervirology* 42:398–404, 1999.

[36] von Muller L, Klemm A, Weiss M, Schneider M, Suger-Wiedeck H, Durmus N, Hampl W, Mertens T, Active cytomegalovirus infection in patients with septic shock, *Emerging Infectious Diseases* 12:1517–1522, 2006.

[37] Wodarz D, Sierro S, Klenerman P, Dynamics of killer T cell inflaction in viral infections, *Interface* 4:533–543, 2007.

[38] Yerly S, Perrin L, Van Delden C, Schaffer S, Thamm S, Wunderli W, Kaiser L, Cytomegalovirus quantification in plasma by an automated real-time PCR assay, *Journal of Clinical Virology* 38:298–303, 2007.