Viral dynamics in transplant patients: implications for disease

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Viral infections cause substantial morbidity and mortality in transplant patients. Quantifying viral loads is widely appreciated as a direct means to diagnose and monitor the course of viral infections. Recent studies indicate that the kinetics of viral load changes rather than single viral load measurements better correlate with organ involvement. In this Review, we will summarise the current knowledge regarding the kinetics of viruses relevant to transplantation including cytomegalovirus, Epstein-Barr virus, the herpes viruses 6 and 7, hepatitis C virus, GB virus C, adenovirus, and the emerging human polyomavirus type BK. We discuss the implications of viral kinetics for organ pathology as well as for the evaluation of antiviral interventions in transplant patients.

Introduction

Viruses can cause organ pathology and disease through direct cytopathic effects of viral replication in host cells as well as through inflammatory processes, killing of infected cells by specific immune effectors, or induction of oncogenic transformation.[1,2] The introduction of molecular genetic tools, particularly PCR, has greatly simplified the sensitive and specific identification of viral agents and PCR is now widely available in the clinical routine. In the quantitative format, PCR allows for the direct measurement of viral loads in patient samples. In fact, monitoring viral loads over time reveals changes with onset and treatment of viral disease. In individuals with naive or impaired immune effectors, or both, viral loads are generally found to be higher and persist for longer periods. Mathematical models fitted to serial viral load measurements allowed parameters such as viral doubling times and half-lives in vivo following antiviral interventions to be estimated.[3] These viral dynamic parameters proved useful for predicting the risk of progression to organ failure and for estimating the efficacy of antiviral interventions.

The era of viral dynamics in vivo was inaugurated in 1995 by investigating HIV-1.[4–6] Since then, viral dynamics have been explored for several agents including chronic hepatitis B and hepatitis C viruses.[7–9] The common hallmark of these viral diseases is progressive organ dysfunction through persistent high-level virus replication in affected patients. In immunocompetent individuals, high-level replication is only transiently observed during acute primary infection, if at all. Chronic viral diseases do not only reflect specific viral characteristics, but also result from the inability of an individual's immune system to sufficiently control and clear the virus.

In transplant patients, impaired antiviral immune control results from deliberate pharmacological immunosuppression to counter immune injury (rejection), and from viral infection of HLA-mismatched allogenic tissues. Both factors are particularly important in the setting of primary infection post-transplant—eg, in donor seropositive/recipient seronegative solid organ transplantation and in recipient seropositive patients after T-cell depletion or donor seronegative haematopoietic stem cell transplantation.[10] Finally, some viruses, such as cytomegalovirus (CMV), appear to actively contribute to a quantitative and qualitative state of immunodeficiency,[11] which is paradigmatic for HIV through infecting and exhausting CD4+ T cells en route to the clinical state of AIDS.[12]

Quantification of HIV in blood samples indicated that high steady-state plasma viral loads following the acute phase of infection correlated with faster progression to AIDS.[13] Treatment with potent inhibitors of HIV replication revealed a multiphasic decline in plasma HIV-RNA, associated with different host cell compartments and biological states of infection (figure 1). Analyses of the declines indicated half-lives of 1–2 days (first phase decline), approximately 14 days (second phase decline), and 90–150 days or more (third phase decline).[6,14–17] Time to eradication was determined by the half-life of the slowest decaying infected cell population. The projected eradication time (to less than one virus) predicted lifetime persistence of HIV despite fully suppressive therapy.[18–20] Viral rebounds after cessation of therapy reflect recruitment of productively infected cells as the source compartment. The short viral half-life implied a high daily turnover (figure 2). The bulk of plasma HIV (more than 99%) is derived from productively infected cells, most of which are activated CD4+ T cells. With a productively infected cell half-life of approximately 1 day,[21] about 50% of those cells need to be produced and cleared

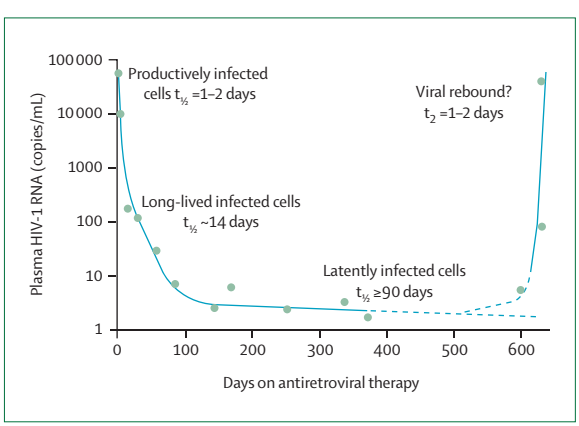

***Figure 1*: HIV kinetics**
Half-lives can be estimated from the slope of the viral load decline after initiation of antiretroviral therapy. The dashed blue line indicates projection of the viral load decay below the limit of detection of the assay. Also depicted is a viral rebound after cessation of antiretroviral therapy. $t_{½}$=half-life. t_2=doubling time.

every day to maintain an apparently stable viral load. A small proportion of plasma virus (less than 1%) appears to come from long-lived infected cells. By contrast, latently infected cells do not contribute to viral loads unless reactivation reverts them into a virus-producing state.

Viral dynamics in transplant patients

In transplant patients, decline of viral loads can be achieved not only by antiviral drugs, but also by modulating (reducing) immunosuppression. Temporary reduction in viral load may occur during surgical procedures—eg, during the short time-span between extraction of an infected graft and reperfusion of a new (uninfected) one. Similar to HIV studies, antiviral drug-induced perturbation experiments have been applied to quantify the "speed" of the viral turnover of three major pathogens in vivo—human CMV, hepatitis C virus (HCV), and Epstein-Barr virus (EBV)—and to link viral dynamics to disease progression. For human herpesviruses (HHV) 6, HHV7, GB virus C (GBV-C; also called hepatitis G virus), and adenovirus, viral load data have been published but the speed of the viral turnover was not established. Here, we have extracted the available data to estimate doubling times and half-lives (table 1).

Human cytomegalovirus

CMV is one of the most significant viral pathogens after transplantation. Without antiviral treatment, the incidence of CMV replication ranges from 40% to 80% and more than half of transplant patients become symptomatic.[23,43] Consensus definitions were established to distinguish between active CMV infection (evidence of replication according to antigen or PCR testing), symptomatic infection defined as CMV syndrome (CMV replication plus fever, weakness, leucopenia, thrombocytopenia), and CMV disease (CMV replication and histological evidence of organ-invasive disease in liver, gastrointestinal tract, lung, etc).[44] These direct effects of CMV replication are distinct from the so-called indirect, part immunologically mediated effects, which include acute rejection episodes, bronchiolitis obliterans, graft vasculopathies, and fungal infections.[11] Major reservoirs of latent CMV infection in vivo are thought to be cells of the myeloid lineage. During active replication, the virus widens its host cell range and may replicate in many cell types including endothelial cells, but also organ-specific cells, which may give rise to specific manifestations in the lungs, liver, intestinal tract, and CNS. Reactivation might involve declining immune surveillance as well as activating stimuli—eg, inflammatory cytokines (tumour necrosis factor α) or ischaemia/reperfusion injury.[45,46]

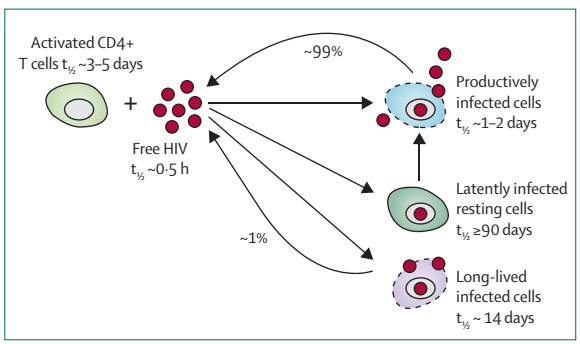

***Figure 2*: HIV compartments**
Associated with the different viral decay half-lives are different host cell compartments and biological states of infection. The figure indicates the respective contributions to the plasma viral turnover. $t_{½}$=half-life.

	Intracellular delay	Transplant patients			Non-transplant patients			Viral generation time	References used to calculate kinetic estimates
		Doubling time	Half-life	R_0*	Doubling time	Half-life	R_0*		
Cytomegalovirus	Approximately 18 h (reference 22)	1·3 days	1–20 days	0·83–1·5	2 days	17 h to 3 days	0·5–1·3	≥3 days	23–26
Epstein-Barr virus	Not well defined	7 h to 3 days	16 h to 3 days	>1–1·4	..	1·7–2·6 h	..	..	27–30
Human herpesviruses 6	5–6 h	≤10 h	21 h	1·5	..		..	≤16 h	31
Human herpesviruses 7	5–6 h	≤22 h	1·8 days	1·2	..		..	≤30 h	31
Hepatitis C virus	≥6 h (reference 32)	7–35 h	0·2–10 h	>2·2	..	1·5–4·6 h	>15	≥8 h	9, 32–35
GB virus C	..	..	2·5–18 days	..	..	..	..	..	36
Polyomavirus type BK	2 days (reference 37)	18 h to 37 days	1–6 h	0·86–2·6	..	..	..	2–3 days	36
Adenovirus	≥16 h (reference 38)	1·3 days	0·05 h	1·4	..	..	..	≥16 h	39, 40
HIV-1	Approximately 1 day (reference 41)	..	..	..	..	0·5 h	2–20	1–2 days	6, 16, 32, 42

..=unknown. Viral doubling time, half-life, and estimated basic reproductive ratio (R_0) calculated from data of transplant and non-transplant patients. *R_0 estimates may be biased to a less than maximum number because viral load slopes are likely to be confounded by the length of the sampling interval, which was often more than or equal to 1–2 weeks.

***Table 1*: Viral kinetics in transplant and non-transplant settings**

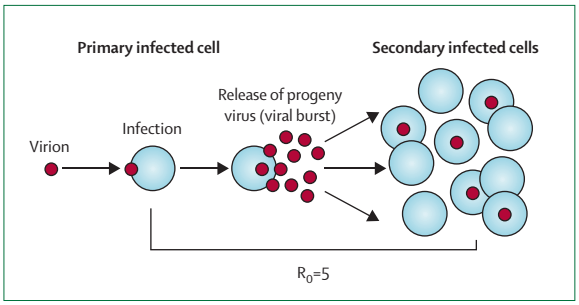

***Figure 3*: Schematic representation of the basic reproductive ratio in infection dynamics**

R_0 quantifies the number of secondary infected cells per primary infected cell at the beginning of an infection when target cells are not yet a limiting factor. During chronic infection, R_0 must be close to 1. R_0 can be calculated from the viral growth rate, *r*, the length of the eclipse phase, *e*, and the average duration over which virus is released, *d*, by the formula $R_0=(1+r*d)Exp(r*e)$. For lytically replicating viruses, the formula simplifies to $R_0=Exp(r*e)$.

The association between CMV loads and clinical manifestations has been investigated. Both faster viral growth rates (ie, a shorter viral doubling time) and slower viral clearance rates (ie, a longer viral half-life) were associated with a higher likelihood of CMV disease. In a study of 127 CMV DNA-positive transplant patients (49 bone marrow, 47 liver, 31 kidney), Emery and colleagues[24] observed that the initial rate of increase of the viral load per mL whole blood correlated well with the risk of developing high viral load peaks and clinical disease. In this study, blood was sampled weekly during the first 3 months post-transplant and retrospectively analysed by quantitative PCR. Fewer than 10% of patients received ganciclovir for pre-emptive therapy. Patients who developed CMV disease (49 patients) had an initial viral doubling time of 2·1 days (range 10 h to 7 days), whereas patients who remained free of CMV disease had a lower doubling time of 3·6 days (range 1·4–23 days). CMV half-life estimates of approximately 17 h are available from five frequently sampled AIDS-patients (median five samples over 21 days) receiving ganciclovir therapy to treat CMV-associated retinitis.[25]

A study by Mattes and colleagues[26] comparing pre-emptive ganciclovir therapy with valganciclovir lent further support to the above estimates. CMV-DNAemia (defined by two consecutive CMV PCR results with at least 200 genomes per mL) was evaluated in 45 transplant patients at least twice weekly for 28 days. 23 patients (11 renal, 12 liver) received intravenous ganciclovir (5 mg/kg twice a day), whereas 22 patients (seven renal, 15 liver) received valganciclovir (900 mg twice a day). In the ganciclovir group, a viral doubling time of 2·0 days before treatment and a half-life of 1·7 days after initiation of therapy were observed. In the valganciclovir group, doubling time was reported as 1·8 days and half-life as 2·2 days. Although viral kinetics in the ganciclovir group were more favourable, differences were not significant.

Additional information on CMV dynamics in transplant patients can be derived from reports of Ghisetti and colleagues[23] and Razonable and colleagues.[47] Liver and heart transplant patients received either oral ganciclovir therapy (1 g three times daily, adjusted for renal function) for 8 weeks,[47] or intravenous ganciclovir (10 mg/kg per day) for 21 days.[23] Razonable and colleagues[47] reported that eight of 25 patients developed CMV disease, but the authors did not provide detectable viral loads 1 week before onset of CMV disease. Within 7 days, CMV loads increased from less than 400 copies per 10^6 peripheral blood leucocytes to a median value of 16 300 copies per 10^6 peripheral blood leucocytes. We used the reported data to estimate a minimum viral load slope of 0·53 per day, which corresponds with a doubling time of 1·3 days or less (consistent with the data reported by Emery et al[24]). Here, it is worth noting that hampered viral replication under therapy was associated with diminished incidence of CMV disease when compared with a placebo control group. When calculating initial CMV doubling time from data provided by Ghisetti and colleagues,[23] we found a doubling time of 1·5 days in symptomatic patients compared with 21 days in asymptomatic patients. From three patients with biweekly sampling,[23] we estimated the following kinetic data: patient 1 (CMV disease), doubling time 2–3·5 days, half-life 4 days; patient 2 (CMV disease), doubling time 1·5–5 days, half-life 2·8–3·8 days. By contrast, patient 3 remained asymptomatic and was not treated with antiviral drugs. In this patient the dynamics seemed to be slow, since doubling time was 6–10 days whereas half-life was 7–12 days.

The relation between CMV clearance and disease recurrence was analysed in a study of 52 transplant patients (35 liver transplants, seven kidney, seven lung, and three others) with viral loads measured at least once per week.[48] CMV half-lives were quantified either after pre-emptive therapy with oral ganciclovir (2 g per day) or with intravenous ganciclovir (5 mg/kg per day), whereas patients with CMV disease were treated with intravenous ganciclovir (10 mg/kg per day). The mean viral half-life was 4·5 days (median 2·5 days, range 1–20 days). The study indicated that the probability of developing CMV disease recurrence was less than 7% in patients with rapid viral declines (half-life 3 days or less), but more than 55% for patients showing only slow CMV declines (half-life 7 days or more).

In infection epidemiology, the basic reproductive ratio (R_0) describes the expansion of a transmittable agent or disease in a susceptible population and serves as a measure to assess intervention strategies including vaccinations.[49] The term may also be useful to quantitatively describe the biology of infectious agents within individuals. Here, R_0 corresponds with the number of secondary infected cells per primary infected cell at the beginning of an infection (figure 3). Expanding viral populations (ie, increasing viral loads) are characterised by $R_0>1$, whereas contracting virus populations (ie, declining viral loads) are characterised

by R_0<1. In patients with persisting viraemia but a stable viral load, R_0=1. A thorough discussion of R_0 in various infectious disease dynamics models has been provided by Lloyd.[50]

To illustrate the use of R_0 for CMV, we assumed an intracellular delay of 18 h according to in-vivo data from an animal model.[22] On the basis of our estimates of the CMV doubling time from the data of Razonable and colleagues,[47] we calculated that R_0 is 1·5 or more during the 7 days between undetectable viral loads and onset of disease. For the three patients detailed above from Ghisetti and colleagues,[23] we estimated that ganciclovir therapy in patient 1 is 24% effective (R_0 decreased from 1·16 to 0·88). In patient 2, the efficacy of the first treatment episode is 22% (R_0 1·11 to 0·87), whereas that of the second treatment episode is 43% (R_0 1·45 to 0·83). For the patients detailed in the study by Mattes and colleagues,[26] we estimated that ganciclovir was 43% effective (R_0 1·29 to 0·74), whereas valganciclovir was 41% effective (R_0 1·33 to 0·79). In patient 3 from Ghisetti and colleagues' study,[23] who remained asymptomatic and did not receive treatment, increasing immune control or decreasing activating stimuli resulted in an efficacy of less than 15% (R_0 1·09 to 0·93).

Taken together, CMV replication dynamics suggest that short doubling times in the order of 2 days seem to predict a higher risk for CMV disease than doubling times of 4 days or more. Conversely, successful intervention for CMV replication and disease was more likely in patients with more rapidly declining CMV loads (half-life less than 4 days). Although these kinetic parameters were observed in patients treated with CMV-specific antiviral drugs, they might include as yet unquantifiable effects of reduced immunosuppression and immune responses. Using R_0, net efficacies of all interventions ranged from 22% to 43%. The use of R_0 may also help to understand cases where antiviral drugs have caused a replacement of the wildtype strain as the prominent species by slower growing drug-resistant strains with reduced fitness and less pathogenic potential.[51]

Epstein-Barr virus

EBV-specific T-cell function has a key role in the control of EBV replication and latency. Intense immunosuppression, the use of T-cell depleting agents for induction or treatment, HLA-mismatches, and primary EBV infection are key risk factors for EBV disease, and particularly, post-transplant lymphoproliferative disorders (PTLD). The pathogenesis of PTLD is complex and involves progression from a polyclonal B-cell proliferation to oligoclonal and monoclonal malignant stages.[52,53] EBV-encoded gene products function as oncogenes driving the proliferation of B cells.[54] Uncontrolled EBV replication might add to the risk for subsequent malignant transformation through extensive infection and recruitment of B cells. Surveillance of EBV loads in plasma, whole blood, and peripheral blood mononuclear cells has been widely used to identify transplant patients at risk for EBV-associated PTLD and to guide therapeutic interventions, which include reducing immunosuppression, chemotherapy, and/or immunotherapy.[55] Only a lesser but variable fraction of the blood viral load is derived from lytic EBV replication, whereas the pathologically more relevant load is derived from transformed host B cells.

No kinetic data are available for EBV in the transplant setting. To approximate EBV kinetics, we selected four papers with sufficient data. In a first step, we extracted the minimum EBV-DNA doubling time and half-life from the data without taking into account episomal or lytic origins (table 2). In a second step, we considered different scenarios of episomal and lytic contributions to EBV load (figure 4).

In the first paper, Smets and colleagues[27] screened 45 paediatric liver transplant patients. In patients with PTLD, immunosuppression was stopped but reintroduced later in some cases. In patients with CMV or EBV disease, aciclovir (30 mg/kg per day) given during the first year was switched to intravenous ganciclovir (10 mg/kg per day) for 14–21 days. From the data reported,[27] we obtained minimum estimates of doubling times and half-lives after primary EBV infection of approximately 2 days (table 2).

	Doubling time	Sampling interval	Half-life	Sampling interval	Intervention
Smets et al (2002),[27] liver transplanted children					
Patient 1	Approximately 2 days	Week 2–4	7 days	Week 8–10	Aciclovir then ganciclovir
Patient 2	≤4 days	Week 2–8	3 days	Week 12–15	Aciclovir then ganciclovir
Patient 3	≤3 days	Week 2–6	1·6 days	Week 6–8	Aciclovir then ganciclovir
Patient 4	≤5 days	Week 2–8	..	..	Aciclovir then ganciclovir
Gärtner et al (2002),[28] stem cell transplantation					
Patient 1	2·7 days	Day 36–63	..	..	Foscarnet, ganciclovir
Patient 3	1·2 days	Day 36–55	..	..	Foscarnet, ganciclovir
Patient 4	2·7 days	Day 70–100	..	..	Foscarnet, ganciclovir, aciclovir
Patient 8	Approximately 21 h	Day 42–54	..	..	Aciclovir
Gustafsson et al (2000),[56] bone marrow transplants					
Patient 1	1·8 days	Day 21–42	..	..	CTL infusion
Patient 2	2·5 days	Day 21–50	..	..	CTL infusion
Patient 3	..	..	≤1 day	Day 147–154	CTL infusion
Patient 4	≤3 days	Day 21–45	..	..	CTL infusion
Mean (SD)	≤2·6 (1·2) days		≤3·2 (2·7) days	..	

..=unknown. EBV-DNA doubing time and half-life was calculated from the indicated sources. The mean (SD) is across all patients. CTL infusion=infusion of ex-vivo expanded EBV-specific cytotoxic T lymphocytes.

Table 2: **Kinetic estimates of EBV calculated from published work**

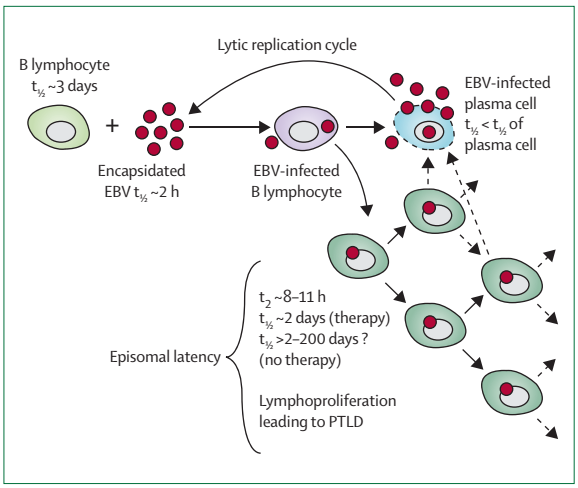

***Figure 4*: B-cell PTLD compartments**
Upon primary infection of uninfected B cells with EBV, infected host cells enter the lymphoproliferative cycle. It is characterised by episomal latency of EBV. Immunocompromised individuals may eventually progress to PTLD. EBV appears to switch to lytic replication if infected host cells differentiate into (short-lived) plasma cells. EBV=Epstein-Barr virus. PTLD=post-transplant lymphoproliferative disorders. $t_{½}$=half-life. t_2=doubling time.

Treatment with antiviral drugs such as aciclovir (1600 mg per day orally or 750 mg per day intravenously), foscarnet (induction 120 mg per day, maintenance 90 mg per day), and ganciclovir (10 mg/kg per day) was used by Gärtner and colleagues[28] who evaluated EBV loads of 59 stem cell transplant patients as a parameter for prediction and monitoring of PTLD. From this study, we estimated a minimum doubling time of about 21 h (table 2). In one adult patient, EBV doubling time was approximately 11 h (day 29–36), and half-life around 16 h (day 57–65, aciclovir discontinued, foscarnet and ganciclovir started). In one paediatric patient with primary EBV infection, doubling time was approximately 23 h (day 28–36) and half-life around 1 day (day 50–59, aciclovir discontinued). Thus, the estimates of the shortest EBV dynamics yielded doubling time of approximately 11 h and half-life approximately 16 h. In these preliminary estimates, no effect of antiviral treatment could be discerned, but the sampling frequency may not have been adequate for further conclusions. Despite antiviral therapy, EBV dynamics were rapid with the shortest viral doubling time similar to B-cell proliferation times of 8–11 h.[57]

Changes in EBV loads after administration of anti-CD20 antibodies (rituximab) were reported by Orentas and colleagues.[29] One paediatric bone marrow transplant patient had—within 1 day—a rapid initial response to rituximab followed by a rapid rebound of EBV load ($1·65\times10^6$ to $2·06\times10^5$ to $7·40\times10^5$ copies per µg DNA).[29] Assuming equally long time intervals for the decay and rebound phase at day 83 (otherwise one estimate would get shorter at the expense of the other one), the half-life was about 4 h, and the doubling time during the viral rebound the same day was around 7 h. The half-life after a second anti-CD20 injection at day 88 was 8 h or less ($1·179\times10^6$ to $1·5\times10^5$ copies per µg DNA). The short half-life of approximately 4 h is similar to median plasma viral load decline half-life of approximately 2 h observed after surgical resection of nasopharyngeal carcinomas.[30]

Adoptive immunotherapy has been applied by Gustafsson and colleagues[56] who studied how infusion of EBV-specific cytotoxic T cells reduced EBV-DNA loads in nine bone marrow transplant patients, thereby diminishing the risk of PTLD. Kinetic parameters estimated from this study were doubling time approximately 1·8 days and half-life 1 day or less (table 2). The two studies[29,56] show that immune therapy is powerful in reducing cell-associated viral loads. However, fast rebounds reported in these studies raise questions about the duration of reponse.

We note that the average doubling time of EBV across different transplant settings and interventions was remarkably robust (mean doubling time 2·6 [SD 1·2] days or less, table 2). To estimate B-cell PTLD dynamics in vivo, we provide preliminary estimates of the daily loss of EBV-infected cells if EBV replication were entirely episomal, if EBV replication were only lytic, or if both modes were mixed (figure 5). 1 µg DNA corresponds with around 150 000 cells (using the standard conversion factor of 6·6 pg DNA per diploid cell).[29] Wagner and colleagues[58] report a median EBV load of 50 copies per µg DNA in immunocompetent (healthy) EBV-seropositive carriers. This corresponds with a frequency of one EBV copy per 3×10^3 cells. Assuming that an adult individual has approximately 10^{11} B cells,[59] this amounts to $3·3\times10^7$ EBV copies per individual. Applying an EBV half-life of 2 days as extracted from the data of Smets and colleagues,[27] we estimate that about $9·7\times10^6$ EBV copies, or 30%, were cleared every day. Assuming a purely episomal mode of EBV-DNA replication in dividing B cells, and only one episome per cell, the turnover is approximately $9·7\times10^6$ cells per day under conditions of stable EBV loads (figure 5). If a cell contains 20 episomes,[60] the daily turnover is 20 times lower. If the daily declining EBV load corresponded with loss of cells alone and was to be balanced by three cell divisions per day,[57] approximately $6·1\times10^4$ to $1·2\times10^6$ cells needed to proliferate. Under steady-state conditions, it is clear that the daily loss of B cells decreases if the half-life of an infected cell increases. Because the fraction of lytically replicating cells also affects the daily loss of B cells, we plotted in figure 5 how a switch from entirely episomal replication over mixed modes to purely lytic replication influences cell loss, assuming that 5000 progeny viruses were released per lysed host cell and a virion half-life of 2 h. The calculated turnover rate for a cell half-life of 33 days and about 12% lytic replication is around four B cells per second (figure 5). For the given viral burst size, a switch from episomal to lytic replication does not much alter estimated B-cell turnover rates as long as the proportion of lytically replicating cells remains at 75% or less.

During the peak of primary EBV infection, average EBV copy numbers are around 2000-fold higher

compared with EBV levels of healthy seropositive carriers.[28] Thus, extrapolations indicate that at peak of infection, 10% or more of all B cells can be EBV positive and that peak body cell loss values can be 10^3 or more times higher than those indicated in figure 5. Considering that the blood contains about 1·4% of B cells,[59] our estimates are in line with values of tonsillar (approximately 135×10^7) and peripheral blood (approximately 175×10^7) EBV-infected cells measured by Laichalk and colleagues.[61]

PTLD sometimes progresses very rapidly leading to death of an individual within 1 month.[28] Our dynamic view of the B-cell PTLD implies that to grow within 30 days after initiation of transplantation-immunosuppression from around 10^7 EBV-positive cells, as reported from healthy EBV-seropositive individuals,[58] to around 10^{11} EBV-positive cells—ie, doubling the abundance of B cells in vivo—the net growth rate (Malthusian parameter) must be approximately 0·31 per day. This corresponds with a net doubling time of approximately 2·2 days (panel and figure 6).

In summary, we find rapid EBV dynamics in the order of hours to around 2 days when comparing kinetic data of cell-free virus in non-transplant patients where the source of viral replication was removed with estimates of cell-associated virus obtained from antibody or cytotoxic-T-lymphocyte-treated transplant patients. The shortest viral doubling times of cell-associated EBV-DNA of 7–11 h match the doubling times of proliferating B cells.[57] Our estimation of the daily EBV-associated B-cell turnover hinged on the assumption of a dynamic steady-state. Under conditions described in figure 5, the turnover in a healthy EBV-positive individual is one to four B cells per second. The variable kinetics of PTLD progression observed in patients can be explained by differences in the net surplus between proliferating cells and destructed cells. This could also explain the rapid doubling of the tumour mass and difficulties with debulking treatment necessitating surgery and chemotherapy. The accumulation of EBV-infected cells could be circumvented by avoiding T-cell depleting protocols or by infusing ex-vivo expanded EBV-specific T cells to check uncontrolled B-cell proliferation.[62,63]

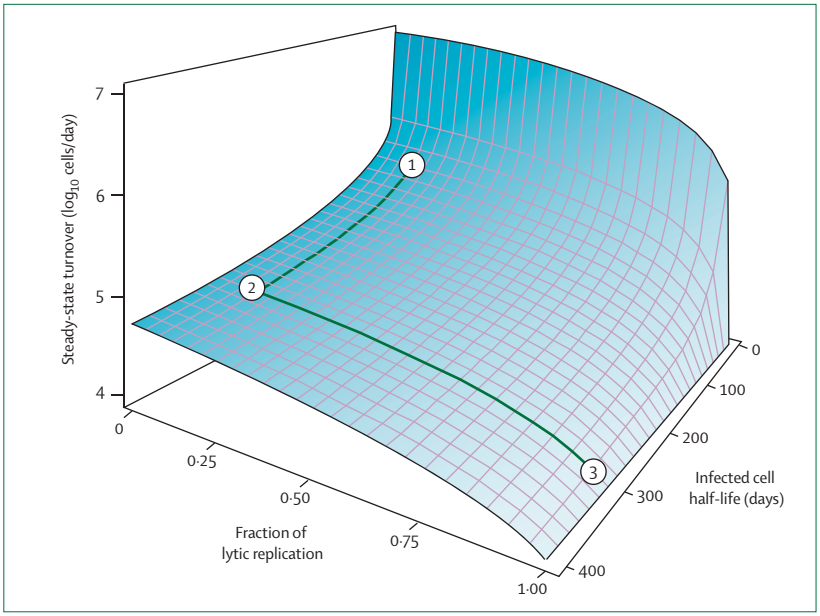

***Figure 5*: Daily loss of B cells in healthy EBV-seropositive individuals**
Steady-state EBV load: equivalent to 50 copies per µg peripheral blood mononuclear cell DNA. Circles indicate selected points: (1) 12% lytic replication, half-life 33 days (corresponding cell loss approximately 5·5 log_{10} cells per day, equivalent to four cells per second); (2) 12% lytic replication, half-life 300 days (corresponding cell loss 4·8 log_{10} cells per day, equivalent to 0·8 cells per second); (3) 96% lytic replication, half-life 300 days (corresponding cell loss approximately 4·2 log_{10} cells per day, equivalent to approximately 0·5 cells per second). Note that changes in the proportion of lytic replication between 0–75% do not substantially alter B-cell turnover rates. We assume that 5000 progeny viruses are released per lytically infected cell; virion half-life 2 h. Total B cells per individual: 10^{11} cells. Colour code shows darker blue=high values; lighter blue=low values. The plot was generated with a two dimensional function $f(x,y)=log_{10}[x(t_{½}p)+y(t_{½}p)]$, with $x(t_{½}p)$ considering cell loss caused by episomal replication and $y(t_{½}p)$ considering cell loss caused by lytic replication. $t_{½}$=half-life.

Other transplant relevant herpesviruses

The majority of adults are seropositive for HHV6 and HHV7. Primary infection usually occurs during early childhood and infected individuals remain persistently infected throughout life. The main target cells of both viruses seem to be CD4+ mononuclear cells and cells of salivary glands. Reactivation in immunocompromised hosts might be responsible for opportunistic diseases. In stem cell transplant recipients, HHV6 has been associated with fever, encephalitis, interstitial pneumonitis, delayed engraftment, and high graft-versus-host disease. The pathological potential of HHV7 in this setting is less well defined. Boutolleau and colleagues[31] assessed the level of viral replication in 78 stem cell transplant patients post-transplant. The median follow-up was 107 days, and post-transplant peripheral blood mononuclear cell samples were collected every 7–14 days. All patients received aciclovir prophylaxis against CMV infection. In a subset of 66 patients, sufficient data for HHV6 and HHV7 kinetic estimates were described.[31] On the basis of median viral load data, we obtained an HHV6 doubling time of 1·6 days (day 0–7) and 3·2 days (day 7–14) and a half-life of 1·9 days (day 14–21). Analysis of the HHV6 growth kinetics of patient 1, who had a tremendous viral expansion followed by a stable viraemia,[31] revealed a rapid increase with a doubling time of 10 h or less over the first 6 days and a doubling time of about 4·5 days between days 6 to 21. Growth kinetics were similar in patients 2 and 3 (doubling time approximately 1·5 days), whereas the viral half-life between days 26 to 33 estimated from patient 2 was 21 h or less.[31] In all three cases, transient thrombocytopenia was observed about 2 weeks after the onset of HHV6 expansion. Additionally, delayed engraftment, cutaneous rash, and partial myelosuppression were associated with elevated viral load measurements.

From the median HHV7 viral load measurements reported by Boutolleau and colleagues,[31] we obtained a doubling time of 1·6 days (day 0–7) and half-life 1·8 days (day 7–14). The viral doubling time of HHV7 between day 0

Panel: Mathematical model of EBV-associated PTLD

A mathematical model was constructed to describe the dynamics of EBV-infected B cells (equation 1) and free virus (equation 2) en route to PTLD.

$d/dtB_v = p*B_v + i*V*B - d*B_v - k*T*B_v$ (equation 1)

$d/dtV = n*d*B_v - c*V$ (equation 2)

The term $p*B_v$ denotes proliferation of EBV-infected B cells, $i*V*B$ denotes infection of uninfected B cells by virus, $d*B_v$ lysis of infected B cells, $k*T*B_v$ killing of infected B cells by immune effectors such as cytotoxic T lymphocytes, $n*d*B_v$ release of viral progeny from lysed host cells, and $c*V$ clearance of free virus. Assuming that the dynamics of free virus is much faster than that of infected cells, we can set $d/dtV=0$, solve for V, and replace V in equation 1. We then obtain a single equation describing the dynamics of EBV-infected B cells, which is

$d/dtB_v = p*B_v + i*(n*d*B_v*1/c)*B - d*B_v - k*T*B_v$ (equation 3).

Factoring out B_v in equation 3 leads to

$d/dtB_v = B_v*[p+(i*n*d*B*1/c) - d - k*T]$ (equation 4).

From equation 4, we obtain the basic reproductive ratio as

$R_0 = p/(d+k*T) + i*n*d*B/(c*[d+k*T])$ (equation 5).

The term $p/(d+k*T)$ denotes the contribution from episomal replication, $i*n*d*B/(c*[d+k*T])$ the respective contribution from lytic replication. If EBV replicates purely episomal (no lysis), R_0 reduces to $p/(k*T)$. To have B_v growing, R_0 must be >1 ($B_v \neq 0$).

The formula for R_0 provides a rationale to discuss the effect of various interventions on growth control of EBV-infected B cells. For a mixed mode of 10% lytic and 90% episomal replication, as shown in figure 6, the depletion of B cells by an anti-B220 antibody would reduce R_0 from 1·16 to 1. Remission of a tumour requires additional simultaneous interventions such as chemotherapy with proliferation inhibitors or the infusion of ex-vivo expanded autologous T cells. For example, a 10% expansion of the EBV-specific T cells would further reduce R_0 to about 0·91 (equivalent to a half-life of 5–6 days).

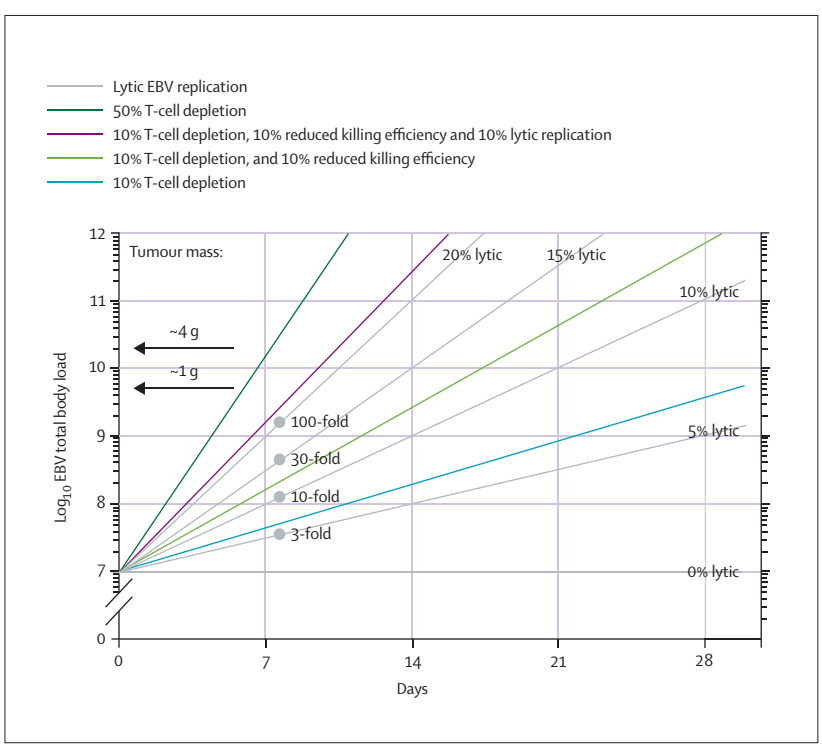

***Figure 6*: Increase of EBV load en route to PTLD**

The horizontal grey line (labelled 0% lytic) indicates the steady-state EBV load of an immunocompetent healthy EBV-seropositive individual (equivalent to about 50 copies per µg DNA[27]). A switch from 100% episomal replication to a mixed mode of 5% lytic and 95% episomal replication increases the viral load by a factor of three within 1 week (see the line labelled 5% lytic). Higher fractions of lytic replication further increase the viral load. Coloured lines show the effect of various interventions on growth control of EBV-infected B cells. The diagram can be used as a graphical calculator to extrapolate the time until a critical tumour mass will be achieved in a patient. 5×10^9 EBV-positive B cells correspond with a tumour mass of about 1 g, 2×10^{10} EBV-positive B-cells to a tumour mass of about 4 g. Parameters at steady-state: p=2·1, i*B=3·3, n=5000, d=0·00016, c=8, k=0·03, T=70, $B_v(0)=1\times10^7$.

to 7 in patient 3 was 22 h or less, the half-life between days 7 to 21 was approximately 2·1 days.[31] In general, viral loads were rather low, often below the limit of detection. No association between HHV7 infection and clinical or biological manifestations was observed.

The reported data suggest rapid dynamics of both HHV6 and HHV7 in stem cell transplant patients despite aciclovir prophylaxis. Preferentially, HHV6 seemed to reactivate in immunosuppressed patients, but this needs further study. CD4+ mononuclear cells, including CD4+ T cells, may be affected by HHV6 and HHV7 infection, but the precise relation of HHV6 and HHV7 kinetics on immune dysregulation requires further study. Individuals with prolonged high viral loads might require early treatment with antiviral drugs such as foscarnet, ganciclovir, or cidofovir.[64,65]

Hepatitis C virus

Liver failure resulting from chronic HCV infection is the main indication of liver transplantation today. The major challenges are complex treatments at substantial costs, but limited efficacy, particularly for genotype 1, which is associated with the poorest prognosis.[66,67] Patients treated with interferon α frequently have depressive complaints. Of the 200 million people infected with HCV, only a minority lives in countries with transplantation centres. In addition to donor organ shortage, reinfection of the allograft is almost inevitable and graft failure because of recurrence of HCV is the most common cause of retransplantation.

Two types of kinetic estimates obtained from non-transplant patients support the hypothesis of a high viral turnover of HCV. In two HIV/HCV-infected patients

undergoing plasma apheresis, the half-life of HCV was 1·7 h and 3 h, as described in Ramratnam and colleagues.[33] During apheresis (duration 78–207 min), each patient had 25–30 viral load measurements. Similarly, rapid HCV kinetics were observed by Neumann and colleagues[9] in 23 HCV-positive patients treated with interferon alfa-2b (5, 10, or 15 million IU interferon alfa-2b for 2 weeks; sampling every 2–4 h for the first 2 days, then daily for 2 weeks).[9] The mean half-life was 2·7 h (range 1·5–4·6 h). The immediate efficacy of interferon alfa-2b was 80% or more, but dropped to 5–10% or less after 2–3 days. Different HCV genotypes have different interferon response elements leading to distinct decay kinetics under therapy.[66] Thus, the duration of therapy should be chosen according to the HCV genotype. The data indicate rapid dynamics of HCV in non-transplant patients.

In transplant patients, HCV is known to reinfect the newly transplanted liver allograft. Frequent sampling during and immediately after the anhepatic phase of liver transplantation provides unique insights into the replication dynamics of HCV. Fukumoto and colleagues[34] presented data of nine liver transplant patients with sampling 1 day before transplantation, then daily for up to 30 days post-transplant. During the first week post-transplant, all patients received antithymoglobulin and a combination of prednisolone, azathioprine, and cyclosporin A or tacrolimus for the next 3 weeks. The mean HCV half-life was about 4 h (range 2–5·2 h). In eight patients, viraemia began to increase around day 3 post-transplant and HCV-RNA levels exceeded the pre-operative values by day 8.

Gracia-Retortillo and colleagues[35] also analysed data with sampling at the beginning and at the end of the anhepatic phase, followed by 4 h intervals for the first day after graft reperfusion, then daily for another 5 days, and weekly sampling for the next 3 weeks. All 20 patients received 0·5–1 g of methylprednisolone during transplantation. Thereafter, 13 patients received cyclosporin A or tacrolimus plus corticosteroids (regimen A), whereas the remaining seven patients received tacrolimus, mycophenolate mofetil, and monoclonal anti-interleukin-2 receptor antibodies (regimen B). During the anhepatic phase (45–207 min), viral loads decreased in 18 of 20 patients. The estimated mean half-life during the anhepatic phase was 2·2 h (range 0·22–10·3 h). However, in two of 20 patients (regimen A), viral loads remained stable during the anhepatic phase, suggesting that the removed graft was not the major source of plasma virus. After graft reperfusion, viral loads decreased for the first 8–24 h with a mean half-life of 3·4 h (range 0·71–12·8 h) in 19 of 20 patients (including the two patients with stable viraemia during the anhepatic phase). Subsequently, a rapid viral increase was observed in ten of 20 patients (eight on regimen A), with a mean doubling time of 13·8 h (range 7–35 h), and pretransplant viral loads were reached by day 4 post-transplant. Four of 20 patients maintained a stable reduced viraemia (all on regimen A), and six patients showed a slower second-phase decline (no quantitative information available). It should be noted that five of six patients with a second-phase decline were on regimen B. 1 week after transplantation, viral load concentrations increased progressively in 15 of 20 patients (regimen A, nine; regimen B, six), and reached a plateau exceeding pretransplant values by the first month post-transplant. The total follow-up time was 24 weeks, but the study did not aim to relate virus kinetics with graft function. When assuming an intracellular delay of 8 h for HCV,[32,35] we estimate R_0 to be in the order of 0·06 during viral contraction (anhepatic phase, half-life 2 h), whereas R_0 is approximately 1·5 during the subsequent viral expansion driven by reinfection of the allograft (doubling time 14 h).

The stable viraemia during the anhepatic phase in two patients detailed in the study by Garcia-Retortillo and colleagues[35] suggests the existence of an extrahepatic replication compartment. Further evidence for extrahepatic HCV replication was obtained by Dahari and colleagues,[32] who analysed data of 30 liver transplant patients (including the 20 patients from the study by Garcia-Retortillo et al). By fitting mathematical models to data beyond the period of the anhepatic phase, evidence for extrahepatic HCV replication was found in more than 50% of the patients. The mean half-life of productively infected extrahepatic cells was estimated to be 2·6 days (range 0·7–5·3 days), and its contribution to the total plasma viral load was approximately 3% (range 0·1–14%).[32] Figure 7 shows the current view of HCV compartmentalisation.

HCV-associated pathology in the setting of transplantation-immunosuppression was studied in 51 renal transplant recipients.[68] Patients were not treated with interferon α or ribavirin, but cyclosporin A was part of the immunosuppressive regimens. Serial biopsies of the native liver suggested that neither HCV-RNA levels nor the duration of infection were correlated with progression to liver fibrosis. This appears to be inconsistent with a dynamic appreciation of disease progression and organ pathology. Unfortunately, detailed HCV kinetic data in renal transplant recipients were not available, which would allow the effect of HCV dynamics or immunopathology to be distinguished. Possible explanations for the discrepancy are (1) a biopsy bias because of the focal nature of the disease, (2) cyclosporin A might inhibit HCV replication by binding to cyclophilin B, a functional regulator of the HCV RNA polymerase NS5B,[69] and (3) immunopathology contributes to liver fibrosis. A confounding effect of cyclosporin A on the data cannot be excluded, because a trend ($p=0·08$) between duration of infection and progression to fibrosis was found.[68] The observation that HCV levels remained substantially elevated after renal transplantation suggests that the immunosuppressive effect of cyclosporin A overrides the inhibitory effect on HCV replication.[68]

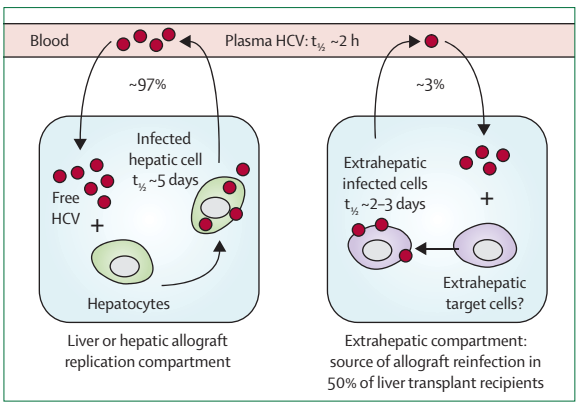

***Figure 7*: HCV dynamics in vivo**
According to reference 32, the bulk of HCV in the plasma (around 97%) comes from replication in the hepatic compartment (left), and around 3% may come from an extrahepatic replication compartment (right). HCV=hepatitis C virus. $t_{½}$=half-life.

Taken together, the data indicate a high daily turnover rate of HCV in vivo of 99% or more. The data point to the liver as the principal site of HCV replication. The abundance of infected hepatocytes after liver transplantation was estimated to be 19%,[70] which compares with values of 30% or less estimated from chronic HCV-infected non-transplant patients.[71] Mathematical models suggest that in 50% or more of liver transplant recipients a second, slowly decaying extrahepatic replication compartment must exist.[32,70] Rapid reinfection of a new allograft with virus from the extrahepatic source is a major concern, which might be amenable to new potent drugs such as protease inhibitors that will be well tolerated before and after liver transplantation. Without antiviral treatment, the kinetics of viral decay and reinfection appear to be rapid, with a better viral control in patients in whom corticosteroids were not part of the immunosuppressive regimen. With treatment, a rapid decline of plasma HCV was associated with an amelioration of liver fibrosis scores.[72] However, this observation does not exclude immunopathology as an important cofactor in liver fibrosis and organ loss.[73]

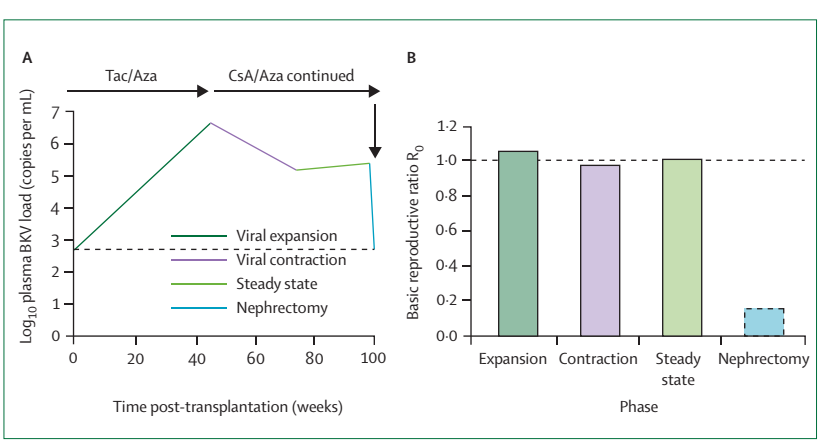

***Figure 8*: BKV load changes in a kidney transplant recipient over 102 weeks post-transplant**
(A) Four phases are depicted. The immunosuppressive regimen is indicated at the top of the panel, transplant nephrectomy is indicated by a vertical black arrow, the detection limit of the assay is indicated by the horizontal dashed line. (B) Quantification of the efficacy of the interventions using the basic reproductive ratio R_0 for the four phases. Upon nephrectomy, R_0 drops to 0·2 or less. The dashed line at R_0=1 indicates the threshold separating viral growth from viral decline. R_0 is calculated according to the formula $R_0=Exp(s*t_d)$, with s denoting the slope of the viral load curve and t_d the intracellular delay. Tac=tacrolimus. CsA=cyclosporin A. Aza=azathioprine.

Polyomavirus type BK

Polyomavirus infection is widespread in the general population with an age-dependent polyomavirus type BK (BKV) seroprevalence ranging from 60% to 90%.[74] Unlike herpesviruses, polyomavirus replication is largely dependent on host cell factors. It does not encode typical antiviral drug targets such as thymidine kinases or viral DNA polymerase. In replication-permissive cells, expression of the viral capsid proteins is followed by virion assembly in the nucleus, which eventually results in host cell lysis to release infectious progeny. By contrast with HIV, where lymphoid tissues in many anatomical sites are infected, graft nephrectomy studies in kidney transplant recipients indicate that the principal site of BKV replication is the renal allograft.[75–77] When manifest as a disease—a newly recognised cause of early allograft loss—it is called polyomavirus associated nephropathy (PVAN).[73,78–81]

The relation between the level of BKV replication, development of PVAN, and graft failure is not well understood.[2,82] First analyses indicate that BKV loads in plasma above 10^4 copies per mL are 93% sensitive and specific for histologically manifest disease.[83] However, false-negative biopsy results presumably caused by the focal nature of the infection have been obtained in around 30% of individuals.[83] In patients clearing BKV load in plasma, PVAN is no longer found histologically. Because no specific antiviral treatment is established, first-line treatment aims at improving the immune control by reducing immunosuppression.

Recently, we explored the dynamics of BKV in three nephrectomised and 12 non-nephrectomised patients receiving reduced immunosuppression.[77] Four different phases of BKV replication kinetics can be distinguished, as shown for one patient (figure 8). During the viral expansion phase between weeks 0 and 45, the BKV load increased from undetectable to a peak value of $3·8x10^6$ copies per mL. During that time the patient received tacrolimus and azathioprine as immunosuppressive therapy. From the slope of 0·025 per day of a fitted regression line, we estimated that the net viral doubling time was 28 days. Assuming an intracellular delay of 2 days from de novo infection of host cells to release of progeny virus,[37] we estimated that R_0 was 1·05 (figure 8). When tacrolimus was replaced by cyclosporin A at week 45 (but azathioprine was continued), a phase of viral contraction lasting 29 weeks was observed. From the slope of –0·015 per day, a net half-life of 45 days could be estimated, and R_0 dropped to 0·97. Over the following 25 weeks, the BKV load remained stable at about 10^5 copies per mL indicating a quasi-steady-state

phase with an R_0 of approximately 1. Because the slope of a fitted line would be close to zero during that time, no meaningful viral doubling time or half-life could be estimated. At week 99, the allograft was removed from the body and the viral load dropped within 1 week to levels below the limit of detection. Assuming that the detection limit represents the minimum decline after nephrectomy, a decay slope of –0·85 per day could be obtained, which translated into a half-life of 20 h or less. R_0 dropped to 0·2 or less after nephrectomy.

In a patient prospectively sampled with high frequency after nephrectomy (every 3 h for the first day), the viral load dropped for 6 h with an average half-life of 1·4 h. The average viral kinetics estimated from 12 patients receiving various combinations of immunosuppressive drugs were: doubling time 5 days (range 18 h to 37 days), half-life 5·5 days (range 5·5 h to 17 days).[77]

The general picture of BKV pathogenesis and PVAN that emerges when combining in-vivo and in-vitro data of BKV from various sources is a short viral half-life, which implies a high viral turnover of more than 99%. In-vitro experiments reported an intracellular delay of 2 days between infection of a host cell and release of infectious progeny virus.[37] Assuming a viral half-life of 1–2 h and a burst size of 10^4 progeny viruses per productively infected cell,[77,84] we estimate that 10^5 to 10^6 renal cells are lost every day to maintain an apparently stable viral load of around 10^6 copies per mL.[77] Because the kidney has only a finite number of host cells (tubular epithelial cells) and a presumably limited regenerative capacity, ongoing viral replication pushes the graft to a state where the target cell pool is eventually exhausted. If, for example, the overall size of the target cell pool were 10^8 cells, extrapolation suggests an infection duration of 3 months to 3 years. Over a period of 25 weeks with a stable viraemia of around 10^5 copies per mL, as shown in figure 8, 50–60 generations of lytically replicating virus follow one another with a cumulative loss of 5×10^6 or more renal cells. The extrapolated graft survival time is 10 years or less. The examples indicate that for a cytopathic virus such as BKV, a stable viraemia in a patient is not sufficient to prevent disease progression.

Our estimate of the cytopathic contribution of BKV to PVAN is solely based on plasma virus data. By not considering viral load data from other compartments such as the urinary tract, where the viral load is usually much higher than in the plasma, it is likely that we underestimate the overall cytopathic contribution of BKV to PVAN. Hence, the projected survival time of an infected graft could well be much shorter than estimated from blood data only. Viral load dynamics of BKV in matched blood and urine samples and mathematical modelling suggest weakly linked replication compartments in vivo (Funk and Hirsch, unpublished data). Additionally, a renal allograft becomes dysfunctional well in advance before the last host cell is destroyed. As the replication kinetics of BKV become better defined, immunotherapeutic approaches should be reconsidered to improve current post-transplant management strategies.[82]

Other transplant relevant virus infections

GBV-C, previously known as hepatitis G virus, was quantified by Berg and colleagues[36] in 12 patients before and daily after liver transplantation for 25–28 days to test whether the liver is the principal site of replication.[36] Although the paper provides quantitative viral load information, decay rates were not calculated. When reanalysing the reported mean GBV-C RNA values from day –1, 1, 2, 3, 7, and 28 post-transplant, we estimated that the GBV-C half-life immediately after hepatectomy was 2·5 days or less. Between days 1 and 7, the respective half-lives were 18, 16, and 12 days. Between days 7 and 28 the viral load increased with a doubling time of 67 days. From the observed decay characteristics, the authors concluded that the liver is not the major site of GBV-C replication. This implies that the true half-life might be shorter than 2·5 days, but also that the contribution of GBV-C to liver pathology is limited. It also offers an explanation for the poor response of GBV-C to interferon-α therapy.[85]

Adenovirus has received attention because stealth adenovirus constructs are used as vectors for transgene delivery in gene therapy and oncolysis. Adenovirus type 5 is rapidly cleared from the blood of mice with a half-life of 2 min or less.[86] The kinetics of adenovirus in human hosts remains obscure. In paediatric transplantation, adenovirus infections cause frequent complications. The initial phase of infection may be local and asymptomatic. Simultaneous isolation of adenovirus from multiple sites (urine, stool, throat swaps) has been associated with occurrence of clinical disease, whereas isolation of adenovirus from plasma is associated with high mortality. Lankester and colleagues[39] quantified adenovirus loads in two paediatric stem cell transplant patients to evaluate the effect of antiviral treatment. From one patient described in the study,[39] we were able to estimate a viral doubling time of 1·3 days (day 48–73). The increasing adenovirus load is remarkable since treatment with cidofovir (5 mg/kg per

Search strategy and selection criteria

Data for this Review were identified by searches of PubMed using combinations of the search terms "transplant", "transplantation", "kinetics", "dynamics", "eclipse phase", and "intracellular delay" together with the names of the viruses described in this article. Further search terms were "EBV episomal", "EBV circular DNA", "EBV linear DNA", "EBV cell free", and "EBV cell associated". We also included references from key articles and from our own files. If no viral kinetic data (doubling times, half-lives) were available, we selected those papers that allowed us to accurately estimate viral kinetics from viral loads reported in the text, in tables, or in figures. Only papers written in English language were considered. The final search date was March, 2006.

day) was initiated on day 59 (17 days after the second transplantation). Because the viral load further increased and clinical symptoms progressed, ribavirin was added to the antiviral treatment at day 66 (loading dose 30 mg/kg per day, maintenance dose 60 mg/kg per day), but its antiviral efficacy on adenovirus is questionable. Unfortunately, the patient died at day 78 because of fulminant adenovirus disease. In another patient, adenovirus was isolated from stool, urine, and throat swabs. However, the patient remained free of adenovirus in the plasma and clinical symptoms were restricted to a temporary form of mild diarrhoea.[39] From a bone marrow transplant patient described by Watzinger and colleagues,[40] we obtained a similar doubling time of 2·1 days (day 154–168). Our analysis shows that rapid dynamics of adenovirus can be observed in some paediatric stem cell transplant patients. Because plasma viraemia precedes onset of clinical symptoms by a median of more than 3 weeks,[87] frequent sampling is required to identify patients at risk of progression to fulminant disseminated disease with organ complication such as pneumonia, hepatitis, encephalitis, or fatal multi-organ failure, and to monitor pre-emptive antiviral interventions.

Conclusion

When looking across various viral infections and different transplant settings, we see that viral replication in vivo is much more rapid than initially thought. Minimum half-lives range from less than 1 h (HCV) to 1–2 days (CMV). A direct consequence of short viral half-lives in vivo is a high daily turnover of viruses ranging from around 50% to more than 99%. Organ pathology depends partly on pathogen-related factors such as cytopathicity, the viral generation time, and turnover rate, but also on the viral load and the strength and timing of a host's immune response (the degree of immunosuppression). To identify potentially harmful infections as early as possible, blood sampling and molecular diagnostic analysis need to be optimised after transplantation and after changing immunosuppressive therapy. Efficacies of antiviral interventions can be quantified and monitored by the basic reproductive ratio R_0. For lytically replicating viruses, it might not be sufficient to achieve a stable viral load in a patient, since host cells are constantly lost and must be replenished to preserve graft function. The kinetic view suggests that disease and graft failure are correlated with the rapid replication dynamics that above a certain threshold exhaust the regenerative capacity and provoke compromising acute and chronic inflammatory responses. For viruses eliciting an only partly replication-dependent or fully replication-independent pathology, such as EBV in PTLD, viral dynamic estimates may prove crucial to optimally interpret viral load data, risk of disease, and efficacy of treatment.

Conflicts of interest

We declare that we have no conflicts of interest.

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