Herpes viruses and tumours in kidney transplant recipients. The role of immunosuppression

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Abstract

Herpes virus infections are frequent in renal transplant recipients. Some herpes viruses are not only responsible for life-threatening infections and renal graft injury but can also increase the risk of malignancy. Three herpes viruses, namely cytomegalovirus (CMV) or human herpes virus 5, Epstein–Barr virus (EBV) or herpes virus 4 and human herpes virus 8 (HHV8), may play an oncogenic role. The oncogenic role of CMV is controversial. However, there is growing evidence showing that CMV can infect cancer cells and favour their resistance to the immune system and chemotherapy. B cells infected by EBV can have uncontrolled proliferation eventually resulting in polyclonal polymorphic or monomorphic post-transplant lymphoproliferative diseases (PTLD), which are particularly frequent in children and in EBV-negative recipients. In some ethnicities, the carriers of HHV8 are susceptible to develop Kaposi’s sarcoma after transplantation. The intensity of immunosuppression therapy plays a critical role in mediating infections from oncogenic herpes viruses. However, the type of drugs used to prevent and treat rejection can influence the risk of virus-related diseases and complications.

In this paper, the possible impact of immunosuppression on post-transplant tumours caused or mediated by herpes viruses will be reviewed.

Cytomegalovirus

Human cytomegalovirus (CMV) is a double-stranded DNA virus belonging to the family β-herpes viridae. It is also called human herpes virus 5. Replication of CMV involves three types of genes: immediate early genes coding for IE proteins; early genes coding for E antigens and late genes coding for L antigens, including the H65 matrix.

CMV is the most common virus infecting renal transplant recipients. There are three forms of CMV infection in transplant patients: primary infection, secondary or reactivated infection and superinfection. The typical CMV disease manifests itself with fever, anorexia, myalgias, headache and malaise. These symptoms usually occur between 1 and 4 months after transplantation; however, the onset of CMV disease may occur later if the patient received a prophylactic treatment with intravenous ganciclovir or valganciclovir. The disease is often associated with anaemia, leucopenia, thrombocytopenia, mild lymphocytosis with atypical lymphocytes and mild hepatitis. Some patients, particularly those affected by primary infection, may develop pneumonia, gastrointestinal complications, encephalitis or myocarditis. Apart from the risk of infection, CMV may favour the development of acute rejection [2], particularly in CMV mismatched (donor+/recipient−) patients [3], and in the long term, may increase the risk of interstitial fibrosis and chronic allograft injury [4,5] through the overproduction of mediators, cytokines, chemokines and allograft outcome. Not only can viruses cause life-threatening infections but they can also cause irreversible renal graft injury. Moreover, there is evidence that cytomegalovirus (CMV) can increase the risk of cancer in patients with acquired immunodeficiency syndrome [1]. The intensity of immunosuppression therapy plays a major role in these events. However, it is also possible that the type of drugs used to prevent and treat rejection can influence the risk of virus-related diseases and complications.

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and growth factors. It has also been shown that CMV infection is associated with atherosclerosis [6] and can accelerate vascular disease in experimental models [7].

The relationship between CMV and cancer is still under investigation. Although CMV is generally not regarded to be an oncogenic virus, there is emerging evidence that the progression of some tumours may be enhanced by oncomodulatory signals produced by regulatory proteins encoded by human CMV. (i) The US28 protein, a G protein-coupled chemokine receptor encoded by CMV, mediates proliferative signalling through the interleukin (IL)-6–Signal Transducer and Activator of Transcription (STAT) axis. It has been proposed that US28 induces proliferation in HCMV-infected tumours by establishing a positive feedback loop through activation of the IL-6–STAT3-signalling axis [8]; (ii) Recruitment of tumour vessels is an integral part of cancer initiation and progression. In rats, the US28 protein can induce vascular endothelial growth factor stimulation and recruitment of tumour vessels, therefore, promoting angiogenesis [9]. Moreover, the expression of US28 promotes development of intestinal dysplasia and cancer in transgenic mice suggesting that CMV infection may facilitate development of intestinal neoplasia in humans [10] (Figure 1); (iii) Resistance to apoptosis is a common feature of cancer cells and represents a relevant mechanism inducing chemoresistance. Experimental studies showed that human CMV can protect tumour cells from apoptosis by the induction of cellular proteins, including AKT, Bcl-2 and ΔNp73α [11]; (iv) Cancer cell migration, invasion and adhesion to the endothelium play important roles during formation of metastases. The CMV-encoded US28 protein can induce arterial smooth muscle cell migration by a ligand-dependent process. This migration might have important implications in the invasion of CMV-infected tumour cells through the increased movement of these cells in and around vascular lesions [12]; (v) The ability to evade recognition by the immune system is essential for cancer cells. UL16, another CMV-encoded protein, mediates the increased protection against cytolytic proteins released by activated natural killer (NK) cells, possibly by a membrane-stabilizing mechanism [13] and (vi) Clinical investigations using highly sensitive techniques for virus detection showed the presence of genome and antigens, of CMV in tumour cells of >90% of patients affected by colon cancer [14], malignant glioblastoma [15], prostate carcinoma [16] or breast cancer [17].

These observations demonstrate that CMV can cause low-grade infections in tumour cells probably sustained by persistent virus replication. Based on the available experimental and clinical data, Michaelis et al. [18] developed the concept of ‘oncomodulation’ to better explain the role of CMV in cancer. Oncomodulation means CMV infects tumour cells and increases their malignancy. What is still to be elucidated is whether CMV establishes persistent virus replication in tumour cells or not.

The type of immunosuppressant may have a different influence on the risk of CMV infection. Randomized controlled trials (RCTs) and meta-analyses showed an increased incidence of CMV infection in transplant recipients, who received induction therapy with antithymoglobulins [19,20], OKT3 [21] or alemtuzumab [22], while the incidence of CMV was comparable with that of placebo in patients who received induction therapy with anti-CD25 monoclonal antibodies [23,24]. A review of 16 studies comparing anti-CD25 monoclonal antibodies to Anti-thymocyte globulin (ATG) showed a 75% increase in malignancy and a 32% increase in CMV disease for patients treated with ATG [25]. Tacrolimus and cyclosporine showed similar incidence and severity of CMV infection in a meta-analysis and meta-regression of RCT [26]. Some investigators reported that mycophenolate mofetil may increase the risk of CMV infection [27] but this was not confirmed by other studies [28]. From a theoretical point of view, the mammalian target of rapamycin (mTOR) inhibitors sirolimus and everolimus could interfere with CMV infection. Actually, the cascade of kinases governed by phosphatidylinositol-3 kinase (PI3-k), which is necessary for viral DNA replication and completion of the viral lytic life cycle is up-regulated by human CMV [29]. By inhibiting mTOR, the downstream effectors of PI3-k, sirolimus and everolimus can inhibit the PI3-k-dependent factors of transduction and CMV replication. However, human CMV can induce mechanisms to maintain the integrity of two mTOR effectors, eucaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP) and eIF4G even when mTOR signalling is inhibited [30]. The anti-CMV activity of mTOR inhibitors may be limited by the fact that in CMV-infected cells, the rictor complex (mTORC2) is drug sensitive for the hyperphosphorylation of 4E-BP, but the raptor complex is not. These data suggest that, during CMV infection, the rictor- and raptor-containing complexes are modified so that their substrate specificities and sensitivities to mTOR inhibitors are altered. This may explain why the inhibitory effects of these drugs may be incomplete [31]. On the other hand, a recent study showed that mTOR is a major regulator of memory CD8 T-cell differentiation and, in contrast to what is expected, sirolimus has immunostimulatory effects on the generation of memory virus-specific CD8 T cells. This molecular pathway regulating memory formation can therefore represent a significant barrier against viral infection [32]. Whatever the mechanisms, a number of studies reported a relatively low incidence and severity of CMV infection in organ transplant recipients receiving mTOR inhibitors. Two RCTs, one comparing

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**Fig. 1.** A simplified overview of the mechanisms through which activated chemokine receptor US28 might act as a viral oncogene and enhance and/or promote CMV-associated tumour progression [8–10].
everolimus versus azathioprine and the other comparing everolimus versus mycophenolate mofetil in cardiac transplant recipients reported a significantly lower incidence of CMV infection in patients assigned to everolimus [33,34] and a meta-analysis of 33 studies reported that the risk of developing a CMV infection in renal transplant recipients treated with an inhibitor of mTOR, either as primary immunosuppressor or in association with a calcineurin inhibitor (CNI), was halved in comparison with patients treated with a CNI and an antimetabolite [35].

There are conflicting data about the impact of CMV on malignancy in transplant recipients. In a study of 40 adult liver transplant recipients, all of whom seronegative for Epstein-Barr virus, 12 developed post-transplant lymphoproliferative disorders (PTLD). In these patients, the significant factor associated with the development of PTLD was CMV disease with a relative risk of 7.3 [36]. Another report showed that in transplant recipients who were seronegative for CMV but received a CMV-positive allograft the risk of PTLD was 4- to 6-fold higher [37]. A retrospective analysis of the Collaborative Transplant Study (CTS) reported that prophyactic anti-CMV immunoglobulin, but not antiviral drugs, prevented the development of early post-transplant non-Hodgkin lymphoma in kidney graft recipients [38]. Another report of CTS showed that the CMV status did not influence the risk of developing PTLD; however, hospitalization caused by CMV disease during the first year post-transplant increased by 6.1 times the risk of PTLD [39]. On the other hand, it has been suggested that the increased number of blood γδ T cells following CMV infection may protect kidney transplant recipients from malignancy since these cells react against CMV-infected cells and tumour epithelial cells in vitro. In a longitudinal case–control study involving 18 recipients, who developed cancer between 2 and 6 years after transplantation and 45 recipients who did not, the median percentage of γδ T cells among total lymphocytes in patients with malignancies was significantly lower compared with that seen in control patients at 6, 12 and 18 months before the diagnosis of cancer. Instead, a γδ T-cell percentage of >4% was significantly associated with a lower incidence of cancer only in recipients who experienced pre- or post-graft CMV infection. In the same paper, a retrospective follow-up of 131 recipients for 8 years revealed that CMV-naive recipients had an ~5-fold higher risk of cancer compared with CMV-exposed patients. [40]. However, the expansion of γδ T cells in CMV-positive patients is puzzling since CMV down-regulates factors involved in the activation of γδ T cells. Moreover, at least in malignant glioblastomas, the level of CMV in the tumour is a strong prognostic factor for patient survival. Patients with tumours and low-grade CMV infection lived three times as long as those with high-grade CMV infection [41].

In summary, experimental and retrospective clinical studies suggest that CMV may promote tumour progression. However, there is no formal evidence supporting the oncogenic role of CMV in transplant recipients. Large prospective studies are needed to better elucidate if the different impact of immunosuppressive drugs on tumours is mediated by their effects on CMV and/or on γδ T cells. While we wait for further data, induction therapy with lymphocyte depleting antibodies should be avoided in CMV-negative patients receiving a transplant from CMV-positive donors since this treatment can increase the risk of CMV disease [19–22] and malignancy [25]. Moreover, CMV-negative transplant patients should preferably receive an immunosuppression based on mTOR inhibitors, which can interfere with CMV replication [33–35] and protect from cancer [42].

**Epstein–Barr virus**

EBV, also known as herpes virus 4, is a latent γ-herpes virus that infects almost 90% of the general population as a consequence of a primary lytic infection in the oropharynxes. The primary infection may be asymptomatic or present as infectious mononucleosis. In healthy subjects, very few B lymphocytes harbour EBV DNA. This pool is maintained, in part, by a lytic infection cycle, which presents the virus for the infection of new cells [43]. Latently infected B cells express only a limited number of genes, such as EBV nuclear antigen-1 (EBNA-1) and latent membrane protein-2 (LMP-2). This restricted gene expression is a mechanism through which the virus evades host responses. Moreover, EBNA-1 is required for the viral DNA to maintain itself in actively dividing lymphocytes and can reduce the surface expression of Major Histocompatibility Complex class I and adhesion molecules, while LMP-2 allows the virus to limit its gene expression in the latency phase [44]. These latently infected B lymphocytes are immortalized through the production of an EBV-encoded gene, BCRF1, which has >80% aminoacid identity with IL-10 [45]. BCRF1 inhibits the synthesis of interferon-γ (IFN-γ) by lymphocytes and NK cells. The inhibition of IFN-γ can favour the outgrowth of EBV-transformed B cells. However, in normal subjects, the immune response to EBV by CD4+ and CD8+ T lymphocytes limit primary EBV infection and control chronic infection control [46].

After transplantation, immunosuppressive therapy disrupts the normal balance between latently infected B-cell proliferation and the EBV-specific T-cell response. Moreover, EBV generates the polypeptide BCRF1, which can induce anergy to donor-specific alloantigens through its suppressive effects on macrophage and T-lymphocyte functions [47], while the role of oncogenes as cofactors is still under investigation. All these factors impair the ability of the immune system to eliminate the virus and lead to the development of PTLD, ranging from polyclonal polymorphic PTLD to more malignant monomorphic PTLD. About 85% of PTLD are represented by B-cell lymphoma and express CD20, while T-cell lymphoma and Hodgkin’s disease are more rare. The role of EBV infection in the pathogenesis of PTLD is supported by evidence that most patients with PTLD are EBV positive [48]. EBV-naïve status and the intensity of immunosuppression play a major role for the development of PTLD [49]. The incidence of PTLD is variable and may depend on the degree of immunosuppression, the number of episodes of acute rejection and a patient’s immune status to EBV. The incidence of PTLD is thought to be bimodal; cases in the first year after solid-
organ transplantation are typically related to EBV. A second peak of incidence occurs >1 year following transplantation and is typically not related to EBV [50].

The type of induction therapy can influence the risk of PTLD. In a multivariate Cox analysis of 38,519 primary kidney transplants from 1997 to 2000, the actual incidence of PTLD was 0.85% in 2713 recipients treated with orthoclone OKT3, 0.81% in 4343 treated with antithymoglobulins, 0.50% in 7800 with anti-IL-2 receptor antibodies such as daclizumab or basiliximab and 0.51% in 23663 recipients with no induction therapy. The Cox model indicated that compared with no induction, the increased risk of PTLD was 72% with monoclonal antibodies, 29% with polyclonal antibodies and 14% with anti-IL-2 receptor induction [51]. Another American survey also reported an almost doubled risk of PTLD in renal transplant recipients treated with antilymphocyte antibodies [52]. Opelz et al. [53] also reported that the standardized incidence ratio of lymphoma compared with a similar normal population was >20 for patients who received induction with powerful antithymocyte globulins versus 9.4 for patients who did not receive induction therapy. It has been pointed out, however, that this effect is related to the more powerful immunosuppression caused by these agents rather than to a specific oncogenic effect [54]. Indirect confirmation of the role played by the intensity of immunosuppression is given by data showing a higher incidence of PTLD in young heart and lung transplant recipients, who usually receive a stronger immunosuppressants [55].

On the same line are the data showing a higher and earlier risk for PTLD in patients who received cyclosporine in comparison with those given azathioprine [56]. An analysis of the United States Renal Data System showed an increased risk of PTLD with tacrolimus than with cyclosporine [57]. An analysis of two large registries (Organ Procurement and Transplantation Network/United Network for Organ Sharing and CTS) showed that MMF was not associated with an increased risk of lymphoma or other malignancies post-renal transplantation, and was even associated with a lower risk in some populations [58]. A report pointed out that belatacept, a co-stimulation blocker developed for primary maintenance immunosuppression, may increase the risk of PTLD in comparison with cyclosporine, particularly in EBV-negative transplant recipients [59].

Since EBV replication occurs in an S-phase-like cellular environment with high cyclin-dependant kinase (CDK) activity, the mTOR signal transduction pathway can be a critical nexus for growth and survival of EBV-infected B cells in PTLD [60]. In vitro studies showed that the mTOR inhibitors sirolimus and everolimus might exert a protective effect against PTLD both by suppressing growth of cells derived from post-transplant lymphoproliferative disorder at allograft-protecting doses [61] and by inhibiting the IL-10 signal transduction pathway and the growth of EBV B-cell lymphomas [62]. Adding sirolimus to EBV-infected lymphoblastoid cell lines derived from patients with PTLD decreased CDK2, CDK3 and CDK4 protein levels and arrested the cell cycle at G1 [63]. These data would suggest a potential role for mTOR inhibitors as a therapeutic option in the treatment of PTLD. Early clinical data suggest clinical activity of mTOR inhibitors in a number of haematological malignancies, including non-Hodgkin lymphoma subtypes, making these drugs potentially attractive as single agents or in combination with conventional cytotoxic and other targeted therapies [64]. In fact, cases of PTLD regression have been reported in renal transplant recipients after conversion from CNI to sirolimus [65–67]. However, it is difficult to determine if the results were actually attributable to the antioncogenic activity of the drug or rather to the reduction of immunosuppression. On the other hand, a study reported that out of 274 paediatric renal transplant recipients treated with a regimen based on basiliximab for induction, steroids cyclosporine and sirolimus 19 patients (6.9%) developed PTLD [68]. These data suggest that the intensity of immunosuppression can overcome the potential protection of mTOR inhibition against EBV+ B-cell lymphoma.

In summary, immunosuppression can favour the replication of EBV-infected B cells leading to polymorphic polyclonal proliferation or monoclonal malignancy. Children and EBV-negative recipients are at higher risk. Induction therapy with antithymocyte antibodies and strong immunosuppression can increase the risk of PTLD. The mTOR inhibitors may interfere with the metabolism of EBV and its replication on B cells.

**Human herpes virus 8**

Human herpes virus 8 (HHV8) is a γ-herpes virus sharing biochemical and biological properties with the herpes viridae family, namely the ability to establish a latent infection that may reactivate with immunosuppression. The distribution of HHV8 is heterogeneous, with a higher prevalence in sub-Saharan and West Africa, in Southern Italy and other Mediterranean areas and in South America. There is now solid evidence linking Kaposi’s sarcoma to HHV8 infection. In fact, HHV8 can be found in sporadic, endemic, human immunodeficiency virus (HIV)-related epidemic and transplant-associated Kaposi’s sarcoma [69]. However, the presence of HHV8 is not sufficient per se to cause the development of Kaposi’s sarcoma. It requires reactivation of HHV8, which can be triggered by compromised host immune response and/or the evasion of infected cells from the host NK cell driven response. Hypotheses for how HHV8 can be oncogenic are wide ranging. The virus might exert either a direct oncogenic effect, as it contains potential oncogenes which are able to transform all the lines in vitro, or mediate the development of neoplasia by interfering with IL-6 and glycoprotein vOx-2 homologues. It has been shown that in HIV-related Kaposi’s sarcoma multiple lesions in the same patient arise from a single clone of cells [70]. The current hypothesis is that each lesion arises from a monoclonal population of circulating progenitor cells, transformed by HHV8, that home to multiple local sites develop into spindle cells and proliferate. Emerging evidence points to a single HHV8 gene, vGPCR, as being essential for Kaposi’s sarcoma development [71].

Kaposi’s sarcoma is an angioproliferative disease starting as a hyperplastic reactive inflammatory and angiogenic process, which may evolve into monomorphic nodules that can be clonal and resemble a sarcoma. Mucocutaneous
lesions can be seen in >90% of cases. Lymphnodes, gastrointestinal tract and lungs can be involved in 20–50% of cases. The prevalence of Kaposis's sarcoma in organ transplant recipients varies between 0.5 and 5% [72], but it rises to 23% in patients who were positive for HHV8 before transplantation [73]. Kaposis's sarcoma may develop in transplant recipients either as a consequence of a reactivated infection in a seropositive patient or as a primary infection in a patient without HHV8-specific antibodies. In the latter case, a primary infection may be transmitted from the donor through the graft [74].

HHV8 encodes a G protein-coupled receptor (vGPCR), which requires CXC chemokines and acts as an oncogene. Moreover, it encodes three viral chemokines (vMIP-I, -II and -III) which act as agonists for CCR3, CCR4 and CCR8. These receptors are preferentially expressed on TH2 and Treg cells. Thus, these chemokines represent a sophisticated strategy to subvert and divert effective anti-viral, antitumour immunity [75].

It is likely that ethnic, genetic and socio-economic factors may contribute to the development of Kaposis's sarcoma, but, although a review of cases of Kaposis’s sarcoma in transplant recipients in the USA reported that Kaposis's sarcoma was not significantly related to use of specific antirejection medications [76], there are few doubts that the intensity of immunosuppression plays a major role. This is well demonstrated by a number of facts: (i) the incidence of Kaposis's sarcoma has tremendously increased after the introduction of cyclosporine [77], (ii) regression of mucocutaneous lesions can be obtained by reducing or even withdrawing immunosuppression [78] and (iii) there is a high risk of recurrence after retransplantation in patients, who had a clinical remission of Kaposis’s sarcoma with reduction/withdrawal of immunosuppression in a previous transplant [79].

Emerging evidence points to a single gene, vGPCR, as being essential for Kaposis's sarcoma development. Of note, the signalling cascade mediated by mTOR is a critical pathway in vGPCR sarcomagenesis [71]. On this basis, the anti-mTOR drugs could exert an inhibitory effect on the replication of the virus and the diffusion of sarcoma. In fact, it has been demonstrated that in a number of cases, the conversion from CNIs to sirolimus or everolimus led to the cure of cutaneous [80,81] and even visceral Kaposis’s sarcoma [82]. This beneficial effect is probably related both to the antiangiogenic activity of these drugs and to their inhibition of mTOR which stimulates protein synthesis and cell cycle progression by activating P70 S6 kinase [81]. In some patients, however, no effect of sirolimus on the outcome of Kaposis’s sarcoma was noted [83].

Apart from Kaposis’s sarcoma, HHV8 can be involved also in some lymphoproliferative disorders such as Castleman disease, HHV8-related lymphoma and primary effusion lymphoma, a very rare type of lymphoma usually confined to the body cavities predominantly in immunosuppressed patients infected with HHV8 [84].

In summary, immunosuppression can favour the replication of HHV8. HHV8-positive patients are at higher risk of developing Kaposis’s sarcoma. The intensity of immunosuppression can favour the development of Kaposis’s sarcoma. The mTOR inhibitors may interfere with the metabolism of EBV and its replication on B cells.

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