

Review Article

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Cellular immune therapy for viral infections in transplant patients

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Stem cell and organ transplantation are considered as the major advances of modern medicine. Unfortunately the success of transplantation is limited by its toxicity and infectious complications as a result of profound immunosuppression. Viral infections are an extremely common and predictable problem in these patients. Antiviral drugs given either prophylactically or as early therapy for patients with detectable viral loads appear to be an effective strategy for reducing viral infections. However, long-term treatment with these drugs is associated with significant toxicity, expense and the appearance of drug resistant virus isolates ultimately resulting in treatment failure. Over the last few years, there is increasing evidence that cellular immune therapies can reverse the outgrowth of haematological malignancies and can also provide therapeutic benefit against lethal viral infections. While the expansion and adoptive transfer of virus-specific T-cells from the healthy donor can be an effective strategy to control viral replication, this is not possible when donors are seronegative or are subsequently inaccessible. Recent studies have demonstrated successful expansion of virus-specific T-cells from seropositive stem cell transplant recipients of a seronegative graft with active virus disease and the long term reconstitution of protective anti-viral immunity following their adoptive transfer back into the patients. Furthermore, this immunotherapeutic strategy has also been extended for multiple pathogens including cytomegalovirus, Epstein-Barr virus, adenovirus and BK polyoma-virus. This approach can be employed to rapidly expand multiple pathogens-specific T cells that can be used for adoptive immunotherapy. Finally, new assays to monitor T cell immunity have been developed which will allow to identify the high risk transplant patients who may develop virus-associated complications post-transplantation and can be given adoptive T cell therapy prophylactically.

Key words Adoptive immunotherapy - CD4 cells - CMV - EBV - PTLN - solid organ transplant - transplant recipients - T cell therapy - viral infection

Introduction

Infectious complications following transplantation remains a major burden on the clinical management of transplant recipients^{1,2}. The incidence of these infectious complications depends on a number of factors. These

include (i) serological status of the recipient/donor, (ii) levels of immunosuppression, (iii) type of organ transplanted, and (iv) anti-rejection therapy (e.g. antibody-mediated depletion of T cells)³. Furthermore, recrudescence of existing viral infections such as

the common herpes virus, cytomegalovirus (CMV) can increase the susceptibility to other opportunistic infections such as bacterial and/or fungal infections^{4,5}. These viruses can also promote reactivation of other latent herpes virus infections such as Epstein-Barr virus (EBV) resulting in uncontrolled proliferation of EBV-infected B cells⁶. Clinical studies have indicated that approximately 30 per cent of infectious deaths after transplantation are associated with the primary viral infection or recrudescence of existing latent infection³. It is now established that the delay in the reconstitution of virus-specific CD8+ and/or CD4+ T cell responses is a critical factor in viral recrudescence and viral disease^{7,8}. Over the last two decades, several groups have developed novel strategies to reconstitute T cell immunity in transplant patients using adoptive immunotherapy⁹⁻¹². These virus-specific T cells are primarily derived from the transplant donor or from the recipient. T cells from HLA-matched healthy volunteers have also been successfully used to treat virus-associated diseases in transplant recipients^{13,14}. This review summarizes the current status of T cell-based immunotherapy to treat virus-associated diseases including EBV, CMV, adenovirus and BK polyomaviruses. We also discuss emerging immune monitoring technologies which will allow us to identify high risk transplant recipients who are likely to develop infectious complications and can be offered adoptive immunotherapy as prophylactic treatment.

EBV associated post-transplant lymphoproliferative disease

EBV is the best known and most widely studied herpesvirus due to its clinical and oncogenic importance^{15,16}. EBV establishes a latent infection in B cells and these cells express an array of latency-associated viral proteins which drive proliferation of virus-infected B cells¹⁷. Extensive analysis of *ex vivo* isolated B cells from virus-infected individuals has shown that EBV gene expression in resting B cells is often restricted to a limited number of genes which allows the virus to escape immune recognition and maintain long-term persistent infection^{18,19}. Occasionally, resident EBV in these B cells is reactivated resulting in the release of infectious virus which is the primary source of transmission of EBV^{20,21}. It is now well established that virus-specific CD8+ and CD4+ T cells play a crucial role in controlling the overall pool of EBV-infected B cells^{15,16}. Any impairment of T cell immunity results in uncontrolled proliferation of EBV-infected B cells^{20,21}. A classic

example of this is seen in transplant setting where the balance between the virus-infected cells and the anti-viral T cell immunity is disrupted as a consequence of immunosuppressive therapy²². This uncontrolled proliferation of EBV-infected cells is referred to as post-transplant lymphoproliferative disease (PTLD) and can be a life threatening clinical complication if not controlled at early stages of manifestation²³⁻²⁵. PTLT in haematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) patients usually have distinct genesis²³⁻²⁷. In HSCT recipients, PTLT generally results from donor B cells, while PTLT in SOT patients is of recipient origin. The clinical symptoms of PTLT include fever, sweats, lymphadenopathy, sepsis and mass lesions in lymph nodes, the spleen and the brain²³⁻²⁷. It is important to mention that PTLT emerging soon after the transplant is invariably positive for EBV^{28,29}, while a large proportion of the PTLT cases which develops 2-5 years or more after transplantation are negative for EBV²⁷. These EBV-negative PTLT are generally more aggressive and are highly resistant to standard treatments including immunotherapies. Many studies have shown that longitudinal monitoring of EBV DNA in the peripheral blood can be used as a biomarker for identifying patients who are at a high risk of developing PTLT^{22,25}.

Traditionally, reduction of immune suppression is considered as the first option to treat PTLT in transplant patients^{30,31}. Development of the second line therapies based on a chimeric murine/human monoclonal antibody directed to the CD20 molecule which is expressed on all B cells, including in PTLT has provided improved outcome for transplant recipients^{31,32}. In fact, the combination of anti-CD20 antibody with chemotherapy (*e.g.* CHOP) has been successfully used to treat more aggressive PTLT, although the toxicity associated with chemotherapy limits its use in paediatric patients or patients with other infectious complications³¹. In spite of successful implementation of these second line therapies, it is now well established that PTLT recurrence is frequently seen in patients who are unable to reconstitute EBV-specific cellular immunity³¹. Anti-CD20 treatment also increases the risk of bacterial and viral infections and PTLT recurrence is occasionally associated with escape from anti-CD20 treatment due to the loss of CD20 expression on malignant cells³³. To overcome these limitations, reconstitution of cellular immunity by adoptive transfer of EBV-specific T cells has been successfully implemented by many groups^{22,34-36}.

Table I. Comparative advantages and disadvantages of emerging technologies for adoptive T cell therapy

T cell therapy technology	Advantages	Disadvantages
<i>In vitro</i> expanded virus-specific T cells (stimulated with synthetic peptides/recombinant protein)	<ul style="list-style-type: none"> • Expands both CD8+ and CD4+ T cells • Defined antigen specificity • No infectious agent in T cell therapy 	<ul style="list-style-type: none"> • Restricted antigen specificity of T cells • Limited by the availability of appropriate peptide epitopes or protein antigens
<i>In vitro</i> expanded virus-specific T cells (stimulated with viral lysate or LCLs)	<ul style="list-style-type: none"> • Expands both CD8+ and CD4+ T cells • Broad coverage of antigen specificity 	<ul style="list-style-type: none"> • Prolonged expansion process (especially when LCLs are used as APC) • Potential risk from infectious virus in T cell therapy
<i>In vitro</i> expanded virus-specific T cells (stimulated with recombinant replication-deficient viral vectors)	<ul style="list-style-type: none"> • Expands both CD8+ and CD4+ T cells • Defined antigen specificity • Rapid availability of T cell therapy 	<ul style="list-style-type: none"> • Restricted antigen specificity of T cells • Limited by the antigens or epitopes included in the recombinant vector • May not expand CD4+ T cells
MHC-peptide multimers selected antigen-specific T cells	<ul style="list-style-type: none"> • Rapid availability of T cell therapy (1-2 days) • Defined antigen specificity • Enhanced <i>in vivo</i> expansion capacity 	<ul style="list-style-type: none"> • Restricted antigen specificity of T cells • Limited by the availability of MHC-peptide multimers • Lack of availability of MHC class II multimers for CD4+ T cells
IFN- γ capture enriched T cells	<ul style="list-style-type: none"> • Rapid availability of T cell therapy (1-2 days) • Defined antigen specificity • Enhanced <i>in vivo</i> expansion capacity 	<ul style="list-style-type: none"> • Restricted antigen specificity of T cells • Limited by the availability of peptides or recombinant antigens
Third party HLA matched virus-specific T cells	<ul style="list-style-type: none"> • Rapid availability of T cell therapy ("off-the shelf") • Defined antigen specificity • Includes both CD8+ and CD4+ T cells 	<ul style="list-style-type: none"> • Limited by the HLA matching of T cells with recipient HLA alleles • Therapeutic efficacy drops with lower HLA matching

LCL, lymphoblastoid cell line; APC, antigen presenting cell; IFN- γ , interferon gamma
Source: Refs 4, 13, 14, 35, 38-46

Viral gene expression analysis of EBV-infected B cells in PTLD has revealed that in the majority of the cases, these cells express a full array of latent antigens including EBV nuclear antigens (EBNA) 1-6 and latent membrane proteins 1 and 2 (LMP1 & LMP2)^{22,29}. This latency phenotype is referred to as type III latency. In contrast, the B cells in late PTLD display type II latency and express EBNA1, LMP1 and LMP2^{29,34}. It is important to consider the pattern of viral gene expression in PTLD before proceeding with *in vitro* expansion of EBV-specific T cells. For PTLD expressing type III latency, EBV transformed lymphoblastoid cell lines (LCLs) are often used as antigen presenting cells (APC) to stimulate EBV-specific T cells. However, these LCLs should not be used as APC to expand T cell to treat PTLD with type II latency since LCLs preferentially expand T cells specific for EBNA2-6 proteins which are not expressed in these PTLD cases. An alternative antigen presentation system based on adenoviral vectors expressing EBNA1, LMP1 and LMP2 epitopes or full-length antigens have been

developed which allows preferential expansion of T cell directed towards these antigens^{37,38} (Table I).

Development of EBV-specific T cell adoptive immunotherapy for EBV-associated PTLD in HSCT recipients was pioneered by Cliona Rooney and Helen Heslop^{39,47}. Their group has successfully used *in vitro* expanded T cells from HSCT donors to treat more than 100 patients⁴⁸. These T cells were expanded using donor-derived LCLs as APC and the expanded cells included both CD8+ and CD4+ EBV-specific T cells. Long-term follow up of these patients has shown that T cell infusion was safe and none of the patients developed *de novo* graft-versus-host disease (GvHD) following adoptive immunotherapy. Most importantly, none of the patients who received T cell therapy as a prophylactic treatment developed PTLD and >75 per cent of the patients with active PTLD showed complete resolution of the disease following adoptive immunotherapy. Rooney and Heslop's group has also used genetically marked T cells to monitor

Table II. Clinical assessment of adoptive T cell therapy for viral infections in transplant patients

Target for T cell therapy	Type of the transplant	Observed serious adverse events related to T cell therapy	Clinical outcome
EBV-PTLD (Prophylactic and therapeutic)	HSCT/ T cell depleted HSCT/ Mismatched HSCT/ Haploidentical HSCT (Number of patients treated to date: 155)	None or local inflammation following adoptive T cell infusion	Prophylaxis: No PTLD detected in 101 patients Therapeutic: CR – 33/54 patients PR – 7/54 patients PD – 14/54 patients
EBV-PTLD (Prophylactic and therapeutic)	SOT (renal/lung and heart and lung) (Number of patients treated to date: 29)	None	Prophylaxis: No PTLD detected in 20 patients Therapeutic: CR – 7/9 patients PR – 2/9 patients PD – 0/9 patients
EBV-PTLD (Third party EBV-specific T cells used as therapeutic)	SOT, HSCT and cord blood (Number of patients treated to date: 57)	1 chronic GvHD and viral reactivation	Therapeutic: CR – 27/57 patients PR – 5/57 patients PD – 22/57 patients 2 patients did not complete T cell therapy
CMV infection and/or disease	HSCT (Number of patients treated to date: 85)	4 grade II 1 grade III	Therapeutic: 62 patients showed complete or partial reduction in CMV replication. 13 no response. Prophylaxis/preemptive: patients required single episode of antiviral therapy (2) or no therapy (7)
CMV infection and/or disease	SOT (Number of patients treated to date: 1)	None	Therapeutic: Transient resolution of CMV infection
Adenovirus	HSCT (Number of patients treated to date: 6)	None	Therapeutic: Reduction in adenoviral DNA in blood and stool
JCV-associated disease	HSCT (Number of patients treated to date: 1)	None	Clearance of viral DNA from cerebrospinal fluid and resolution of neurological symptoms

CR, complete response; PR, partial response; PD, progressive disease; EBV-PTLD, Epstein-Barr virus – post-transplant lymphoproliferative disease; CMV, cytomegalovirus; JCV, John Cunningham virus, SOT, solid organ transplant; HSCT, haematopoietic stem cell transplant; GvHD, graft vs host disease

Source: Refs 9-14, 34, 35, 39-41, 43, 47-49, 51, 54-58

long-term survival of adoptively transferred T cells. These analyses have shown that these infused T cells can survive up to 9 years^{39,47}. These observations have been successfully reproduced by multiple clinical centers⁴⁹⁻⁵² (Table II). More recently, an alternative protocol for the isolation of polyclonal EBV-specific T cells using an interferon gamma (IFN- γ) capture technique (Meltenyi-biotech, Germany) has been successfully used to achieve reconstitution of antiviral T-cell immunity after HSCT⁵³ (Table II).

Extension of this successful implementation of adoptive immunotherapy in the HSCT setting to SOT recipients was a major challenge. In this setting,

PTLD arises from recipient B cells and there was a general dogma that underlying immunosuppression may inhibit expansion of EBV-specific T cells from these patients. However, studies carried out by our group showed that it was possible to expand T cells from SOT recipients and these T cells consistently showed strong EBV-specificity, including reactivity through defined epitopes in spite of concurrent immunosuppressive therapy, and no alloreactivity toward donor alloantigens³⁵. Further, adoptive transfer of these autologous EBV-specific T cells into SOT recipients with active PTLD was coincident with complete regression of the PTLD. An extensive analysis

of T cell responses after the completion of adoptive immunotherapy revealed that the reconstitution of T cells directed towards EBV latent antigens rather than lytic antigens was crucial in preventing recurrence of PTLD⁴⁹. Subsequent studies by Heslop and colleagues and other groups have successfully reproduced these observations and have shown that adoptive transfer of autologous EBV-specific T cells can be used to treat SOT recipients with high viral load or active PTLD⁵⁹⁻⁶¹ (Table II). The overall success of T cell-based adoptive immunotherapy is less impressive in SOT recipients when compared to HSCT recipients. This is probably due to the *in vivo* loss of adoptively transferred T cells in SOT recipients as these patients continue to receive high dose immunosuppression which can compromise the long-term survival of these effector cells.

One of the major limitations of autologous or donor-derived T cell therapy is that the process of generating these effector cells often takes many weeks to months. This limits the use of this therapy in a therapeutic setting where the patients often succumb to progressive disease before the T cells are ready for infusion. Ideally, a T cell therapy which can be offered as “off-the-shelf” treatment would be more suitable for these patients. Crawford and colleagues have developed an alternative strategy which involves the use of third-party HLA matched T cells from healthy volunteers which can be stored as a T cell bank^{14,54,55,62,63} (Table I). Using this approach, these authors achieved an overall response rates of >50 per cent with the best response observed in patients that matched most HLA class I alleles with the third party T cells (Table I). Similar outcomes have also been reported by other groups in SOT patients who failed to respond to the conventional therapies. Leen and colleagues^{40,64} have developed a novel T cell expansion protocol which allows expansion of antigen-specific T cells against multiple viral infections including EBV, CMV and adenovirus. This group has successfully used third party multi-virus specific T cells to treat multiple transplant patients with EBV-associated PTLD with a response rate of 60 per cent.

CMV-associated disease in transplant patients

CMV is another member of the human herpesvirus family that persists for life the following the primary infection. It is also one of the most important infectious pathogens in clinical transplantation^{4,5}. Acquisition of primary CMV infection or reactivation of latent infection causes significant morbidity and can cause deleterious effects on engrafted organs. Extensive studies in healthy

virus carriers have indicated that immune control of CMV infection is critically dependent on both innate and adaptive immune responses⁶⁵. Impairment of these immune regulatory pathways due to prolonged immunosuppression, antibody-mediated depletion of T cells and induction of alloreactive immunity due to MHC mismatch can disrupt the balance between the host immune system and the virus⁵. Invasive CMV disease once affected more than one third of recipients who received a transplant from a seropositive donor. These recipients are at the highest risk of CMV disease, while the seronegative recipients of seronegative donors are at the lowest risk and seropositive recipients having medium risk⁶⁶⁻⁶⁹. Several studies have demonstrated that early ganciclovir therapy (anti-viral prophylaxis) reduces the incidence of early CMV pneumonitis from 13 to 2 per cent, and the overall rates of CMV disease from 33 to 6 per cent⁶⁹. The “health cost” of this aggressive approach, however, is a syndrome, referred to as “late CMV”, in which the patients develop disease after 100 days, rather than in the engraftment period (typically in the first 2 months)^{69,70}. Late CMV is seen in as many as 5 to 18 per cent of all at-risk patients, depending on the series reported⁷¹. This effect may be due to an attenuated form of the disease, myelosuppressive toxicity of ganciclovir, and/or directly immunosuppressive effect of ganciclovir⁷². Drug resistance is an increasing concern for patients undergoing long-term ganciclovir therapy⁷³. Studies have suggested that the early replication kinetics during an active infection are predictive of future outcome and can be used to identify patients destined to reach virus loads that result in pathology⁷⁴. However, these predictive algorithms often fail to identify patients at high risk of CMV disease and some of these patients develop drug-resistant CMV-associated complications⁷⁵.

There is an increasing realization that new predictive biomarkers can improve the clinical management of CMV infection in transplant patients^{7,68,76}. This information can also be exploited for the development of novel therapeutic tools⁷⁷. Over the last decade our knowledge of CMV immune regulation has dramatically improved and it is now well established that CMV-specific T cell immunity plays a critical role in preventing CMV disease in transplant patients⁴. This knowledge has been exploited to develop new diagnostic tools and therapeutic strategies. Several groups have used *ex vivo* quantitative and qualitative analysis of CMV-specific T cells to assess transplant patients’ ability to control CMV infection^{7,8,78,79}.

These studies have allowed us to (i) identify high risk transplant patients who may develop late-onset CMV viraemia and disease following antiviral prophylaxis; (ii) distinguish patients who are at an increased risk of progression to CMV-associated disease versus patients who spontaneously clear viral infection; and (iii) identify patients who are at a higher risk of developing recurrent viral reactivation and disease following a course of anti-viral therapy. In spite of these promising outcomes, translation of these findings to the clinical settings for diagnostic application was constrained due to the highly complex nature of the techniques used for the analysis of virus-specific T cells. These techniques require specialized equipment such as a flow cytometer or ELISpot reader. Not all hospital laboratories are equipped with such facilities, and such assays have to be carried out in a reference laboratory. Furthermore, shipping of clinical samples can compromise cell viability and turnaround time for results is very long. We have developed a simple whole blood diagnostic test which overrides many of the limitations of research laboratory-based assays⁷⁷. This assay (referred to as QuantiFERON-CMV) allows rapid assessment of CMV-specific T cell immunity in transplant patients and can be carried out in any hospital-based laboratory. More importantly, a number of independent studies in SOT patients carried out in the US and Europe have shown that the QuantiFERON-CMV assay can be successfully used to not only identify high-risk transplant patients but to also predict patients who may develop recurrent CMV reactivation after the completion of prophylaxis⁷⁸⁻⁸⁰.

Development of predictive diagnostic tools based on immune monitoring provides a unique opportunity to exploit this information to complement immune-based therapies for clinical management of CMV complications in transplant patients. CMV-specific CD8+ T cell based adoptive immunotherapy in bone marrow transplant patients was first pioneered by Riddell *et al*⁵⁶ who successfully used this strategy to treat patients with active CMV disease. Follow up analysis of these patients revealed that although the adoptively transferred T cells survived for a few weeks *in vivo*, these virus-specific CD8+ T cells dramatically declined in patients who were unable to reconstitute a concomitant virus-specific CD4+ T cell response. These observations clearly highlighted the importance of CD4+ T cells in the long-term maintenance of the CMV-specific CD8+ T cell response⁵⁷. These observations were strongly supported by the studies carried out by Peggs and colleagues⁹ who demonstrated that adoptive

transfer of CMV-specific CD8+ and CD4+ T cells was coincident with rapid and long-term expansion of CMV-specific T cells *in vivo*. The importance of reconstitution of CD4+ T cells was further emphasized by Einsele *et al*¹⁰, who successfully used CMV-specific CD4+ T cells to treat drug-resistant CMV infection in HSCT recipients. Most of these studies used live CMV lysate to expand antigen-specific T cells which remained a significant safety concerns. Micklewaite and colleagues⁴¹ developed an alternative strategy based on synthetic peptides to expand CMV-specific T cells. This approach allows rapid expansion of T cells, although this technology is limited by the availability of appropriate peptide epitopes for specific HLA alleles (Table II). Other groups have used replication-deficient viral vectors encoding CMV antigens or epitopes to expand T cells which are directed against virally-encoded antigens expressed at the different stages of infection (*e.g.* early, immediate early and late)^{42,81}.

As discussed for EBV-specific T cell therapy, expansion of CMV-specific T cells requires prolonged *in vitro* culture which often limits the application of adoptive immunotherapy for patients who have systemic end organ CMV disease and require urgent intervention. To overcome this potential roadblock, Cobbold and colleagues⁴³ have used MHC-peptide tetramers to isolate CMV-specific T cells from HSCT donors and, adoptively transferred these cells (without any *in vitro* manipulation) in nine HSCT recipients. Longitudinal follow up analyses showed massive *in vivo* expansion of CMV-specific T cells in these patients and 8/9 HSCT recipients successfully cleared CMV infection following the infusion of these cells⁴³. In spite of the high success rate of this approach, lack of availability of clinical grade MHC-peptide tetramers for multiple HLA alleles limits its potential use in clinical setting. Another approach involves capture of CMV-specific T cells using cytokine secretion following stimulation with viral peptide epitopes or recombinant protein. This approach has been assessed by many groups to treat HSCT recipients and follow up analysis demonstrated that the majority of the patients showed *in vivo* expansion of antigen-specific T cells and significant reduction of viral load^{5,9-11} (Table I). Although adoptive immunotherapy has been successfully implemented in the HSCT setting^{9-11,67}, extension of this strategy to treat CMV infection/disease in SOT patients has remained unexplored. While expanding T-cells from the SOT recipient seems an attractive alternative, there are many potential problems. These include activating a

cytotoxic T lymphocyte (CTL) response *in vitro* from an individual receiving immunosuppressive drugs, and the risk of expanding allospecific T-cells that may trigger graft rejection when adoptively transferred. These factors have led to the perception that adoptive immunotherapy in SOT patients is unlikely to be successful and poses a significant risk to the transplant recipient. However, a single report based on a lung transplant patient with ganciclovir-resistant CMV-pneumonia, showed that adoptive transfer of autologous CMV-specific T cells inhibited viral replication as confirmed by extensive longitudinal immunological monitoring¹², but after 4 wk, the infection reappeared and persisted at a low level even after a second T-cell infusion. The authors argued that this could be the consequence of the late differentiated phenotype of the infused T cells and therefore, their insufficient longevity *in vivo*. Recently, we have developed a novel protocol for expanding CMV-specific T-cells in SOT recipients with active CMV disease and have successfully infused these cells into a lung transplant patient with active drug-resistant CMV disease (unpublished observation). A critical aspect of this work is that while SOT patients with active CMV disease have circulating CMV-specific T-cells, these are largely non-functional. Importantly, this anergic phenotype can be reversed by re-stimulating these cells *in vitro*, in the absence of the immunosuppressive environment.

Adenovirus-associated diseases in transplant patients

Adenoviruses (AdV) are double stranded DNA viruses and are often associated with self-limiting gastrointestinal, respiratory or conjunctival disease in healthy immunocompetent individuals⁸². However, AdV infection in paediatric HSCT recipients can cause severe respiratory disease, hepatitis, and colitis. In addition, haemorrhagic cystitis and adenoviral keratoconjunctivitis are also seen in severely immunocompromised HSCT recipients^{83,84}. Clinical manifestation of AdV infection in SOT recipients depends on the type of transplanted organ^{82,85,86}. For example, in lung transplant acute AdV infection causes flu-like symptoms including necrotizing pneumonia or alveolar damage, while in liver transplant recipients AdV infection results in hepatitis and can involve other gastrointestinal organs. Although the antiviral drug such as cidofovir, is commonly used to treat AdV infection, this drug has modest efficacy and causes significant toxicity including nephrotoxicity and neutropenia⁸². It is important to mention here that the use of antiviral

drugs for adenovirus is not supported by prospective randomized clinical trials, and none of the currently available drugs have been formally approved by the regulatory agencies for the treatment of adenoviral infection or disease^{87,88}.

As with many other viral infections in transplant recipients, reduction in immunosuppressive therapy often results in resolution of AdV infection which emphasizes the importance of immune reconstitution to prevent post-transplant AdV-associated diseases^{89,90}. Earlier studies in human volunteers have shown that both humoral and cellular immunity play a critical role in preventing AdV infection⁸⁹. Antibody responses directed towards viral capsid and fiber proteins provide lifelong protection against viral infection. Although adaptive cellular immunity against AdV includes both CD8+ and CD4+ T cells, *in vitro* depletion of CD4+ T cells alone can compromise the lymphoproliferative response^{38,44,91}. Extensive analysis of these CD4+ T cells has shown that these effector cells produce IFN- γ and display strong cytolytic function against virus-infected cells^{38,44,92}. *Ex vivo* analysis of immune responses of HSCT recipients has shown that reconstitution of AdV-specific T cells is crucial for clearance of viral infection in these patients. Clinical correlative studies have shown that the reduction in CD3+ T cell counts (< 25/ μ l) or failure of an AdV-specific T cell response after AdV infection (CD3+ T cells < 300/ μ l within 2 wk of AdV detection) is often associated with a poor outcome^{87,88}.

Considering the importance of T cell immunity in preventing AdV-associated disease in transplant recipients, adoptive immunotherapy using donor-derived T cells in HSCT recipients has emerged as an attractive strategy^{45,89,93,94} (Table II). One of the major challenges for the development of adoptive immunotherapy is that there are multiple serotypes of AdV, although T cell directed towards epitopes derived from the hexon protein which is conserved in multiple serotypes may overcome this potential limitation. Feuchtinger and colleagues⁹⁴ used the IFN- γ capture technology to enrich AdV-specific T cells from the peripheral blood of HSCT donors and these cells were adoptively transferred (without any further *in vitro* manipulation) into the transplant recipients with systemic AdV infection. To capture these antigen-specific T cells, peripheral blood mononuclear cells were stimulated overnight with adenovirus lysate and then labelled with IFN- γ capture antibody. T cells producing IFN- γ were isolated using cliniMACS

technology and these cells included both CD8+ and CD4+ T cells specific for AdV. Follow up analyses of these patients showed that these T cell infusions were safe with minimal side effects and of the six evaluable patients five showed a significant decrease of adenoviral DNA in peripheral blood⁹⁵. More importantly, this T cell infusion was coincident with the reconstitution of AdV-specific T cells *in vivo*. Subsequent studies by Leen and colleagues⁹⁶ from Baylor College of Medicine⁹⁴ have also shown that the adoptive immunotherapy with AdV-specific T cells can be safely used in the recipients of HLA-matched related, matched unrelated, and haploidentical HSCT recipients. This group has conducted two different clinical trials where multi-virus specific T cells including AdV-specific T cells have been used as therapeutic products. These T cell therapies were manufactured using monocytes and LCLs transduced with AdV vectors as antigen presenting cells.

Polyomavirus-associated diseases in transplant recipients

The human BK polyomavirus (BKV) and JC (John Cunningham) polyomavirus (JCV) infections are ubiquitous in human population with seroprevalence of >90 per cent⁹⁷. These viral infections are acquired in early childhood and primary infection is generally asymptomatic or may show clinical manifestation of mild respiratory symptoms⁹⁸. Polyomaviruses can persist as latent infection in circulating lymphocytes, in the brain and predominantly in the urogenital system. Drug-induced immunosuppression can often lead to the reactivation of these viruses⁹⁹. BKV is associated with two major clinical complications in transplant recipients. These include polyomavirus-associated nephropathy in renal transplant patients and polyomavirus-associated haemorrhagic cystitis in HSCT recipients^{100,101}. These clinical symptoms are seen in 1-15 per cent of transplant recipients. Clinical symptoms of JCV reactivation in immunosuppressed patients are referred to as multifocal leucoencephalopathy^{100,102}. There are no specific drugs which can be used to treat BKV and JCV in transplant recipients. Hirsch and colleagues¹⁰³ have recently shown that reduction in immunosuppression can be successfully used to manage BKV and JCV-associated diseases in transplant patients. Furthermore, reconstitution of cellular immunity against JCV induced by highly active antiretroviral therapy (HART) was coincident with the control of JCV-associated multifocal leucoencephalopathy in immunosuppressed individuals^{58,104}. Blyth and colleagues⁴⁶ have used

overlapping peptides from BKV to expand virus-specific T cells. These T cells showed strong functional activity including cytolytic function and included both CD4+ and CD8+ T cells. The efficacy of these effector cells in a formal clinical setting still remains to be assessed. There is only one case report of a 14 yr old HSCT recipient with JCV-associated multifocal leucoencephalopathy who was infused with donor-derived JCV-specific T cells stimulated with overlapping peptides spanning the JCV-encoded VP1 and large T antigen¹⁰². Follow up analysis of this patients showed that adoptive transfer of JCV-specific T cells cleared the viral DNA from cerebrospinal fluid and dramatic improvement in neurological symptoms was observed (Table I).

Concluding remarks

The development of novel strategies to expand or isolate virus-specific T cells in combination with *ex vivo* monitoring of T cell immunity provides a unique opportunity to implement these emerging tools in clinical settings. To achieve this goal, it will be important to establish high quality facilities to manufacture these cellular therapies within the hospital settings so that these therapies can be provided to the transplant patients at an early stage rather than when the virus-associated diseases are difficult to manage. It is also important to appreciate that the uncontrolled use of anti-viral drugs often leads to post-therapy complications including graft rejection and emergence of drug-resistant viruses. Implementation of immune monitoring technologies in the routine clinical management of transplant patients will help identify high risk patients and limit the use of anti-viral drugs. Furthermore, patients who are at higher risk of developing clinical complications can be treated with a combination approach which will include anti-viral drugs and adoptive immunotherapy.

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