Editorial

The coming of age of adoptive T-cell therapy for viral infection after stem cell transplantation

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The success or failure of allogeneic stem cell transplantation (SCT) in curing a variety of hematological disorders, centers to a large extent on the quality of the immune recovery of the donor graft. As well as exerting a graft-versus-malignancy effect, critical in achieving cure of hematological malignancies, donor T lymphocytes confer protective immunity against resident viruses that reactivate during the period of profound immunosuppression following the graft. As they engraft alloreacting donor T lymphocytes can cause life-threatening graft versus host disease (GVHD). Prevention and treatment of GVHD with immunosuppressive agents delays immune recovery and further extends the post transplant period the recipient is at risk from viral complications. Despite progressive improvements in SCT over nearly 50 years success of allogeneic SCT is still constrained by the failure to adequately and rapidly install donor immunity to viruses and malignancy without GVHD. Indeed even in the most favorable well-matched donor-recipient combinations viral infection, graft versus host disease and relapse are the principle causes of death after SCT, which may reach 50% in the case of malignant diseases.

Viral reactivation predominantly occurs in the first 3-6 months after SCT. The most clinically important are the herpes viruses; cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human herpes virus 6 (HHV6); the polyoma viruses BK and JC virus, and Adenovirus (Ad). Depending upon the immune competence of the recipient, reactivation of these viruses can range from asymptomatic viremia to overt disease. CMV, HHV6 and Ad can cause fatal pneumonias. HHV6 can cause cytopenias and encephalitis and Ad can cause fatal hepatitis. EBV reactivation (in the donor B cells) causes a potentially life-threatening post-transplant lymphoproliferative disorder (PTLD) and BK and JC virus cause hemorrhagic cystitis and progressive multifocal leucoencephalopathy respectively. In recent years treatment of viral complications after SCT has improved in part because of the introduction of new antiviral agents and in part from the preemptive use of antivirals at the onset of viremia, which has notably reduced mortality from CMV disease. The common human herpes virus is highly susceptible to acyclovir, and the antivirals foscarnet, ganciclovir and valganciclovir have efficacy against CMV and HHV6. Cidofovir is moderately effective at controlling Ad and the CD20 monoclonal antibody rituximab is usually effective at controlling EBV PTLD. Nevertheless viral reactivation still has unfavorable consequences for the recipient—despite treatment, patients can still die of refractory virus infection, and antivirals can cause renal failure, and cytopenias. Aside from the morbidity from viral reactivation are the economic burden of greater hospital inpatient days and the significant cost of antiviral therapy (1). There is therefore still a need to improve the management of reactivating viruses, both to reduce morbidity and mortality and also to reduce the cost of the transplant.

A logical approach to managing post transplant viral reactivation is to restore effective antiviral immunity with infusions of virus-specific T lymphocytes from the donor. Provided the donor is already immune to the virus it has been possible for many years to select and expand T lymphocytes recognizing viral antigens. Evidence that CMV-specific donor T-cell clones could transfer immunity

to CMV after SCT was first demonstrated in 1992 by Riddell, Greenberg and colleagues in Seattle (2). Infusions of lymphocytes exposed to CMV-infected fibroblasts cloned and expanded in culture were used to successfully treat patients with CMV reactivation. However this approach was cumbersome, lengthy, and not applicable beyond an investigational setting. Shortly after, Rooney and Heslop demonstrated similar successes using donor-derived EBV specific T cells (3,4).

Subsequent to the demonstration of these proofs of principle studies, a series of important conceptual and technical developments has led to clinically practical cell therapies to control of reactivating viruses. Most research has focused on T-cell therapy for reactivating CMV and EBV (5,6). An important achievement has been the generation of cells for infusion, free of alloreacting donor cells, without recourse to generating pure CMV-reactive T-cell clones. This can be achieved most simply by isolating CMV specific T cells from donor blood products by selecting CMV pp65 peptide activated T cells by interferon-gamma capture (7) or targeting T cells with CMV antigen bound to HLA class I multimers (8,9) but the technique is limited by HLA class I restriction of the selected T cells. In the absence of CD4 T-cell help, pure CD8 infusions persist poorly in the recipient (2).

An alternative approach to avoiding GVHD from T-cell infusions is to generate T-cell lines stimulated by viral antigens. During the culture period where virus specific T cells expand manyfold, the alloreactive capacity and risk of GVHD is lost. Over the last decade several groups have broadened the scope and clinical applicability of virus specific T-cell therapy to a point where the approach is more readily available for general adoption by the transplant community (6,9-11). Key developments in the development of practically applicable adoptive T-cell therapy are described below.

Development of multivirus specific CTL (EBV, Ad, CMV)

Since CMV, EBV, and Adv are the leading causes of viral-associated mortality in the post-SCT period, initial multi virus specific T-cell approaches focused on these three viruses. The development of the clinical grade adenovirus vector Ad5f35 expressing the immunodominant CMV antigen pp65 transgene, permitted transduction of donor-derived dendritic cells or EBV-transformed B cells (lymphoblastoid cell lines or LCL) as antigen presenting cells (APCs) to stimulate and expand multi virus specific T cells. In a dose-escalation study, 26 patients received T cells at doses ranging from 5×10^6 to 1×10^8 cells/m^2, without toxicity or GVHD (12). All patients who received the T cells as prophylaxis were protected against CMV, EBV, and Adv and the majority of patients with viral reactivations at the time of T-cell infusion cleared the virus(es) after a single dose of T cells without pharmacotherapy. A follow-up trial used Ad5f35-transduced LCL to produce bispecific T cells targeting EBV and Adv, which were infused into 13 patients as prophylaxis or treatment of EBV and Adv following SCT. All patients were protected against EBV and Adv but Adv-specific CTLs were not detectable except in the setting of Adv infection (13). Subsequently, Ad5f35pp65 transduced dendritic cells were used to produce CMV and Adv-specific CTLs, which were clinically effective in 12 patients who received infusions following SCT (14). The same group published a follow up report 50 patients following who received tri-viral (CMV, EBV, Adv-specific) CTLs post SCT. These T-cell products were generated either using DCs pulsed with the HLA-A2 restricted CMV peptide NLVPMVATV (n=10), or using Ad5f35pp65-transduced donor DCs as APC (n=40) to stimulate and expand virus specific T cells. After T-cell infusion, only five of the 50 patients developed CMV reactivation but only one of these 5 patients required pharmacotherapy for antiviral control (15).

Simplifying the manufacturing methodology

More recently, protocol advances have validated the use of either DNA plasmids or 15-mer peptide pools encompassing viral antigens to pulse APC, thereby avoiding the use of gene modified APC, thus removing the expense and the potential safety and regulatory barriers associated with use of viral vectors. Further, use of gas-permeable rapid-expansion (G-Rex) bioreactors has further simplified CTL culture. Gerdemann *et al*. combined two advances (transfection of DC with plasmids and the G-Rex culture expansion) to develop a rapid-protocol to generate T-cell products at clinically relevant numbers within only 10-12 days. These rapidly expanded T cells provided effective antiviral protection in 10 patients without GVHD following SCT (16).

The most recent study from the same group further modified this rapid protocol to produce 5-virus specific CTLs targeting EBV, CMV, Adv as well HHV6, and BK virus infections in a single T-cell product (17). Forty-eight

donor-derived multi virus specific T cells were generated. Fourteen of the lines recognized all five of the component viral targets, while 35 (73%) recognized three or more viruses as determined by IFN-ELISpot assay. These multi virus specific T cells were used to treat 11 patients following SCT. Three patients were treated prophylactically and remained free of viral infections. The remaining eight patients were treated for 18 viral reactivations, and all of them experienced partial or complete responses against CMV, EBV, Adv, BKV and HHV6. Only one patient failed to clear their virus infection, which was a BK virus infection in a patient who received a CTL line that did not display BK reactivity. Hence, these VSTs proved safe in all subjects and produced an overall 94% virological and clinical response rate that was sustained long-term.

Broadening applicability

Third-party CTL use

Until recently, the selection or culture of anti-pathogen CTLs was dependent on the presence of pathogen-specific memory T cells in donor blood. These protocols are however not applicable for recipients when the donors are pathogen-naïve or for recipients where it is not possible to go back to the donor to obtain blood for T-cell manufacture (e.g., umbilical cord blood). One answer to this problem is the use of "off-the-shelf" T cells derived from eligible third-party donors. Haque *et al.* were the first to validate this strategy, using partially matched EBV-CTLs for PTLD in the setting of solid organ transplantation and SCT. They demonstrated a response rate of 64% at 5 weeks and 52% at 6 months, with outcomes correlating to the degree of HLA match between the CTL donor and recipient. Similarly, the group at Memorial Sloan Kettering Cancer Center (MSKCC) successfully treated two patients with refractory EBV-PTLD following cord blood transplantation (CBT) with third-party EBV-specific CTLs (18). Most recently, the group at Baylor College of Medicine (BCM) utilized a bank of 32 CTL lines specific for EBV, CMV, and Adv and in a multicenter study treated 50 patients with refractory viral infections (19). Infusion of these multi virus third party CTL resulted in partial or complete anti-viral responses in 74%, 78%, and 67% of patients with CMV, Adv, and EBV respectively with minimal toxicity. This represented a dramatic improvement from the standard therapy response rate as seen in the eight patients for whom a matched line could not be found, who had a mortality rate of 75%. Based on this success, the groups at BCM, are now exploring the use of T cells targeting five viruses in the third party setting.

CTL manufacture from pathogen-naïve donors

One limitation of the third party approach is that the infused T cells fail to persist long term with maximal persistence documented at approximately 6 weeks post infusion. Therefore, in an attempt to infuse T-cell products from virus naïve donor sources, which will reconstitute long term antiviral immunity, several groups have shown that T cells targeting a single virus can be expanded from virus naïve donors including umbilical cord blood and adult seronegative donors (20-22). In an effort to broaden this approach, Hanley *et al.* demonstrated that multi virus specific T cells could be produced from the 20% fraction of cord blood units using donor-derived dendritic cells (DCs) and LCL transduced with the *Ad5f35pp65* vector as APC (23). The resulting cell lines had specific antiviral activity against CMV, EBV, and Adv and have been infused to 12 recipients of cord blood grafts with evidence of efficacy without toxicity in an ongoing trial (NCT00880789).

Future developments

The concurrent administration of antiviral T cells in the setting of immunosuppressive agents is a common event after SCT. In particular the use of steroids can render T-cell transfusions ineffective. The ability to confer resistance to steroids in adoptively transferred T cells would be a practical advantage step forward. For example T cells could be engineered to overexpress 11β-Hydroxysteroid dehydrogenases type 2 (11β-HSD2), which converts active GC, cortisol, to inactive cortisone, thereby inducing steroid resistance (24). Alternatively, glucocorticoid induced apoptosis in T cells could be reduced by blocking Nfil3 which lies downstream of the corticoid receptor (25).

An interesting observation of leukemic remission following administration of multi virus specific T cells (mVST) raises the possibility of a favorable cross-reactivity of antiviral T cells with malignancy. Further research to define the basis of this cross-reactivity is of interest in extending the therapeutic application of VST (26).

Finally, the pepmix approach to generating mVST is a robust technology whose limits have yet to be reached. Gerdemann and colleagues have already generated MVST recognizing seven viruses (27) and there is no obvious limit to the number of virus antigens that could be incorporated

in this technology, opening the way to targeting other viruses complicating SCT such as the respiratory syncytial virus, and influenza. Similar pepmix approaches are also being developed to target tumor antigens as a means to prevent or treat leukemia relapse after SCT (28) or even HIV (29). As the field advances it is therefore foreseeable that the use of virus specific T cells will become a powerful new therapy for virus infections not only for patients following allogeneic SCT but also for other at-risk immune compromised populations.

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