

* CHAPTER 14

Immunotherapy post-transplant

* 14.1 Immunotherapy post-transplant for infections

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1. Introduction

The main issue regarding immunotherapy of infections after allogeneic haematopoietic stem cell transplantation (HSCT) is human cytomegalovirus (HCMV) infection. Therefore, this Chapter will focus on the experiences made with immunotherapy for HCMV infection. However, the techniques described here have been applied to immunotherapy of other viral infections such as adenovirus and EBV infection.

2. HCMV infection after allogeneic stem cell transplantation

HCMV infection continues to be one of the most important and life threatening complications after allogeneic HSCT.

To improve T-cell immunity against HCMV and other infections in bone marrow transplant patients different strategies have been explored. In general, HCMV-specific T-cells can be selected from the donor, and be transferred to the patient either with or without *in vitro* expansion, or the HCMV-specific T-cells can be activated and expanded *in vivo* by stimulation with antigen presenting cells (APCs) loaded with specific proteins or peptides.

3. Isolation and infusion of HCMV specific CD8⁺ T-cells

Riddell et al. and Walter et al. have demonstrated that adoptive immunotherapy by transfer of HCMV-specific CD8⁺ T-cell clones into patients at risk of HCMV disease protected the patients from HCMV-related complications (1, 2). $1-2 \times 10^9$ HCMV-specific CD8⁺ T-cells were administered and these cells were detectable in patients' blood for at least 8 weeks. Patients were protected against HCMV disease although HCMV-specific CTLs declined progressively in patients who did not develop a concomitant HCMV-specific CD4⁺ T_H response.

Analyses using the new technologies developed for determining antigen-specific T-cells (e.g. intracellular cytokine staining or staining with tetrameric HLA Class I /peptide complexes) have contributed to a substantial improvement in our understanding of the role and function of immune responses *in vivo*. The use of peptide-HLA multimers facilitates the visualisation and isolation of antigen-specific CTLs (3). CD8⁺-T-cells that bind multimeric HLA complexes can be isolated to high purity using magnetic beads or FACS sorting (4–6). Newly developed multimeric HLA complexes, binding reversibly to the T-cell receptor, offer the opportunity of selecting unmanipulated antigen specific CTLs (7). Thus, phenotypical analysis with MHC-peptide multimers, functional assays as well as multimer-based enrichment protocols can now be used in the setting of adoptive T-cell therapy. The transfer of HCMV-specific CTLs freshly isolated from peripheral blood might be superior to

the *in vitro* expansion and manipulation of T-cells. The *in vitro* expansion may increase the expression of the pro-apoptotic FAS molecule (CD95) and reduce telomere length of specific T-cells, leading to a shorter survival of the adoptively transferred T-cells. In addition, the prolonged *in vitro* culture of T-cells is cumbersome when performed under GMP conditions (requirements for sterility, media, cytokines, serum, etc.).

4. Adoptive transfer of HCMV-specific CD4⁺ T_H-cells for the treatment of HCMV infection

The safety and efficacy of cellular therapy with HCMV-specific CD8⁺ lymphocytes in immunocompromised patients has been documented. These studies demonstrated the role of HCMV-specific CD4⁺ cells in maintaining an HCMV-specific CTL response. Several studies have outlined the significance of antiviral effector functions of T_H cells in maintaining CTL responses after adoptive transfer and their capacity to produce antiviral cytokines (8).

We therefore evaluated the infusion of HCMV-specific CD4⁺ T_H-cells to treat patients with HCMV viraemia resistant to antiviral chemotherapy after allogeneic HSCT (9). The patients enrolled in this study showed a documented lack of an HCMV-specific CD4⁺ TH and an HCMV-specific CTL response. We generated HCMV-specific T-cell lines for adoptive transfer by 4 repetitive weekly stimulations of donor lymphocytes with HCMV lysate *in vitro*. No side effects occurred during and after infusion of the cells, even in patients receiving their graft from a donor, mismatched in 1 to 3 HLA antigens with the stem cell graft recipient. Reconstitution of HCMV-specific CTL responses could be demonstrated following the transfusion of HCMV-specific CD4⁺ T-cell lines. Five out of 8 patients cleared the viral infection following a single infusion of HCMV-specific CD4⁺ T-cells, one other patient after a second infusion. These findings show that HCMV-specific adoptive immune transfer is a therapeutic option in patients with reactivated HCMV-infection after HSCT. Even the infusion of low numbers of CD4⁺ HCMV-specific T-cell lines was found to be successful in some of these patients.

Another issue which has to be addressed is the role of CD4⁺ T-cells in the direct control of infection. It has been shown that highly differentiated CD4⁺ T-cells, specific for HCMV pp65, mediate antiviral effector functions (10): HCMV-specific CD27⁻ CD4⁺ T-cells degranulate when they encounter a cognate antigen and the number of CD4⁺ T-cells which contain granzyme A, granzyme B and perforin raises during the maturation process of these cells. Furthermore, target cells bearing a distinct HCMV pp65-derived MHC Class II-restricted epitope were killed by CD4⁺ T-cells from an individual in which degranulation occurred in a subset of cells with a high frequency of perforin. Overall, it could be demonstrated that mature HCMV-specific

CD4⁺ T-cells have functional features which equal antiviral CD8⁺ T lymphocytes. Taken together, the transfer of HCMV-specific CD4⁺ T-cells is not only a promising option for providing helper cell support for pre-existing HCMV-specific CTLs but may also exert effector functions which directly contribute to a persistent virus control.

5. Infusion of HCMV-specific CD4⁺ and CD8⁺ T-cells

The opportunity to generate cellular products which contain both CD4⁺ and CD8⁺ arises from the provision of selection protocols based upon cytokine secretion. Our group has performed a study which avoids the restrictions in the generation of virus-specific T-cell lines for adoptive transfer into allogeneic HSCT recipients without affecting the function of the generated T-cell lines (11). The IFN- γ secretion assay, which conforms to current GMP-regulations, was used for the enrichment of virus-specific CD4⁺ and CD8⁺ T-cells. One important step to stimulate both cytotoxic and helper cells in one procedure was to evaluate the optimal stimulus. First, we compared HCMV-Ag only in eliciting a combined HCMV-specific CD4⁺ and CD8⁺ T-cell response to concomitant usage of HLA matched HCMV-specific MHC-I epitopes and HCMV-Ag. Stimulation of HCMV-specific CD4⁺ and CD8⁺ T-cells was similarly effective when using HCMV-antigen compared to the stimulation with HCMV-antigen and peptides, as assessed by intracellular cytokine staining (ICC).

The number of HCMV-specific T-cells required for an effective adoptive transfer and their composition in respect to CD4⁺/CD8⁺ ratio required for either prevention and/or treatment of HCMV-viraemia after allogeneic stem cell transplantation is not yet defined. The Seattle group and others demonstrated that adoptive transfer of up to $5 \times 10^9/m^2$ CD8⁺ T-cell clones could reconstitute HCMV-specific immune responses (1, 2). In our hands, the combined generation of HCMV-specific CD4⁺ and CD8⁺ cells resulted in an average of 1.3×10^8 HCMV-specific stimulated, selected and expanded T-cells from 8/8 randomly selected HCMV-seropositive donors in 10 days from one single 500 mL collection of blood, utilising feeder cells, media and cytokines compatible with current GMP regulations. The specificity of this GMP-grade product could be clearly demonstrated by detection of intracellular cytokine production after antigen-specific stimulus and lysis of HCMV-infected target cells by the HCMV-directed CTL lines. Furthermore, we were able to show that the generated HCMV-specific T-cells do not represent terminally differentiated CD4⁺ and CD8⁺ T-cells as stimulation led to several cell divisions as demonstrated by dilution of CFSE dye and a corresponding cell expansion. Thus, adoptive transfer of the generated T-cells into HSCT recipients may allow further *in vivo* expansion, if T-cells are stimulated by HCMV-Ag presenting cells *in vivo*.

6. Vaccination with HCMV peptide loaded DCs

Mature dendritic cells (DCs) are the most potent APCs, with the ability to initiate and boost primary immune responses. Mature DCs can be generated *in vitro* from peripheral blood monocytes by culturing in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 4 (IL-4) and tumour necrosis factor α (TNF- α). Vaccination with peptide pulsed DCs was shown to be feasible and effective in inducing tumour-specific T-cell responses and was able to induce regression of metastatic disease in a minority of patients.

In our trial (12) HCMV seropositive patients who underwent an allogeneic-HSCT with a graft from a HCMV seronegative donor received a vaccination with HCMV peptide loaded mature DCs in addition to the routinely applied pre-emptive antiviral chemotherapy. The DCs were pulsed with nonamer peptides from the HCMV protein pp65, restricted by the HLA-class I elements A1, A2, A3, A11, A68 and B7. The DC vaccination study was designed as a phase I/II trial. The primary objectives were to evaluate tolerability and safety. Therefore, the acute local and systemic side effects of the vaccination procedure were analysed as well as the induction or aggravation of acute or chronic graft versus host disease (GvHD) or any other autoimmune phenomenon. As a secondary objective, the efficacy of the DC vaccination was documented by analysing the HCMV specific T-cell response and the long-term control of HCMV infection. Because all the patients with HCMV infection or reactivation at the time of vaccination received additional anti-viral chemotherapy, the short-term control could not be analysed. Our DC vaccination trial recruited HCMV seropositive HSCT recipients, who are at a high risk for HCMV disease without a HCMV specific CTL response. Patients at high risk for HCMV disease were defined as patients with prolonged (more than 4 weeks) antiviral chemotherapy prior to day 100, patients receiving a T-cell depleted (*in vivo* by ATG or *ex vivo* by CD34 selection) graft and HCMV seropositive patients with a seronegative stem cell donor.

In all patients, DC vaccination was well tolerated without any local or systemic side effects. No induction or aggravation of acute or chronic GvHD was observed in any of the patients receiving DC vaccination for chemotherapy refractory HCMV infection. DC vaccination was shown to induce HCMV specific T-cell responses with antiviral activity even in a patient who was infected by a HCMV strain resistant to ganciclovir, foscavir and cidofovir. In conclusion, HCMV specific DC vaccination is a feasible and effective immunotherapy among recipients of an allogeneic haematopoietic stem cell transplant and will be further tested in a multicentre study.

7. Vaccination with recombinant modified vaccinia Ankara (MVA)

The usage of full-length antigens expressed from MVA is a powerful immunotherapeutic tool applicable regardless of an individual's HLA type. It has been shown that dual HCMV antigen pp65/pp150 expressing MVA can be strongly recognised *in vitro* by PBMC from CMV positive healthy subjects with HLA A*1101, HLA A*6801, HLA A*0301 and HLA B*0702 haplotypes. Furthermore, evaluation of immunogenicity indicates that pp65-IE1-MVA additionally can induce robust primary immune response to both antigens in HLA A2.1 transgenic mice (13, 14).

Since MVA has an exceedingly large capacity for foreign DNA, potentially multiple disease targets could be incorporated into one vaccine, thereby reducing manufacturing costs and dosing to patients.

Table 1: Treatment options for HCMV infection after allogeneic HSCT

Adoptive immunotherapy with HCMV-specific T-cells	Vaccination Based on Dendritic cells	Vaccination Based on Modified Vaccinia Virus (MVA)
Adoptive immunotherapy with HCMV specific cytotoxic T-cells has shown to be effective in improving the elimination of HCMV. A detailed summary of different strategies when performing adoptive immunotherapy is provided in Table 2	Study using HCMV-peptide pulsed dendritic cells to treat HCMV infections after allogeneic HSCT. This strategy is feasible and improves control of HCMV in allografted patients (12)	Preclinical studies have been performed and demonstrate the feasibility and efficacy of vaccinia-based HCMV vaccines in mice (13, 14)

8. Conclusions

Antigen specific T-cells are essential to the control of reactivation or primary infection with HCMV or other viral infections. Immunotherapy offers an attractive tool to improve immune reconstitution in these patients, leading to control of viral replication without apparent side effects. This may reduce the usage of potentially toxic antiviral chemotherapy (adverse effects such as myelo- or nephrotoxicity) and circumvent the increasingly reported problems of the development of antiviral drug resistance.

Stimulation and expansion conditions have to be improved to generate T-cell lines containing not only terminally differentiated effector cells but also central-memory T-cells, which are essential to build up a memory T-cell response in the recipient (15). Further controlled trials with adoptive transfer of virus-specific T-cells versus

Table 2: Clinical application of HCMV-specific immunotherapy

Year (Ref.)	Strategy	Results
Riddell S. 1992 (2)	Infusion of HCMV-specific cytotoxic T-cell clones	First trial that demonstrated a successful transfer of HCMV-specific CTL isolated from bone marrow donors, propagated <i>in vitro</i> , and adoptively transferred to immunodeficient bone marrow transplant recipients. The transfer was safe, and reconstitution of HCMV-specific CTL responses could be documented.
Riddell S. 1995 (1)	Infusion of HCMV-specific cytotoxic T-cell clones	Fourteen patients received intravenous infusions of HCMV-specific T-cell clones from their donors. In total, 56 infusions (4/patient) of HCMV-specific cytotoxic-T-lymphocyte clones were performed without any major side effects and no viraemia or HCMV disease was observed in any patients receiving adoptive immunotherapy.
Einsele H. 2002 (8)	Infusion of HCMV-specific polyclonal CD4 ⁺ T-cell lines	HCMV load dropped significantly in all 7 evaluable patients, with a maximal reduction after a median of 20 days (range, 5-31 days). Anti-HCMV cellular therapy was successful in 5 of 7 patients, whereas in 2 of 7 patients, who received an intensified immune suppression at the time of or after T-cell therapy, only transient reductions in virus load were obtained.
Mackinnon S. 2003 (6)	Adoptive cellular therapy with virus-specific T-cell lines	Polyclonal HCMV-specific T-cell lines were generated for the treatment of 16 patients. A massive <i>in vivo</i> expansion of HCMV-specific CTLs was observed leading to a recovery of virus-specific T-cell responses. 50% of the treated patients (8/16) did not require a further treatment with antiviral drugs.
Moss P. 2005 (5)	Direct isolation and infusion of HCMV specific CTL using HLA peptide multimeric complexes	Reduction of HCMV viraemia in all treated patients and a complete clearance of HCMV infection in 8 patients, including one patient who had a prolonged history of HCMV infection that was refractory to antiviral therapy. HCMV-specific CD8 ⁺ T-cells were detected in all patients within 10 d of infusion.

Table 3: Current projects**“Adoptive immune therapy of chemotherapy-refractory CMV-infection with Streptamer-selected T-cells after allogeneic bone marrow- or peripheral blood stem cell transplantation”***Correspondence to:*

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Although the adoptive transfer of *in vitro* expanded T-cells is effective its application has several drawbacks. It is very time consuming and laborious to prepare the high cell numbers needed for transplantation, especially since patients with an acute CMV-infection may not allow to wait for the preparation of the cells. In addition the costs of such a treatment are extraordinary high, raising the question if such a medication could be broadly introduced into clinical practice. Due to these drawbacks multimer-based direct isolation of antigen-specific T-cells has evolved. The use of low cell numbers without the need of time consuming *in vitro* expansion greatly improves the adoptive transfers of T-cells.

Even though this approach has advanced adoptive transfer protocols, multimer-based T-cell isolation still suffers from several disadvantages. The multimer reagents bind to the T-cell receptor during the isolation procedure and thus stimulate the respective T-cell. This stimulus is likely to cause apoptosis in the isolated cells, interfering with an effective T-cell transplantation. In addition the multimer reagents are transferred together with the T-cells to the patient, which may cause toxic or immunogenic side effects.

Recently the Streptamer technology was developed to overcome these problems (7). Streptamers are reversible multimers which are unlikely to interfere with T-cell function since Streptamer reagents can be rapidly dissociated from the T-cell receptor. In addition adoptively transferred cells do not contain Streptamer.

Primary endpoints of this study are acute infusion related toxicities, such as anaphylactic reaction, and the development of GvHD.

Secondary endpoints are the HCMV-load measured by quantitative PCR and the reconstitution of HCMV specific cellular immunity.

To assess the side effects (primary endpoint) of the treatment, all patients undergo the following examinations before and 1, 2, 4 weeks then monthly (up to 6 month) after transplantation: physical examination, Karnofsky-score, bodyweight, body size, surface area, clinical grading of GvHD (Seattle-scheme), serum chemistry, coagulation tests, and differential blood count.

To assess the efficacy of the adoptive T-cell therapy (secondary objective) the viral load and the frequency of the HCMV specific T-cells will be monitored before and 1, 2, 4 weeks then monthly (up to 6 month) after adoptive T-cell transfer.

continued

“Adoptive immunotherapy of chronic HCMV infection post allogeneic HSCT – DC vaccination”*Correspondence to:*

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Dendritic cell (DC) vaccination is a pathogen specific and highly immunostimulatory approach to fight different infections and malignancies, but has never been evaluated in the setting of allogeneic HSCT. The risk of GvHD still inhibits the use of immunostimulatory therapies such as DC vaccination.

We completed a phase I/II study including 24 allogeneic HSCT recipients at high risk for HCMV disease to analyse feasibility and efficacy of vaccination with HCMV peptide loaded DC. No acute side effects were observed and we could demonstrate a significant clinical benefit in comparison to our control group. Furthermore, an induction or expansion of HCMV-specific CTL could be observed in 5 patients after DC vaccination.

To evaluate efficacy of this vaccination strategy, we set up a consecutive study with the goal to vaccinate another 50 patients.

“Adoptive immunotherapy of chemotherapy-refractory CMV or EBV infection using CD4⁺ and CD8⁺ T-cells selected by cytokine-secretion assay”*Correspondence to:*

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Goals:

To assess toxicity and efficacy of cytokine-capture based selection of CMV- or EBV-specific CD4⁺ and CD8⁺ T cells in patients with refractory CMV/EBV infection after allo-HSCT.

pre-emptive or prophylactic antiviral drug administration are needed to define the role of cellular immunotherapy in the treatment algorithms of infections in the immunocompromised host.

Although availability of clinical grade reagents for the selection of antigen specific T-cells has largely improved in the last few years, we still need to learn more about the best composition of a cellular product used for adoptive transfer. There is still controversy about the benefit of transferring different T-cell subsets for adoptive immunotherapy: namely, should we transfer only CD4⁺ T-cells or CD8⁺ T-cells or a combination of both.

In addition, various methods of cell isolation and/or expansion result in different stages of T-cell senescence. Freshly isolated and specifically selected T-cells have greater expansion potential *in vivo* when compared to repetitive *in vitro* stimulated T-cells. In contrast specific stimulation *ex vivo* might be more effective in depleting alloreactive T-cells from the T-cell product.

In view of the many different methods of isolating and generating T-cells, future studies have to define the best isolation procedures, the optimal T-cell subpopulations to be used for antiviral T-cell therapy and the differentiation/activation stage or stages of specific T-cells to be preferentially applied. In addition we will have to define the optimal cell dose depending on viral load and immunosuppression of the patient for each of these different cell populations.

According to established protocols for the generation of sufficient numbers of donor-derived virus-specific T-cells for adoptive immunotherapy, the donor has to be antigen-experienced. Thus, alternative strategies for priming virus-specific T-cell responses are highly warranted.

Because no relevant adverse effects were observed in our first DC vaccination trial in allogeneic HSCT recipients and because induction and expansion of HCMV-specific T-cell responses leading to viral control was observed in some of the patients, we will further evaluate and try to improve the efficacy of DC vaccination post allogeneic HSCT in a larger cohort of patients.

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Mutiple Choice Questionnaire

To find the correct answer, go to <http://www.esh.org/ebmt-handbook2008answers.htm>

1. **Why might the transfer of "unmanipulated" virus-specific T-cells (without *in vitro* expansion) be more efficient in control of viral infection when compared to *in vitro* expanded cells?**
 - a) The *in vitro* expansion decreases the expression of the pro-apoptotic FAS molecule (CD95) and therefore leads to a shorter survival of the transferred cells
 - b) The telomere length of specific T-cells after *in vitro* expansion is reduced, possibly leading to a shorter survival of the adoptive transferred T-cells
 - c) *In vitro* expanded cells have a greater risk of inducing GvHD compared to directly selected cells
 - d) The TCR of the selected cells is blocked by the selecting agent

- 2. Which risk of adoptive immunotherapy post allogeneic SCT is potentially reduced by long term *ex-vivo* culture?**
- a) Acute transfusion reactions
 - b) GvHD
 - c) Contamination
 - d) Costs
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- 3. Which statement is correct regarding an MVA-based vaccines?**
- a) Multiple antigens can be included in one single vaccine
 - b) MVA soon will be the standard in immunotherapy post-transplant due to a high evidence level based on multiple randomised controlled trials
 - c) These vaccines could be offered only to a limited number of patients because only patients with distinct HLA haplotypes can be vaccinated
 - d) MVA has a small capacity for foreign DNA
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- 4. What is the relevance of HCMV-specific T_H cells in immunotherapy post-transplant?**
- a) HCMV-specific T_H cell responses are not necessary for the maintenance of an adequate pathogen-specific immune responses. Transfer of a CTL response is always sufficient
 - b) Mature HCMV-specific CD4⁺ T-cells have functional features which equal antiviral CD8⁺ T-lymphocytes and are important for maintenance of transferred CMV-specific CD8⁺ T-cells
 - c) T_H infusions have been demonstrated to cause severe transfusion related reactions
 - d) Generation of T_H cells is much more expensive and time consuming than generation of CD8⁺ clones
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- 5. Which *is not* a potential risk of the transfer of CMV-specific T-cells?**
- a) Alloreactivity
 - b) Increase in viral load
 - c) Transfer of fungal infections
 - d) Development of antiviral drug resistance