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Clinical-scale isolation of 'minimally manipulated' cytomegalovirus-specific donor lymphocytes for the treatment of refractory cytomegalovirus disease

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Abstract

Background aims. Reactivation of cytomegalovirus (CMV) after hematopoietic stem cell transplantation remains a major cause of morbidity despite improved antiviral drug therapies. Selective restoration of CMV immunity by adoptive transfer of CMV-specific T cells is the only alternative approach that has been shown to be effective and non-toxic. We describe the results of clinical-scale isolations of CMV-specific donor lymphocytes with the use of a major histocompatibility (MHC) class I peptide streptamer-based isolation method that yields minimally manipulated cytotoxic T cells of high purity. *Methods.* Enrichment of CMV-specific cytotoxic T lymphocytes (CTLs) was performed by labeling 1×10^{10} leukocytes from a non-mobilized mononuclear cell (MNC) apheresis with MHC class I streptamers and magnetic beads. Thereafter, positively labeled CMV-specific CTLs were isolated through the use of CliniMACS (magnetic-activated cell sorting), and MHC streptamers were released through the use of d-biotin. The purity of enriched CMV-specific CTLs was determined on the basis of MHC streptamer staining and fluorescence-activated cell sorting. *Results.* A total of 22 processes were performed with the use of five different MHC class I streptamers. The median frequency of CMV-specific CTLs in the starting apheresis product was 0.41% among CD3+ T cells. The isolation process yielded a total of 7.77×10^6 CMV-specific CTLs, with a median purity of 90.2%. Selection reagents were effectively removed from the final cell product; the CMV-specific CTLs displayed excellent viability and cytotoxicity and were stable for at least 72 h at 4°C after MNC collection. *Conclusions.* Clinical-scale isolation of "minimally manipulated" CMV-specific donor CTLs through the use of MHC class I streptamers is feasible and yields functional CTLs at clinically relevant dosages.

Key Words: *adoptive T-cell transfer, CliniMACS, CMV-specific T cells, MHC streptamer technology*

Introduction

Despite the advent of new antiviral drugs and improvements in immune surveillance protocols, cytomegalovirus (CMV) reactivation in immunocompromised patients remains associated with grave clinical complications and considerable morbidity

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and mortality (1–3). Administration of new antiviral drugs diminishes the risk of early-onset CMV disease after chemotherapy and hematopoietic stem cell transplantation (HSCT) but is frequently associated with substantial organ toxicity, for example, nephrotoxicity, the development of late-onset CMV disease (4–8), impaired immune reconstitution leading to fungal infections and bacterial sepsis (2,9) and, as a result of viral resistance, is often non-effective (4,10).

Because CMV reactivation arises from impaired CMV-specific T-cell immunity, various adoptive transfer protocols have been developed to restore cellular immunity against CMV (11–13).

The potential of adoptive transfer of CMV-specific cytotoxic T lymphocyte (CTL) clones to restore antiviral immunity after allogeneic stem cell transplantation was first shown by Ridell *et al.* (11).

This approach was based on selective propagation of human CMV-specific CTL clones from bulk donor lymphocytes *in vitro,* thus exploiting the benefits of donor lymphocyte infusion by transferring CMV-specific T cells contained within the graft while minimizing the risk of an acute graft-versus-host disease (aGvHD). Although this approach is effective, its application has several drawbacks. It is very time-consuming and laborious to prepare suitable cell numbers necessary for an effective restoration of CMV immunity. Patients with an acute CMV infection need immediate medical treatment within days, which does not comply with a protocol of *in vitro* propagation of T-cell clones for several weeks. Hence, such an *in vitro* approach is only effective when performed pre-emptively.

Because only a minority of immunocompromised patients have resistant CMV disease, and thus the majority of cell products will never be used, such an approach is by far too costly and labor-intensive to be broadly introduced into the clinic. Moreover, the potential risks for patients, including cross-contamination or introduction of infectious agents into the product during processing, must be considered, as well as the manufacturing process that qualifies the cell product as an advanced therapy medicinal product (ATMP) with all the regulatory complexities associated with this product rating.

To overcome these difficulties regarding the generation of antigen-specific T cells and its clinical use, various approaches have been established aiming for the direct *ex vivo* selection of virus-specific T cells on the basis of magnetic-activated cell sorting (MACS) technology.

The cytokine secretion assay (CSA) is based on the capture—by means of bi-specific antibodies—of cytokines, such as interferonγ, on the surface of the memory/effector T cells that are induced to produce and secrete cytokines on *in vitro* stimulation with the respective virus-peptides (14). Thus, after stimulation (4–6 h), virus-reactive T cells can be labeled selectively according to the bound cytokines and subsequently enriched by MACS (15,16). With the use of recombinant protein or peptide-pools consisting of overlapping peptides spanning an entire immunodominant protein, the CSA had already been successfully used to simultaneously enrich CMV-, Epstein-Barr virus–specific and adenovirus-specific CD4+ T-helper cells and CD8+ CTLs irrespective of human leukocyte antigen (HLA) restriction for therapeutic approaches. Yet, these enrichment protocols were typically followed by further *in vitro* culture (12,17–20).

The second approach for the enrichment of virus-specific T cells uses the direct labeling of antigen-specific T cells by means of multimeric peptide class I major histocompatibility (MHC) complex staining and subsequent sorting.

Tetrameric MHC class I peptide complexes are soluble complexes of four synthetic biotinylated β_2-microglobulin/MHC monomers loaded with an antigenic peptide and streptavidin. By use of multimerization, an exponential increase in avidity is created to generate stable binding of MHC tetramer

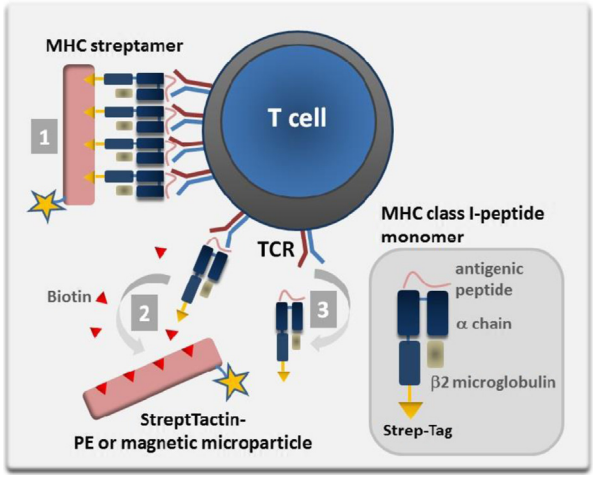

Figure 1. Schematic view of the reversible MHC streptamer technology. MHC class I peptide streptamer complexes are soluble complexes of four synthetic Strep-tag labeled β_2-microglobulin/MHC class I monomers loaded with an antigenic peptide. By use of multimerization with StrepTactin, an exponential increase in avidity is created to generate stable binding of MHC streptamers to its cognate TCRs (1). Labeling of StrepTactin, which functions as "backbone" for the streptamer complex, with fluorochromes or magnetic microparticles allows specific visualization and/or enrichment of antigen-specific CTLs in combination with FACS or MACS. The multimeric peptide MHC class I streptamers can easily be disassembled by the addition of d-biotin because the affinity of d-biotin to the Strep-tag binding sites significantly exceeds the affinity of Strep-tag to StrepTactin (2). Because of their low affinity, peptide-MHC class I monomers dissociate from their cognate TCR spontaneously within seconds, leaving "untouched" T cells (3).